Distiller's dried grains with solubles (DDGS) are not to blame for low-quality Baby Swiss cheese

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ABSTRACT

Late-blowing in Swiss cheese, a result of unwanted gas production, is unacceptable to consumers, and causes economic loss to manufacturers. Cheese processors have raised concerns that feeding dried distillers grains with solubles (DDGS) to cows leads to this defect, in part, because of *Clostridial* spores. We hypothesized that spores in DDGS would affect the quality of milk and baby Swiss cheese by promoting late-blowing defects. Thirty healthy multiparous and mid-lactation Holstein cows were fed total mixed ration containing DDGS (0, 10 and 20%; 10 cows per treatment group) by dietary dry matter (DM) in a 3 × 3 Latin square design. One complete milking from all cows within a treatment was collected and pooled for baby Swiss cheese, twice within each month of the three-month study. Additionally, individual milk samples from three milkings of a day were collected weekly for proximate analysis. Incubation in modified reinforced clostridium lactate medium tubes containing milk, cheese, TMR, or manure showed gas formation. Conversely, the DDGS used in our study was not a source of gas-producing spores. Feeding 20% DDGS decreased milk fat percent and increased the solids nonfat, protein, and lactose percent of milk. After 60 days ripening, baby Swiss cheese had typical propionic acid Swiss cheese aroma. Regardless of dietary treatment, pinholes, slits, and cracks were seen throughout most cheeses. Feeding of DDGS increased the amount of long-chain unsaturated fatty acids and decreased short-chain and most medium-chain fatty acids in the baby Swiss cheese. Although feeding cows diets with DDGS modified milk composition, and subsequently cheese composition, DDGS was not a source for gas producing spores or for quality defects in Swiss cheese. Rather, the gas-producing spores likely originate from environment or the cow herself.

INTRODUCTION

The late-blowing defect in Swiss-style cheese varieties can be described as appearance of undesirable slits, cracks, splits or blown areas in the cheese. Late-blown cheese is unacceptable to consumers and high-speed slicing operations. Such cheeses cannot be sold at full value and thus lead to economic losses to cheese producers. Reports on late-blowing in cheeses have raised concerns that feeding distillers grains to dairy herds contributed to the late-blowing in cheese (Houck et al., 2007).

Eye formation in Swiss cheese is considered desirable when it is formed by carbon dioxide produced as a result of fermentation by starter cultures (Propionibacterium *freudenrichii* subsp. *shermanii*). However, contamination by non-starter bacteria such as *Clostridium tyrobutyricum* or *C. butyricum* could lead to the production of hydrogen gas (H_2) and hydrogen sulfide gas (H_2S), in addition to carbon dioxide (CO_2), butyric acid, butanoic acid, and acetic acid, by utilizing lactate substrate (Fox et al., 2000; Frohlich-Wyder and Bachmann, 2007). H_2 gas is less soluble in the cheese body when compared with CO_2, thus leading to

production of unforeseen large openings in the cheese body. Excessive gas (CO_2 and H_2) elevates the gas pressure higher than the structure of curd can withstand, leading to blowing or exploding in the cheese body (Hutkins, 2006). Excessive gas also leads to variation of eye size (forming too large, too small, or too numerous eyes) and the resultant cheese body has eyes that are usually split and/or cracked, leading to split defects or late-blowing defects. Unforeseen gas formation in the cheese, such as CO_2, H_2, or H_2S is usually associated with unclean and strong atypical off-flavors (Frohlich-Wyder and Bachmann, 2007, Cakir and Clark, 2009). Another theory by Hettinga et al. (1974) linked Propionibacterium strains that are active at low temperatures to the eyes formed by late-fermentation after the cheese is moved from the warm room to the cold room. The temperature difference when the cheese is moved from the warm room to the cold room increases the rigidity, resulting in a brittle cheese body. Any further gas production by non-starter Propionibacteria in the cold room could also contribute to slit formation because the cheese body is incapable of accommodating gas bubbles.

Indeed, various researchers have proposed a number of hypotheses to explain the mechanism of split formation. These hypotheses include 1) the elasticity of cheese and contribution of proteolysis (Johnson, 2001), 2) secondary fermentation where starter or non-starter bacteria produce gas during and/or after the normal warm room ripening causing splitting and irregular eyes (Park at al., 1967, Hettinga et al., 1974, Hutkins, 2006, Frohlich-Wyder and Bachmann, 2007), 3) presence of *Clostridium tyrobutyricum* spores (Dasagupta and Hull, 1989; Steffen et al., 1993; Frohlich-Wyder and Bachmann, 2007), low quality feed with high Clostridial spore count acting as source of contamination in milk and in the cow environment (Dasagupta and Hull, 1989; Houck et al., 2007).

