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Effect of substituting soybean meal and canola cake with grain-based dried distillers grains with solubles as a protein source on feed intake, milk production, and milk quality in dairy cows

C. Gaillard,* M. T. Sørensen,* M. Vestergaard,* M. R. Weisbjerg,* A. Basar,* M. K. Larsen,† H. Martinussen,‡ U. Kidmose,§ and J. Sehested*[1]
*Department of Animal Science and
†Department of Food Science, Aarhus University, 8830 Tjele, Denmark
‡SEGES, 8200 Aarhus N, Denmark
§Department of Food Science, Aarhus University, 5792 Aarslev, Denmark

ABSTRACT

The growth of the bioethanol industry is leading to an increase in the production of coproducts such as dried distillers grains with solubles (DDGS). Both corn-based DDGS and grain-based DDGS (gDDGS; defined as originating from grain sources such as barley, wheat, triticale, or a mix, excluding corn) appear to be relevant sources of feed and protein for dairy cows. To date, most of the studies investigating DDGS have been performed with corn-based DDGS. The objectives of this study were to determine the effects of the proportion of gDDGS in the diet on feed intake, milk production, and milk quality. The present experiment involved 48 Holstein cows in a replicated 3 × 3 Latin square design with 3 grass-based dietary treatments consisting of 4, 13.5, and 23% gDDGS on a dry matter (DM) basis (L, M, and H, respectively) as a replacement for a concentrate mix. The concentrate mix consisted of soybean meal, canola cake, and beet pulp. Dry matter intake and energy-corrected milk yield were not affected by the proportion of gDDGS in the diet. Daily milk yield decreased with the H diet compared with the L and M diets. The percentage of fat in milk was higher when cows were fed the H diet compared with the L and M diets, whereas milk fat yield was not affected by dietary treatment. The M diet had a higher percentage of protein in milk compared with the L and H diets. Milk protein yield was similar for the L and M diets; however, it decreased for the H diet. Milk taste was not affected by the proportion of gDDGS in the diet or when milk was stored for 7 d. Linoleic acid and conjugated linoleic acid *cis*-9,*trans*-11 in milk increased with increasing proportion of gDDGS. To conclude, gDDGS can replace soybean meal and canola cake as a protein source in the diet of dairy cows. Up to 13.5% of the diet may consist of gDDGS without negatively affecting milk production, milk quality, or milk taste. When gDDGS represents 23% of dietary DM, milk production is reduced by 1.6 kg/d, whereas energy-corrected milk production is numerically reduced by 1 kg.
Key words: dairy cow, dried distillers grains with solubles, protein source, coproduct

INTRODUCTION

The growth of the bioethanol industry is resulting in increased production of coproducts, such as dried distillers grains with solubles (**DDGS**). The composition and quality of DDGS vary (Belyea et al., 2010) depending on the type of feedstock used (typically corn or another type of grain, such as wheat, barley, or triticale), the processing steps used during ethanol production, and the subsequent mixing and drying of distillers grains and solubles (Azarfar et al., 2012; Li et al., 2012; Pedersen et al., 2014). It has been documented that DDGS is a relevant feed for dairy cows because it is high in CP protein and fiber; however, thus far, most experiments have been conducted using corn-based DDGS (**cDDGS**) in combination with corn silage-based diets (De Boever et al., 2014; Pedersen et al., 2014). The high content of CP in both cDDGS (271–364 g/kg of DM; Pedersen et al., 2014) and the other grain-based DDGS (**gDDGS**; wheat DDGS with 303–383 g of CP/kg of DM; Pedersen et al., 2014) makes DDGS an interesting alternative feed protein source. The proteins in DDGS are moderately resistant to ruminal degradation and are a good source of RUP (55.6 and 59.3% of CP for wheat and wheat-and-corn gDDGS, respectively, and 69.8% of CP for cDDGS; De Boever et al., 2014). Christen et al. (2010) tested 4 different sources of feed protein: soybean meal, high-protein cDDGS, cDDGS, and canola

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[1]Corresponding author: jakob.sehested@anis.au.dk

meal. The diets were formulated to be isonitrogenous at 16% CP and isolipidic at 4.7% fat. Christen et al. (2010) found that DMI, milk yield, protein yield, and fat yield were similar for the 4 treatments. Oba et al. (2010) arrived at a similar conclusion when comparing 4 different sources of protein: triticale-based DDGS, cDDGS, soybean meal, and canola meal. Results for the effects of DDGS on fat content in milk are variable; however, most of the studies reported no changes in milk fat content when cows were fed DDGS diets compared with other diets (Kleinschmit et al., 2006; Janicek et al., 2008). Overall, cDDGS had no negative effect on milk yield and milk composition (Christen et al., 2010; Oba et al., 2010; Benchaar et al., 2013).

