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Evaluation of Dried Distillers Grains with Solubles as a Partial Replacement of Barley Silage or Barley Grain in Diets for Lactating Dairy Cows

By

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DEDICATION

This thesis is dedicated to my husband Zhenzhou Wu for his love and support.

ABSTRACT

Feeding value of dried distillers grains with soluble (DDGS) as an energy source for lactating dairy cows was evaluated in two studies. A diet in which barley grain was replaced by DDGS at 20% of dietary dry matter (DM) did not affect milk yield but tended to increase rumen pH compared with the control diet. Diets in which barley silage was replaced by DDGS at 20% of dietary DM increased milk yield and decreased chewing time compared with the control diet in both studies, but decreased rumen pH and milk fat concentration in the second study, and the inclusion of alfalfa hay in place of barley silage at 10% of dietary DM did not alleviate those depressions. In conclusion, DDGS can be used as an energy source as a partial replacement of barley grain or barley silage in diets for lactating dairy cows.

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LIST OF ABBREVIATIONS

BCS	body condition score
BW	body weight
CP	crude protein
DDGS	dried distillers grains with solubles
DG	DDGS partially replacing barley silage diet
DG+AH	DDGS and alfalfa hay partially replacing barley silage diet
DIM	days in milk
DM	dry matter
DMI	dry matter intake
ECM	energy corrected milk
EE	ether extract
LF	low forage diet
LG	low grain diet
MUN	milk urea nitrogen
NDF	neutral detergent fiber
NFC	non fiber carbohydrate
NE_L	net energy for lactation
NEm	net energy for maintenance
OM	organic matter
PEF	physically effective factor
TMR	total mixed ration
SEM	standard error of the mean
SFA	saturated fatty acids
UFA	unsaturated fatty acids

CHAPTER 1. LITERATURE REVIEW

1.1 Introduction

Dried distillers grains with soblubles (DDGS) is a by-product of the ethanol industry. There are varieties of feedstock used for ethanol production depending on the geographical location and the availability, such as corn, wheat, sorghum, and barley. Unlike the United States or eastern Canada where corn is primarily used as a substrate for ethanol production, the primary feedstock used in western Canada is wheat. However, as a result of the fluctuation in price of wheat, corn has been often partly mixed with wheat for ethanol production.

The booming ethanol industry has resulted in large amounts of pure wheat DDGS or the blend of wheat and corn DDGS as a feedstuff for animals in western Canada. The DDGS is high in crude protein, crude fat and digestible NDF content but low in starch content. Based on these characteristics, DDGS can be used as a partial replacement of protein feedstuff, forage or grain in diets for dairy cows. Although DDGS has been primarily used as a protein source, there are little data available about feeding value of DDGS as a partial replacement of forage or grain.

In this chapter, processes for DDGS production and its chemical composition as well as factors affecting the chemical composition are discussed. Secondly, feeding value of DDGS in dairy cattle is extensively reviewed. Use of DDGS may alter dietary concentration of starch, fat or forage NDF and these parameters affect milk fat concentration (Eastridge, 2009), which is an important component determining the milk price for dairy producers. In order to have a good understanding about the use of DDGS as a partial replacement of barley silage or barley grain, dietary factors affecting milk fat production are reviewed.

1.1.1 DDGS production

The basic process of ethanol production involves the enzymatic hydrolysis of starch to sugars and the fermentation of sugars to ethanol by yeast. The process from grain to ethanol is generally initiated with dry milling process. The clean grain passes through a grain milling system to be milled into fine powder and then is mixed with water and an amylase enzyme. The mixtures are cooked to liquefy starch. The mash from cooker is cooled and the secondary enzyme gluco-amylase is added to completely convert starch into sugars. Then yeast is added to the mash to ferment these sugars. The fermented mash passes through a distillation system where the ethanol is separated from the solids and water.

After the distillation of ethanol, the residue called whole stillage contains everything that is not fermented in the process. The whole stillage can be divided into two parts by centrifuge. The solid is wet distillers grains and may be dried. The thin stillage represents the soluble portion and can be condensed by evaporation to produce distillers solubles, which can be fed alone or added to the dried distillers grains to produce DDGS or added to the wet distillers grains to produce wet DGS.

1.1.2 The chemical composition of DDGS

The corn distillers solubles is high in CP (18.8%) and fat content (21%) but low in NDF content (5.3%) according to the data from previous studies (Cruz et al., 2005; Sasikala-appukuttan et al., 2008). Compared with corn distillers solubles, the fat concentration of corn distlillers grains is lower ranging from 8.5% (Schingoethe et al., 1999) to 15.4% (Ham et al., 1994) with an average of 12.5% on a DM basis. The CP concentration of corn distillers grains can be as high as 39.5% (Schingoethe et al., 1999) and averages at 31.4% on a DM basis. Similarly, the NDF concentration of corn distillers grains is also high ranging from 42% (Al-Suwaiegh et al., 2002) to 58% (Schingoethe et al., 1999) with an

average of 46.9% on a DM basis.

DDGS is the combined product of distillers solubles and distillers grains, therefore, the composition of DDGS is affected by the ratio of distillers solubles to distillers grains and the chemical compositions of the two by-products. In general, DDGS has almost three-fold greater concentrations of fiber, crude protein and fat than its original grain due to the removal of starch by the fermentation process (Widyaratne and Zijlstra, 2006). Fat concentration of corn DDGS ranges from 9.7% (Anderson et al., 2006) to 10.8% (Kleinschmit et al., 2006) with an average of 10% on a DM basis (NRC, 2001). The NDF concentration of corn DDGS is high and ranges from 32% (Anderson et al., 2006) to 44% (Kleinschmit et al., 2006) with an average of 38% (NRC, 2001). The CP concentration of corn DDGS ranges from 24.6% (Stein et al., 2006) to 34.4% (Anderson et al., 2006) with an average of 29.0% (NRC, 2001). Similar to the corn grain, corn DDGS has poor amino acid profiles and especially low in lysine concentration (Grings et al., 1992), which limits its extensive use as a protein supplement in diets for dairy cows.

Wheat DDGS has not been evaluated as extensively as corn DDGS. Based on the available research data, generally wheat DDGS is lower in fat concentration and higher in CP concentration than corn DDGS. The fat concentration of wheat DDGS ranges from 2.9% (Widyaratne and Zijlstra, 2006) to 9.9% (Penner et al., 2009) with an average of 5.0% on a DM basis. The CP concentration of wheat DDGS ranges from 34.0% (Emiola et al., 2009) to 45.8% (Gibb et al., 2008) with an average of 38.6%, and the lysine concentration is low (Widyaratne and Zijlstra, 2006). There is a large variation in the NDF concentration for wheat DDGS ranging from 25.9% (Dong et al., 1987) to 54.1% (McKinnon and Walker, 2008) with an average of 37.0%.

Nuez Ortin and Yu (2009) compared wheat DDGS, corn DDGS, and the

blend of wheat and corn DDGS. The results showed that wheat DDGS has the lowest (1.94 Mcal/kg DM) and corn DDGS (2.35 Mcal/kg DM) has the highest energy values (NE_{L3X}) while the energy value of blended DDGS (2.06 Mcal/kg DM; 70% wheat- and 30% corn-DDGS) is between the values of wheat- and corn-DDGS. The energy value of wheat DDGS is similar to corn grain while the energy value of corn DDGS is higher than corn grain, indicating both wheat DDGS and corn DDGS can be used as a partial replacement of corn grain in ruminant diets. Nuez Ortin and Yu (2009) also reported other differences between the characteristics of wheat DDGS and corn DDGS. Wheat DDGS is higher in fractions of non-structural carbohydrate (23.8 vs. 8.9% of DM) and rapidly degradable free sugar content (18.2 vs. 4.3% of DM), and higher in the CP degradability (90.0 vs. 68.1% of DM) but lower in NDF degradability (63.5 vs. 79.4% of DM) than corn DDGS when incubated in the rumen for 48 h.

1.1.3 Factors affecting chemical composition of DDGS

The chemical composition of DDGS varies greatly (Belyea et al., 1989 and 1998). Generally, the variation can be caused by the factors discussed below. First, the type of feedstock used for ethanol production affects the chemical composition of DDGS. In addition, the chemical composition may differ among varieties of the same type of grain. Rasco et al. (1987) observed different CP concentration of DDGS produced from soft white wheat Tyee and soft white Hill 81 with 19.6% and 38.4%, respectively. Secondly, the variation can result from the inconsistent processing such as temperature and duration of drying, and the amount of residual starch. During the drying process, protein can be severely damaged if it is overheated. Lysine is especially susceptible to heat damage because the ε-amino group can bind easily with the reducing sugars in a Maillard reaction. As a result, digestibility of amino acids in heat-dried DDGS was lower than the digestibility of freeze-dried DDGS, particularly for lysine

(Martinez-Amezcua and Parsons, 2007). Further, the nutritional value of DDGS can be greatly affected by the amount of distillers solubles added back to distillers grains (Martinez-Amezcua et al., 2007). Due to the variations above, the chemical composition of DDGS may differ greatly among ethanol plants or within plants (Spiehs et al., 2002). Therefore, it is necessary to analyze the chemical composition of different lots of DDGS before diet formulation.

1.2 The use of DDGS in diets of dairy cattle

In this section, corn DDGS is referred as DDGS unless it is specified otherwise. Use of DDGS can be divided into three categories: as a protein source, as an energy source and as a partial replacement of forage in diets for dairy cattle.

1.2.1 As a protein source

Effect on milk yield. Due to the high protein content, DDGS is commonly used as a dietary N source for dairy cows. Generally, milk yield was increased (Kleinschmit et al., 2006; Anderson et al., 2006) or not affected (Sasikala-Appukuttan et al., 2008; Leonardi et al., 2005a) when DDGS was fed as a partial replacement of corn and soybean meal (SBM) at 20% of dietary DM or less. The increase in milk production could be attributed to the higher RUP content (Anderson et al., 2006) of DDGS, the increased dietary energy density (Kleinschmit et al., 2006), or the increase in DMI (Nichols et al., 1998; Owen and Larson, 1991). But the response of milk production cannot be always attributed to the increase in DMI. In some studies, milk yield was increased for cows fed DDGS even if DMI was not affected (Cruz et al., 2005; Anderson et al., 2006). The DMI was decreased by feeding wet distillers grains at 30% of dietary DM (Schingoethe et al., 1999; Birkelo et al., 2004), but the milk yield was still maintained. In contrast, the milk yield was decreased with the reduction of DMI when up to 35% DDGS was included in the diet, which could be associated with low digestibility of the diet (Owen and Larson, 1991).

The reduction in milk yield by feeding DDGS diets may also be partly attributed to its poor amino acid profiles (especially the low lysine concentration) or the increased unavailable protein (ADIN) as a result of heat-damage (Van Horn et al., 1985). The greater ADIN content was associated with poor animal performance; milk yield was 0.85 kg/d less for cows fed the DDGS with 21% ADIN than those fed the DDGS containing 13% or 17% ADIN (Powers et al., 1995).

Effect on milk fat. In most studies (Nichols et al., 1998; Leonardi et al., 2005a; Birkelo et al., 2004), concentration of milk fat was not affected by partially replacing corn and soybean meal with DDGS while the milk fat yield increased as a result of the higher milk yield. Milk fatty acid profiles showed that concentrations of short and medium chain fatty acids decreased and concentrations of long chain fatty acids increased. As a result, there was no overall difference in milk fat concentration (Leonardi et al., 2005a). In contrast, Abdelqader et al. (2009) reported decreased milk fat concentration by feeding DDGS in place of soy hull and high-protein dried distillers grains in diets. This reduction was attributed to the inhibition of de novo fatty acids synthesis by *trans*-10, *cis*-12 CLA in the mammary gland.

Effect on milk protein. Feeding DDGS often decreased milk protein concentration (Kleinschmit et al., 2006; Schingoethe et al., 1999). This is possibly attributed to heat damage during the drying process, deficiency of lysine, or reduced microbial protein synthesis due to deficiency in rumen RDP. Lower concentrations of milk urea nitrogen (MUN) and rumen NH_3-N were observed in some studies (Kleinschmit et al., 2006; Sasikala-Appukuttan et al., 2008) and attributed to the lower RDP content of DDGS than SBM (NRC, 2001).

Effect on rumen fermentation. Total VFA concentration was lower (Kleinschmit et al., 2006; Owen and Larson, 1991; Schingoethe et al., 1999) when

DDGS was used to replace corn and SBM in diets for dairy cows. This might be caused by the reduced amount of starch when corn grain was partially replaced by DDGS. Less concentration of acetate (Sasikala-Appukuttan et al., 2008; Cruz et al., 2005) might have resulted from the inhibitory effects of high concentrations of long chain unsaturated fatty acids on fiber digestion (Palmquist and Jenkins, 1980). There was no difference in rumen fermentation between cows fed distillers grains with solubles in the form of dry or wet (Al-Suwaiegh et al., 2002).

It was concluded that DDGS is effective as SBM as a dietary protein source at maintaining milk production of dairy cows when the dietary inclusion rate is less than 20%.

1.2.2 As an energy source

The high energy value of DDGS (Nuez Ortin and Yu, 2009) indicated that DDGS could be an alternative to grain as a dietary energy source for dairy cows. But there were limited studies to evaluate the feeding value of DDGS relative to grain as an energy source. Grings et al. (1992) linearly increased the dietary inclusion of DDGS (0, 10, 20 and 30%) in place of corn grain until it was totally replaced in the diets. Feeding more DDGS in the diets increased the dietary CP concentration from 13.9 to 20.3%, and increased both protein and energy intakes. The yields of milk and milk protein were increased linearly with the increasing dietary inclusion of DDGS. Although in this study DDGS was used to replace corn grain, it was the main source of dietary protein due to the high CP concentration of DDGS and the absence of other high-protein feedstuffs in the diets. As such, the greater milk yield can be attributed to greater dietary protein concentration independent from effects of feeding DDGS.

In contrast, Penner et al. (2009) compared the effects of DDGS to barley grain with similar dietary protein concentration across the experimental diets but DDGS replaced canola meal and soybean meal more than barley grain in the diets.

Therefore, no studies in the literature evaluated the specific effects of DDGS as a partial replacement of grain as an energy source in diets for dairy cows.

1.2.3 As a partial replacement of forage

Use of DDGS as a partial replacement of forage is of interest due to the high NDF content of DDGS. As the particle size of DDGS is smaller than that of forages, DDGS was low in physical effectiveness at stimulating chewing. Clark and Armentano (1993) compared the effectiveness of NDF from DDGS with that from alfalfa haylage (AH) for cows in mid-lactation. The AH diet consisted of 43.6% haylage and 56.4% concentrate mix on a DM basis. The DDGS diet consisted of 30.9% haylage, 12.7% DDGS, and 56.4% concentrate mix on a DM basis. The diets were similar in dietary CP and fat concentrations. Replacing alfalfa haylage with DDGS increased DMI, which might be caused by the smaller particle size (Kononoff et al., 2003) of DDGS diet but it was not determined in their study. Cows fed the DDGS diet increased yields of milk and milk protein by 1.90 and 0.09 kg/d, respectively, compared with those fed the AH diet, which could be attributed to the higher DMI and dietary energy availability for milk protein synthesis. The milk fat concentration was not affected by treatment with the average of 3.29%, but milk fat yield was higher (1.04 vs. 0.99 kg/d) for cows fed the DDGS diet than the AH diet due to the higher milk yield. These results suggested that DDGS can be used to substitute alfalfa haylage without negatively affecting milk production.

