PROGRESS REPORT

PROJECT TITLE: **Reduced Fat DDGS Feeding: Investigating Impact on Milk Composition and Cheese Quality.**
PROJECT NUMBER:
REPORTING PERIOD: **2016**
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1.) PROJECT ACTIVITIES COMPLETED DURING THE REPORTING PERIOD. (*Describe project progress specific to goals, objectives, and deliverables identified in the project workplan.*)

Objectives:

1) Investigate impact of lactose, sulfate, and thiosulfate on quality of baby Swiss cheese
   a. No significant effect of dietary treatment was observed on milk components (other than protein) or quality of Baby Swiss cheese. Because we found no treatment effect, further research on the effect of lactose, sulfate and thiosulfate on Baby Swiss cheese is not warranted and will not be evaluated.

2) Determine quality of baby Swiss cheese produced from cows fed reduced-fat DDGS
   a. Quality of cheese made from milk of cows fed reduced-fat DDGS did not differ significantly from cheese made from milk from cows fed a control diet containing no DDGS.

3) Determine economic impacts of feeding reduced-fat DDGS on feed efficiency
   a. Milk production parameters and feed efficiency have been assessed and are reported in this document. An economic analysis has yet to be completed but will be completed before the final report.

2.) IDENTIFY ANY SIGNIFICANT FINDINGS AND RESULTS OF THE PROJECT TO DATE.

Thirty-five multiparous lactating Holstein dairy cows were assigned to one of two dietary treatment groups. Rations were formulated to be isonitrogenous and isoenergetic and to contain similar available amino acid concentrations. Ration one (control) was a standard corn/corn silage/hay based ration supplemented with soybean meal as a protein source. Ration two was formulated using the same base ration as control but with 20% of the dry matter being a reduced-fat distillers grain with solubles (RF-DDGS, Poet Biorefining, Jewell, IA) containing approximately 6.5% fat in place of SoyPlus (Dairy Nutrition Plus, Des Moines, IA) (Table 1).

Ration two (DDGS) was supplemented with lysine to make diets similar in available limiting amino acids. Cows were fed each diet in a two-period, two-treatment crossover design. Each experimental period lasted 35 days, and cows were fed individually using Calan gates (American Calan, Northwood, New Hampshire), allowing for measurement of individual feed intake. Additionally, individual milk production was recorded daily by using a Boumatic milking system (Boumatic LLC, Madison, WI). Weekly, after a 14-day acclimation period, individual milk samples were collected at the three milkings, for proximate analyses and other components (i.e., milk urea nitrogen, somatic cell count) (Performed by using official NIR methods at Dairy Lab Services, Dubuque, IA). During the final week of each experimental period rumen fluid was collected for volatile fatty acid (VFA) concentration analyses, approximately four hours post-feeding following milking to collect at the time of maximal VFA production. In addition to rumen fluid, during the final week of each experimental period, blood was collected for metabolic profiling.

CHEESE MAKING

For cheese making, milk from one complete milking of each treatment group (control or reduced-fat DDGS) was collected, two to three times during weeks three and four, during each of the three 35-day periods. The milk cans and dump buckets were washed with automatically diluted Ecolab® Oasis Enforce (St. Paul, MN) and sanitized with automatically diluted Ecolab® Mikroklene® (St. Paul, MN) iodine-based sanitizer. The morning milking (approximately 6:30 am) was collected during the farm's usual milk collection routine. Teats were sanitized with 1000 ppm chlorine predip (ECAcept technology, Zurex PharmAgra LLC, Middleton, WI) and wiped dry with individual towels before collecting milk from each cow by the Boumatic milking system (Boumatic, Madison, WI). Milk from two groups of six cows was collected by re-routing the Boumatic line into a dump bucket. After the milk of two cows fed the same diet filled a dump bucket, it was dumped through cheesecloth, into a labeled milk can, for transport, at ambient temperature, to the ISU Center for Crops Utilization Research (CCUR) pilot plant in the Food Sciences Building at Iowa State University (Ames, IA) within 20 min of collection of milk from the last cow. The milk cans were immediately weighed and tested for fat, protein and lactose prior to further processing (within 60 min) by using a LactiCheck Milk Mini Analyzer (Page and Pederson Inc, Hopkinton, MA). Those who collected milk at the dairy farm showered and changed into clean clothes before participation in cheese making to minimize additional external contamination of milk to be used for cheese production.