When testing the effects of increasing the proportion of cDDGS in the diet (0, 10, 20, and 30% of DM) at the expense of corn and soybean meal, Benchaar et al. (2013) found that milk yield, DMI, and milk protein yield increased with increasing proportion of cDDGS, whereas milk fat yield was not affected by the proportion of cDDGS. The meta-analysis of Hollmann et al. (2011), based on 16 studies, reported an increase in milk yield with increasing proportion of cDDGS in the diet, peaking at 1.2 kg of additional milk/d at 21% cDDGS of diet DM basis. Milk fat concentration was not affected by dietary cDDGS when the diet contained less than 21% of cDDGS (Hollmann et al., 2011). Reported effects of cDDGS on milk fat content have been variable among studies, making it difficult to define the optimum inclusion level of cDDGS in the diet. Leonardi et al. (2005) found no change in milk fat content when the proportion of cDDGS increased from 0 to 15% of dietary DM. Overall, the inclusion of cDDGS, up to 20% of dietary DM, would increase milk yield and maintain milk components (Leonardi et al., 2005; Anderson et al., 2006; Kleinschmit et al., 2006). Janicek et al. (2008) also found no negative effect on lactation performance when including up to 30% cDDGS of diet DM basis; however, above 30% inclusion, the DMI and milk yield decreased (Owen and Larson, 1991; Kalscheur, 2005). Lysine was the most limiting AA for milk protein synthesis when cDDGS replaced soybean meal (Owen and Larson, 1991; Kleinschmit et al., 2006).

In Europe and Canada, wheat and grain blends are commonly used as substrates for bioethanol production (De Boever et al., 2014), and in Northern Europe, gDDGS are exclusively used in dairy feeds. Few studies have focused on the inclusion of gDDGS in a feed ration for dairy cows. Triticale-based DDGS seems to have the same advantages as cDDGS (Oba et al., 2010) and does not impair the productivity of lactating dairy cows (Greter et al., 2008), encouraging further investigations into the use of gDDGS. To our knowledge, the inclusion of gDDGS as a protein feed in a grass-clover-based diet for dairy cows, as used in Northern Europe, has not been studied yet. The present experiment involved 3 grass-clover-based diets with different ratios of 2 feed protein sources: gDDGS (originating from triticale, wheat, and barley) and a soybean–canola mix. The objective was to determine the effects of increasing the proportion of gDDGS in the diet on feed intake, milk production, and milk quality. We hypothesized that the inclusion of gDDGS at the levels tested would not have negative effects on milk production, milk quality, or milk taste.

MATERIALS AND METHODS

Experimental Facilities and Animals

The experiment was approved by the Animal Experiments Inspectorate under the Danish Veterinary and Food Administration and was carried out from March to May 2013 at the Danish Cattle Research Centre at Aarhus University, Foulum, Denmark. A total of 48 Danish Holstein cows (18 primiparous and 30 multiparous) were included in the experiment. The animals were housed as one group in a loose housing system with slatted floors and cubicles with mattresses and sawdust as bedding. Cows had free access to water and automatic feed bins (RIC system, Insentec, Marknesse, the Netherlands). The automatic milking unit (**AMU**; DeLaval AB, Tumba, Sweden) was equipped with a device for delivering and recording the amount of concentrate and refusals.

Experimental Design

The experimental animals were blocked according to parity (primiparous and multiparous), milk production (average of 38 ± 9 kg of milk/d), and DIM (average of 88 ± 78 DIM when starting the experiment) and randomly assigned to treatments within blocks. The experiment was organized as a replicated 3 × 3 Latin square design with 3 dietary treatments. Sampling occurred during the third week of each period. The cows received a partially mixed ration (**PMR**) ad libitum in automatic feeders. Feed was added to feeders 4 times/d to minimize feed sorting effect. Cows also received restricted amounts of concentrate in the AMU (3 kg of concentrate/d). If a cow ate less than the daily 3 kg of concentrate allowed in the AMU, the amount not eaten (up to 1.5 kg) was allowed on top of the 3-kg allowance on the following day. Each group of cows had access to one third of the available automatic feeders for the PMR, with an average of 2 cows/feeder. During diet rotation, the cows kept the same feeders to avoid any perturbation effect. The composition of the 3 diets is

presented in Table 1. The PMR was based on silages (grass-clover and corn), rolled barley, and sodium hydroxide–treated whole wheat. The PMR also contained varying proportions of 2 protein feeds: (1) a concentrate mixture based on commonly used protein feeds for dairy cows in Northern Europe (soybean meal, canola cake with oil extracted by mechanical pressure, and dried beet pulp; referred to as "mix") or (2) gDDGS (Agrodrank 90; Lantmännen Agroetanol, Norrköping, Sweden) based on triticale (25% DM), wheat (55% DM), and barley (20% DM). The mix feed resembled gDDGS with respect to CP, NDF, and OM digestibility. The gDDGS substituted the mix in increasing proportions, consisting of 4, 13.5, and 23% of dietary DM (**L**, **M**, and **H** diets, respectively). The chemical composition of the mix and gDDGS is shown in Table 2.