However, the total chewing time (651 vs. 757 min/d) was decreased by feeding the DDGS diet compared with the AH diet. Also molar proportion of acetate was decreased (61.6 vs. 66.3 mol/100mol) and molar proportion of propionate was increased (22.9 vs. 18.5 mol/100mol) by feeding the DDGS diet compared with the AH diet. The decreased chewing time indicated that DDGS was poor in physical effectiveness at stimulating chewing activity as compared to

alfalfa haylage and thereby altered rumen fermentation.

Janicek et al. (2008) substituted DDGS for both forage and concentrate ingredients in diets for dairy cows in early lactation. The control diet consisted of 50% forage and 50% concentrates. Four experimental diets varied in DDGS inclusion (0, 10, 20, and 30%). The DMI increased linearly (22.4, 23.0, and 24.0 vs. 21.4 kg/d) with increasing dietary inclusion of DDGS compared with the control diet. The proportion of fine particles (< 1.18 mm) was greater for the DDGS diets (59.4, 63.8, and 68.3%, respectively for 10, 20, and 30% DDGS diets) than the control diet (54.8%). The milk yield was also increased (27.4, 28.5, 29.3, and 30.6 kg/d) as the dietary inclusion of DDGS increased. The concentrations of milk fat and milk protein were not affected by treatment, averaged at 3.66% and 3.17%, respectively, but the yields of milk fat and milk protein were linearly increased as a result of the higher milk yield. The MUN concentration was lower with greater dietary inclusion of DDGS, which might reflect the lower RDP content of DDGS. However, DDGS replaced concentrates more than barley silage in their study. Additionally, this study did not evaluate effects of partially replacing forage with DDGS on rumen fermentation and chewing activity, which would be very likely affected by the reduced dietary particle size (Kononoff et al., 2003).

Penner et al. (2009) found that feeding wet blend of corn and wheat distillers grains in place of barley silage at 10% of dietary DM had no effect on DMI. But the treatment increased yields of milk and milk protein without affecting milk fat yield. However, the milk fat concentration and chewing time were decreased by treatment. This result indicated that the physical effectiveness of NDF from DDGS is lower at stimulating chewing than NDF from barley silage. The authors suggested that it may increase the risk of rumen acidosis when DDGS was used as a partial substitution of forage.

1.3 Factors affecting milk fat production

The partial replacement of barley silage or barley grain with DDGS affects dietary concentration of fat, starch, and physical effective fiber, and these factors are related to milk fat production. Therefore, the effects of dietary fat, starch and physical effective fiber on milk fat are to be reviewed.

1.3.1 Dietary fat

It is a common practice to increase the dietary energy density by adding fat in diets for lactating dairy cows. Dietary fat supplementation often affects DMI (NRC, 2001). Fat supplementation at 2% and 4% of dietary DM increased the dietary fat concentration from 3% of control diet to 5% and 7%, respectively (Onetti et al., 2001). In the study of Onetti et al. (2001), cows in mid lactation fed the high fat diets had lower milk yield and milk fat concentration compared with those fed the control diet. The decreased milk yield was likely attributed to the reduction in DMI (Delbecchi et al., 2001; Onetti et al., 2001) or the tendency of reduced digestibility of nutrients, which is associated with the higher dietary fat content (Khorasani et al., 1992). In contrast, milk yield of cows in early lactation was increased by supplementing whole roasted soybeans, which increases dietary fat concentration from 2.8% to 6.8% (Knapp and Grummer, 1991). The greater milk yield for high fat diets might be attributed to the higher dietary energy density with no negative effects on DMI (Knapp et al., 1991).

There are two main sources of fatty acids in milk; one is de novo synthesis in the mammary gland and the other is pre-formed fatty acids from the blood circulation. The fatty acids with chain length of 4 to 14 primarily derive from de no synthesis. Fatty acids with chain length greater than 16 primarily derive from the blood circulation. The C16 originates from either source (Grummer, 1991). The concentration and yield of milk fat was not affected by feeding protected canola seed (Delbecchi et al., 2001; Khorasani et al., 1991).

These studies observed concentrations of long chain fatty acids in milk increased but concentrations of short and medium chain fatty acids decreased for cows fed the high fat diets, which may be attributed to the inhibition of de novo fatty acid synthesis or the dilution by increased pre-formed long chain fatty acids from the dietary source. Onetti et al. (2001) reported both concentration and yield of milk fat were decreased by supplementing fat in diets. Concentration of *trans*-10 C18:1 in milk increased, which was often associated with milk fat depression and may be a marker for altered rumen biohydrogenation pathway (Lock et al., 2007). But no difference was observed in the concentration of *trans*-10, *cis*-12 CLA, which was identified as a potent inhibitor of milk fat synthesis (Peterson et al., 2003; Lock et al., 2007). In contrast, Abdelqader et al. (2009) found decreased milk fat concentration with the addition of DDGS, which was attributed to the inhibition of de novo fatty acid synthesis by *trans*-10, *cis*-12 CLA.

Griinari et al. (1998) proposed that there were two conditions for milk fat depression: reduction in rumen pH and presence of unsaturated long chain fatty acids. Low rumen pH allows the accumulation of biohydrogenation intermediates in the rumen, which may inhibit milk fat synthesis. Therefore, the inconsistent effects of dietary fat on milk fat synthesis may be attributed to factors affecting rumen pH as discussed in the following section.

1.3.2 Dietary starch

Starch is the major energy source for lactating dairy cattle. The starch fermentability in the rumen may affect milk fat production. Herrera-Saldana et al. (1990) compared five cereal grains and ranked the degradability of starch in the rumen as following: oats > wheat > barley > corn > sorghum. Corn and barley are most commonly used as animal feedstuffs, and many studies have compared corn and barley grains in diets for dairy cows. Milk fat concentration or yield was decreased when cows were fed barley in place of corn (Casper et al., 1990;

Khorasani et al., 1994; Silveira et al., 2007a), but other studies found no difference in milk fat concentration (Bilodeau, 1989; Casper and Schingoethe, 1989).

The inconsistent effects of grain type on milk fat production may be explained by different dietary starch concentrations. Some studies reported that starch concentration of diet had no effect on milk fat concentration (Beauchemin et al., 1997; Silveira et al., 2007b: Cabrita et al., 2007). In those studies, treatment had no effect on milk yield either. Oba and Allen (2003a) found decreased milk fat concentration when cows were fed high starch diets (31% of DM) versus low starch diets (21% of DM). In this study, cows fed high starch diets had greater milk yield, which might account for the lower milk fat concentration by a dilution effect.

In the rumen, microbial attachment is the first step to digest starch in grain. Microbes have to overcome obstacles of seed coat, protein matrix surrounding starch granules, and crystallized starch granules. Therefore, whole grain needs to be processed prior to feeding in order to improve its utilization by animals. The process of ensiling high moisture corn (HMC) exposes the grain to heat, moisture, and pressure that degrades the endosperm structure to be in a semicrystalline arrangement. As a result, the HMC increased starch digestibility (Oba and Allen, 2003b; Krause and Combs, 2003; Krause et al., 2002) compared with dry corn. The milk fat concentration was either reduced (Krause and Combs, 2003) or not affected (Krause et al., 2002) by replacing dry corn with HMC. The inconsistent response may be attributed to the difference in dietary starch concentration: 36.7% (Krause and Combs, 2003) and 27.3% (Krause et al., 2002), respectively. Oba and Allen (2003a) demonstrated that milk fat concentration was decreased by replacing HMC for dry corn in high starch diets (31%) but not affected in low starch diets (21%).

Physical processing can breakdown the pericarp and decrease the particle size of grain. In the review of Dehghan-banadaky et al. (2007), milk fat concentration was reduced for cows fed pelleted grains versus the control diet, which was attributed to the higher rate and extent of starch digestion in the rumen. In contrast, in some studies, milk fat concentration was not affected by feeding grain with increased starch fermentability (Yang et al., 2000; McGregor et al., 2006). The inconsistent effect may be attributed to the differences in dietary forage allocation as there was an interaction between grain processing and dietary forage allocation for milk fat concentration. Yang et al. (2001) reported that the decline in milk fat concentration by feeding processed grain was greater for cows fed the diets containing 25% forage (from 3.89 to 3.69%) compared with the diets containing 55% forage (from 3.99 to 3.86%). In addition, processing method affects animals' response in milk fat production. Compared with dry rolled barley grain, feeding pelleted barley grain decreased milk fat concentration and milk fat yield of cows in mid lactation (Gozho and Mutsvangwa, 2008). This was probably because the pelleted barley grain was processed more extensively and starch was more rapidly fermented in the rumen than dry rolled barley grain.

1.3.3 Dietary physically effective fiber

Physically effective NDF (peNDF) is a term to describe the physical characteristics of NDF that affect the ability of a diet stimulating chewing activity (Mertens, 1997), which integrates particle size and dietary NDF content. The peNDF content is affected by the forage NDF content and particle size of diet.

Forage NDF. Feeding less forage NDF is expected to reduce rumen pH and milk fat concentration (Allen and Grant, 2000), which may be a result of inadequate amount of physically effective NDF needed to maintain chewing activity for cows fed a low forage diet. Milk fat concentration of dairy cows increased from 3.45 to 3.82% as dietary forage ratio increased from 35 to 60%

(Yang and Beauchemin, 2007). Feeding less forage NDF had a greater negative effect on milk fat concentration for cows in late lactation than those in early lactation (Kennelly et al., 1999; Khorasani and Kennelly, 2001) possibly because cows in early lactation can mobilize their body fat to meet the demand for milk fat synthesis.

Dietary particle size. Particle size of forage affects physical effectiveness of forages at stimulating chewing. The decreased forage particle size decreased chewing time, rumen pH, and milk fat concentration (2.90 vs. 3.07%; Krause and Combs, 2003). However, some studies found no difference in milk fat concentration for cows fed diets with decreased forage particle size (Krause et al., 2002; Kononoff and Heinrichs 2003; Yang and Beauchemin, 2007). The lack of response to shorter forage particles suggested that those diets provided sufficient fiber for dairy cows to maintain milk fat production.

There was an interaction between the forage particle size and source of forage for milk fat concentration. The decrease in milk fat concentration tended to be greater for cows fed the diets containing mixture of corn silage and alfalfa silage than the diets based on alfalfa silage only (Krause and combs, 2003). The higher starch content of corn silage possibly exacerbated the negative effect of decreasing forage particle size on milk fat synthesis.

Use of non-forage fiber sources (NFFS). The characteristics of NFFS are high in NDF concentration (NRC, 2001) with 47.3%, 30~40%, 66.6%, 48.3% and 38% for beet pulp, corn gluten feed, soybean hull, whole cotton seed hull and DDGS, respectively. Because of high NDF concentration, NFFS is often used as a partial replacement of forages in diets for dairy cows. However, compared with forages, NFFS has a shorter particle size and higher specific gravity resulting in shorter retention time in the rumen (Allen and Grant, 2000).

When NFFS was used as a partial replacement of forages in diets for

lactating dairy cows, generally milk fat concentration was depressed (Boddugari et al., 2001; Weidner and Grant, 1994) possibly due to decreased chewing time and rumen pH (Boddugari et al., 2001; Harvatine et al., 2002). However, those responses were not consistent.

In the study conducted by Kononoff and Heinrichs (2003), cows in early lactation were fed control diet containing 57% corn silage. When corn silage was replaced by cottonseed hull at 7.8% of dietary DM, the proportion of fine particles in the diet increased. But, the reduction in dietary particle size did not affect rumen pH or milk fat concentration, which may be a result of the higher NDF intake. The effects of wet corn gluten feed (WCGF) as a partial replacement of alfalfa haylage was evaluated by Allen and Grant (2000) using cows in early lactation. The control diet contained 65% alfalfa silage and the replacement of alfalfa silage with WCGF at 25% of dietary DM increased milk yield with no effect on milk fat concentration. However, cows fed the WCGF diet spent less time chewing compared with those fed the control diet due to poor physical effectiveness of WCGF, and the rumen pH was also reduced by feeding the WCGF diet.

Reduction in particle size of TMR by partially replacing forages with NFFS likely decreases chewing time but not necessarily decreases rumen pH and milk fat concentration. The discrepancy may be explained by the amount of forages replaced by NFFS. In the basal diet containing 54% silage, Boddugari et al. (2001) linearly increased the dietary inclusion of WCGF (8, 16, and 24% of dietary DM) as a partial replacement of silage mixture (50% corn silage and 50% alfalfa silage) to determine the optimum amount of WCGF in the diets. Replacing forage with WCGF at 24% of dietary DM decreased ruminating time and milk fat concentration, but not at the lower inclusion rates, indicating that the diets maintained physical effective NDF to some extent but failed to provide the critical

amount beyond a certain point.

The results of previous studies indicate that the physical effectiveness of NFFS at stimulating chewing is not high compared with that of forage NDF, but does not necessarily decrease rumen pH and milk fat concentration. Milk fat concentration may be reduced only when the supply of physically effective NDF is lower than a critical minimum threshold.

Because NDF is highly digestible, NFFS can also replace concentrate as an energy source. Several studies have been conducted to investigate the effects of feeding NFFS in low forage diets for lactating dairy cows. In the basal diet containing 30% haylage (Clark and Armentano, 1993), feeding DDGS, whole cottonseed or alfalfa hay in place of corn and soybean increased milk fat concentration from 3.16% for cows fed the basal diet to 3.27%, 3.34%, and 3.30% for cows fed DDGS, whole cottonseed and alfalfa hay diets, respectively. However, chewing time was increased only by feeding alfalfa hay and whole cottonseed but not by feeding DDGS. In another study (Allen and Grant, 2000), in which the basal diet contained 40% alfalfa silage, milk fat concentration was 2.90% for cows fed the basal diet. Feeding wet corn gluten feed in place of corn, soybean and soy pass at 25% of dietary DM did not increase chewing time or milk fat concentration. In contrast, feeding alfalfa silage at 25% of dietary DM increased chewing time and milk fat concentration to 3.25%.

These results suggested that NFFS may not increase physically effectiveness of diets at stimulating chewing activity compared with that of forages, but may increase milk fat concentration. When NFFS was used as a partial replacement of concentrates, generally milk yield and milk fat concentration was maintained (Boddugari et al., 2001; Voelker and Allen, 2003) or increased (Mansfield et al., 1994). Soy hull can replace corn grain at 30% of dietary DM (Ipharraguerre and Clark, 2003) to supply energy without negatively

affecting milk production. As mentioned before, DDGS can be used as a partial replacement of corn grain and soybean meal at 20% of dietary DM with maintained or increased milk yield. The NFFS serves as a good source of energy for high producing dairy cows due to the highly digestible NDF content or high ether extract content (DDGS or cottonseed). In addition, feeding NFFS in place of grain reduces the risk of rumen acidosis (Stone, 2004) because of the lower starch content for NFFS compared with grain.

