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Feeding reduced-fat dried distillers grains with solubles to lactating Holstein dairy cows does not alter milk composition or cause late blowing in cheese

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Abstract

Feeding dried distillers grains with solubles (DDGS) to lactating dairy cows has been implicated as a cause of late blowing defects in the production of Swiss-style cheeses. Our objectives were (1) to test the effect of feeding reduced-fat DDGS (RF-DDGS; ~6% fat) to lactating dairy cows on the composition of milk and on the suitability of the milk for production of baby Swiss cheese and (2) to evaluate the effect of diet on cow lactation performance. Lactating Holstein dairy cows were fed both dietary treatments in a 2 × 2 crossover design. Cows were housed in a 48-cow freestall pen equipped with individual feeding gates to record feed intake. The control diet was a corn, corn silage, and alfalfa hay diet supplemented with mechanically expelled soybean meal. The experimental diet was the same base ration, but 20% (dry matter basis) RF-DDGS were included in place of the expelled soybean meal. The RF-DDGS diet was additionally supplemented with rumen-protected lysine; diets were formulated to be isoenergetic and isonitrogenous. Cows were allowed ad libitum access to feed and water, fed twice daily, and milked 3 times daily. For cheese production, milk was collected and pooled 6 times for each dietary treatment. There was no treatment effect on milk yield (35.66 and 35.39 kg/d), milk fat production (1.27 and 1.25 kg/d), milk fat percentage (3.65 and 3.61%), milk protein production (1.05 and 1.08 kg/d), lactose percentage (4.62 and 4.64%), milk total solids (12.19 and 12.28%), and somatic cell count (232.57 and 287.22 × 103 cells/mL) for control and RF-DDGS, respectively. However, dry matter intake was increased by treatment, which implied a reduction in feed efficiency. Milk protein percentage also increased (3.01 and 3.11%), whereas milk urea nitrogen decreased (14.18 and 12.99 mg/dL), indicating that protein utilization may be more efficient when cows are fed RF-DDGS. No differences in cheese were observed by a trained panel except cheese appearance; control cheese eyes were significantly, but not practically, larger than the RF-DDGS cheese. These results indicate that RF-DDGS can be effectively used in the rations of lactating Holstein cows with no deleterious effects on milk production and composition and metrics of the physiology of the cow (i.e., blood glucose and nonesterified fatty acids); however, feeding RF-DDGS increased dry matter intake, which decreased feed efficiency. Finally, feeding RF-DDGS did not negatively influence quality and suitability of milk for production of baby Swiss cheese.

Keywords

Efficiency, dried distillers grains with solubles, milk fat, protein utilization, sensory

Disciplines

Dairy Science | Food Chemistry | Food Science | Molecular, Genetic, and Biochemical Nutrition

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E. D. Testroet

Interpretive summary

Holstein dairy cows were fed a control diet not containing reduced-fat distillers grains with solubles (RF-DDGS), or a diet that contained 20% of the dry matter (DM) as RF-DDGS. Milk composition was not affected when cows were fed RF-DDGS, except for an increase in milk protein percentage. Cows fed either diet performed equally well, but cows fed RF-DDGS had improved nitrogen efficiency. These results show that cows can be fed 20% RF-DDGS without a loss in performance. Furthermore, milk from cows fed RF-DDGS was used to produce baby Swiss cheese that did not differ in quality from cheese made from milk of cows fed the control diet.

RUNNING HEAD: RF-DDGS, COW PERFORMANCE, AND CHEESE QUALITY

Feeding reduced-fat dried distillers grains with solubles to lactating Holstein dairy cows does not negatively influence cow performance or cause late blowing in cheese