Recordings

Individual daily feed intake was summed from PMR and concentrate intake recorded at each visit to the Insentec feeder and the AMU, respectively. All feeds were sampled weekly and stored at −20°C until pooled, and a representative sample was drawn for chemical analysis. Individual milk yield was recorded at each visit to the AMU and summed to obtain daily milk yield. Daily milking frequency was also recorded. Individual milk samples were collected weekly by the AMU using a modified automatic sampler (XMS, DeLaval; Løvendahl and Bjerring, 2006). Individual milk samples were taken over a 48-h period, starting on Sunday at noon and finishing on Tuesday at noon. The individual samples were preserved with bronopol and kept cold (4°C) until analyzed for fat, protein, lactose, and cells. Within the same period 1 milk sample was taken, frozen immediately, and kept below −18°C until analysis for fatty acids (**FA**). For sensory analysis, milk was sampled manually from the milking robot on Tuesday of the third week of an experimental period. Samples of approximately 5 L of milk from each of 6 cows per treatment were taken. These samples were pooled to obtain one composite sample per treatment and used for sensory analysis. A similar sampling was done the week before (on Wednesday of the second week of an experimental period) to obtain stored milk (7 d) for testing.

The ECM yield (3.140 MJ/kg) was calculated using the equation of Sjaunja et al. (1991):

$$\text{ECM} = \text{milk yield} \times [(38.3 \times \text{fat} + 24.2 \times \text{protein} + 15.71 \times \text{lactose} + 20.7)/3.140],$$

Table 1. Composition of the 3 diets[1] containing grain-based dried distillers grains with solubles (gDDGS) fed to Holstein cows

Item, % DM	L	M	H
PMR[2]			
gDDGS	4.0	13.5	22.8
Concentrate mix			
Soybean meal	7.4	3.6	0
Canola cake	5.5	2.7	0
Dried beet pulp	5.5	2.7	0
Barley	3.8	3.8	3.8
Wheat, NaOH	4.9	4.9	4.9
Grass-clover silage	15.3	15.3	15.2
Corn silage	45.7	45.7	45.5
Automatic milking unit concentrate	7.9	7.8	7.8
PMR chemical composition			
CP	16.3	16.5	16.8
NDF	33.0	33.2	33.4
Starch	28.4	28.6	28.6
Crude fat	3.3	3.5	3.7
Sugars	2.5	2.0	1.5
Ash	5.7	5.6	5.4
Digestible OM[3]	84.0	83.5	83.0

[1]L = diet containing 4% gDDGS; M = diet containing 13.5% gDDGS; H = diet containing 23% gDDGS.

[2]PMR = partially mixed ration.

[3]The digestible OM in % DM comes from the in vivo digestibility (%OM) calculated with the equations presented in Volden (2011) using the enzyme digestible OM (82.7 for rapeseed cake, 99.4 for soybean meal, 91.1 for barley, 99.6 for wheat NaOH, 88.3 for DDGS, 93.3 for dried beet pulp) and in vitro OM digestibility (76.8 for grass-clover silage, 71.9 for corn silage) measurements.

Table 2. Chemical composition of the concentrate mix and grain-based dried distillers grains with solubles (gDDGS) diets

Item	Mix	gDDGS[1]
DM, %	90	91
Ash, % DM	7.4	5.6
CP, % DM	33.5	33.9
Crude fat, % DM	4.8	6.7
NDF, % DM	21.7	23.9
ADF-N, % DM	0.2	0.6
ADF-N, % N	4.2	10.6
Starch, % DM	0.8	2.6
Sugars, % DM	9.3	4.3
Digestible OM, %	92.5	86.8
AA, g/kg of DM		
Alanine	15.5	13.4
Arginine	23.6	13.9
Asparagine	35.2	17.5
Cysteine	6.1	6.6
Glutamine	58.8	84.2
Glycine	15.8	13.7
Histidine	9.2	6.8
Isoleucine	16.2	11.9
Leucine	25.6	21.2
Lysine	20.9	7.9
Methionine	5.5	4.9
Phenylalanine	16.2	14.8
Proline	18.3	29.7
Serine	17.9	15.8
Threonine	14.6	10.6
Valine	18.4	15.4

[1]AgroDrank 90 (Lantmännen Agroetanol, Norrköping, Sweden) based on triticale (25% DM), wheat (55% DM), and barley (20% DM).

with ECM and milk yield in kilograms and fat, protein, and lactose in grams per kilogram.