1.3.4 Sorting behavior

Cows prefer to sort for grain component and against long feed particles of TMR (Leonardi and Armentano, 2003). Such behavior can lead to less fiber intake than the amount expected and increased the risk of rumen acidosis (Stone, 2004). Cows fed long alfalfa hay diet had lower milk fat concentration (2.96 vs. 3.17%) compared with those fed short alfalfa hay diet (Onetti et al., 2004), which may be a result of sorting against long particles.

It was demonstrated that cows sorted for fine particles and against long particles to a greater extent when cows were fed a low forage diet (50.7%) compared with those fed a high forage diet (62.3%; DeVries et al., 2007). This might be because there was more concentrate in the low forage diet. Another possibility was due to the higher DM content of a low forage diet compared with a high forage diet. Leonardi et al. (2005b) reported that sorting against long particles was reduced by adding water to decrease dietary DM content from 80.8 to 64.4%. In this study, the diet contained 30% hay which was easily sorted. The reduced sorting against long particles tended to increase NDF intake and milk fat concentration without negative effects on milk production. In contrast, for the diet containing 54% haylage, addition of water to decrease the dietary DM content from 57.6 to 47.9% did not reduce sorting against long particles (Miller-Cushon and DeVries, 2009). These inconsistent responses imply that addition of water

affects sorting activity to a greater extent if a basal diet is drier and sorted easily.

Frequent feed delivery (i.e., twice per day vs. once per day) reduces the extent of sorting (DeVries et al., 2005). As such, increasing feeding frequency from 2 to 4 daily may increase milk fat concentration or milk fat yield (Yang and Varga, 1989; Shabi et al., 1999).

1.4 Summary

Feeding value of corn DDGS relative to SBM in diets for dairy cows has been well documented. The high CP concentration and the low degradability in the rumen (Firkins et al., 1984) have made DDGS as an alternative protein feed to partially replace SBM in diets for dairy cows. However, poor amino acid profiles of DDGS, especially the low lysine concentration (Grings et al., 1992), and the risk of heat-damaged protein (Van Horn et al., 1985) limited the inclusion of DDGS up to 20% of dietary DM as a protein source in diets for lactating dairy cows.

The DDGS can be used as a partial replacement of forages in diets for lactating dairy cows, but particle size of DDGS is shorter than forages thus low in physical effectiveness (Clark and Armentano, 1993). Dairy cows require adequate physically effective fiber to stimulate chewing activity. Because the chewing activity stimulates secretion of saliva to help buffer fermentation acids produced in the rumen, risk of rumen acidosis is reduced (Krause et al., 2002). Feeding value of wet distillers grains as a partial replacement of barley silage was evaluated for dairy cows (Penner et al., 2009), but wet feed is not convenient to transport and store. Consequently, it can be used locally only by animal producers who are close to an ethanol plant. In contrast, DDGS can be used more widely because it is easier to transport and store. Therefore, research is warranted to evaluate the effects of partially replacing forages with DDGS in diets for lactating dairy cows.

High fat content and the highly digestible NDF of DDGS (Getachew et al., 2004) encourage the use of DDGS as an energy source or as a partial replacement of grain in diets for dairy cows. DDGS was fed as a replacement of grain in a previous study (Grings et al., 1992). But, effect of feeding DDGS was confounded by different dietary CP concentration. Therefore, further research is warranted to evaluate effects of partially replacing barley grain with DDGS on productivity of dairy cows using iso-nitrogenous diets.

Feeding DDGS as a partial replacement of barley silage or barley grain would be encouraged if it increases the profitability of dairy operations. Therefore, an economic analysis is conducted based on feeding cost and milk income in Chapter 4.

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CHAPTER 2. EFFECTS OF PARTIALLY REPLACING BARLEY SILAGE OR BARLEY GRAIN WITH DRIED DISTILLERS GRAINS WITH SOLUBLES ON RUMEN FERMENTATION AND MILK PRODUCTION OF LACTATING DAIRY COWS[*]

2.1 Introduction

Dried distillers grains with solubles (DDGS) is high in CP concentration and has been commonly used as a dietary protein source for lactating dairy cows. In addition to the high CP concentration, DDGS is also high in NDF content ranging from 32% (Anderson et al., 2006) to 44% (Kleinschmit et al., 2006) with an average of 38% (NRC, 2001). Due to the high NDF content, DDGS may be used as a partial replacement of forage for ruminants. However, physical characteristics such as small particle size and high particle density result in a lower physical effectiveness compared with forages (Clark and Armentano, 1993). There are currently a limited number of studies evaluating the potential of using DDGS as a partial replacement for forage. Penner et al. (2009) reported an increase in milk production for dairy cows fed wet wheat/corn distillers grains as a partial replacement of barley silage, but also reported decreased milk fat concentration and total chewing activity. These data imply that partial replacement of barley silage with distillers grains may predispose cows to rumen acidosis. However, to our knowledge there is no study examining ruminal fermentation when DDGS is included as a partial replacement of forage in diets for dairy cows.

In addition to a high NDF concentration, the NDF from DDGS is highly digestible (Getachew et al., 2004), and the NE_L value of DDGS is high; 1.94 and 2.35 Mcal/kg DM for wheat- and corn-DDGS, respectively (Nuez Ortin and Yu, 2009). As such, DDGS may serve as an energy source partially replacing grain in

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diets for lactating dairy cows. In addition, as starch concentration of DDGS is lower than grain, partially replacing grain with DDGS in diets for lactating dairy cows is expected to decrease the risk of rumen acidosis. Use of DDGS as a substitute for corn grain was studied in a previous study (Grings et al., 1992). However, effects of feeding DDGS were confounded by different dietary CP concentration in their study. There is little data available to assess the feeding value of DDGS as an energy source for dairy cows.

The objective of this study was to evaluate the effects of partially replacing barley silage or barley grain with DDGS on DMI, milk yield and milk composition, chewing activity and rumen fermentation of lactating dairy cows.

2.2 Materials and methods

2.2.1 Animals, diets and experimental design

This experiment was conducted at the Dairy Research and Technology Center at the University of Alberta. All procedures were pre-approved by the Faculty Animal Policy and Welfare Committee at the University of Alberta and conducted according to the guidelines of the Canadian Council of Animal Care (Ottawa, Ontario, Canada).

Six multiparous lactating Holstein cows, each fitted with a ruminal cannula, were used. Cows were blocked by stage of lactation, and assigned to one of three dietary treatments in a replicated 3 × 3 Latin square design balanced for carryover effects. Stage of lactation was used as a blocking variable because three cows were in mid lactation (76 ± 26 DIM; 605 ± 49 kg of BW) and the other three cows were in late lactation (244 ± 41 DIM; 726 ± 67 kg of BW). Each period consisted of a 15-d diet adaptation period and a 6-d data and sample collection period. The treatments were control (**CON**: 45% barley silage, 5% alfalfa hay, and 50% barley-based concentrate mix), low forage (**LF**) and low grain (**LG**) diets, in which barley silage or barley grain was replaced by DDGS at 20% of dietary DM,

respectively (Table 2.1). Experimental diets contained different amounts of canola meal, corn gluten meal, beet pulp and urea as an effort to make experimental diets iso-nitrogenous. The DDGS was produced from 70% corn and 30% wheat (Husky Energy, Lloydmister, SK, Canada). The same batch of DDGS was used throughout the study. Diets were formulated according to NRC (2001) to meet the nutritional requirements for a 670 kg cow producing 40 kg of milk/d with 3.5% milk fat and 3.2% milk protein. Cows were housed individually in tie stalls and allowed to exercise for 2 h daily throughout the experiment except for weekends and during sample collection periods. Cows were fed experimental diets as TMR once daily at 0800 h and had free access to fresh water. Animals were fed at 105 to 110% of expected feed intake. The amounts of feed offered and refused were recorded daily during sample collection periods. Samples of feed ingredients and orts were collected daily during sample collection periods and composited by period for feed ingredients, and by period and by cow for orts. The DM concentrations of barley silage and alfalfa hay were determined twice weekly and used to adjust dietary formulation if necessary. Dietary forage NDF concentration was 24.2, 14.6, and 24.4%, and dietary starch concentration was 27.7, 23.7, and 17.1% for the CON, LF, and LG diets, respectively (Table 2.2).

Cows were milked twice daily at 0400 and 1600 h. Milk was sampled at both milkings on d 19, 20, and 21 of each period. Cows were weighed after the morning milking on two consecutive d immediately prior to the start of experiment and on the last 2 d of each period. Body condition score was determined by two experienced individuals separately at the beginning of the experiment and at the end of each period with five-point scale (1= thin and 5= fat; Wildman et al., 1982), and averaged.

2.2.2 Chewing activity and sorting behavior

Chewing activities were monitored for 24 h on d 16 of each period.

Eating and ruminating activities were recorded every 5 min and each activity was assumed to continue for the entire 5-min interval between observations. Total chewing time was calculated as the sum of eating time and ruminating time. The sorting index was calculated as the ratio of actual intake to expected intake for particles retained on each sieve of Penn State Particle Separator (Leonardi and Armentano, 2003). A sorting index of 100, greater than 100, and less than 100 indicate no sorting, sorting for, and sorting against, respectively.

2.2.3 Rumen pH and rumen fermentation

Rumen pH was measured every 30 s for 72 h using an industrial electrode (model S650-CDHF, Sensorex, Garden Grove, CA) that was positioned within the ventral sac using weights. The electrode was linked to a pH data logger (model M1b-pH-1KRTD; Dascor, Escondido, CA) as described in detail by Penner et al. (2006). Rumen fluid was collected every 9 h over a 72-h period starting on d 16 of each experimental period (i.e., 0900 and 1800 h on d 16; 0300, 1200, and 2100 h on d 17; and 0600, 1500 and 2400 h on d 18). Rumen digesta were collected from the cranial, ventral, and caudal regions, and strained through a perforated material immediately after collection and placed on ice. The filtrate was centrifuged at 4ºC at 3,000 × *g* for 20 min, and composited to yield one sample per cow per period. Samples were stored at -20ºC until analysis.

2.2.4 Solid passage rate

The passage rate of digesta from the rumen was estimated using Cr-mordanted fiber as solid marker according to Udén et al. (1980). On d 19 of each period, approximately 6 kg of rumen digesta were collected via the ruminal cannula. Subsequently, 100 g of Cr-mordanted fiber was mixed evenly with the collected rumen digesta and placed into several different locations of the rumen. Ruminal digesta samples were collected as previously described at -1, 0.5, 1, 2, 3, 6, 9, 12, 18, 24, 36, 48, and 72 h after the dose of Cr-mordanted fiber and solid

samples were stored at -20ºC until analysis. For analysis, samples were thawed and dried in a forced air oven at 55ºC for 72 h, and ground to pass through a 1-mm screen (Thomas-Wiley, Philadelphia, PA). Samples were digested according to the procedure of Williams et al. (1962), and analyzed by an atomic absorption spectrophotometer (AA240FS, Varian Inc, US). The Cr concentration was fitted to the one compartment model (Grovum and Williams, 1973):

$$Y_t = Y_0 \times e^{-kt}$$

where Y_t is the concentration of Cr at time t (mg/kg); Y_0 is the concentration of Cr at time 0 (mg/kg); t is the sampling time after marker dosing (h); and k is the passage rate of Cr (%/h).

2.2.5 Apparent total tract digestibility

Fecal samples were collected from the rectum every 9 h over a 72-h period on d 16, 17, and 18 of each experimental period (at the same time as rumen fluid collection). Samples were composited by cow and by period, dried in forced air oven at 55ºC for 72 h, and ground to pass through a 1-mm screen (Thomas-Wiley, Philadelphia, PA). Indigestible NDF was used as an internal marker to calculate apparent total tract digestibility (Cochran et al., 1986). The indigestible NDF concentration of feed ingredients, orts, and fecal samples were determined by incubating samples in the rumen for 120 h using nitrogen free polyester bags (5 × 10 cm, pore size = 50 μm; R510, Ankom Technology, Macedon, NY).

2.2.6 Blood metabolites

Blood samples were collected from the coccygeal vessel using a vacutainer tube (Fisher Scientific Company; Franklin Lakes, NJ, USA) containing Na heparin every 9 h over a 72-h period starting on d 16 of each experimental period (at the same time as rumen fluid collection). Blood samples were centrifuged at 4ºC at 3,000 × *g* for 20 min. Plasma was then harvested and

samples were composited to yield one sample per cow per period, and stored at -20ºC until analysis.

2.2.7 Sample analysis

Particle size distribution of feed ingredients and orts was determined using the Penn State Particle Separator (Lammers et al., 1996). The dietary particle size distribution was calculated from the particle size distribution of individual feed ingredients and their dietary inclusion rate. The physically effective factor (PEF) was defined as the proportion of particles retained on 19- and 8-mm sieves.

The composited samples of feed ingredients and orts were dried in a forced air oven at 55ºC for 48 h to determine DM concentration. Dried samples were then ground to pass through a 1-mm screen using a Wiley mill (Thomas-Wiley, Philadelphia, PA) for chemical analysis. Analytical DM concentration was determined at 135ºC for 2 h (AOAC, 2002; method 930.15). The OM concentration was determined by oxidizing the dry sample in a muffle furnace for 2 h at 600ºC (AOAC, 2002; method 942.05). The NDF concentration was determined according to the method of Van Soest et al. (1991) using amylase and sodium sulfite. The CP concentration was determined using Leco (Leco FP-2000 N Analyzer; Leco instrument Inc., St. Joseph, MI, USA). The starch concentration was measured by an enzymatic method described by Karkalas (1985) after samples were gelatinized with sodium hydroxide and starch was hydrolyzed with industrial amylase; glucose concentration was measured using a glucose oxidase/peroxidase enzyme (No. P7119; Sigma, St. Louis, MO) and dianisidine dihydrochloride (Sigma, No. F5803). Absorbance was determined with a plate reader (SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA). Ether extract concentration was determined using a Goldfisch extraction apparatus with petroleum ether (Labconco, Kansas City, MO; Rhee, 2005).

Milk samples were analyzed for fat, CP, lactose, and MUN by infrared spectroscopy (AOAC, 2002; method 972.16; MilkoScan 605, Foss North America, Brampton, Ontario, Canada) at the Alberta Central Milk Testing Laboratory. Period composite samples, prepared based on the yield of milk fat from each milking, were stored at -20ºC until fatty acid analysis. Lipids were extracted from the milk samples by the procedure described by Folch et al. (1957). The fatty acids were derivatized using methanolic base (Supelco, Bellefonte, PA, U.S.A.) and quantified using a gas chromatography (Varian 3400, Varian Chromatography Systems, Walnut Creek, CA) with a flame ionization detector. Separation of the fatty acid methyl esters (**FAME**) was performed using a SP-2560 fused silica capillary column (100m × 0.25 mm internal diameter, with 0.25 µm film thickness; Supelco, Bellefonte, PA,USA). Helium was used as the carrier gas with a head pressure of 30 psi. The initial column temperature was set at 45ºC and held for 4 min, increased to 175ºC at the rate of 13ºC/min and held for 27 min. It was finally increased to 215ºC at the rate of 4ºC/min and held for 35 min. The initial injector temperature was set at 50ºC and held for 0.2 min. Subsequently, the injector temperature increased at a rate of 150ºC/min to 230ºC and held for 88.6 min. The detector temperature was held at 230ºC. Peak integration was performed using the Galaxie Chromatography Data System (Varian Chromatography Systems, Walnut Creek, CA). The individual fatty acids were identified using the FAME standard #463 (Nu Chek Prep, Elysian, MN). Each fatty acid was reported as g/100g of total fatty acids.