With ethanol production being a major industry in the Midwest, utilization of protein-rich dried distillers grains with solubles (DDGS) in animal feeding is inevitable and generally considered economical. Consequently, understanding the effects of DDGS inclusion in the diet of dairy cows on quality markers of milk that contribute to cheese production is of high priority to cheese processors in the Midwest. The objective of this research was to investigate the impact of feeding diets with three concentrations of DDGS (0%, 10% and 20% dietary DM) to dairy cows on milk and cheese composition, eye formation and gas-forming spores present in milk and cheese.

MATERIALS AND METHODS

Experimental Design

Thirty healthy multiparous and mid-lactation Holstein cows from the Iowa State University Dairy Farm (Ames, IA) were selected and stratified by days in milk (DIM) and parity to one of the three dietary treatment groups with 10 cows in each treatment group in a 3×3 Latin Square with repeated measures. The diets were formulated to be isonitrogenous (16.5% crude

protein) and isoenergetic. The treatments diets were (1) total mixed ration (TMR) with no DDGS, (2) TMR with 10% DDGS by dietary DM, and (3) TMR with 20% DDGS. Cows were trained to use Calan® gates (American Calan Inc., Northwood, NH), and each cow had *ad libitum* access to food and water. After each treatment period, the cows were switched to the next diet, such that each cow served as her own control for a total of three treatments. Individual milk samples from all the three milkings from each cow were collected and pooled to represent an entire milking from that individual cow every week on Tuesday and proximate analysis was performed on Wednesday. To minimize carryover effects, the first 14 days of each 28-day period were not included in the milk proximate analyses.

Proximate Analysis

The milk from each cow was collected throughout one entire milking by a Boumatic milking system (Boumatic, Madison, WI), then poured into blue 60 mL snap cap bottles (Fisher Scientific, Pittsburgh, PA), transported to the laboratory within 30 min of collection, and refrigerated (~4°C). Proximate analyses (percent lactose, fat, and protein) then were performed on the individual milk samples in duplicate by using a Lacticheck-01 RapiRead Milk Analyzer (Page & Pedersen Intl. Ltd., Hopkinton, MA). The chilled milk samples were inverted gently inside the blue snap cap bottles to homogenize any separated cream on the cap of the bottle and tempered to room temperature (22 ± 2^{o}C) before analyzing. Introduction of air bubbles was avoided while mixing the sample to minimize error in the readings from the Lacticheck™ milk analyzer.

Milk Collection for Cheese Making

Milk from one complete milking of each treatment group (0%, 10%, 20% DDGS).was collected twice during each of the three 28-day periods. For cheese-making, the morning milking (approximately 6:30 am) was collected. Typically, milk from two groups of 10 cows each on Friday and 1 group of 10 cows on Saturday were collected and pooled for use in cheese making trials. The milk cans were washed with automatically diluted Ecolab® Oasis Enforce (St. Paul, MN) and sanitized with automatically diluted Ecolab® Mikroklene® (St. Paul, MN) sanitizer or manually diluted 100 to 150 ppm Clorox® (Oakland, CA) solution. At the dairy farm, the teats were sanitized 1000 ppm Chlorine predip (ECAcept technology, Zurex PharmAgra LLC, Middleton, WI) and wiped dry with individual towels before collecting milk from each cow. Milk was collected in sanitized dump buckets and transferred into sanitized, labeled aluminum milk cans. The milk cans were then transported, at ambient temperature, to the 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 placed in the walk-in cooler (4^{0}C$\pm$2^{0}C) until further processing (within 60 min).

Those who collected milk at the dairy farm were requested to take a shower or change to clean clothes before participation in cheese making to minimize additional external contamination of milk to be used for cheese production.

Prior to proximate analysis, the milk was agitated, filtered through cheesecloth, and weighed before sampling for proximate analysis. 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 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).