Laboratory Analysis

Feed Analysis. The DM content of grass-clover and whole-corn silages was determined weekly by drying feed samples in a forced-air oven at 60°C for 48 h. The results were used for weekly adjustments of diet recipes. All feed samples were milled through a 1-mm screen before chemical analysis. Ash content was analyzed by weighing after combustion at 525°C for 6 h (AOAC International, 2000). Crude protein was calculated based on the analysis of total N according to the Dumas principle (Hansen, 1989) using a Vario MAX CN (Elementar Analysesysteme GmbH, Hanau, Germany). Crude fat was analyzed by Soxhlet extraction with petroleum ether after hydrolyzing with HCl. Sugar was analyzed by the Luff-Schoorl method (European Community, 2012; 71/250/EEC). Starch was analyzed by an enzymatic colorimetric technique (Knudsen et al., 1987). The NDF was determined using the Fibertec M6 system (Foss Analytical, Hillerød, Denmark) using heat-stable amylase to remove starch followed by neutral detergent boiling and was reported as being ash free (Mertens et al., 2002). Determination of in vitro digestibility of OM in forages was performed by 48-h anaerobic incubation in diluted rumen fluid followed by 48-h incubation of insoluble material with pepsin HCl solution (Tilley and Terry, 1963). Rumen fluid was harvested from 3 dry cows fed a ration consisting of 4 kg of artificially dried grass hay/d, 2 kg of barley straw/d, and 2.8 kg of concentrate on maintenance level/d. The in vitro digestibility of the concentrates was determined by first incubating the samples with pepsin HCl solution for 24 h, after which the samples were heated to 80°C for 45 min, treated for 24 h with enzyme mixture at 40°C, and then further incubated for 19 h at 60°C (Weisbjerg and Hvelplund, 1993). Nitrogen bound in ADF-N was determined in replicates using the Fibertec system (Tecator AB, Höganäs, Sweden) for acid-detergent destruction (Van Soest, 1963) followed by N quantification in the filtered residue by modified Kjeldahl method (AOAC International, 2000). Feed samples were analyzed for AA (cysteine, methionine, alanine, arginine, asparagine, glutamine, glycine, histidine, isoleucine, leucine, lysine, ornithine, phenylalanine, proline, serine, threonine, and valine) according to the method described by Mason et al. (1980). Briefly, feed samples of the mix and DDGS were mixed with an oxidation solution containing performic acid in a flask and sealed with an airtight film in a refrigerator at 0°C. After 16 h, a hydrolysis mixture was added to the flask and boiled for 23 h at 110°C in order for the hydrolysis to take place. The hydrolysis mixture was then filtered through a 0.22-μm membrane filter and transferred to a Biochrom 30 AA analyzer (Laborservice Onken, Gründau, Germany) for analysis via ion exchange chromatography. Serine, valine, and isoleucine are prone to oxidation with the addition of acid during the hydrolysis step; therefore, they were corrected with a factor of 1.06 (Rudemo et al., 1980). Buffer soluble N was determined as N in the supernatant after incubation in a borate–phosphate buffer according to Åkerlind et al. (2011).

Milk Composition and Sensory Analysis. Milk samples were cooled immediately to 4°C and subsequently analyzed for overall milk composition (fat and protein) using a CombiFoss 4000 (Foss Electric A/S, Hillerød, Denmark). The milk samples for sensory analysis were pasteurized in a water bath at 65 ± 2°C for 7 min. Afterward, the samples were cooled in an ice bath and stored at 4°C for 24 h before carrying out descriptive sensory analysis, as described in Maciel et al. (2016). To summarize, a trained panel of 9 assessors attended a discussion and a training session (2 h each) before the sensory evaluation, during which they were introduced to reference samples as described by Hedegaard et al. (2006) and Maciel et al. (2016). A list of 12 sensory descriptors including aroma (cardboard, stored, metallic, and creamy), appearance (color saturation and yellowness), flavor or taste (faded, metallic, cardboard, creamy, and sweetness), and mouth feeling (creaminess) was agreed upon by the panelists before the evaluation. During training and the sensory evaluation, the milk samples were randomly served in small plastic beakers with lids (Abena A/S, Aabenraa, Denmark) in amounts of approximately 50 mL after being kept at 12°C for 1 h. The ratings were directly recorded electronically (Fizz software, 2.30C, Biosystemes, Couternon, France). Training and sensory evaluation were conducted in accordance with the International Organization for Standardization (ISO, 1993) and carried out in a sensory laboratory fulfilling the requirements provided by the American Society for Testing and Materials (ASTM International, 1986). For the training session, 4 milk samples that varied in sensory quality were used. For the sensory evaluation, fresh and stored milk samples from the L, M, and H treatments were served in small plastic beakers with lids to each assessor in 3 replicates (18 samples total). The order of the samples was randomized for each assessor. The fresh samples were stored for 1 d and stored samples were stored for 7 d at 5°C. The 6 samples were evaluated twice within 3 wk.

FA Profile. The FA analysis of milk was performed based on Larsen et al. (2013), where fat was separated from milk by centrifugation and FA were methylated

using sodium methylate. The FAME were quantified using external standards (FAME mix C_4–C_{24}, Supelco, Bellefonte, PA, and GLC 469 methyl ester standard, Nu-Chek Prep Inc., Elysian, MN), and the concentrations were calculated in grams per kilogram of identified milk FA.

Statistical Analysis

Feed Intake, Milk Production, and FA Profile. The effects of diet, parity, and period on daily milk yield (kg/d), ECM (kg/d), fat (% and kg), protein (% and kg), milking frequency, PMR intake (kg of DM/d), concentrate intake at AMU (kg/d), and FA (g/100 g of FA) were analyzed by the model

$$Y_{ijkl} = \mu + D_i + P_j + (DP)_{ij} + T_k + C_{ijl} + \varepsilon_{ijkl},$$

where μ is the overall mean. The model includes the effects of the ith diet D (i = L, M, and H), the jth parity P (j = primiparous, multiparous), and the kth period T (k = 1, 2, or 3); $(DP)_{ij}$ denotes the 2-way interaction; C_{ijl} is the random effect of the lth cow within i treatment and j parity; and ε_{ijkl} is the residual error.