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ABSTRACT

Feeding dried distillers grains with solubles (DDGS) to lactating dairy cows has been implicated as a cause of late blowing defects in production of Swiss-style cheeses. Our objectives were to test the impact of feeding reduced-fat dried distillers grains with solubles (RF-DDGS; ~6% fat) to lactating dairy cows on the composition of milk and on the suitability of the milk for production of baby Swiss cheese, and to evaluate the impact of diet on cow lactation performance. Lactating Holstein dairy cows were fed both dietary treatments in a 2 × 2 crossover design. Cows were housed in a 48-cow free stall pen equipped with Calan gates to record individual feed intake. Diet one (**control**) was a corn/corn silage/hay diet supplemented with SoyPlus®. Diet two was the same base ration, but 20% (DM-basis) reduced-fat dried distillers grains with solubles (**RF-DDGS**) were included in place of SoyPlus®. The RF-DDGS diet was

additionally supplemented with rumen protected lysine; diets were formulated to be isoenergetic and isonitrogenous. Cows were allowed *ad libitum* access to feed and water, fed twice daily, and milked three times daily. For cheese production, milk was collected and pooled six times for each dietary treatment. There was no treatment effect on milk yield (35.66 and 35.39 kg/day, control and RF-DDGS, respectively), milk fat production (1.27 and 1.25 kg/day), milk fat percentage (3.65 and 3.61%), milk protein production (1.05 and 1.08 kg/day), lactose percentage (4.62 and 4.64%), milk total solids (12.19 and 12.28%), and somatic cell count (232.57 and 287.22 × 10^3 cells/mL). Milk protein percentage, however, was increased (3.01 and 3.11%) and milk urea nitrogen decreased (14.18 and 12.99 mg/dL), indicating that protein utilization may be more efficient when cows are fed RF-DDGS supplemented with lysine. Additionally, DMI and milk yield were unaffected by treatment. Consequently, there was no treatment effect on any measures of feed efficiency. No differences in cheese were found by a trained panel, except cheese appearance; control cheese eyes were significantly (but not practically) larger than the RF-DDGS cheese. These results indicate that RF-DDGS with supplemental lysine can be effectively utilized in the rations of lactating Holstein dairy cows without negatively influencing production parameters or the physiology of the cow. Finally, feeding RF-DDGS did not negatively influence quality and suitability of milk for production of baby Swiss cheese.

Keywords: efficiency, DDGS, milk fat, protein utilization, sensory

INTRODUCTION

Feeding of traditional (full-fat) corn dried and wet distillers grains with solubles (**DGs**) to ruminant animals has been studied and reviewed extensively (Klopfenstein et al., 2008; Schingoethe et al., 2009). However, results are mixed, with some studies reporting no changes in production parameters (Anderson et al., 2006; Sasikala-Appukuttan et al., 2008) and others reporting altered milk composition or yield, either positively or negatively, when DG are fed (Kleinschmit et al., 2006; Abdelqader et al., 2009). One problem with feeding traditional DGs is that they contain approximately 13% fat, which is composed of mainly unsaturated fatty acids. This problem is multi-faceted; inclusion of DGs can result in diets that contain concentrations of fat that exceed five percent, which can inhibit fiber digestion (Zinn, 1989). Secondly, problems can arise relating to the great concentration of unsaturated fatty acids in DGs that remain after the fermentation process. Unsaturated fatty acids are toxic to rumen microbes (Maia et al., 2007) and undergo biohydrogenation in the rumen because the rumen has a great capacity for reducing unsaturated hydrocarbons. Incomplete biohydrogenation of these hydrocarbons, however, can result in the production of bioactive forms of conjugated linoleic acid (*trans*-10 *cis*-12 CLA), which inhibit *de novo* lipogenesis in the mammary gland (Baumgard et al., 2001). The aforementioned problem can result in milk fat depression, that is characterized by decreased milk fat without concomitant alteration in concentrations of other milk components (Bauman and Griinari, 2001). With the relatively recent improvements in oil extraction from DGs (Majoni et al., 2011) because of the economic value of the corn oil extracted, traditional high fat DGs probably will be less available in the future. Indeed, personal conversations with regional farmers have indicated that reduced-fat dried distillers grains with solubles (**RF-DDGS**; ~6% fat) are typically the only form of DGs available. While the lower energy content of RF-DDGS is a

concern for producers of monogastric animals, it could be an advantage for dairy producers by allowing them to include greater concentrations of this typically economical protein source. Research has shown positive results of lactation performance when RF-DDGS are included in the rations of lactating dairy cows (Mjoun et al., 2010; Castillo-Lopez et. al., 2014; Ramirez-Ramirez et al., 2016).