Measured percentage fat and protein were used to standardize milk to the target fat:protein ratio (0.88±0.05). If the fat:protein ratio was not 0.88±0.05, the milk was separated and standardized, and cream or skim from the milk collected from the same experimental cows were added to raise or lower the ratio, respectively. Milk was separated using a Type LWA 205 Westfalia Separator (219 rpm in 2.5 dial setting, Dusseldorf, Germany). Pooled standardized milk from each dietary treatment was poured into a labeled cheese vat and heat treated (63°C, 2 min) by delivering steam-heated water to the jacketed vat, with gentle agitation. After heat treatment, the milk was gradually cooled to 33°C by running cold water in the jacketed vat, and with gentle agitation of the milk.

Baby Swiss cheese was made by using CHOOZIT 60 (0.16 g/45 kg milk, DuPont ™ Danisco®, New Century, KS), and CHOOZIT eyes (0.06 g/ 45 kg milk, DuPont ™ Danisco®,). Coagulant (6 mL/45 kg of milk, DCI Supreme, Dairy Connection Inc., Madison, WI) was diluted with cold water to a ratio of 1:40 and added with slow agitation for one minute. The cheese curd was allowed to set for approximately 30 min, tested for firmness visually, and manually cut with 12-cm wire curd knives. About 25% of vat volume of whey was initially removed, followed by constant stirring and addition of water (3 to 5% of the vat volume) at 33°C; the forework proceeded for 35 min at 33°C. Gradually, the curds were cooked by increasing the temperature to 40°C over a 15-min period, and then to 46°C over a 10-min period by adding steam to the jacket of the vat. Warm water (~10 % of the vat volume) was added at 44°C to facilitate the rise in temperature of the cheese to 46°C(±1°C)., where the curds were held for 42 min (postwork). After postwork, and at a target pH of 6.4, whey was removed.

Cheese curds were collected into perforated stainless steel Longhorn hoops. Towers were pressed under whey by using a 7 kg weight for 15 min. The whey was drained completely and the cheese block was pressed for 1 hr with 11 kg, 1 hr with 23 kg, and an additional 3 hr with 35 kg of weights. Curd pH was measured (Accumet® Basic AB15, Fisher Scientific Inc, Pittsburgh, PA), the press was removed, and cheese was fermented in an empty basin for an additional 5 to 8 hr at 28°C ± 3°C. The pressing time was based on the time required for the pH of the cheese to drop from 6.4 to 5.25 (±0.5). Brining was carried out in saturated brine containing 23% NaCl and 0.38% $CaCl_2$, for up to 7 to 9 hr (depending on block weight (approximately 30 min/kg cheese)). Cheese blocks were vacuum-packed in clear vacuum seal bags (Fisher Scientific Inc, Pittsburgh, PA) with a Koch vacuum packing machine (Koch Equipment LLC©, Kansas City, MO). Cheeses were stored at 10±1°C for 7 days (Pre-cool), 22±3°C for 21 days (warm room), and 4±1°C for 60 days (cold room). Cheeses were analyzed for composition and sensory quality after at least 60 days aging. The cheeses were sliced systematically for analysis and photographs. Representative photographs are included in Figures 2 – 5.

A descriptive sensory analysis panel, composed of 6 trained panelists, evaluated the quality of the cheeses. Panelists were recruited from students of the Department of Food Science and Human Nutrition who would be available in the summer of 2016. Training consisted of 5 hours of initial training, followed by an additional hour of refreshing of training between the first and second official tasting period (separated by a month). Clear description of each sensory term and references were provided. Panelists were trained to evaluate baby Swiss cheese in relation to set quality standards, which served as anchors during training sessions. Baby Swiss cheese should have a mild nutty (roasted hazelnut) and propionic acid aroma and flavor character with little to no apparent sour/lactic acid taste. Other than a slight bitter aftertaste, baby Swiss should clean up, leaving no fruity, fermented, rancid yeasty or other foreign flavors on the palate. Eyes should be completely round, from 1/8 to 1/4 inch in diameter (hole punch size; smaller than a penny; Figure 6). Panelists were provided individual samples, cut to a standard size with a template, in zipper-lock bags, along with water and grapes for cleansing palate (Figure 7).