Milk was subsequently HTST pasteurized with a UHT/HTST Electric model 25HV Hybrid pasteurizer (MicroThermics®, Releigh, NC). The pasteurization temperature and time was set at 73.5°C for 15 sec (flow rate 4 L/min). Upon exit from the pasteurizer (~37°C), milk entered one of two cheese vats and was 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 cultures of *Streptococcus thermophilus SSC 17 (*Chr Hansen, Milwaukee, WI*), Lactobacillus helveticus (*DuPont ™ Danisco®, Madison, WI), and *Propionibacterium freudenrichii* subsp*. shermanii* (Chr Hansen PS-1, Milwaukee, WI). Coagulant (DSM Maxiren®, The Neatherlands) was diluted with cold water to a ratio of 1:40 and added at 4 mL/45 kg of milk and the cheese curd was allowed to set for 25 to 30 min. The curd was 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 jackets 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 47.8°C, where the curds were held for 42 min (postwork). After postwork, and a target pH of 6.4, whey was removed. Cheese curds were collected into perforated cheese molds made by drilling holes in 5-gallon plastic storage basin containers. Blocks were pressed under whey by using the same style plastic basin filled with water (16 kg hydraulic weight) for 15 min. The whey was drained completely and the cheese block was pressed for about 5 hr. The pH of the curd was measured (Accumet® Basic AB15 (Fisher Scientific Inc, Pittsburgh, PA)) after 2 hr and the curd block was flipped. The press was removed, and cheese was fermented for an additional 8 to 10 hr at 28°C $\pm$ 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. Brining was carried out in saturated brine containing 23% NaCl and 0.38% $CaCl_2$, for 12 hr. Cheese blocks were vacuum-packed in clear vacuum seal bags (Fisher Scientific Inc, Pittsburgh, PA) with a gas flash vacuum packing machine (Koch Equipment LLC©, Kansas City, MO). Cheeses were stored at 10°C for 7 days (Pre-cool), 22±3°C for 21 days (warm room), and 4°C for 60 days (cold room). Cheeses were analyzed for composition, quality, and

spores after 60 days in the cold room. The cheeses were cut into pieces for analysis, and pictures were taken (Fig 1).

Staff at the Agricultural Utilization Research Institute (AURI; Marshal, MN) carried out the cheese proximate analysis. Standard methods were followed for measuring different components of cheese (moisture: AOAC 926.08, protein: AOAC 2001.14, ash: AOAC 935.42, fat: AOAC 933.05, calcium: AOAC 991.25, sodium: AOAC 991.25, and Tbars: J. Am. Oil Chem. Soc. 1960, 37, 44-48). AURI staff also carried out the cheese fatty acid profile analysis (AOCS Ce 2 – 66, Ce 1j – 07).

Spore Testing on DDGS, TMR, Milk, Cheese and Manure

DDGS, TMR (with and without DDGS), milk, baby Swiss cheese, and manure were evaluated for the presence of gas-producing spores by observing gas formation in 50 mm Durham tubes (Fisher Scientific, Pittsburgh, PA) placed in 20 mL glass test tubes containing modified reinforced clostridium media with lactate (RCM–lactate, Dasagupta and Hull, 1989) within 48 hr. The modified RCM-lactate media was made by mixing beef extract (10 g), yeast extract (3 g) (BD chemicals, Sparks MD), sodium chloride (5 g), L-cysteine (0.5 g), soluble starch (1 g), (Fisher Scientific, New Jersey NJ), tryptone (10 g), sodium lactate syrup (10 mL), sodium acetate.$3H_2O$ (8 g) (Sigma Aldrich, St. Louis MO), and agar (2 g) (BD chemicals, Sparks MD) in 1 L distilled water and was subsequently autoclaved.

Twenty-five g of sample were mixed in 225 mL of 0.1% peptone water by using a stomacher 400C (Seward®, Daive FL) for 30 sec. From the stomached sample, about 10 to 20 mL of liquid sample was transferred to a 30 mL glass test tube, heated to 80°C for 10 min in a water bath and cooled immediately after 10 min to kill most of the vegetative cells and to enrich spores. One mL of the heat-shocked sample was transferred to a clean autoclaved test tube with 9 mL modified RCM-lactate broth in a Durham tube and stored at 35°C in anaerobic conditions by using GasPak (BD Chemicals, Sparks, MD). The experiment was conducted in duplicate. Gas formation in the samples was observed by checking tubes for the presence of gas after 24 to 48 hr.