The results were presented in tables containing the least squares means (± standard error of the mean) and the P-values of the overall F-tests for mixed effects. The analyses were performed with the MIXED procedure of SAS for Windows (version 9.3, SAS Institute Inc., Cary, NC).

Sensory Analysis. To study the effect of the 3 diets on the sensory attributes of fresh and stored milk samples, a 3-way ANOVA was applied; the fixed effects were diet, storage duration (0 or 7 d), and 2-way diet × storage interaction. The assessor was also included as a random factor. To reveal the differences between the milk samples, the Bonferroni method was used for post hoc testing (Næs et al., 2010).

RESULTS AND DISCUSSION

DDGS Quality

The mix and gDDGS were similar in DM. However, numerically the mix contained more ash and sugars than gDDGS but less starch, NDF, ADF-N, CP, and crude fat (Table 2). The composition of gDDGS was in accordance with the range of values indicated for wheat DDGS by Olukosi and Adebiyi (2013), taking into account several data sets of DDGS composition (wheat and corn). The concentrations of AA were on average lower in gDDGS than in the mix. In particular, lysine was much higher in the mix than in gDDGS (20.9 vs. 7.9 g/kg of DM). Only cysteine, glutamine, and proline were higher in gDDGS compared with the mix (Table 2). The AA profile of gDDGS in the present study was within the ranges observed in De Boever et al. (2014) for wheat DDGS and blend DDGS. Compared with cDDGS, wheat DDGS has a higher content of CP and a lower fat content (Pedersen et al., 2014). Because literature is scarce on the effects of gDDGS on production variables of dairy cows, the following results are carefully compared with those observed by feeding cDDGS despite the chemical composition of cDDGS and gDDGS being quite different (Oba et al., 2010).

Effect of DDGS on Feed Intake

The DMI of PMR ($P = 0.35$) and AMU concentrate ($P = 0.98$) was not affected by substituting the mix with increasing proportions of DDGS in the diet (Table 3). This is similar to the findings of Christen et al. (2010) and Kleinschmit et al. (2006) using cDDGS. Christen et al. (2010) tested 4 different kinds of protein sources, including cDDGS, and found no significant difference in total DMI between the cows fed cDDGS and those fed soybean meal or canola meal. Kleinschmit et al. (2006) fed a 20% cDDGS diet to Holstein cows and found no difference in DMI compared with cows fed ground corn and soybean meal instead of cDDGS. Liu et al. (2000) and Powers et al. (1995) reported similar results. However, the effects of feeding DDGS on DMI have been variable between studies. Benchaar et al. (2013), Nichols et al. (1998), and Janicek et al. (2008) observed greater DMI for cows fed cDDGS (cDDGS was included from 10 to 30% of dietary DM). Conversely, Birkelo et al. (2004) reported an 11% decline in DMI when cDDGS was included at 31% of dietary DM. Anderson et al. (2006) reported a tendency for lower DMI for cows fed 20% cDDGS compared with cows fed the control diet. These differences can be attributable to several experimental factors (e.g., the inclusion rate of cDDGS and type of forage or concentrate) and differences in ruminal and intestinal degradability among DDGS sources, reflecting the large variation in the quality of DDGS (Kleinschmit et al., 2007; Hollmann et al., 2011; Benchaar et al., 2013). In the present study, the proportion of starch and forages was similar among the 3 diets (Table 1), which can explain the similar DMI for the 3 diets. This is supported by the meta-analysis of Hollmann et al. (2011), which included 16 peer-reviewed publications. Hollmann et al. (2011) reported that DMI was not influenced by the proportion of cDDGS included in the diet but was linearly related to the proportion of starch and was affected by the proportion of forage in the diet. In the present

study, the 16 cows of a group had access to 8 feeders with the attributed ration. A potential shortcoming of that approach is that cows might not have consumed the formulated PMR if sorting occurred. Feed-sorting behavior, in favor of small particles and against longer forage particles, is commonly observed in cows fed mixed rations (Miller-Cushon and DeVries, 2017). This behavior can result in an unbalanced intake of nutrients, decrease the nutritive value of the ration (DeVries et al., 2005), and alter rumen fermentation, which can affect digestion efficiency and production (Sova et al., 2013). However, the effects of sorting behavior are not consistent. Some studies reported a decrease in milk production with increasing sorting (Rabelo et al., 2003; Sova et al., 2013), whereas other associated sorting with greater efficiency of milk production from peak milk yield to peak DMI (DeVries et al., 2011). In the present experiment, sorting behavior was minimized by feeding the cows 4 times/d. Indeed, it has been shown that sorting behavior decreases with increasing feeding frequency (DeVries et al., 2005; Endres and Espejo, 2010; Sova et al., 2013) and can even be avoided when feeding occurs 3 times/d (Hart et al., 2014). Leonardi and Armentano (2007) and Miller-Cushon and DeVries (2010) also reported an increase in sorting when the amount of refusal increased, indicating the importance of the amount fed. Finally, Miller-Cushon and DeVries (2017) hypothesized that the shape of the feeder may also influence the ability of cattle to sort, as sorting would occur more easily in a flat feed bunk as opposed to a more enclosed manger because of the possibility of pushing feed around and away on a flat bunk surface. In our case Insentec feeders were used, which could also contribute to the control of sorting.