Regarding appearance, the baby Swiss cheeses were slightly atypical. Compared to the ideal, the cheeses were characterized by a high number (overset) of very small to small eyes (0.32 cm to 0.64 cm in size), many of which were irregular in shape (including frog mouth, collapsed, and rarely, cabbage), and the distribution was slightly uneven. DDGS cheeses were not significantly different from control for any attribute except size of eyes (Table 6). Mean score for eye size of control cheeses were closer to ideal than DDGS cheeses ($P < 0.05$). Eyes exhibited a typical glossy appearance, but some exhibited a wet (free whey) appearance. Although gas formation appeared normal in most cheeses, several cheeses exhibited checks, picks, and rarely, spilts or a blowhole.

PRODUCTION PARAMETERS AND MILK COMPONENTS

In contrast with our previous research utilizing full-fat DDGS (~13% fat), milk components were not negatively affected by incorporation of reduced-fat DDGS at a 20% by dry matter (DM) inclusion rate. Total milk fat production per day did not change (control 1.30 kg/day and DDGS 1.26 kg/day; $P = 0.18$) nor did milk fat percentage (control 3.70% and DDGS 3.63%; $P = 0.18$) (Table 2). Milk protein percentage (control 3.05% and DDGS 3.15%; $P < 0.0001$) increased significantly but total milk protein (control 1.08 kg/day and DDGS 1.07 kg/day; $P = 0.13$) was unaffected (Table 2). Both total lactose production (control 1.67 kg/day and DDGS 1.65 kg/day; $P = 0.78$) and percentage milk lactose (control 4.78% and DDGS 4.78%; $P = 0.78$) did not change as a result of the treatment diet (Table 2). In addition, total percentage milk solids (control 12.32% and DDGS 12.37%; $P = 0.58$) were not altered (Table 2). When cows were fed DDGS, milk urea nitrogen (12.78 mg/dl) was lower in concentration than control (14.14 mg/dl; $P < 0.0001$) (Table 2) indicating that, when taken with milk protein %, reduced-fat DDGS may support better protein utilization. Additionally, blood glucose concentration (control 53.51 mg/dl and DDGS 55.27 mg/dl; $P = 0.31$) (Figure 1) did not differ between treatments.

Feeding TMR containing reduced-fat DDGS at a 20% inclusion rate increased ($P = 0.02$) feed intake of cows fed DDGS compared with the control TMR (46.5 vs 45.6 ± 0.5 kg/d as fed, respectively) (Table 3). Milk production was not altered ($P = 0.92$) by feeding DDGS compared with control TMR (36.0 vs 35.9 ± 0.5 kg/d, respectively) (Table 3). Nor did feeding DDGS affect milk production when milk was normalized for energy (energy corrected milk; ECM; $P = 0.85$) or fat (fat corrected milk; FCM; $P = 0.25$) (Table 3). Consequently, efficiency of milk production, measured as kilograms of milk produced per kilogram of feed consumed daily was not altered when using raw milk ($P = 0.11$) or using the ECM value ($P = 0.13$) (Table 3). Only expressing milk production on a FCM basis resulted in a decrease ($P = 0.02$) in FCM production efficiency in cows fed the DDGS TMR compared with cows fed the control TMR (0.81 vs 0.83 ± 0.01 kg FCM/kg feed intake, respectively) (Table 3). Finally, rumen fluid pH was not altered ($P = 0.48$) when cows were fed DDGS (6.55 ± 0.06) compared with control TMR (6.50 ± 0.06) (Table 3). The flavor and body and texture of all baby Swiss cheeses produced in the study were typical, with no significant diet effects ($P > 0.05$). Control and DDGS cheeses were characterized by very low levels of acid, flat, and unclean flavors, with low bitterness (Table 4). Control and DDGS cheeses were neither weak or pasty but instead moderately curdy and slightly mealy/grainy (Table 5). A moderate but significant negative ($P < 0.00005$) correlation was observed between weak and curdy (-0.52) and curdy and pasty (-0.45); a strong negative correlation was observed between amount of eyes and eye distribution (-0.74). A moderate but significant positive ($P < 0.0005$) correlation was demonstrated between mealy and curdy (0.47) and pasty and flat (0.53).