Statistical Analysis

During the feeding trials, the cows diagnosed with mastitis or any other sickness were removed from the study. The study started with 30 cows in period one but decreased to 25 by the end of the study. All statistical analysis was carried out by using SAS 9.3 (Cary, NC). A mixed model was used with diet, period, parity, and diet by trial interaction as fixed effects, and days in milk as covariate. Analysis was done as repeated measures with date as the repeated statement and cow nested within diet and trial as the subject for proximate analysis of milk and the repeated statement of date with the subject being an individual cheese quality measure

(e.g., pH, moisture). To minimize the carryover effects, first two weeks of each period was considered as an acclimation period and were not included in the statistical analysis. Tukey-Kramer multiple pairwise comparison adjustment was used to identify significant differences at *(P* < 0.05).

RESULTS AND DISCUSSION

Milk Proximate Analysis

After excluding the first two weeks of data from each period, milk samples were collected from each cow for a total of 10 times throughout the entire experiment. Milk protein% and SNF% increased significantly (P < 0.05) with 20% dietary inclusion of DDGS. These findings were supported by our previous research (Testroet et al., 2015) where the treatments included 10 and 25% DDGS in TMR. The same trend of increase in protein percentage was not observed by previous studies (Anderson et al., 2006, Kleinschmit et al., 2006) when cows were fed with 20% DDGS. The milk yield in those studies, however was increased by the 20% DDGS treatment, which could have diluted in the protein and decreased the protein concentration. In our study, the protein yield remained unaffected by the DDGS treatments with a non-significant decrease in milk yield. This change could have concentrated the protein in the milk, which lead to significantly higher protein in milk from 20% DDGS treatment.

The milk from cows fed 10% and 20% DDGS had significantly lower fat percentage (P< 0.05) compared to the milk from cows fed 0% DDGS. The same pattern of depression in milk fat percentage and daily milk fat yield was not observed in the previous studies that fed DDGS to dairy cows (Anderson et al., 2006, Kleinschmit et al., 2006). However, milk fat content could be highly influenced by dietary fat content. Dairy nutritional experts limit the dietary inclusion of DDGS to 20% (Anderson et al., 2006) because increased unsaturated fatty acids (i.e., the corn oil in DDGS) in the diet of a ruminant could lead to milk fat depression. Indeed, Testroet et al., (2015) showed that feeding unsaturated dietary fats from 10% and 25 % DDGS treatment caused significant (P< 0.05) milk fat depression, which supported the results of our current study.

Lactose percentage of the milk increased significantly with 10% and 20% DDGS inclusion (*P* <0.01), which supports the findings of Tanaka et al. (2011) who observed higher (P < 0.05) lactose content in milk when fed 20% DDGS compared with control group. The US Grains Council (2012) also reported a non-significant (P > 0.05) increase in lactose content when cows were fed 10% DDGS. The increase in lactose may be relevant with respect to eye formation, although the present study did not directly evaluate the impact of lactose on eye formation. Lactose is the primary substrate for starter cultures used in the cheese production process. Lactose is split into glucose and galactose by the starter cultures (S. *thermophilus*, L. *helveticus*), and metabolized during the maturation of cheese. Alterations in the concentration of lactose

available for metabolism by these microbes may modify the maturation and subsequent properties of cheese. It is possible that excess lactose provides substrate for secondary fermentation and late-blowing.

Eye formation in Swiss cheese depends upon its appropriate physiochemical and mechanical properties. If the milk itself contained higher lactose, then it is likely that the lactose content at the start of fermentation was also high. Hutkins (2006) mentioned that it is very important that the cheese curds contain the right amount of lactose after the cooking process for the final pH to be 5.2. A pH of less than 5.2 would increase the chances of fracturing because of excessive gas production or incidence of blind cheese (Lawrence et al., 1984). A pliable and elastic curd mass is required for accommodation of eyes in the cheese network. Finally, proper eye formation depends on the presence of seeding points for eye nucleation, proper acidification and calcium solubilization, molding and pressing, elastic cheese body, low storage temperature to reduce plasticity of cheese body (Guggisberg et al., 2015).

Milk and Cheese Analysis

Because protein in cheese milk slightly increased with inclusion of DDGS (Table 1), fat:protein ratio of raw milk decreased with inclusion of DDGS (data not shown). Standardized fat:protein ratio, however, were all within 0.03 of target (0.88) among treatments (data not shown). Baby Swiss cheese standards of identity require a minimum 45% fat in solids and maximum 43% moisture (ATCP, 1985). The lack of significant differences ($p > 0.05$) in cheese pH, moisture, fat, sodium and salt in moisture based upon diet (Table 2) is expected because the goal was to perform procedures consistently among batches of cheese.