Effect of DDGS on Milk Production and Composition

Milk yield and milking frequency decreased with increasing proportion of gDDGS in the diet (Table 3; $P < 0.004$), whereas ECM was not affected ($P = 0.09$; Table 3). There was a diet × parity interaction for milk fat content ($P = 0.001$), indicating that for the M diet the primiparous cows had a lower percentage of fat in milk than the multiparous cows fed the same diet (3.8 vs. 4.1 ± 0.1%); we have no explanations for this result. Total milk fat yield was not affected by diet. Total milk protein yield was similar between the L and M diets, whereas it decreased for the H diet (1.27 vs. 1.22 ± 0.03 kg). Milk protein content increased for the M diet compared with the L and H diets. This resulted in an unchanged fat yield with increasing proportion of gDDGS in diet but a slight decrease in milk protein yield with the H diet ($P = 0.05$) compared with the L and M diets.

Christen et al. (2010), Mjoun et al. (2010), and Liu et al. (2000) reported no differences in milk production between the cDDGS diet and the control diet (soybean meal or canola meal diets). However, Benchaar et al. (2013), Nichols et al. (1998), and Anderson et al. (2006) showed that DDGS inclusion in the diet increased milk production. For Benchaar et al. (2013), this increase is mainly attributable to the increase in DMI with proportion of DDGS in the diet. In the present study, the decrease in milk yield observed with the addition of DDGS in the diet might be partly attributable to the decrease of milking frequency from 2.80 to 2.63 milkings/d from the L to the H diet.

Anderson et al. (2006) reported that feeding cDDGS to dairy cows did not affect milk fat content if

Table 3. Milk yield, ECM, fat in milk, protein in milk, milking frequency, intake of partially mixed ration, and intake of concentrates at the milking robot

	Diet[2]			Parity			*P*-value		
Item[1]	L	M	H	Primiparous	Multiparous	SEM	Diet	Parity	Diet × parity
Intake PMR, kg/d	20.3	20.4	20.1	17.8	22.2	0.3	0.35	<0.01	0.31
Intake AMU, kg/d	2.39	2.39	2.39	2.43	2.35	0.04	0.98	0.20	0.44
Milk, kg/d	37.3[a]	37.0[a]	35.7[b]	32.4	40.9	1.2	<0.01	<0.01	0.90
ECM, kg/d	36.7	36.7	35.7	32.0	40.7	1.0	0.09	<0.01	0.64
Milking frequency	2.80[a]	2.75[a]	2.63[b]	3.18	3.53	0.12	0.05	0.30	0.95
Fat, %	3.89[a]	3.92[a]	4.01[b]	3.89	3.99	0.09	0.05	0.57	<0.01[3]
Fat, kg/d	1.43	1.44	1.42	1.24	1.62	0.05	0.74	<0.01	0.11
CP, %	3.45[a]	3.48[b]	3.43[c]	3.46	3.44	0.04	0.02	0.73	0.43
CP, kg/d	1.27[a]	1.27[a]	1.22[b]	1.11	1.39	0.03	<0.01	<0.01	0.65

[a–c]Different letters within a row indicate significant difference between treatments ($P < 0.05$).

[1]PMR = partially mixed ration fed at the automatic feed bins (Insentec, Marknesse, the Netherlands); AMU = automatic milking unit.

[2]L = diet containing 4% grain-based dried distillers grains with solubles (gDDGS); M = diet containing 13.5% gDDGS; H = diet containing 23% gDDGS.

[3]Cows had a lower percentage of fat in milk than the multiparous cows fed the same diet (3.8 vs. 4.1 ± 0.1%).

diets contained the adequate amount of fiber. In the present experiment we had a 61:39 forage:concentrate ratio and around 33% dietary NDF content in all 3 diets. Christen et al. (2010) and Benchaar et al. (2013) found that the inclusion of cDDGS in dairy cow rations decreased milk fat, which contradicts our finding. To our knowledge, fat addition slightly increases milk fat at low fat supplementation and slightly decreases milk fat at higher fat supplementations. Where the inflexion point is depends on FA composition in feed (the more saturated, the higher the top point), but often other feed characteristics are more important for milk fat.