CONCLUSIONS

In summary, these findings demonstrate that feeding of DDGS had little effect on cheese quality. Instead, cheese make procedures (temperature control, moisture removal, brine incorporation into curd, aging conditions) had more of an impact on cheese quality attributes. Significant cheese by production day interaction effects were noted for most sensory attributes

(Table 7), but very few trends stand out. These findings, additionally, demonstrate that feeding RF-DDGS did cause a decrease in FCM efficiency as a result of an increase in DMI, however, when ECM efficiency was calculated (accounting for fat, protein, and lactose concentration in milk) no difference in feed efficiency resulted. These results indicate that reduced-fat DDGS can be effectively fed at a 20% (DM) inclusion rate without having negative effects on milk components, blood glucose, or ECM milk efficiency and that protein utilization may be improved when cows are fed RF-DDGS.

Table 1.
Diet composition.

Ingredient, % Dry Matter	Control	DDGS
Corn silage	35.13	31.57
Alfalfa hay	23.09	20.74
Whole cotton seed	8.03	7.21
Ground corn	14.41	15.13
RF-DDGS	0.00	19.45
Soy Plus[1]	13.51	0.54
Quality Liquid Feeds[2]	3.81	3.42
USA Lysine[3]	0.00	0.11
Vitamin and mineral premix	3.81	3.42

[1] Dairy Nutrition Plus, Des Moines, IA.
[2] Quality Liquid Feeds, Dunlap, IA. Custom vitamin and mineral supplement.
[3] Kemin Industries, Des Moines, IA.

Table 2.
Effects of feeding RF-DDGS to lactating Holstein dairy cows on milk components.

	Treatment		
Item	Control	DDGS	*P*-Value
Milk yield, kg/day	35.9	36.0	0.92
Milk fat, kg/day	1.30	1.26	0.18
Milk fat, %	3.70	3.63	0.18
Milk protein, kg/day	1.08	1.07	0.13
Milk protein, %	3.05	3.15	<0.0001
Lactose, kg/day	1.67	1.65	0.78
Lactose, %	4.78	4.78	0.78
Milk solids, %	12.32	12.37	0.58
Milk urea nitrogen, mg/dl	14.14	12.78	<0.0001

Table 3.

Effects of feeding RF-DDGS to lactating Holstein dairy cows on body weight, plasma non-esterified fatty acid (NEFA) concentration, rumen pH, dry-matter intake (DMI), milk production, and milk production efficiency.

	Treatment			
Item	Control	DDGS	SEM	*P* - Value
Body wt gain or loss, kg	11.9	17.0	2.5	0.10
NEFA, μeq/L	163.1	158.1	6.3	0.51
Rumen pH	6.55	6.49	0.06	0.37
DMI, kg/d	45.6	46.5	0.49	0.02
Milk, kg/d	35.9	35.6	0.5	0.92
ECM, kg/d[1]	37.0	37.1	0.4	0.85
FCM, kg/d[2]	36.8	36.5	0.4	0.25
Milk efficiency, kg milk/kg DMI	0.81	0.79	0.01	0.11
ECM efficiency, kg ECM/kg DMI	0.83	0.82	0.01	0.13
FCM efficiency, kg FCM/kg DMI	0.83	0.81	0.01	0.02

[1] **Energy-corrected milk; (0.327×Milk)+(12.95×Milk Fat)+(7.65×Milk Protein).**
[2] **Fat-corrected milk; (0.432×Milk)+(16.23×Milk Fat).**

Table 4.
Flavor means and *P*-values

Flavor	**Mean Control**	**Mean DDGS**	**SEM**	***P* - Value**
Acid	1.01	1.05	0.19	0.840
Bitter	3.82	3.47	0.46	0.450
Flat	1.35	1.61	0.31	0.411
Unclean	1.43	1.86	0.39	0.260

Table 5.
Body and Texture means and *P*-values

Body and Texture	**Mean Control**	**Mean DDGS**	**SEM**	***P* - Value**
Curdy	7.17	6.85	0.43	0.471
Mealy/Grainy	5.56	6.47	0.57	0.110
Pasty	0.75	0.98	0.29	0.445
Weak	1.03	1.24	0.25	0.409

Table 6.
Appearance of eyes means and *P*-values

Appearance	**Mean Control**	**Mean DDGS**	**SEM**	***P* - Value**
Amount	7.77	7.98	0.48	0.664
Distribution	4.08	3.56	0.62	0.392
Gloss	6.77	6.86	0.31	0.793
Shape	8.39	8.23	0.15	0.722
Size	**6.41**	**5.71**	**0.70**	**0.027**
Gas Formation	3.71	4.57	0.86	0.318

Table 7.
Cheese by production day summary for significant interaction effects for flavor and body and texture attributes.