Although no formal sensory evaluation panel was conducted on the cheese, after about 90 days of ripening (including 7 days of cool storage, 21 days of warm room, and 60 days of cold room), informal sensory evaluation by our research team revealed that baby Swiss cheeses had a typical propionic acid Swiss cheese aroma. However, more lactic acid aroma than expected in typical baby Swiss was present in cheeses from 10% and 20% diets when compared with aroma in the control cheese. The greater than expected lactic acid aroma was likely because lactose percentage was significantly higher in milk from the 10% and 20% DDGS-fed cows than the control milk (Table 1). Additionally, some cooked eggy H_2S aroma was apparent in the 10% and 20% DDGS cheeses. Because spoilage organisms such as *Clostridium tyrobutyricum* and *C. butyricum* are capable of metabolizing lactose into H_2S, H_2, butyrate and CO_2 (Fox et al., 2000; Cakir and Clark, 2009), it was thought that the aroma was a sign of Clostridial contamination.

Eye distribution and cheese body and texture were atypical in both control and treatment cheeses. Although proximate analysis revealed that all cheeses met the standard of identity for Swiss cheese (Table 2), all cheeses were less rubbery and more moist than typical baby Swiss. Slits (Figure 1A), pin-holes (Figure 1B), cracks (Figure 1B), checks (Figure 1C), and splits (Figure 2D) were evident in all cheeses. Glossy, round eyes were rarely found (Figure 1A)

beyond period one, regardless of dietary treatment. Blindness was also occasionally exhibited, most typically within ¼-inch (0.64 cm) of the cheese surface. Several cheeses exhibited long longitudinal cracks or blown areas, which tended to appear along curd junctions, preventing a tight, closed body (Figure 1), but mechanical openings were not present. The defects may have resulted from overly-high rate of CO_2 production in the warm room, coupled with a high rate of diffusion out of the cheese body (Guggisberg et al., 2015).

Moisture content of the cheeses was higher than those reported by White et al. (2003). The difference in moisture may partially explain the body defects in the cheeses, though moisture was within standards. Higher moisture content may lead to loss of elastic nature of cheese; accelerated proteolysis can lead to a brittle cheese body in the later ripening stage, which enables crack formation (Fröhlich-Wyder and Bachmann, 2007; Guggisberg et al., 2015). White et al., (2003) noticed reduced splitting in cheese with lower moisture content. Aging time is also sometimes associated with splitting of cheese. White et al., (2003) observed more splitting as the ripening time increased up to 120 days. This is an unlikely explanation for the defects found in the present study because the total cheese ripening time was not more than 90 days.

On average, final cheese pH tended to be slightly lower than 5.2 (Table 2), perhaps because of over-active cultures resulting from low salt-to-moisture ratio (mean 1.1-1.2 % S/M) Mean calcium concentration (7.5-7.8 mg/g) was slightly lower than typical Swiss cheese (7.9 mg/g). Because calcium is a structural component of cheese matrix, the lower calcium, potentially lost because of the lower pH, may also partially explain the cheese softness and resulting body defects. Solubilisation of calcium due to acidification is one of the factors that influences eye defects in Swiss cheese (Guggisberg et al., 2015). On the other hand, White et al. (2003) did not notice any correlation between the factors such as pH, fat, protein, and calcium contents in split and non-split cheese.

Cheese fatty acid profile revealed that long chain unsaturated fatty acids increased with DDGS feeding (Table 3). All short (C4, C6, C8, C10) and medium-chain (C14, C16, C18) saturated fatty acids decreased significantly ($P<0.05$). No changes were seen in C15, C17, C19 and C20, which were found only in trace amounts. The amount of C12 increased significantly with DDGS feeding, but 10% DDGS cheese had more than did control and 20% DDGS cheese. All long-chain mono-unsaturated fatty acids increased significantly (9c-C14:1, 9c-C16:1, 6t-C18:1, 11t-C18:1, 9c-C18:1 and 11c-C18:1) except 9t-C18:1, 6c-C18:1 and 8c-C20:1. Poly-unsaturated fatty acids remained unchanged (9c, 12c, 15c-C18:3, 8c, 11c, 14c-C20:3 and 5c, 8c, 11c, 14c-C20:4) or increased (9c, 12c-C18:2).