Additionally, there are mixed reports about the impact of feeding DGs on cheese quality (Houck et al., 2007; Manimanna Sankarlal et al., 2015). The inclusion of DDGS in the rations of lactating dairy cows has been implicated as a cause of late blowing defects both by the scientific community and the dairy community, including dairy producers (Personal conversation; Houck et al., 2007). Our research group investigated the effects on the quality of baby Swiss cheese of feeding full-fat DDGS to dairy cows and found no differences in the quality of baby Swiss cheese when cows were fed a conventional total mixed ration (TMR) or a TMR containing full-fat DDGS (~ 13% fat; Manimanna Sankarlal et al., 2015).

To extend our DDGS research, our objectives were to investigate the effects of feeding RF-DDGS (~ 6% fat) to lactating Holstein dairy cows on feed efficiency, the composition of milk produced, and the quality of baby Swiss cheese produced from that milk compared with a control TMR that does not include RF-DDGS. On the basis of our previous research, we hypothesize that feeding RF-DDGS to lactating Holstein dairy cows will not adversely affect feed efficiency, milk composition, or quality of baby Swiss cheese made from the milk of those cows.

MATERIALS AND METHODS

Animals and Diets

All procedures were approved by the Iowa State University Animal Care and Use Committee (IACUC). Thirty-six multiparous mid-lactation Holstein dairy cows (body weight 680 ± 11 kg, 52.25 ± 27.34 DIM) were assigned to one of two dietary treatment groups (n = 18 per group) in a 2 × 2 crossover design. Each experimental period lasted 35 days, with the first 14-d used as an acclimation period. Rations were formulated to meet NRC requirements, to be isonitrogenous and isoenergetic (Tables 1 and 2), and to contain similar intestinally available amino acid concentrations. Ration one (control) was a corn/corn silage/hay based TMR supplemented with SoyPlus® (Dairy Nutrition Plus, Ralston, IA) as a protein source. Ration two was formulated by using the same base ration as the control but with 20% of the dry matter being RF-DDGS (Poet Biorefining, Jewell, IA) containing approximately 6.0% fat in place of SoyPlus® (Table 1). The RF-DDGS ration was supplemented with rumen-protected lysine (Kemin, Des Moines, IA) to make diets similar in available lysine (Table 1). The cows were fed individually by using Calan gates (American Calan, Northwood, NH), allowing for measurement of individual feed intake. Cows were housed together at the Iowa State University Dairy Farm (Ames, IA) in a 48-cow, free-stall pen and individually fed twice daily (0800 h and 1600 h) to allow for approximately 10% refusal. Refusals were weighed daily and recorded. Feed ingredients in the TMR were mixed by using a Patz V615 mixer (Patz Corporation, Pound, WI). Cows were allowed *ad libitum* access to food and water, except during their three daily milkings (8 h apart). Initially, cows were allowed to adapt to using the Calan gates before start of the acclimation period. Additionally, individual milk production was recorded daily by using a Boumatic milking system (Boumatic LLC, Madison, WI).

Sample Collection and Analyses

Because the first 14-d of each experimental period were an acclimation period, those data were excluded from analyses. Feed samples were collected three times per experimental period after the acclimation period, and proximate analyses were done by wet chemistry (Dairylands Lab, Arcadia, WI). Fiber (acid detergent) was quantified by AOAC Official Method 973.18 (1996) and lignin by AOAC Official Method 973.18; ether extract was determined by using AOAC Official Method 945.16 and AOAC Official Method 920.39; feed fatty acids were quantified by using the method described by Sukhija and Palmquist (1988); nitrogen was quantified by using AOAC Official Method 990.0; minerals were determined by ICP-MS by using AOAC Official Method 985.0 and AOAC Official Method 2011.14; NDF was determined as described by Mertens (2002); AD-ICP was determined by using AOAC Official Method 973.18 and AOAC Official Method 990.03; ash was determined by AOAC Official Method 942.05; dry matter was determined by using NFTA Method 2.1.4. After the 14-d acclimation period, individual milk samples were collected weekly from all three milkings to represent a 24-h lactation period. Individual milk samples (three from each test day) were sent for proximate analyses and assay of milk urea nitrogen and somatic cell counts (Dairy Lab Services, Dubuque, IA).