Plasma glucose concentration was measured using a glucose oxidase/peroxidase enzyme and dianisidine as described above. A commercial kit was used to determine the plasma concentration of insulin (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA). The concentration of plasma urea N was determined enzymatically (Fawcett and Scott, 1960). Rumen fluid

samples were thawed and centrifuged at 4ºC at 26,000 × *g* for 15 min. The supernatant was used for quantifying VFA concentration by gas chromatography according to the method described by Khorasani et al. (1996). Rumen ammonia N concentration was determined using a spectrophotometer (UV/VIS Spectrophotometer, V-530, Jasco Corporation, Japan) as described by Fawcett and Scott (1960).

2.2.8 Calculations and statistical analysis

The energy corrected milk (ECM) yield was calculated according to the equation described by Tyrrell and Reid (1965): ECM = [0.327 × milk yield (kg) + 12.95 × fat yield (kg) + 7.2 × protein yield]. Total digestible nutrient (TDN) was calculated from apparent total tract DM digestibility according to NRC (2001), with the modifications described by Penner and Oba (2009). The TDN was then used to calculate dietary NE_L according to NRC (2001). The net energy required for maintenance was calculated as NE_M (Mcal/d) = 0.08 Mcal/kg of $BW^{0.75}$, and NE_L was calculated according to NRC (2001) with the observed milk yield and concentrations of milk fat, milk CP, and milk lactose according to NRC (2001): NE_L (Mcal/d) = Milk yield × (0.0929 × milk fat + 0.0547 × milk protein + 0.0395 × milk lactose).

Data were analyzed using the fit model procedure of JMP (version 7.0.2, SAS) according to the following model:

$$Y_{ijkl} = \mu + P_i + S_j + T_k + C(S)_{k(l)} + e_{ijkl}$$

where μ is overall mean, P_i is fixed effect of period, S_j is fixed effect of stage of lactation, T_k is fixed effect of treatment, $C(S)_{k(l)}$ is random effect of cows nested in the stage of lactation, e_{ijkl} is residual. The interaction of stage × treatment had been included in the initial model, but it was removed because the interaction was not significant for primary response variables. Pre-planned orthogonal contrasts were used to compare treatment means of CON vs. LF and

CON vs. LG. Treatment effects were declared significant at $P \leq 0.05$ and a tendency was declared at $0.05 < P \leq 0.10$.

2.3 Results

2.3.1 Intake, passage rates and digestibility

Intakes of DM ($P = 0.01$), CP ($P = 0.01$) and EE ($P < 0.01$) were higher for cows fed the LF diet than those fed the CON diet (Table 2.3). The starch intake was not different between cows fed the LF and CON diets. There was no difference in DMI between cows fed the LG and CON diets. However, cows fed the LG diet had higher intake of NDF ($P = 0.01$) and EE ($P < 0.01$) but lower intake of starch ($P < 0.01$). The solid passage rate tended to be lower ($P = 0.08$) for cows fed the LF diet than those fed the CON diet. The apparent total tract digestibility of DM ($P = 0.03$), OM ($P = 0.05$), CP ($P = 0.03$), and EE ($P < 0.01$) were higher but starch digestibility was lower ($P = 0.01$) for cows fed the LF diet compared with those fed the CON diet. The apparent total tract digestibility of NDF was not affected by the LF diet. Cows fed the LG diet had lower total digestibility of DM ($P = 0.02$) and starch ($P < 0.01$), whereas digestibility of NDF ($P = 0.01$) and EE ($P < 0.01$) was greater compared with the CON diet. The digestibility of OM and CP was not different between cows fed the LG and CON diets.

2.3.2 Chewing activity and sorting behavior

Eating, ruminating and the total chewing time (min/d) were not affected by treatment (Table 2.4). However, cows fed the LF diet tended to spend less time eating ($P = 0.07$), and had less ruminating time ($P = 0.01$) and chewing time ($P < 0.01$) per unit of DMI (min/kg DMI) compared with those fed the CON diet. Similarly, cows fed the LG diet had shorter eating time ($P = 0.05$) and total chewing time ($P = 0.01$), and tended to have shorter ruminating time ($P = 0.09$)

than cows fed the CON diet. For cows fed the LF diet, total chewing time per unit of NDF intake (min/kg NDF intake) was also lower (P = 0.02) than those fed the CON diet. When barley grain was partially replaced by DDGS, eating (P = 0.04), ruminating (P = 0.02) and total chewing (P < 0.01) time per unit of NDF intake was reduced.

For particles retained on the 19-mm sieve, the sorting index was less than 100 (P < 0.05) for all three diets, indicating that all animals sorted against long particles (Table 2.4). However, cows fed the LF diet sorted against long particles to a less extent compared with those fed the CON diet (P = 0.05).

2.3.3 Rumen pH and rumen fermentation

The daily mean, minimum, maximum rumen pH, duration of pH < 5.8 or pH < 5.5, and the area below pH 5.8 or 5.5 were not affected by feeding the LF diet (Table 2.5). However, cows fed the LG diet tended to have higher minimum (P = 0.10) and maximum (P = 0.07) rumen pH than cows fed the CON diet. For cows fed the LG diet, the daily minimum rumen pH was 5.85, but the duration and area below pH 5.8 or 5.5 were greater than zero caused by variations among animals. Total VFA concentration, the molar proportions of individual VFA, and the concentration of rumen NH_3-N did not differ among treatments.

2.3.4 Plasma metabolites and milk production

Plasma concentrations of urea and glucose were not affected by treatment, averaging 12.5 and 64.3 mg/dL, respectively (Table 2.6). The concentration of insulin tended (P = 0.10) to be higher for cows fed the LF diet compared with those fed the CON diet, whereas it was not affected by feeding the LG diet.

Milk yield was 3.4 kg/d higher (P = 0.01) for cows fed the LF diet than those fed the CON diet (Table 2.7). Cows fed the LF diet had greater (P < 0.01) ECM yield compared with those fed the CON diet. The yields of milk protein and lactose were also greater (P = 0.01) for cows fed the LF diet compared to those

fed the CON diet. Milk protein concentration tended to be higher (P = 0.07) for cows fed the LF diet than those fed the CON diet. There were no differences in milk yield or milk composition between cows fed the LG and CON diets, but cows fed the LG diet had 2 kg/d greater (P < 0.01) ECM yield compared with those fed the CON diet. The feed efficiency, expressed as the ratio of milk yield to DMI, was not affected by treatment, but when expressed as the ratio of ECM to DMI (P = 0.01) or of ECM to NE_L intake (P < 0.01), feed efficiency was lower for cows fed the LF diet. The changes in BW and BCS were not affected by treatment.

Concentration of C16 in milk fat was lower for cows fed the LF diet compared with those fed the CON diet (Table 2.8). Feeding the LF diet tended to decrease (P = 0.10) the proportion of saturated fatty acids (SFA) and to increase the proportion of unsaturated fatty acids (UFA) compared with the CON diet. Cows fed the LG diet had greater (P = 0.01) concentrations of long chain fatty acids and lower (P < 0.01) concentration of C16, and tended to have lower (P = 0.08) concentrations of short and medium chain fatty acids compared with those fed the CON diet. In addition, the proportion of SFA in milk fat was decreased whereas the proportion of UFA in milk was increased by feeding LG diet (P = 0.04).

2.3.5 Energy balance

Compared with the CON diet, NE_L intake was increased (P < 0.01) by feeding the LF diet but was not affected by the LG diet (Table 2.9). Energy output as milk production was greater (P = 0.01) for both LF and LG diets relative to the CON diet. The resulting net energy balance was higher (P < 0.01) for cows fed the LF diet compared with those fed the CON diet but did not differ between cows fed the CON and LG diets.

2.4 Discussion

Previous research has indicated that the response of cows to feeding dietary high-fiber byproducts is largely affected by the type of carbohydrate source (forage or grain) being replaced (Ipharraguerre and Clark, 2003). As a non-forage fiber source (NFFS), DDGS contains highly digestible NDF (Getachew et al., 2004), thus the feeding value of DDGS as a replacement of either forage or grain is of interest. Partial replacement of barley silage with wet distillers grains increased milk yield but decreased milk fat concentration (Penner et al., 2009). However, in their study, the dietary allocation of barley grain was increased by 4% in the diet containing wet distillers grains. Therefore, the treatment effect on milk production is confounded by the different dietary allocation of barley grain. Grings et al. (1992) linearly increased the dietary inclusion of DDGS (0, 10, 20 and 30%) by replacing corn grain, but diets containing DDGS also linearly increased dietary CP concentration (13.9, 16.0, 18.1, and 20.3%). As such, effects of feeding DDGS on animal responses were confounded by different dietary CP concentration. To increase our understanding of DDGS as an energy source, the current study was undertaken to evaluate effects of DDGS as a partial replacement for barley silage or barley grain in diets for lactating dairy cows. The experimental diets were formulated to be iso-nitrogenous using feed ingredients other than forage or grain to minimize confounding effects of different dietary CP concentration.

2.4.1 DDGS as a partial replacement of barley silage

In this study, barley silage was replaced by DDGS at 20% of dietary DM without the change in dietary allocation of barley grain to minimize the confounding effects of diet fermentability on animal responses. Feeding the LF diet increased milk yield by 3.4 kg/d. The higher milk yield is likely attributed to the greater DMI (+3.6 kg/d) and NE_L intake (+8.8 Mcal/d) for cows fed the LF diet compared with those fed the CON diet. Penner et al. (2009) also observed

greater milk yield by replacing barley silage with wet distillers grains at 10% of dietary DM, and attributed the higher milk yield to a possible increase in metabolizable protein flow to the small intestine. This may also partially explain the higher milk yield in the current study as apparent total tract digestibility of CP was greater for cows fed the LF diet compared with the CON diet.

The greater DMI for cows fed the LF diet as observed in our study is in agreement with Janicek et al. (2008), in which DDGS was used as a partial replacement of corn silage and concentrates at 10, 20, and 30% of dietary DM. In that study, cows linearly increased DMI as dietary allocation of DDGS increased. Allen and Grant (2000) suggested that the inclusion of NFFS as a partial replacement for forage reduced dietary particle size and increased DMI due to a faster passage rate. In contrast, we observed that cows fed the LF diet had higher DMI but tended to have a slower passage rate compared with those fed the CON diet. These discrepancies suggested that the greater DMI cannot be attributed to faster passage rate and a reduced physical fill in our study. This was supported by the increased total tract digestibility of most nutrients for cows fed the LF diet; greater DMI associated with faster passage rates would generally decrease digestibility of nutrients (Tyrrell and Moe, 1975; Colucci et al., 1982).

Although a partial replacement of barley silage with DDGS decreased dietary forage NDF content, both concentration and yield of milk fat were not affected. Past studies demonstrated that cows fed a low forage diet (Yang and Beauchemin, 2007) or a diet with shorter particle size (Krause and Combs, 2003) reduces milk fat concentration. Cows fed the LF diet had lower chewing time (min/kg DMI) compared with those fed the CON diet, which is in agreement with other studies using NFFS as a partial replacement of forage fiber (Clark and Armentano, 1997; Allen and Grant, 2000; Penner et al., 2009). However, regardless of the reduced chewing time, rumen pH was not different between

cows fed the LF and CON diets. This is possibly because rumen pH is not determined only by dietary forage NDF concentration, but also by other factors such as fermentability of diets (Yang and Beauchemin, 2009). Although forage NDF was 14.6% for the LF diet, the dietary NFC concentration was 34.8%. According to NRC (2001), dietary forage NDF concentration can be decreased to 15% if dietary NFC concentration is 36% or less.

2.4.2 DDGS as a partial replacement of barley grain

Using DDGS as a partial replacement for barley grain did not affect milk yield despite a 10% unit reduction in the dietary starch concentration. This result indicates that the DDGS used in the current study can be used as an alternative energy source to partially replace barley grain in diets for lactating dairy cows. The high energy content of DDGS may be attributed to the highly digestible NDF and the high EE content. Nuez Ortin and Yu (2009) reported the 48-h in situ NDF digestibility of corn- and wheat-DDGS was 79.4% and 63.5%, respectively. In addition, EE intake and the total digestibility of EE were also higher for cows fed the LG diet than those fed the CON diet. Collectively, these factors contributed to greater NE_L intake and milk energy output for cows fed the LG diet relative to the CON diet.

Milk fat yield or concentration was not affected by the LG treatment in the present study. Past studies showed that milk fat concentration was not affected (Boddugari et al., 2001; Voelker and Allen, 2003a; Leonardi et al., 2005) or increased (Mansfield et al., 1994; Ipharraguerre et al., 2002) without affecting milk yield when cows were fed NFFS in place of grain. Milk fat production can be affected by the concentration of dietary UFA (Griinari et al., 1998). Fat in corn DDGS was high in concentrations of C18:1 and C18:2 (Sasikala-Appukuttan et al., 2008), and the inclusion of 20% DDGS in the present study has likely increased the dietary concentrations of long chain fatty acids and UFA. Feeding a high UFA

diet, particularly in combination with low rumen pH alters biohydrogenation pathway in the rumen and allows for the accumulation of intermediates (Griinari et al., 1998), such as *trans*-10, *cis*-12 CLA, which inhibits de novo fatty acid synthesis in mammary gland (Peterson et al., 2003). Although cows fed the LG diet tended to have increased daily minimum and maximum rumen pH compared with those fed the CON diet, they had lower concentration of C16, and tended to have lower concentration of short and medium chain fatty acids. However, the concentration of long chain fatty acids in milk was higher for the LG treatment compared with the CON, reflecting greater supply of dietary long chain fatty acids with the substitution of DDGS for barley grain. The reduction in short and medium chain fatty acids might have been compensated by the increased long chain fatty acids absorbed from dietary source in the present study, and resulted in no difference in milk fat concentration between cows fed the LG and CON diets. Similar changes in milk fatty acid profile were observed in other studies (Schingoethe et al., 1999; Leonardi et al., 2005).

Cows fed the LG diet tended to have higher rumen pH compared with those fed the CON diet possibly because diet fermentability was lower for the LG diet. Starch content is lower for DDGS than grain as starch in grain is almost completely removed by ethanol production (Widyaratne and Zijlstra, 2006). However, rumen pH is also affected by chewing activity as it stimulates the secretion of saliva to buffer fermentation acids produced in the rumen (Mertens, 1997). It is noteworthy that cows fed the LG diet had lower chewing time (min/kg DMI) compared with the CON diet. We expected that cows fed the LG diet would maintain chewing activity because dietary forage NDF content and the particle size distribution were similar between the LG and CON diets. Although the concentrates used for LG and CON diets were further separated by using the additional 1.18-mm aperture sieve (Kononoff et al., 2003), LG concentrate had

76.9% particles retained on 1.18-mm sieve whereas the CON diet had 69.5%. Therefore, it is not clear why feeding the LG diet decreased chewing time compared with the CON diet. It has been suggested that the use of DDGS as partial replacement of grain may reduce the risk of rumen acidosis in high producing dairy cows (Stone, 2004). However, Beliveau and McKinnon (2009) reported the substitution of wheat DDGS for barley grain had no effect on daily mean rumen pH in finishing beef cattle. Therefore, effects of feeding DDGS as a partial replacement of grain on rumen pH warrants further investigation.