Spore Testing

Although modified RCM-lactate is typically used to observe for gas formation by *C. tyrobutyricum* spores, any gas-producing organism (e.g., Propionibacterium) that is capable of

utilizing lactate as the sole carbon source and produce gas can grow in this medium. Therefore, RCM-lactate may not be considered a highly selective media (Wehr and Frank, 2004). However, the heat-shocking (80°C for 10 min) step was followed in order to induce spore germination and eliminate vegetative cells, as the main focus of the research is to identify gas formation by spores. Additionally, the samples were inoculated in the test tubes containing Durham tubes and allowed to incubate under strict anaerobic conditions, thus inhibiting obligate aerobes. All these steps were performed to make the medium as selective as possible and to ensure that the conditions would support the growth of Clostridia spores over other microbes.

No gas formation by spores was observed when RCM-lactate medium was inoculated with DDGS. The DDGS used in the feeding study was likely devoid of spores because of the high temperatures (up to 426.6°C) associated with the DDGS drying process (Pfizer Animal Health, Lactrol Technical Information Pamphlet). Although Pedersen et al. (2004) did not find microorganisms in the wheat WDG samples collected directly out of the distillation column, storage in unclean environment could possible lead to increases in microbial loads. Under anaerobic conditions, gas formation in RCM-lactate medium was observed in all samples of baby Swiss cheese, manure and the TMR. These results suggest that the TMR and/or or contamination during milking were sources for gas forming species and not DDGS.

Herlin and Christiansson (1993) suggested that spores make their way into the milk during the milking process through manure-contaminated teats. Butyric acid bacteria and their spores are naturally present in the soil, make their way into feed, and act as a source of contamination in feed and eventually in milk (Vissers et al., 2006). One of the major spore-forming butyric acid producing bacteria, C. *tyrobutyricum* is known to be propagated via feed and contaminates milk at the farm level (Houck et al., 2007). Halligan and Fryer (1976) and Dasgupta and Hull (1989) reported large numbers of spores in manure from cows fed silage containing *C. tyrobutyricm*. Other Clostridial species, such as *C. beijerinckii,* could also end up in cheese, as they are common anaerobic spore-formers found in milk (Klijn et al., 1995). Preventing contamination of raw milk and removing spores from contaminated milk via bactofugation are steps to avoid economic losses because of late-blowing defect, but bactofugation can lead to inferior cheese (Fox et al. 2000; Guggisberg et al., 2015).

Because all cheeses, including cheeses from cows not fed DDGS, exhibited body and texture defects, these findings do not implicate DDGS, but rather spores not originating from DDGS, as the likely causative agent for the defects observed. However, we cannot rule out the potential role of increased lactose concentration and changes in fatty acid profile on baby Swiss quality defects.

CONCLUSION

Feeding 20% DDGS as part of a TMR decreased percentage of fat and increased percentage of lactose and protein in milk. The DDGS source used for this research did not

contain gas-forming spores, yet TMR, manure, milk and cheese contained gas-forming spores, demonstrating that the source of the contamination of the milk and cheese by spores was not the DDGS but rather the environment. Because all cheeses, including cheeses from cows not fed DDGS, exhibited body and texture defects, these findings do not implicate DDGS, but rather spores not originating from DDGS, as a contributing agent for the defects observed.

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Table 1. Summary of proximate analysis conducted on milk collected every week from individual cows during DDGS feeding study.

Item	0% DDGS	10% DDGS	20% DDGS	SEM	P -VALUE	
					Diet	Period
Fat, %	3.45[a]	2.94[b]	2.67[b]	0.09	<0.0001	0.110
Lactose, %	5.07[b]	5.15[a]	5.17[a]	0.02	0.007	0.650
Protein, %	3.58[b]	3.62[ab]	3.65[a]	0.02	0.041	0.002
SNF[1], %	9.33[b]	9.47[ab]	9.55[a]	0.06	0.004	0.118

[a, b] -Values not connected by the same superscripts are significantly different ($P < 0.05$).

Table 2. Summary of chemical analysis conducted on all baby Swiss cheeses made from milk of cows fed total mixed ration (TMR) and partial substitution of TMR with 10 to 20% dried distillers grains with solubles (DDGS).