Our response for milk protein content is difficult to interpret because it has not been reported previously. Anderson et al. (2006) found that milk protein content was similar for cows fed cDDGS (10 and 20% of dietary DM) and cows fed a control diet without cDDGS. Concerning our decrease in total milk protein yield for the H diet, Janicek et al. (2008) and Anderson et al. (2006) both found that milk protein yield increased from 10 to 30% of dietary DM with increasing proportion of cDDGS in the diet. In both studies this increase was attributed to the increase of DMI leading to more energy available for milk protein synthesis. In our case, as DMI was not affected by the diet, it explains the similar yields of total milk protein between the L and M diets but does not explain the decrease observed while feeding the H diet.

Effect of DDGS on Milk Quality

Milk FA. The proportion of gDDGS in the diet influenced the composition of FA in milk. As presented in Table 4, the saturated FA (C4–C14 and C16) were not affected by dietary gDDGS level. Only the proportion of C4 increased slightly (from 5.49 to 5.69 ± 0.08 g/100 g; $P = 0.05$), and C12 and C14 decreased (from 4.75 to 4.50 ± 0.1 for C12, $P = 0.02$; from 12.87 to 12.25 ± 0.12 for C14, $P < 0.01$) with increasing proportion of gDDGS in the diet. Oleic acid was not affected by the proportion of gDDGS in the diet ($P = 0.68$). The largest relative difference was observed for the proportion of linoleic acid and CLA *cis*-9,*trans*-11 in milk, increasing with dietary proportion of gDDGS ($P < 0.01$). Leonardi et al. (2005) and Anderson et al. (2006) also found that feeding cDDGS slightly increased the amount of CLA *cis*-9,*trans*-11 in milk. These results indicate that there is no concern regarding FA composition of milk when feeding gDDGS at low or high levels under the conditions in the present study. The

Table 4. Proportion of fatty acids (g/100 g) in milk of lactating dairy cows fed grain-based dried distillers grains with solubles (gDDGS)

	Diet[2]			Parity			*P*-value		
Fatty acid[1]	L	M	H	Primiparous	Multiparous	SEM	Diet	Parity	Diet × parity
C4	5.50[a]	5.60[ab]	5.70[b]	5.58	5.58	0.08	0.05	0.98	0.38
C6	2.90	2.90	2.90	2.91	2.95	0.04	0.36	0.53	0.68
C8	1.70	1.70	1.70	1.73	1.76	0.03	0.69	0.53	0.51
C10	4.20	4.20	4.00	4.06	4.17	0.08	0.13	0.45	0.29
C11	0.10	0.11	0.10	0.10	0.10	0.01	0.27	0.79	0.01*
C12	4.70[a]	4.70[a]	4.50[b]	4.59	4.72	0.10	0.02	0.45	0.18
C13	0.15	0.16	0.15	0.15	0.15	0.01	0.27	0.67	0.06
C14	12.80[a]	12.50[a]	12.20[b]	12.55	12.53	0.12	<0.01	0.92	0.77
C14:1	1.10	1.10	1.10	1.13	1.03	0.04	0.19	0.20	0.02*
C4–C14	31.90	31.60	31.20	31.42	31.72	0.30	0.07	0.58	0.59
C15	1.20	1.20	1.20	1.26	1.21	0.03	0.17	0.43	0.09
C16	30.20	29.70	29.80	29.63	30.23	0.30	0.09	0.35	0.60
C16:1	1.50	1.50	1.50	1.51	1.53	0.05	0.23	0.81	0.45
C17	0.57	0.58	0.57	0.58	0.57	0.01	0.55	0.93	0.91
C11–C17	2.10	2.10	2.00	2.06	2.04	0.05	0.26	0.51	0.07
C18	8.70	8.90	8.70	8.76	8.93	0.10	0.10	0.49	0.27
C18:1 *trans*-6	0.32[a]	0.33[ab]	0.34[b]	0.34	0.32	0.01	0.01	0.09	0.47
C18:1 *trans*-9	0.21	0.21	0.21	0.22	0.20	0.01	0.16	0.08	0.13
C18:1 *trans*-11	1.52[a]	1.68[b]	1.77[c]	1.75	1.56	0.04	<0.01	0.02	0.30
C18:1 *cis*-9	17.60	17.50	17.70	17.83	17.37	0.29	0.68	0.37	0.60
C18:2	2.40[a]	2.70[b]	3.00[c]	2.79	2.65	0.05	<0.01	0.16	0.67
C18:3	0.56[a]	0.56[a]	0.53[b]	0.56	0.54	0.01	<0.01	0.20	0.05
CLA *cis*-9,*trans*-11	0.61[a]	0.63[a]	0.71[b]	0.69	0.60	0.02	<0.01	0.01	0.83

[a–c]Different letters within a row indicate significant difference between treatments ($P < 0.05$).

[1]Primiparous cows had 0.11, 0.12, and 0.09 g of C11/100 g of milk and 1.17, 1.17, and 1.06 g of C14:1/100 g of milk in the L, M, and H diets, respectively. The multiparous cows had 0.10 g of C11/100 g of milk and 1.06, 0.99, and 1.05 g of C14:1/100 g of milk in the L, M, and H diets, respectively.

[2]L = diet containing 4% gDDGS; M = diet containing 13.5% gDDGS; H = diet containing 23% gDDGS.