Cheese and production day[1]	Bitter	Curdy	Mealy/ grainy	Pasty	Weak	Amount	Distribution	Gas Formation
A1.day1	4.27[abc]	**11.05[a]**	**10.52[a]**	0.28b	0.30b	**8.88a**	3.16abc	1.78a
C1.day1	3.52[abc]	8.63[abc]	5.90[bcd]	0.50b	0.57b	**9.23a**	3.20abc	3.58a
A3.day2	**5.71[c]**	7.40[bc]	4.64[bcd]	0.42b	1.04b	**9.77a**	**1.93c**	4.23a
C2.day2	**5.64[c]**	**5.86[cd]**	**2.13[d]**	1.34b	1.85ab	7.65ab	3.89abc	6.44a
A2.day3	4.24[abc]	9.22[ab]	5.18[bcd]	0.33b	0.40b	**8.98a**	**1.63c**	**7.86a**
C3.day3	3.13[ab]	8.48[abc]	5.89[bcd]	0.36b	1.39b	**5.13b**	**6.73a**	5.19a
A1.day4	3.85[abc]	7.30[bc]	6.85[abc]	0.29b	0.39b	7.02ab	4.28abc	6.74a
C1.day4	3.45[abc]	8.09[abc]	6.64[abc]	0.36b	0.52b	7.55ab	2.33bc	1.59a
A2.day5	3.22[ab]	**3.08[d]**	**4.11[cd]**	**3.94a**	**3.36a**	**5.45b**	6.62ab	**7.76a**
C2.day5	4.55[b]	8.92[abc]	**8.36[ab]**	1.00b	0.43b	**8.78a**	3.89abc	3.22a
A3.day6	**2.19[a]**	6.43[bcd]	6.13[bcd]	1.03b	1.70b	6.58ab	4.03abc	3.58a
C3.day6	**2.32[a]**	7.28[bc]	4.69[bcd]	0.41b	0.58b	8.10ab	2.68abc	2.88a
A1.day7	**2.36[a]**	8.10[abc]	7.61[abc]	0.45b	0.64b	7.68ab	2.87abc	2.81a
A2.day7	**2.12[a]**	7.08[bc]	6.50[abc]	0.57b	1.21b	8.26ab	**2.13c**	2.78a
P - value	0.01	<0.0001	<0.0001	<0.0001	<0.0001	0.001	0.005	0.03

[1]A = Control, C = Control

[a, b, c] Items in a row not sharing a common superscript differ, $P < 0.05$

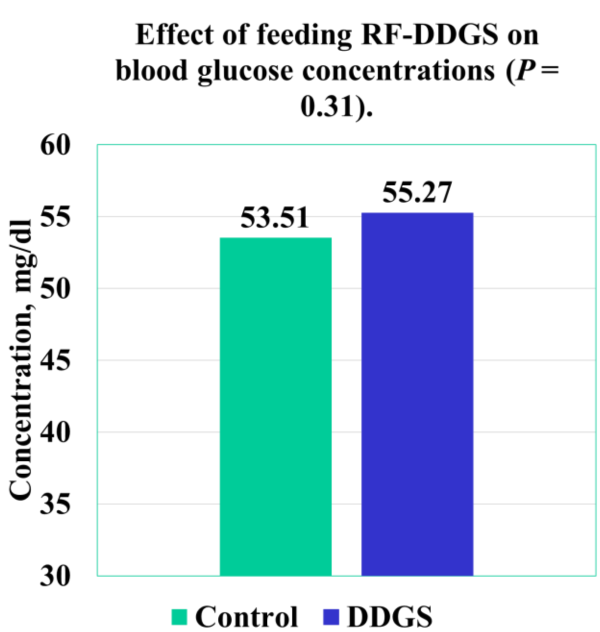

Figure 1.

Figure 2. Representative photograph of baby Swiss cheese from milk of cows fed control diet in period 1.

Figure 3. Representative photograph of baby Swiss cheese from milk of cows fed DDGS diet in period 1.