2.4.3 Effects of feeding DDGS on nutrients digestibility

The higher digestibility of NDF for cows fed the LG diet is in agreement with Birkelo et al. (2004) who reported that the apparent total tract NDF digestibility increased (60.6 vs. 49.2%) when cows were fed wet distillers grains in place of corn grain and soybean meal. The greater NDF digestibility may be attributed to the tendency of higher rumen pH for cows fed the LG diet that contained less starch and NFC than the CON diet. Despite of the greater NDF digestibility, the DM digestibility was decreased by feeding the LG diet and this may be partially attributed to the lower starch digestibility as well as lower dietary starch content. Diets containing DDGS (LF and LG diets) decreased starch digestibility, and this might have resulted from the low amylolytic activity in the rumen of cows fed low starch diets (Oba and Allen, 2003). The growth of amylolytic bacteria is affected by the dietary starch content (Cotta, 1988). Further, Voelker and Allen (2003b) also observed that rumen starch digestibility decreased from 42.2 to 9.7% when high moisture corn was replaced by beet pulp at 24% of dietary DM in diets of lactating dairy cows, but total tract starch digestibility was not affected due to a compensatory starch digestion in the intestines in their study. The greater fat digestibility for the LF and LG diets is consistent with the finding of Vander Pol et al. (2009); total tract fat digestibility was increased from 72.5 to

81.0% when 40% of corn-based concentrate was replaced by wet distillers grains with solubles. The greater fat digestibility may be also attributed to the higher dietary EE content (Palmquist and Conrad, 1978). The higher EE digestibility for animals fed higher dietary EE content was also reported by Smith et al. (1993). These results indicate that digestibility of nutrients can be affected by inclusion of DDGS in diets.

2.5 Conclusion

A partial replacement of barley silage with DDGS increased DMI and yields of milk, milk protein and lactose of lactating dairy cows. Despite the lower dietary forage NDF content, no adverse effects on rumen pH and rumen fermentation were observed in this study. A partial replacement of barley grain with DDGS tended to increase rumen pH but did not affect milk yield. In conclusion, DDGS can be used as a partial replacement of forage or grain in diets for lactating dairy cows, and considered as an alternative energy source when forage is in short supply or when grain is not available at reasonable costs.

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Table 2.1. Ingredients of experimental diets

Ingredients, % of DM	Diet[1]		
	CON	LF	LG
Alfalfa hay	5.1	5.0	5.1
Barley silage	44.6	24.8	44.6
DDGS[2]	…	20.1	20.1
Rolled barley	35.2	35.1	15.1
Canola Meal	3.1	…	…
Corn gluten meal	5.6	0.4	0.5
Beet pulp	2.5	10.8	11.1
Urea	0.1	0.2	…
Premix[3]	1.0	1.0	1.0
Limestone	1.0	1.1	0.8
Salt	0.5	0.5	0.5
Magnesium oxide	0.1	0.1	0.1
Dicalcium phosphate	1.2	0.9	1.1

[1] CON = Control; LF = Low forage; LG = Low grain.

[2] DDGS: a blend of 70% corn- and 30% wheat-based dried distillers grains with soluble.

[3] Contained 0.10% Ca; 0.60% P; 11.50% Na; 0.30% Mg; 10 mg/kg F; 80 mg/kg I; 5000 mg/kg Zn; 31000 mg/kg Mn; 1170 mg/kg Cu; 6.2 mg/kg Co; 1265 KIU/kg vitamin A; 142 KIU/kg vitamin D; 3800 IU/kg vitamin E.

Table 2.2. Nutrient composition and particle size distribution of experimental diets

Item	Diet[1]					
	CON (n = 3)		LF (n = 3)		LG (n = 3)	
	Mean	SD	Mean	SD	Mean	SD
Composition, %DM						
DM (% As fed)	55.5	0.9	67.1	1.6	55.5	1.0
OM	91.0	0.9	90.7	1.8	91.1	0.5
CP	18.8	0.8	19.6	0.5	18.8	0.6
NDF	36.0	0.4	33.0	1.8	38.2	1.1
Forage NDF	24.2	0.3	14.6	0.4	24.4	0.5
Starch	27.7	0.3	23.7	1.6	17.1	1.4
Ether extract	2.0	0.6	3.4	0.2	3.5	0.4
NFC[2]	34.3	1.4	34.8	2.1	30.7	0.3
Particle size distribution, % (as fed)						
> 19 mm	6.2	1.6	5.0	1.1	6.2	1.6
19 - 8 mm	41.1	3.0	28.0	2.2	41.1	3.0
< 8 mm	52.7	4.4	67.1	3.2	52.7	4.4
PEF[3]	0.47	0.04	0.33	0.03	0.47	0.04

[1] CON = Control; LF = Low forage; LG = Low grain.

[2] NFC: = 100 – (NDF% + CP% + Ether extract% + Ash%).

[3] PEF = physically effective factor determined as the proportion of particles retained on 19- and 8-mm sieves (Lammers et al., 1996).

Table 2.3. Effects of partially replacing barley silage or barley grain with DDGS in diets for lactating cows on feed intake and apparent total tract digestibility

Item	Diet[1]			SEM	*P* value	
	CON (n = 6)	LF (n = 6)	LG (n = 6)		CON vs. LF	CON vs. LG
Intake, kg/d						
DM	22.4	26.0	23.7	0.5	0.01	0.11
OM	20.4	23.5	21.6	0.4	< 0.01	0.07
CP	4.2	5.1	4.5	0.1	0.01	0.19
NDF	8.0	8.5	8.9	0.2	0.09	0.01
Starch	6.5	6.3	4.5	0.1	0.32	< 0.01
EE	0.4	0.9	0.9	0.03	< 0.01	< 0.01
Solid passage rate, %/h	3.6	2.3	2.9	0.5	0.08	0.30
Digestibility, %						
DM	65.6	67.7	63.3	0.6	0.03	0.02
OM	69.3	71.0	68.5	0.5	0.05	0.28
CP	68.3	70.6	66.7	0.6	0.03	0.11
NDF	52.6	53.2	55.1	0.6	0.50	0.01
Starch	96.7	95.3	94.1	0.2	0.01	< 0.01
EE	62.6	78.4	80.0	1.8	< 0.01	< 0.01

[1] CON = Control; LF = Low forage; LG = Low grain.

Table 2.4. Effects of partially replacing barley silage or barley grain with DDGS in diets for lactating cows on chewing activity and sorting behavior

Item	Diet[1]			SEM	*P* value	
	CON (n = 6)	LF (n = 6)	LG (n = 6)		CON vs. LF	CON vs. LG
Time, min/d						
Eating	303	250	226	37	0.30	0.15
Ruminating	563	518	518	23	0.20	0.21
Total chewing[2]	866	768	744	41	0.13	0.07
Time, min/kg DMI						
Eating	13.7	9.8	9.5	1.3	0.07	0.05
Ruminating	25.4	20.0	22.1	1.2	0.01	0.09
Total chewing	39.1	29.7	31.6	1.7	< 0.01	0.01
Time, min/kg NDFI						
Eating	38.6	29.7	25.1	3.8	0.14	0.04
Ruminating	71.7	61.1	58.4	3.4	0.06	0.02
Total chewing	110.3	91.0	83.5	4.7	0.02	< 0.01
Sorting index[3]						
> 19 mm	80.5	91.0	85.0	3.2	0.05	0.35
19-8 mm	101.5	103.0	103.2	0.8	0.21	0.17
< 8 mm	101.0	99.5	99.0	0.7	0.16	0.07

[1] CON = Control; LF = Low forage; LG = Low grain.

[2] Sum of time on eating and ruminating time.

[3] Sorting index above 100 indicates sorting for particles and below 100 indicates sorting against particles (Leonardi and Armentano, 2003).

Table 2.5. Effects of partially replacing barley silage or barley grain with DDGS in diets for lactating cows on rumen pH and rumen fermentation

Item	Diet[1]			SEM	*P* value	
	CON (n = 6)	LF (n = 6)	LG (n = 6)		CON vs. LF	CON vs. LG
Rumen pH						
Mean	6.21	6.17	6.39	0.08	0.72	0.13
Minimum	5.50	5.51	5.85	0.13	0.96	0.10
Maximum	6.86	6.78	7.00	0.05	0.32	0.07
Area, pH × h/d						
pH < 5.8	2.1	3.3	1.5	0.9	0.40	0.66
pH < 5.5	0.3	0.8	0.4	0.3	0.31	0.73
Duration, h/d						
pH < 5.8	3.9	4.7	1.8	1.1	0.64	0.21
pH < 5.5	0.8	1.3	0.8	0.5	0.45	0.91
Total VFA, m*M*	130.7	131.9	136.8	4.6	0.86	0.38
Molar proportion, mol/100 mol						
Acetate	60.2	60.6	60.5	1.4	0.81	0.88
Propionate	24.1	22.4	23.1	2.1	0.58	0.74
Isobutyrate	0.95	0.95	0.89	0.04	0.93	0.35
Butyrate	10.8	11.7	11.6	0.7	0.37	0.43
Isovalerate	1.25	1.44	1.25	0.13	0.33	0.98
Valerate	2.24	2.20	2.11	0.16	0.85	0.57
Rumen NH_3-N, mg/dL	13.4	14.5	14.2	0.7	0.29	0.47

[1] CON = Control; LF = Low forage; LG = Low grain.

Table 2.6. Effects of partially replacing barley silage or barley grain with DDGS in diets for lactating cows on plasma metabolite concentrations

Item	Diet[1]			SEM	*P* value	
	CON (n = 6)	LF (n = 6)	LG (n = 6)		CON vs. LF	CON vs. LG
Urea-N, mg/dL	11.8	12.6	13.0	0.9	0.54	0.36
Glucose, mg/dL	63.2	65.7	64.0	1.4	0.23	0.67
Insulin, μIU/mL	11.7	14.5	10.2	1.0	0.10	0.32

[1] CON = Control; LF = Low forage; LG = Low grain.

Table 2.7. Effects of partially replacing barley silage or barley grain with DDGS in diets for lactating cows on milk production and milk composition

Item	Diet[1]			SEM	*P* value	
	CON (n = 6)	LF (n = 6)	LG (n = 6)		CON vs. LF	CON vs. LG
Yield, kg/d						
Milk	33.0	36.4	34.7	0.7	0.01	0.14
ECM[2]	33.1	35.1	35.1	0.4	<0.01	< 0.01
Fat	1.14	1.14	1.22	0.04	1.00	0.14
Crude protein	1.05	1.18	1.10	0.02	0.01	0.14
Lactose	1.46	1.63	1.55	0.04	0.01	0.13
Composition						
Fat, %	3.53	3.29	3.61	0.11	0.14	0.65
Crude protein, %	3.26	3.31	3.25	0.02	0.07	0.66
Lactose, %	4.34	4.42	4.39	0.04	0.21	0.37
MUN, mg/dL	13.9	14.6	15.4	0.9	0.59	0.25
Feed efficiency						
Milk yield/DMI	1.45	1.39	1.47	0.03	0.25	0.70
ECM/DMI	1.46	1.35	1.49	0.02	0.01	0.42
ECM/NE_L intake	0.92	0.80	0.95	0.02	< 0.01	0.21
BW, kg	690	691	690	4	0.84	0.20
BW change, kg/d	0.56	0.33	0.66	0.38	0.68	0.85
BCS change, /21d	0.15	0.15	0.10	0.15	1.00	0.48

[1] CON = Control; LF = Low forage; LG = Low grain.

[2] ECM = [0.327 × milk yield (kg) + 12.95 × fat yield (kg) + 7.2 × protein yield]; Tyrrell and Reid, 1965.

Table 2.8. Effects of partially replacing barley silage or barley grain with DDGS in diets for lactating cows on milk fatty acids profile

Item	Diet[1]			SEM	*P* value	
	CON (n = 6)	LF (n = 6)	LG (n = 6)		CON vs. LF	CON vs. LG
g/100 g of total fatty acids						
C4:0	0.44	0.38	0.42	0.02	0.06	0.51
C6:0	1.21	1.14	1.23	0.07	0.35	0.87
C7:0	0.07	0.09	0.06	0.01	0.26	0.42
C8:0	1.16	1.12	1.13	0.05	0.61	0.64
C9:0	0.09	0.11	0.08	0.01	0.09	0.06
C10:0	2.96	3.09	2.83	0.15	0.54	0.56
C11:0	0.39	0.42	0.34	0.01	0.10	0.01
C12:0	3.94	4.28	3.67	0.15	0.14	0.24
C14:0	12.9	12.8	11.9	0.3	0.97	0.07
C14:1	1.15	1.09	0.89	0.13	0.76	0.20
C15:0	1.44	1.79	1.33	0.09	0.03	0.43
C16:0	32.9	30.1	29.2	0.6	0.01	0.01
C16:1	2.53	2.58	2.2	0.1	0.72	0.04
C17:1	0.24	0.24	0.20	0.02	0.82	0.05
C18:0	8.76	8.38	10.97	0.35	0.46	0.01
C18:1 t	1.71	3.47	2.58	0.65	0.09	0.37
C18:1 c	20.5	20.1	22.5	0.85	0.72	0.14
C18:1	22.2	23.5	25.1	0.7	0.23	0.23
C19:0	0.15	0.18	0.18	0.01	0.12	0.12
C18:2	2.68	3.48	3.10	0.21	0.03	0.20
C20:0	0.08	0.11	0.12	0.02	0.32	0.22
C20:1	0.20	0.18	0.21	0.02	0.47	0.47
C18:3	0.39	0.36	0.36	0.01	0.06	0.03
CLA 9/11	0.50	0.73	0.73	0.09	0.12	0.11
C22:0	0.02	0.01	0.02	0.01	0.36	0.64
C20:3 w6	0.15	0.19	0.17	0.01	0.04	0.22
C20:4	0.17	0.18	0.16	0.01	0.30	0.48
Short and medium (C <16)	26.0	26.7	24.1	0.6	0.44	0.08
C16[2]	35.5	32.7	31.4	0.6	0.01	< 0.01
Long (C > 16)	35.6	37.6	41.3	1.0	0.18	0.01
SFA[3]	71.2	68.7	68.0	0.9	0.10	0.04
UFA[3]	28.8	31.3	32.0	0.9	0.10	0.04

[1] CON = Control; LF = Low forage; LG = Low grain.
[2] The sum of C16:0 and C16:1.
[3] SFA: saturated fatty acids; UFA: unsaturated fatty acids.