*Diet × parity interaction was significant ($P < 0.05$) for these fatty acids.

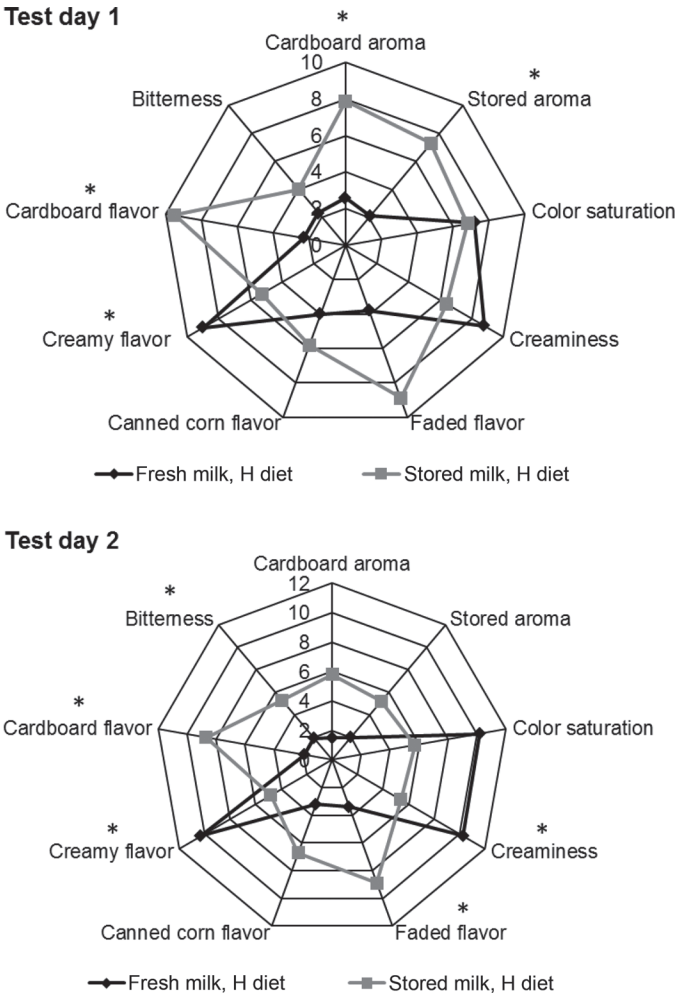

Figure 1. Cobweb plot of the sensory profiles of fresh and stored milk from cows fed the high-DM (H) diet (13.5% DM of the diet containing grain-based dried distillers grains with solubles). Intensity scale: 0 (low) to 15 (high). Asterisks indicate significant differences based on F-test ($P < 0.05$).

effect of feeding DDGS on linoleic acid was expected because this is the main FA in DDGS (Schingoethe et al., 2009) and our results are similar to those reported by Testroet et al. (2015). However, in Testroet et al. (2015), other C18 FA were affected. This effect could be related to a reduced fat yield (lower milk yield in combination with lower milk fat content; Testroet et al., 2015) as opposed to the constant fat yield between treatments in our study.

Sensory Analysis. Two sensory evaluations were carried out during 2 different days. The sensory descriptors that differed between the 6 samples were different regarding the evaluation day. Because there were 3 wk between the evaluation days, it might indicate that there are some differences in milk quality between the 2 d. The biggest differences in sensory quality were observed between fresh and stored milk samples (Figure 1). No significant differences in any of the descriptors were seen between the 3 fresh milk samples. Only the cardboard aroma and creamy flavor differed significantly between the 3 stored samples at 1 of the sensory sessions. In particular, the stored sample for the M diet was characterized as having a higher intensity of cardboard and stored aroma, faded flavor, cardboard flavor, and bitterness compared with 1 or 2 of the fresh samples for at least 1 of the sensory sessions. In contrast, the stored sample for the M diet was lower in creamy flavor and creaminess compared with at least 1 of the fresh samples. This indicates that gDDGS can be included in the diet without any degradation in milk flavor. Raw milk is spontaneously oxidized within 5 d of collection, which leads to an oxidized flavor in milk (Timmons et al., 2001). This could explain some of the differences observed between our fresh and 7-d stored milk samples. Testroet et al. (2015) hypothesized that greater DDGS inclusion in the ration would contribute to the development of the oxidized flavor in milk due to an increase in unsaturated FA in milk. However, the results of their study indicated that DDGS had no negative effect on the milk flavor, which is in accordance with our study.

CONCLUSIONS

Substituting soybean meal and canola cake with gDDGS, up to 13.5% of dietary DM, did not affect feed intake, milk production, or fresh milk sensory quality. When feeding 23% of dietary DM as gDDGS, milk yield decreased by 1.6 kg/d. Linoleic acid and CLA *cis*-9,*trans*-11 in milk increased with increasing proportion of gDDGS in the diet; however, milk taste was not influenced by the proportion of DDGS in the diet. To conclude, gDDGS can replace soybean meal and canola cake in a grass-cover-based diet for high-yielding dairy cows.

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