Figure 4. Representative photograph of baby Swiss cheese from milk of cows fed control diet in period 2.

Figure 5. Representative photograph of baby Swiss cheese from milk of cows fed DDGS diet in period 2.

Figure 6. Evaluation of eye size conducted by trained panelist using hole-punched washable plastic square and penny.

Figure 7. Set up of cheese as presented to trained panelists for evaluation.

Remaining research

Milk fatty acids have been analyzed and quantified but results have yet to be compiled. Additionally, economic analyses on the effect of feeding reduced-fat DDGS to dairy cows have yet to be completed. Finally, feed has yet to be analyzed for nutritional characteristics (e.g., proximate analysis). However, nearly all proposed research has been completed and in the upcoming quarter we will be working on further disseminating the results of this project.

If additional funding were made available, the following proposed research (not initially promised as part of the work) could be conducted on samples collected from the work described above. We propose further research regarding the reason for these changes because of the increased milk protein percentage and the decreased milk urea nitrogen. We propose to quantify concentrations of rumen fluid VFAs, estimate digestibility of feed, and estimate the efficiency of extraction of amino acids by the mammary. Additionally, we propose to describe the microbiome of both the rumen and the feces. Identifying shifts in bacterial populations will help us to elucidate the observed increase in milk protein percentage from cows fed RF-DDGS. All of the proposed measures will help us to explain the changes that occur when cows are fed RF-DDGS with regards to increased dietary protein utilization.

3.) CHALLENGES ENCOUNTERED. (*Describe any challenges that you encountered related to project progress specific to goals, objectives, and deliverables identified in the project workplan.*)

A number of challenges were encountered with regard to completing the research reported herein. First, difficulty in obtaining feed of the proposed fat content proved to be impossible and the project had to be redesigned. The originally proposed research project was designed to compare a full-fat DDGS (~13% fat) to both a reduced-fat DDGS (~3-4% fat) and a control diet. However, when feed was ordered and analyzed it was determined that the "full-fat DDGS" was much lower in fat concentration than anticipated (~8% fat) and the reduced-fat DDGS was much greater in fat concentration (~6-7% fat). Unfortunately, at the time this discrepancy in fat concentration was discovered we were 21 days into the feeding trial. Because the concentrations of fat in the two types of DDGS were so similar it was decided that gleaning any differences in effects of feeding the two types of DDGS would be unlikely. After conferring with Minnesota Corn Growers research personnel, it was agreed upon that the design would be simplified and only compare the reduced-fat DDGS to a control diet. Therefore, the original three period, three treatment experiment was changed to a two treatment and two period crossover design (control versus reduced-fat DDGS). It, however, did cause a considerable delay to change the experimental design thus resulting in considerably more cost incurred with regard to animal usage and labor used to provide animal care.

The necessary re-design of the experiment delayed our research considerably (i.e., the trial lasted five months instead of three). This delay added considerably to the cost of the animal and labor portion of this research. Despite the challenges that occurred, we successfully completed the feeding trial and cheese making portion of this project on April 5th, 2016.

4.) FINANCIAL INFORMATION (*Describe any budget challenges and provide specific reasons for deviations from the projected project spending.*)

The obstacles reported in section three resulted in a greater than expected labor cost and on-farm cost which has placed financial strain on successful completion of additional research both originally proposed and not originally proposed.

5.) EDUCATION AND OUTREACH ACTIVITES. *(Describe any conferences, workshops, field days, etc. attended, number of contacts at each event, and/or publications developed to disseminate project results.)*

1. Clark, S. 2016. Impact of Distillers Grains on Cheese Quality. Invited oral presentation. Presented at the 2016 Distillers Grains Technology Council Conference. St. Louis, MO.

2. M. R. O'Neil, E. D. Testroet, D. C. Beitz, and S. C. Clark. 2016. Feeding lactating Holstein dairy cows reduced-fat dried distillers grains with solubles I: Production parameters. Poster. Presented at the 2016 Corn Utilization Technology Conference, St. Louis, MO.

3. Testroet, E. D**.,** M. R. O'Neil, D. C. Beitz, and S. C. Clark. 2016. Feeding lactating Holstein dairy cows reduced-fat dried distillers grains with solubles II: Milk composition. Poster. Presented at the 2016 Corn Utilization Technology Conference, St. Louis, MO.