Table 2.9. Effects of partially replacing barley silage or barley grain with DDGS in diets for lactating cows on calculated energy intake, expenditure, and balance

Item	Diet[1]			SEM	*P* value	
	CON (n = 6)	LF (n = 6)	LG (n = 6)		CON vs. LF	CON vs. LG
NE_L[2], Mcal/kg	1.58	1.71	1.58	0.04	0.03	0.90
NE_L intake, Mcal/d	35.5	44.3	37.3	0.9	< 0.01	0.22
NE_L output, Mcal/d	22.1	23.4	23.5	0.3	0.01	0.01
NE_M output, Mcal/d	10.8	10.8	10.8	0.04	0.84	0.20
Total NE output, Mcal/d	32.8	34.2	34.3	0.3	0.01	0.01
NE balance, Mcal/d	2.67	10.1	2.96	0.74	< 0.01	0.79

[1] CON = Control; LF = Low forage; LG = Low grain.

[2] Dietary NE_L: was calculated from actual total tract digestibility according to NRC (2001).

CHAPTER 3. EFFECTS OF FEEDING ALFALFA HAY ON CHEWING, RUMEN pH, AND MILK FAT CONCENTRATION OF DAIRY COWS FED WHEAT DRIED DISTILLERS GRAINS WITH SOLUBLES AS A PARTIAL SUBSTITUTE FOR BARLEY SILAGE*

3.1 Introduction

In western Canada, wheat is the main feedstock used for ethanol production and wheat-based dried distillers grains with solubles (**DDGS**) is a commonly available by-product feedstuff for animals. Wheat DDGS is high in NDF concentration ranging from 25.9% (Dong et al., 1987) to 54.1% (McKinnon and Walker, 2008) with an average of 37.4%. In addition to the high NDF content, the NDF from DDGS is highly digestible in the rumen (Nuez Ortín and Yu, 2009). Therefore, wheat DDGS can be considered as a non-forage fiber source (**NFFS**), and used as a partial replacement of forage in diets for lactating dairy cows. However, the physical effectiveness of DDGS at stimulating chewing is lower than forages (Clark and Armentano, 1993). Penner et al. (2009) observed that dairy cows decreased chewing time and milk fat concentration when barley silage was partly replaced with corn/wheat wet distillers grains.

When dairy cows were fed high NFFS diets, dietary inclusion of alfalfa hay in place of silage often increases chewing time (Allen and Grant, 2000), milk fat concentration (Smith et al., 1993), and milk yield (Mullins et al., 2009). In our previous study (Chapter 2), cows fed a high NFFS diet, in which barley silage was replaced by DDGS at 20% of dietary DM, maintained rumen pH and milk fat concentration, and the experimental diets contained alfalfa hay. As such, we hypothesized that feeding alfalfa hay would prevent the reduction in milk fat concentration when cows are fed a high DDGS diet.

The objective of this study was to determine the effects of feeding alfalfa

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hay on chewing time, rumen fermentation and milk fat concentration when DDGS was fed as a partial replacement of barley silage in diets for lactating dairy cows.

3.2 Material and methods

3.2.1 Animals, diets and experimental design

The current study was conducted at the Dairy Research and Technology Center of University of Alberta. All procedures were pre-approved by the Faculty Animal Policy and Welfare Committee at the University of Alberta and conducted according to the guidelines of the Canadian Council of Animal Care (Ottawa, Ontario, Canada).

Thirty multiparous lactating Holstein cows (220 ± 51 DIM; 632 ± 65 kg of BW) were used in this study. Of the 30 cows, 6 were previously fitted with a ruminal cannula. Cows were assigned to one of three dietary treatments in a 3 × 3 Latin square design balanced for carryover effects. Each period consisted of an 18-d diet adaptation period and a 3-d data and sample collection period. Data from one cannulated cow were removed from the study because she dried off during the second period. One intact cow was removed during the third period due to same reason but the data collected from this cow during the first and second periods were included for statistical analysis. Each period consisted of an 18-d diet adaptation period and a 3-d data collection period. The treatments were control (**CON**: 50% barley silage and 50% concentrate mix on a DM basis; Table 3.1), a diet where barley silage was replaced by DDGS at 20% of dietary DM (**DG**), and a diet where barley silage was replaced by DDGS and alfalfa hay at 20 and 10% of dietary DM, respectively (**DG+AH**). Water was added to the DG and DG+AH diets and mixed evenly to make DM concentration similar across treatments. Diets were formulated according to NRC (2001) to meet or exceed the nutritional requirements for a 650 kg cow producing 36 kg of milk/d with 3.5% milk fat and 3.2% milk protein. All diets were formulated to contain similar crude

protein concentration using variable amounts of beet pulp, corn gluten meal and urea in the diets.

Cows were housed individually in tie stalls and allowed to exercise for 2 h daily throughout the experiment except for weekends and during sample collection periods. Cows were fed experimental diets as a TMR once daily at 0730 h and had free access to fresh water. Feed was offered at 105 to 110% of expected feed intake. Samples of TMR, feed ingredients and feed refusals were collected daily during sample collection periods and composited by period for TMR and feed ingredients, and by period and by cow for refusals. The DM concentration of barley silage and alfalfa hay was determined twice weekly and dietary formulation was adjusted if necessary. The dietary DM concentration was similar among treatments as water was added to the DG and DG+AH diets (Table 3.2). Dietary forage NDF concentration (DM basis) was 26.3, 16.1, and 15.2% for the CON, DG and DG+AH diets, respectively.

Cows were milked twice daily at 0400 and 1600 h. Milk was sampled from both am and pm milkings on d 19, 20, and 21 of each period. Cows were weighed after the morning milking on two consecutive d immediately prior to the start of experiment and on the last 2 d of each period. Body condition score was determined by two experienced individuals separately at the beginning of the experiment and at the end of each period using a five-point scale (1= thin to 5= fat; Wildman et al., 1982), and averaged.

3.2.2 Chewing activity and sorting behavior

Chewing activities were monitored for 24 h on d 20 of each period. Eating and ruminating activities were recorded every 5 min and each activity was assumed to continue for the entire 5-min interval between observations. Total chewing time was calculated as the sum of eating time and ruminating time. Sorting index was calculated as the ratio of actual intake to expected intake for

particles retained on each sieve of Penn State Particle Separator (Leonardi and Armentano, 2003). A sorting index of 100, greater than 100, and less than 100 indicate no sorting, selective consumption, and selective refusals, respectively.

3.2.3 Rumen pH and rumen fermentation

Rumen pH was measured every 30 sec for 72 h using the LRC rumen pH data logger system (Dascor, Escondido, CA) as described in detail by Penner et al. (2006). Rumen fluid was collected every 9 h over a 72-h period to account for diurnal variation (i.e., 0900 and 1800 h on d 19; 0300, 1200, and 2100 h on d 20; and 0600, 1500 and 2400 h on d 21). For each rumen fluid sampling, rumen digesta was collected from cranial, ventral, and caudal sacs, and strained through a perforated material and placed on ice immediately after collection. The filtrate was centrifuged at 3,000 × *g* at 4ºC for 20 min. Samples were composited for one sample per cow for each period, and stored at -20ºC until analysis.

3.2.4 Sample analysis

Particle size distribution of feed ingredients and feed refusals was analyzed using Penn State Particle Separator (Lammers et al., 1996). The dietary particle size distribution was calculated from particle size distribution of individual feed ingredients and their dietary allocation. Physically effective factor (PEF) was defined as the proportion of particles retained on 19- and 8-mm sieves.

The composited samples of TMR, feed ingredients and feed refusals were dried at 55ºC for 48 h in a forced air oven (V-31 STD, Style II, Despatch Industries INC, Nashua, Mississauga, ONT) to determine DM concentration. Dried samples were then ground through a 1-mm screen using a Wiley mill (Thomas-Wiley, Philadelphia, PA). The samples were analyzed for concentrations of analytical DM (AOAC, 2002; method 930.15), OM (AOAC, 2002; method 942.05), NDF (Van Soest et al., 1991; Method A), and starch (Silveira et al., 2007). Concentration of CP was determined using Leco (Leco FP-2000 N

Analyzer; Leco instrument Inc., St. Joseph, MI, USA), and ether extract concentration was determined using a Goldfisch extraction apparatus with petroleum ether (Labconco, Kansas City, MO; Rhee, 2005).

Rumen fluid samples were thawed and centrifuged at 4ºC at 26,000 × *g* for 15 min. The supernatant was analyzed for VFA concentration by a gas chromatography as described by Khorasani et al. (1996), and for ammonia N concentration using a spectrophotometer (UV/VIS Spectrophotometer, V-530, Jasco Corporation, Japan) as described by Fawcett and Scott (1960). Milk samples were analyzed for milk fat, CP, lactose, and MUN by infrared spectroscopy (AOAC, 2002; method 972.16; MilkoScan 605, Foss North America, Brampton, Ontario, Canada) at the Alberta Central Milk Testing Laboratory. Milk samples were composited for one sample per cow per period based on milk fat yield from each milking, and stored at -20ºC until fatty acid analysis. Lipids were extracted from the milk samples by the procedure described by Folch et al. (1957). Fatty acids were derivatized using methanolic base (Supelco, Bellefonte, PA, U.S.A.) and quantified using a gas chromatography (Varian 3400, Varian Chromatography Systems, Walnut Creek, CA) with a flame ionization detector. Separation of the fatty acid methyl esters (**FAME**) was performed using a SP-2560 fused silica capillary column (100 m × 0.25 mm internal diameter, with 0.25 µm film thickness; Supelco, Bellefonte, PA, USA). Helium was used as the carrier gas with a head pressure of 35 psi. The initial column temperature was set at 45 ºC and held for 4 min, increased to 175ºC at the rate of 13ºC/min and held for 27 min. It was finally increased to 215ºC at the rate of 4ºC/min and held for 45 min. The initial injector temperature was set at 50ºC and held at 0.2 min, and then increased to 230ºC at the rate of 150ºC/ min and held for 84.2 min. The detector temperature was set at 230ºC. Peak integration was performed using the Galaxie Chromatography Data System (Varian Chromatography Systems, Walnut Creek,

CA). Individual fatty acids were identified with the FAME standard #463 (Nu Chek Prep, Elysian, MN). Conjugated linoleic acid isomers were identified using standards from Matreya (Matreya, Inc., PA). Concentration of each fatty acid was reported as g/100g of total fatty acids.

3.2.5 Statistical analysis

All data were analyzed using the Proc Mixed procedure of SAS (version 9.1; SAS Institute Inc., Cary, NC) according to the following model:

$$Y_{ijkl} = \mu + P_i + T_j + S_k + C(S)_{l(k)} + e_{ijkl}$$

Where μ is overall mean, P_i is fixed effect of period, T_j is fixed effect of treatment, S_k is fixed effect of square, $C(S)_{l(k)}$ is random effect of cow nested in square, e_{ijkl} is residual. If overall treatment effect is significant ($P < 0.05$), treatment means were separated by Bonferroni t-test. Treatment effects were declared significant at $P \leq 0.05$ and tendency was declared at $0.05 < P \leq 0.10$.

3.3 Results

3.3.1 Feed intake and chewing activity

Dry matter intake was higher ($P < 0.01$) for cows fed the DG and DG+AH diets than those fed the CON diet with no difference between the DG and DG+AH diets (Table 3.3). Eating time (min/d) and total chewing time were shorter ($P < 0.01$) for cows fed the DG and DG+AH diets than those fed the CON diet, but ruminating time was not affected by treatment (Table 3.3). Eating, ruminating, and total chewing time per unit of DMI were consistently decreased ($P < 0.01$) by feeding the DG or DG+AH diet compared with the CON diet.

3.3.2 Rumen fermentation and sorting behavior

Daily mean rumen pH was lower ($P < 0.01$) for cows fed the DG and DG+AH diets than those fed the CON diet, but the daily maximum pH was not different across the treatments (Table 3.4). The duration that rumen pH was below

5.8 and 5.5 were longer ($P < 0.01$) for cows fed the DG and DG+AH diets than those fed the CON diet. The area that rumen pH was below pH 5.8 ($P < 0.01$) was also higher for cows fed the DG and DG+AH diets compared with those fed the CON diet. The molar proportions of acetate and isobutyrate were lower ($P < 0.01$) and the molar proportion of propionate was higher ($P < 0.01$) for cows fed the DG and DG+AH diets compared to those fed the CON diet. The ratio of acetate to propionate was higher ($P < 0.01$) for cows fed the CON diet than for the DG and DG+AH diets. The concentration of rumen NH_3-N tended to be lower ($P = 0.10$) for cows fed the DG and DG+AH diets than the CON diet.

Cows fed the CON diet sorted against long particles ($P < 0.05$; Table 3.5) but cows fed the DG diet did not sort, and those fed the DG+AH diet sorted for long particles ($P < 0.05$).

3.3.3 Performance and fatty acids profile

Milk yield was greater ($P < 0.01$) for cows fed the DG and DG+AH diets compared with the CON diet (Table 3.6). Milk fat concentration was lower ($P < 0.01$) for cows fed the DG and DG+AH diets compared with those fed the CON diet, but milk fat yield was not affected by treatment. Milk fat concentration was lower ($P < 0.01$) for the DG+AH diet compared with the DG diet. The yields of milk protein and lactose were greater ($P < 0.01$) for cows fed the DG and DG+AH diets while there were no differences in the concentrations of milk protein and lactose compared with those fed the CON diet. The concentration of MUN was lower ($P < 0.01$) for DG and DG+AH treatments than CON, and for DG+AH compared with DG treatment.

Concentrations of short and medium chain fatty acids (C < 16) were not affected by treatment but the concentration of long chain fatty acids (C > 16) was increased ($P < 0.01$) by feeding the DG and DG+AH diets. The concentration of C16 was lower ($P < 0.01$) for cows fed the DG and DG+AH diets than the CON

diet. The concentration of *trans*-10 C18:1 in milk fat was higher (P = 0.03) when cows were fed the DG+AH diet compared to those fed the CON diet, but was not affected by feeding the DG diet (Table 3.7). Cows fed the DG+AH diet tended to have greater (P = 0.06) concentrations of unsaturated fatty acids (**UFA**) in milk than those fed the CON diet but there was no difference in the concentrations of UFA between cows fed the DG and CON diets. The concentration of *trans*-10, *cis*-12 conjugated linoleic acid (**CLA**) in milk was not different among treatments.

3.4 Discussion

3.4.1 The effects of DDGS as a partial replacement of barley silage

Non-forage fiber sources are high in NDF content, but have a low physical effectiveness at stimulating chewing activity due to their small particle size (Clark and Armentano, 1993). Many studies have been conducted to investigate effects of feeding NFFS as a partial forage replacement for lactating dairy cows. Partial replacement of forage with NFFS often reduces milk fat concentration (Boddugari et al., 2001; Weidner and Grant, 1994) by decreasing chewing time and rumen pH (Boddugari et al., 2001; Harvatine et al., 2002). Penner et al. (2009) reported that chewing time and milk fat concentration were decreased by feeding wet distillers grains as a partial replacement of barley silage.