		0% DDGS	10% DDGS	20% DDGS	SEM	*P* - VALUE	
Items	Units					Diet	Period
pH		5.15	5.14	5.13	0.02	0.795	0.146
Moisture	%	41.28	41.67	41.36	0.39	0.803	0.956
Fat	%	30.18	29.70	29.50	1.39	0.945	0.780
Fat in solids	%	51.37	50.92	50.33	2.19	0.945	0.735
Protein	%	23.83	23.60	24.13	0.89	0.920	0.679
Ash	%	3.40	3.41	3.59	0.11	0.610	0.615
Calcium	mg/g	7.40	7.49	7.84	0.23	0.597	0.880
Sodium	mg/g	4.79	4.74	4.98	0.11	0.508	0.375
Salt	%	1.22	1.21	1.27	0.02	0.497	0.356
Salt in Moisture	%	2.94	2.90	3.07	0.03	0.208	0.173

Table 2. Fatty acid composition of baby Swiss cheese from milk of cows fed total mixed ration (TMR) and partial substitution of TMR with 10 to 20% dried distillers grains with solubles (DDGS).

Fatty acids	Control	10% DDGS	20% DDGS	S.E.		
					Diet	Period
C4:0	2.25a	1.98b	1.97b	0.02	<0.0001	0.6856
C6:0	1.82a	1.45b	1.36c	0.01	<0.0001	0.2822
C8:0	1.08a	0.83b	0.8b	0.01	<0.0001	0.3276
C10:0	2.48a	1.85b	1.66c	0.01	<0.0001	0.0014
C12:0	2.61a	2.25b	2.08b	0.06	<0.0001	0.0279
C14:0	9.91a	8.61b	7.95c	0.04	<0.0001	0.0066
C15:0	0.88a	0.76b	0.73c	0.008	<0.0001	<0.0001
C16:0	26.87a	24.6b	23.61c	0.08	<0.0001	0.0052
C17:0	0.46	0.4	0.4	0	.	.
C18:0	10.86	10.7	10.86	0.11	0.5191	<0.0001
C19:0	0.2a	.	.	0	.	.
C20:0	0.13a	0.11ab	0.1b	0.005	0.0017	<0.001
9c-C14:1	0.8a	0.93b	0.98b	0.01	<0.0001	0.767
9c-C16:1	1.2a	1.48b	1.58c	0.02	<0.0001	0.7921
6t-C18:1	.	0.46a	0.58b	0.006	<0.0001	<0.0001
9t-C18:1	.	.	.	.	.	.
11t-C18:1	2.32a	4.16b	5.91c	0.04	<0.0001	<0.0001
6c-C18:1	1.07a	1.11b	1.1ab	0.01	0.0429	<0.0001
9c-C18:1	17.84a	20.76b	21.13b	0.15	<0.0001	<0.0001
11c-C18:1	0.55a	0.6b	0.6b	0	0.0004	<0.0001
8c-C20:1	0.1	0.1	0.1	.	.	.
9c,12c-C18:2	2.27a	2.55b	2.95c	0.01	<0.0001	<0.0001
9c,12c,15c-C18:3	0.26a	0.2b	0.23ab	0.01	0.0012	0.1837
8c,11c,14c-C20:3	.	.	.	.	.	.
5c,8c,11c,14c-C20:4	0.12a	0.1b	.	0	0.1012	0.5376

[1]nomenclature expressed as number of carbons: number of double bonds

A.  B 

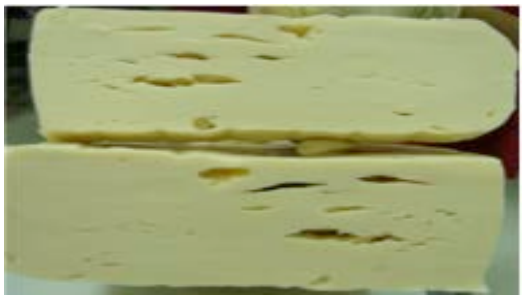
C.  D 

Figure 1. Representative images of defects in experimental baby Swiss cheese made from the milk of cows fed total mixed ration (TMR) and partial substitution of TMR with 10 to 20% dried distillers grains with solubles (DDGS). A. cheese exhibits a few eyes and slits B. cheese exhibits pin-holes and cracks C. cheese exhibits checks D. cheese exhibits splits throughout the cheese body