In our study, particles retained on the 19- and 8-mm sieves were reduced for diets containing DDGS in place of barely silage, and DG and DG+AH treatments decreased chewing time, rumen pH, and milk fat concentration. As particle size of diets affects chewing activity (Mertens, 1997), decreased chewing time for cows fed DDGS diets can be attributed to the smaller particle size of DDGS relative to barley silage. Decreased chewing activity may have resulted in less secretion of saliva (Bowman et al., 2003), and low rumen pH for cows fed the DG diet. Duration of rumen pH below 5.8 was 3.9 h longer for cows fed the DG

diet than the CON diet. Milk fat yield was not affected by treatment, but milk fat concentration was decreased by feeding the DG diet compared with the CON diet. Although concentrations of short and medium chain fatty acids (C < 16) were not affected by treatment, concentration of C16, which partially comes from the de novo synthesis (Grummer, 1991), was decreased by feeding the DG diet. Low concentrations of C16 may be caused by the inhibition of de novo fatty acid synthesis or resulted from the increased concentrations of long chain fatty acids in milk. However, in the present study, the concentration of *trans*-10, *cis*-12 CLA in milk fat, an inhibitor for de novo synthesis of fatty acids in the mammary gland (Peterson et al., 2003; Lock et al., 2007), was not affected by feeding DDGS diet compared to CON diet.

3.4.2 Effects of inclusion of alfalfa hay in DG diet

Dietary inclusion of alfalfa hay in place of silage often increased chewing time (Allen and Grant, 2000) and milk fat concentration (Smith et al., 1993) when cows were fed high NFFS diets. In our previous study (Chapter 2), feeding DDGS in place of barley silage at 20% of dietary DM did not affect rumen pH or milk fat concentration, but the experimental diets contained alfalfa hay. Therefore, we hypothesized that dietary inclusion of alfalfa hay would alleviate the reductions in chewing time, rumen pH, and milk fat concentration that are often observed when cows were fed high NFFS diets (Allen and Grant, 2000; Smith et al., 1993). However, in the present study, addition of alfalfa hay in place of barley silage did not increase chewing time and rumen pH. The milk fat concentration was even reduced by feeding the DG+AH diet compared with the DG diet.

Our findings indicate that physical effectiveness of high NFFS diets may not be increased by dietary inclusion of alfalfa hay. However, Allen and Grant (2000) reported increased chewing time by replacing alfalfa silage with alfalfa

hay at 19% of dietary DM for cows fed high wet corn gluten feed diet. In the present study, the dietary inclusion of alfalfa hay was 10% and might not be high enough to stimulate chewing activity. The lack of stimulatory effect of alfalfa hay on chewing time may be also attributed to similar dietary PEF between the DG and DG+AH diets. In our study, although the proportion of diet particles retained on the 19-mm sieve of Penn State Particle Separator was greater for the DG+AH diet compared with the DG diet (6.0 ± 0.96 vs. 1.8 ± 0.75 %), these long particles may have little marginal impacts on chewing time compared with those retained on the 8-mm sieve. This observation is in agreement with Allen (1997); forage particle size affects total chewing time to a less extent if a mean sieve aperture size exceeds 3 mm.

Although rumen pH was similar between the DG and DG+AH treatments, milk fat concentration was lower for cows fed the DG+AH diet. Because there was no difference in milk fat yield, the treatment effect can be partly attributed to the dilution of milk fat by numerically higher milk yield for cows fed the DG+AH diet. In milk fat, there was an increase in the concentration of *trans*-10 C18:1 for cows fed the DG+AH diet compared with the CON diet. *Trans*-10 C18:1was previously considered to inhibit de novo fatty acid synthesis in the mammary gland and depress milk fat concentration (Griinari et al., 1998). However, a recent study (Lock et al., 2007) demonstrated that *trans*-10 C18:1 has no effect on milk fat synthesis. But, the greater concentration of *trans*-10 C18:1 in milk fat may indicate greater accumulation of *trans*-10 C18:1 in the rumen, suggesting that the biohydrogenation pathway was altered for cows fed the DG+AH diet. However, the concentration of *trans*-10, *cis*-12 CLA in milk fat was not affected by treatment. Therefore, it is not certain whether or not de novo fatty acid synthesis in the mammary gland was inhibited by feeding the diets containing DDGS.

In a previous study (Weidner and Grant, 1994), cows fed a diet

containing soy hulls and alfalfa hay decreased milk fat concentration compared with those fed a diet containing soy hulls without alfalfa hay. Milk fat concentration often decreases for cows fed diets containing long-chopped forages, which was attributed to sorting against long particles (Kononff and Heinrichs, 2003; Onetti et al., 2004). The greater DM concentration of DDGS relative to barley silage diets would have caused sorting against long particles; however, in the current study, we added water to the DG and DG+AH diets, which likely allowed the fine particles to stick to larger particles (Miller-Cushon and DeVries, 2009) and thus decreased sorting for fine particles. Leonardi et al. (2005) reported that the extent of sorting against long particles was reduced when the DM concentration of a hay-based diet was decreased from 80.8% to 64.4% by addition of water. In contrast, sorting against long particles was not reduced as DM concentration of a silage-based diet decreased from 57.6% to 47.9% by addition of water (Miller-Cushon and DeVries, 2009). These results imply that the addition of water likely prevents sorting if an initial diet is dry and easily sorted. In the present study, DM concentration of the DG+AH diet was decreased from 70% to 48% by addition of water, and cows fed the DG+AH diet did not sort against long particles, and sorting behavior does not explain lower milk fat concentration for the DG+AH treatment compared with the DG treatment. It is also noteworthy that cows fed the DG+AH diet actually sorted for long particles. This might be explained as an effort to alleviate low rumen pH. This is in agreement with the report that cows sorted for long feed particles as an attempt to meet physically effective fiber requirement when cows experience low rumen pH (Keunen et al., 2002; Beauchemin and Yang, 2005; DeVries et al., 2008).

3.5 Conclusion

Partial replacement of barely silage with wheat DDGS may increase DMI, and milk and milk protein yields of lactating dairy cows, but may decrease

chewing time, rumen pH, and milk fat concentration. Compared to the DG diet, cows fed the DG+AH diet decreased milk fat concentration but did not affect other response variables measured in this study. Dietary inclusion of alfalfa hay may not alleviate the reductions in chewing time, rumen pH, and milk fat concentration that occur when DDGS partially replaces forage in diets for lactating dairy cows.

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Table 3.1. Ingredients of experimental diets

Component	Diet[1], % of DM		
	CON	DG	DG+AH
Barley silage	49.5	30.2	19.8
Alfalfa hay	...	...	10.2
DDGS[2]	...	20.7	20.4
Concentrate mix[3]	36.7	36.8	36.7
Beet Pulp	4.6	12.1	12.9
Corn gluten meal	8.2	…	...
Urea	1.0	0.2	...

[1] CON: control; DG: 20% DDGS replacing barely silage; DG+AH: 20% DDGS + 10% alfalfa hay replacing barley silage.

[2] DDGS: wheat-based dried distillers grains with solubles.

[3] Concentrate mix including: 45.9% rolled corn, 45.9% rolled barley, 2.2% limestone, 0.8% dicalcium phosphate, 0.6% magnesium oxide, 1.6% NaCl, 3.0% premix (Contained 0.10% Ca; 0.60% P; 11.50% Na; 0.30% Mg; 10 mg/kg F; 80 mg/kg I; 5000 mg/kg Zn; 31000 mg/kg Mn; 1170 mg/kg Cu; 6.2 mg/kg Co; 1265 KIU/kg vitamin A; 142 KIU/kg vitamin D; 3800 IU/kg vitamin E).

Table 3.2. Nutrient composition and particle size distribution of experimental diets

Item	Diet[1]					
	CON (n = 3)		DG (n = 3)		DG+AH (n = 3)	
Composition, %DM	Mean	SD	Mean	SD	Mean	SD
DM (as fed)	49.3	1.0	49.1	1.4	48.0	1.1
OM	89.9	0.9	91.8	0.9	90.6	0.9
CP	20.3	0.8	20.8	0.5	20.3	0.6
NDF	37.0	0.4	35.6	0.9	34.9	1.1
Forage NDF	26.3	0.3	16.1	0.5	15.2	0.4
Starch	27.4	0.3	25.2	1.4	24.1	1.4
Ether extract	2.6	0.3	2.5	0.3	2.4	0.4
NFC[2]	30.0	1.4	33.0	2.1	33.0	0.3
Particle size distribution, % (as fed)						
> 19 mm	3.0	0.6	1.8	0.8	6.0	1.0
19 - 8 mm	36.5	2.0	22.3	2.5	16.7	1.1
< 8 mm	60.5	2.4	75.8	6.0	77.3	5.1
PEF[3]	0.39	0.03	0.24	0.02	0.23	0.02

[1] CON: control; DG: 20% DDGS replacing barely silage; DG+AH: 20% DDGS + 10% alfalfa hay replacing barley silage.

[2] NFC = 100 – (NDF% + CP% + Ether extract% + Ash%).

[3] PEF = physical effectiveness factor determined as the proportion of particles retained on 19- and 8-mm sieves (Lammers et al., 1996).

Table 3.3. Effects of partially replacing barley silage with DDGS or DDGS plus alfalfa hay in diets for lactating dairy cows on DMI and chewing activity

Item	Diet[1]			SEM	*P* value
	CON (n = 29)	DG (n = 29)	DG+AH (n = 28)		
DMI, kg/d	20.1[b]	23.1[a]	22.7[a]	0.3	< 0.01
Time, min/d					
Eating	280[a]	234[b]	234[b]	9	< 0.01
Ruminating	482	468	474	12.1	0.37
Total chewing[2]	762[a]	702[b]	708[b]	14.4	< 0.01
Time, min/kg DMI					
Eating	14.1[a]	10.2[b]	10.4[b]	0.4	< 0.01
Ruminating	24.3[a]	20.5[b]	21.1[b]	0.6	< 0.01
Total chewing	38.3[a]	30.7[b]	31.5[b]	0.8	< 0.01

[a,b]Least square means with different superscripts differ significantly ($P < 0.05$).

[1] CON: control; DG: 20% DDGS replacing barely silage; DG+AH: 20% DDGS + 10% alfalfa hay replacing barley silage.

[2] The sum of eating time and ruminating time.

Table 3.4. Effects of partially replacing barley silage with DDGS or DDGS plus alfalfa hay in diets for lactating dairy cows on rumen fermentation of ruminally cannulated cows

Item	Diet[1]			SEM	*P* value
	CON (n = 5)	DG (n = 5)	DG+AH (n = 5)		
Rumen pH					
Minimum	5.28	5.09	5.07	0.05	0.06
Mean	6.11[a]	5.88[b]	5.84[b]	0.05	< 0.01
Maximum	6.87	6.87	6.77	0.04	0.12
Duration, h/d					
pH < 5.8	7.3[b]	11.2[a]	12.0[a]	0.9	0.01
pH < 5.5	3.6[b]	6.9[a]	7.4[a]	0.6	< 0.01
pH < 5.2	1.2	2.8	2.5	0.5	0.13
Area, pH × h/d					
pH < 5.8	2.4[b]	4.6[a]	4.7[a]	0.4	< 0.01
pH < 5.5	0.8	1.8	1.8	0.3	0.06
pH < 5.2	0.1	0.4	0.3	0.1	0.25
Total VFA, m*M*	114.5	123.3	132.5	5.0	0.11
VFA molar proportions, mol/100 mol					
Acetate	62.1[a]	59.2[b]	58.0[b]	0.8	< 0.01
Propionate	20.9[b]	24.1[ab]	27.3[a]	1.1	0.02
Isobutyrate	0.9[a]	0.7[b]	0.5[b]	0.04	< 0.01
Butyrate	11.8	12.5	10.6	0.8	0.35
Isovalerate	1.6[a]	1.0[ab]	0.7[b]	0.2	0.01
Acetate / Propionate	3.0[a]	2.5[b]	2.2[b]	0.1	< 0.01
Rumen NH_3-N, mg/dL	22.8	15.4	11.6	3.0	0.10

[a,b]Least square means with different superscripts differ significantly ($P < 0.05$).

[1] CON: control; DG: 20% DDGS replacing barely silage; DG+AH: 20% DDGS + 10% alfalfa hay replacing barley silage.

Table 3.5. Effects of partially replacing barley silage with DDGS or DDGS plus alfalfa hay in diets for lactating dairy cows on sorting behavior

Sorting index[2]	Diet[1]			SEM	*P* value
	CON (n = 29)	DG (n = 29)	DG+AH (n = 28)		
>19.0 mm	90.9[c]	100.5[b]	104.9[a]	1.3	< 0.01
19.0 to 8.0 mm	96.6[b]	98.5[a]	96.0[b]	0.5	< 0.01
< 8.0 mm	102.6[a]	100.5[b]	100.5[b]	0.2	< 0.01

[a,b]Least square means with different superscripts differ significantly ($P < 0.05$).

[1] CON: control; DG: 20% DDGS replacing barely silage; DG+AH: 20% DDGS + 10% alfalfa hay replacing barley silage.

[2] A sorting index above 100 indicates sorting for particles, and a sorting index below 100 indicates sorting against particles (Leonardi and Armentano, 2003).

Table 3.6. Effects of partially replacing barley silage with DDGS or DDGS plus alfalfa hay in diets for lactating dairy cows on milk yield and milk composition, change of BW and BCS

Item	Diet[1]			SEM	*P* value
	CON (n = 29)	DG (n = 29)	DG+AH (n = 28)		
Yield, kg/d					
Milk	24.5[b]	27.3[a]	28.1[a]	1.1	< 0.01
Fat	0.95	0.96	0.92	0.04	0.53
Crude protein	0.88[b]	0.99[a]	1.01[a]	0.03	< 0.01
Lactose	1.11[b]	1.24[a]	1.29[a]	0.05	< 0.01
Milk composition, %					
Fat	3.92[a]	3.60[b]	3.38[c]	0.12	< 0.01
Crude protein	3.66	3.67	3.64	0.05	0.76
Lactose	4.52	4.52	4.55	0.03	0.55
MUN, mg/dL	21.3[a]	16.0[b]	13.9[c]	0.52	< 0.01
BW change, kg/d	0.25[b]	1.17[a]	1.23[a]	0.11	< 0.01
BCS change, /21 d	0.06[b]	0.23[a]	0.11[a]	0.04	0.02

[a-c]Least square means with different superscripts differ significantly ($P < 0.05$).

[1] CON: control; DG: 20% DDGS replacing barely silage; DG+AH: 20% DDGS + 10% alfalfa hay replacing barley silage.

Table 3.7. Effects of partially replacing barley silage with DDGS or DDGS plus alfalfa hay in diets for lactating dairy cows on milk fatty acids profile of ruminally cannulated cows

Item	Diet[1]			SEM	*P* value
	CON (n = 5)	DG (n = 5)	DG+AH (n = 5)		
g/100 g of total fatty acids					
C4:0	1.39	0.94	1.25	0.22	0.40
C5:0	0.32	0.31	0.30	0.02	0.64
C6:0	1.85	1.79	1.53	0.15	0.35
C7:0	0.05	0.07	0.05	0.01	0.33
C8:0	1.12	1.17	1.04	0.15	0.82
C9:0	0.08	0.07	0.14	0.02	0.05
C10:0	3.60	3.44	3.26	0.21	0.57
C11:0	0.39	0.39	0.37	0.02	0.63
C12:0	4.27	4.14	3.82	0.20	0.33
C14:0	12.6	12.3	12.0	0.30	0.10
C14:1	1.51	1.33	1.55	0.14	0.47
C15:0	1.55	1.65	1.76	0.11	0.37
C16:0	31.9[a]	29.0[b]	28.5[b]	0.4	< 0.01
C16:1 *t*	0.43	0.49	0.54	0.03	0.05
C16:1 *c*	2.38	1.68	2.08	0.47	0.42
C18:0	7.30	8.49	6.96	0.50	0.11
t-6 or *t*-8 C18:1	0.18	0.28	0.32	0.08	0.55
t-9 C18:1	0.20	0.32	0.31	0.06	0.36
t-10 C18:1	0.36[b]	0.60[ab]	1.14[a]	0.15	0.03
t-11 C18:1	1.14	1.52	2.65	0.46	0.13
c-12 C18:1	0.27[b]	0.43[ab]	0.56[a]	0.04	< 0.01
c-9 C18:1	18.6	19.3	18.5	0.40	0.38
c-7 C18:1	0.51	0.58	0.78	0.08	0.11
C19:0	0.18	0.23	0.26	0.02	0.17
t-9, *t*-12 C18:2	0.15	0.14	0.13	0.02	0.68
c-9, *c*-12 C18:2	3.00[b]	4.03[a]	4.36[a]	0.21	< 0.01
c-9, *t*-11 C18:2	0.56	0.62	0.94	0.13	0.13
t-10, *c*-12 C18:2	0.03	0.03	0.04	0.01	0.11
C18:3 w3	0.28[b]	0.34[b]	0.41[a]	0.02	< 0.01
C18:3 w6	0.05	0.06	0.15	0.06	0.46
C20:0	0.16[a]	0.15[ab]	0.12[b]	0.01	0.03
C20:1 w12	0.18	0.16	0.15	0.01	0.04
C20:1 w15	0.05[b]	0.07[ab]	0.09[a]	0.01	0.02

C20:3 w6	0.13	0.15	0.17	0.01	0.07
C22:0	0.06[a]	0.05[b]	0.04[b]	0.01	< 0.01
C20:4	0.17	0.16	0.15	0.01	0.34
Short and medium (C <16)	30.2	28.9	28.3	0.6	0.18
C16[2]	35.2[a]	31.8[b]	31.6[b]	0.4	< 0.01
Long (C > 16)	34.6[b]	39.2[a]	40.1[a]	0.6	< 0.01
SFA[3]	68.3	65.6	62.6	1.3	0.06
UFA[3]	31.7	34.4	37.4	1.3	0.06

[a,b]Least square means with different superscripts differ significantly ($P < 0.05$).

[1] CON: control; DG: 20% DDGS replacing barely silage; DG+AH: 20% DDGS + 10% alfalfa hay replacing barley silage.

[2] C16: the sum of C16:0, C16:1 *t*, and C16:1 *c*.

[3] SFA: saturated fatty acids; UFA: unsaturated fatty acids.

CHAPTER 4. GENERAL DISCUSSION

4.1 Summary of findings

The first experiment investigated effects of replacing barley silage (LF) or barley grain (LG) with a blend of corn and wheat DDGS at 20% of dietary DM on DMI, chewing activity, rumen fermentation, and milk production using six ruminally cannulated lactating Holstein cows. Cows fed the LF diet had greater DMI and milk yield compared with those fed the CON diet. The yields of milk protein and milk lactose were greater for cows fed the LF diet but milk fat yield was not affected. The total chewing time per d was not affected, but chewing time per unit of DMI (min/kg of DMI) was decreased for the LF treatment. This was probably due to reduced intake of long particles compared with the CON diet. The LF diet did not affect rumen pH and duration that rumen pH below 5.8. It was concluded that partial replacement of barley silage with DDGS can increase milk yield of lactating dairy cows without negatively affecting rumen fermentation and milk fat production. Cows fed the LG diet tended to increase minimum and maximum rumen pH, which was attributed to the high NDF and low starch concentration of the LG diet. However, DMI, milk yield and milk composition were not affected by feeding the LG diet. Barley grain also can be partially replaced by DDGS in diets for lactating dairy cows without negative effects on milk production.

The second experiment was conducted to investigate effects of feeding wheat DDGS as a replacement of barley silage (DG) at 20% of dietary DM on chewing time, rumen fermentation and milk fat concentration of lactating dairy cows. Another objective was to determine the effects of inclusion of alfalfa hay in place of barley silage at 10% of dietary DM (DG+AH) on the response variables mentioned above. Thirty cows in late lactation, six of which were ruminally cannulated, were used in this study. Cows fed DG and DG+AH diets had greater

DMI and yields of milk, milk protein, and milk lactose, as well as greater BW and BCS gain. But no difference was observed between cows fed DG and DG+AH diets except for milk fat concentration, which was significantly lower for cows fed DG+AH compared with those fed DG. However, milk fat yield was not affected by dietary treatment. The chewing time was shorter for cows fed DG and DG+AH diets than those fed CON diet, and subsequently decreased minimum and mean rumen pH, and increased the duration and area that rumen pH below 5.8. It was concluded that partially replacing barley silage with DDGS can improve productivity of lactating dairy cows, but it may also decrease chewing time, rumen pH, and milk fat concentration. The inclusion of alfalfa hay did not increase the portion of particles that are retained on the screen of 8-mm apertures or greater. The results may indicate that inclusion of alfalfa hay was not effective at stimulating chewing activity of cows fed a high DDGS diet.

4.2 Inconsistent effects of replacing DDGS for barley silage

Effects of partially replacing barley silage with DDGS on rumen pH and milk fat concentration were not consistent between the two studies. In the first experiment, DDGS replaced barley silage at 20% of dietary DM without negative effects on rumen fermentation and milk fat concentration. However, the similar dietary change decreased rumen pH and milk fat concentration for the second study.

The discrepancy may be partially attributed to the difference in the characteristics of DDGS; blend of corn and wheat DDGS (70% corn and 30% wheat) was used for the first study while 100% wheat DDGS was used in the second study. The wheat DDGS was higher in rapidly degradable free sugar content (18.2 vs. 4.3% on a DM basis; Nuez Ortín and Yu, 2009). Therefore, the diet with wheat DDGS might be fermented at a faster rate than the diet with the blend of corn and wheat DDGS, and might have resulted in accumulation of VFA

and decrease in rumen pH. In addition, the inconsistent results may be attributed to differences in the capacity of VFA absorption by ruminal epithelial cells between animals used in the two experiments as the rate of VFA absorption is also expected to affect rumen pH (Allen, 1997).

For both studies, we did not observe any differences in concentration of *trans*-10, *cis*-12 conjugated linoleic acid in milk, which was identified as a potent inhibitor of de novo milk fat synthesis (Peterson et al., 2003). In addition, milk fat yield was similar among treatments, indicating that milk fat was diluted by the higher milk yield for cows fed DDGS in the second study. Further, lactation stage may also account for the different responses. In the first experiment, three cows were in early lactation and three cows were in late lactation while all cows used in the second experiment were in late lactation. It was reported that the decreased ratio of forage to concentrate had a greater effect on milk fat production of cows in late lactation than those in early lactation (Kennelly et al., 1999; Khorasani and Kennelly, 2001) because cows in early lactation could mobilize their body adipose tissue to meet the demand for milk fat synthesis (McNamara, 1991). As such, dietary and animal factors together may explain inconsistent effects of feeding DDGS as a partial replacement for barley silage on rumen pH and milk fat concentration.

4.3 Future research

The dietary inclusion of DDGS was recommended not to exceed 20% of dietary DM when it was used as a dietary protein source because low protein digestibility (Owen and Larson, 1991) or poor amino acid profile of DDGS (Grings et al., 1992) may negatively affect milk yield. However, our results showed that total tract digestibility and milk yield were increased by replacing barley silage with DDGS at 20% of dietary DM. Dietary inclusion of DDGS may be increased further by partially replacing both concentrates and barley silage, for

example 30% DDGS replacing for 20% concentrates and 10% barley silage. There are at least three advantages for this strategy. First, it lowers the risk of rumen acidosis compared with diet formulation to increase dietary starch concentrations. Secondly, milk production would be increased as a result of the higher energy value of DDGS relative to barley silage. Additionally, the feed cost may be reduced if DDGS is fed in place of other expensive feedstuffs. The optimum inclusion of DDGS in diets for lactating dairy cows warrants further investigations.

Recently, greenhouse gas production from animal feeding has become a public concern. There are varieties of nutritional strategies proposed to address this issue, such as increasing the ratio of grain in the diet, supplementation of lipids (Beauchemin et al., 2009) or Monensin (Odongo et al., 2007) in diets. Partially replacing barley grain with corn DDGS was also found to reduce the methane emission by ruminants (McGinn et al., 2009). In our study, we found that feeding wheat DDGS in place of barley silage decreased rumen pH and increased the propionate to acetate ratio in rumen, which was associated with a reduction of methanogenesis (Russell, 1998). As such, these results suggest feeding DDGS as a partial replacement of forage can be a new approach to reduce methane production by dairy cows. It is worth conducting further research to investigate the effect of partially replacing barley silage with DDGS on methane production of dairy cattle. However, there is another environmental issue; increased phosphorous excretion may be a concern with the use of DDGS in ruminant diets due to the high phosphorous concentration of DDGS. Therefore, it is very important to consider both pros and cons associated with feeding DDGS: reducing methane production and increasing the phosphorus excretion to run-off water.

4.4 Economic analysis

Cost of primary feedstuffs used in the present studies in Edmonton in

December 2009 is shown in Table 4.1. The costs of DDGS and premix of minerals and vitamins were assumed to be $200/T and $1,000/T, respectively. The calculated costs of diets were $236, $231, and $205/T for CON, LF, and LG treatments, respectively. Assuming that diets were fed at 110% of expected feed intake, feeding costs were $5.8, $6.6, and $5.3/cow/d (Table 4.2) for the CON, LF, and LG diets, respectively. In Alberta, producers receive approximately $0.70/hL of milk. The milk income was $23.1, $25.5, and $24.3 with the net income of $17.3, $18.9, and $19.0 /cow/d by feeding the CON, LF, and LG diets, respectively. The results indicate that, compared with feeding the CON diet, the profitability of dairy operations was increased by feeding DDGS as a partial replacement of barley silage (+ $1.6/cow/d) or barley grain (+ $1.7/cow/d).

Net income generated by feeding DDGS as a substitute for barley silage or barley grain decreases as the price of DDGS increases (Figure 4.1). Break-even point in the price of DDGS is $483 or $517/T for a diet in which DDGS replaces barley silage or barley grain, respectively.

4.5 General conclusion and industry perspective

The characteristics of DDGS as a protein feed have been well documented in the literature, but the information about the feeding value of DDGS as a replacement of barley silage or barley grain in diets of dairy cows was limited. The present study explored the characteristics of DDGS as a high NDF and high energy feedstuff, and provided industry with very valuable information that DDGS could be used as an alternative feed to partially substitute barley silage or barley grain in diets for dairy cows.

The results presented in this thesis provides alternative approaches in nutritional management of lactating dairy cows, especially under the situation that the supply of forage is in short or the quality of forage is poor. Additionally, if feeding DDGS increases the concentration of UFA in milk, this may be also

considered as an advantage for human health (Hu and Willett, 2002).

As a summary, replacement of barley silage with DDGS at 20% of dietary DM can increase DMI and yields of milk, milk protein, and milk lactose as well as the concentrations of UFA. Substituting DDGS for barley grain at 20% of dietary DM can maintain milk yield without affecting milk composition but increased the concentrations of UFA in milk. Additionally, it tended to increase rumen pH and thereby reduce the risk of rumen acidosis of high producing dairy cows. These data indicate that DDGS can be a good alternative to forage or grain as an energy source in diets of lactating dairy cows, and potentially improve the profitability of dairy operations.

4.6 References

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Table 4.1. Cost of individual feed ingredients and CON, LF, and LG diets, $/T on DM basis

	Cost	Cost of diets[1]		
		CON	LF	LG
Barley silage	100	45.0	25.0	45.0
Alfalfa hay	152	7.6	7.6	7.6
Barley grain	229	80.2	80.2	34.4
Canola Meal	371	11.5	…	…
Corn gluten meal	798	44.7	2.8	4.0
Beet pulp	348	8.4	37.6	38.2
DDGS	200	…	40.0	40.0
Urea	865	0.9	1.7	…
Mineral and vitamin premix	1000	38.2	36.5	35.4
Total	…	236	231	205

[1] CON: control; DG: 20% DDGS replacing barely silage; DG+AH: 20% DDGS + 10% alfalfa hay replacing barley silage.

Table 4.2. Feed cost and milk income of feeding CON, LF, and LG diets

	Diets[1]		
	CON	LF	LG
DMI, kg/d	22.4	26.0	23.7
Feed cost[2], $/d	5.8	6.6	5.3
Milk yield, kg/d	33.0	36.4	34.7
Milk income[3], $/d	23.1	25.5	24.3
Net income[4], $/d	17.3	18.9	19.0

[1] CON: control; DG: 20% DDGS replacing barely silage; DG+AH: 20% DDGS + 10% alfalfa hay replacing barley silage.

[2] Feed cost = DMI × 1.1 × cost of diets.

[3] Milk income = milk yield × $0.70/kg.

[4] Net income = milk income – feed cost.

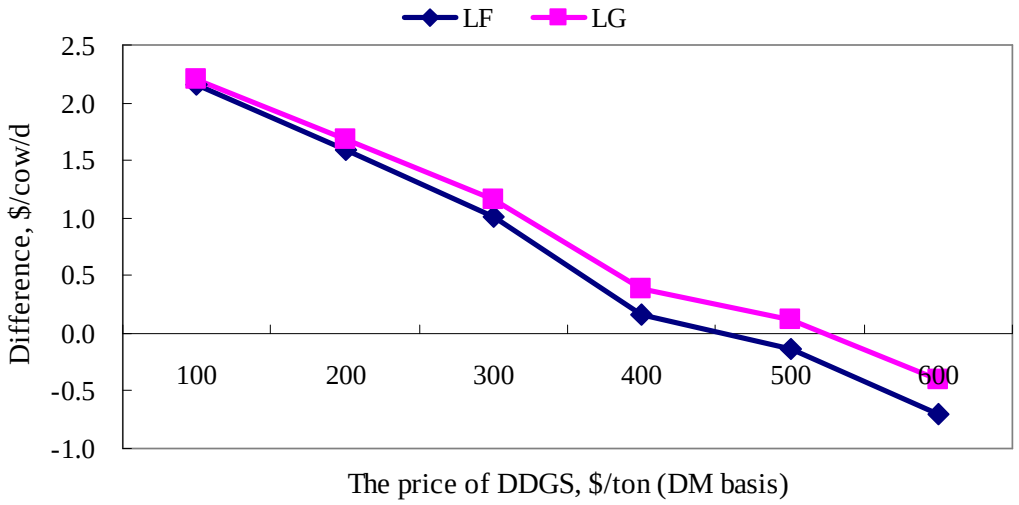

Figure 4.1. Effects of DDGS price on the difference in net income generated between a cow fed the CON diet and a cow fed a diet in which DDGS replaces barley silage (LF) or barley grain (LG) at 20% of dietary DM.