Blood samples were collected during the final week of each period by jugular venipuncture and placed into lithium heparinized vacutainers for NEFA quantification and into fluoridated vacutainers for blood glucose analysis (Becton, Dickson, and Company, Franklin Lakes, NJ). Blood glucose was assayed by following the manufacturer's protocols (Wako Autokit Glucose, Wako Diagnostics, Richmond, VA). Blood NEFA were quantified by following manufacturer's

147 protocol (Wako HR Series NEFA-HR, Wako Diagnostics, Richmond, VA). Rumen fluid was
148 collected via esophageal tube approximately four h post-feeding coinciding with maximal
149 volatile fatty acid (VFA) production. The first few hundred milliliters of rumen fluid were
150 discarded to limit salivary contamination. The next volume of rumen fluid was strained through
151 cheese cloth and frozen at -20°C. Rumen pH was measured immediately on-farm after
152 collection.

153 ***Cheese Making***

154 During each of the three 35-d periods, milk from one complete milking (collected at
155 approximately 0630 h) of each treatment group (control or RF-DDGS) was collected for cheese
156 making two to three times during weeks three and four (n = 14 cheeses). The milk cans and
157 dump buckets were washed with automatically diluted Ecolab® Oasis Enforce (St. Paul, MN)
158 and sanitized with automatically diluted Ecolab® Mikroklene® (St. Paul, MN) iodine-based
159 sanitizer. Teats were sanitized with 1000 ppm chlorine predip (ECAcept technology, Zurex
160 PharmAgra LLC, Middleton, WI) and wiped dry with individual towels before collecting milk
161 from each cow by the Boumatic milking system (Boumatic, Madison, WI). Milk from two
162 groups of six cows was collected by re-routing the Boumatic line into a clean dump bucket.
163 After the milk of two cows fed the same diet filled a dump bucket, it was dumped through
164 cheesecloth into a labeled milk can. Milk was transported at ambient temperature to the ISU
165 Center for Crops Utilization Research (CCUR) pilot plant in the Food Sciences Building at Iowa
166 State University (Ames, IA) within 20 min of collection of milk from the last cow. The milk cans
167 were immediately weighed and tested for fat, protein, and lactose prior to further processing
168 (within 60 min of collection) by using a LactiCheck Milk Mini Analyzer (Page and Pederson Inc,
169 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.

If the fat:protein ratio was not 0.88±0.05, the milk was separated and standardized; cream or skim from the milk collected from the same experimental cows was added to raise or lower the ratio to 0.88±0.05, respectively. Milk was separated by using a Type LWA 205 Westfalia Separator (219 rpm in 2.5 dial setting, Dusseldorf, Germany). Approximately 68 kg of standardized milk from each dietary treatment was poured into a labeled cheese vat and heat-treated (63°C for 2 min, to decrease the initial load of bacteria) by delivering steam-heated water to the jacketed vat while the milk was gently agitated. After heat-treatment, the milk was gradually cooled to 33°C by running cold water in the jacketed vat while the milk was gently agitated.

Baby Swiss-style cheese was made by adding 0.32g (± 0.03) CHOOZIT TA 60 (*Streptococcus salivarius* ssp. *thermophilus*, DuPont™ Danisco®, New Century, KS) and 0.12 g (± 0.02) CHOOZIT eyes (*Propionibacterium freudenfeichii* ssp. *shermanii*, DuPont™ Danisco®) per 100 kg of 33°C milk. Microbial coagulant (13 mL/100 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 1 min after 30 min of ripening. The cheese curd was allowed to set for approximately 30-min, tested for firmness manually and visually, and manually cut with 12-cm wire curd knives. After a 5-min heal, the 35-min forework period of intermittent gentle stirring occurred. About 25% of the vat volume of whey was removed (pre-draw), followed by constant stirring and addition of water (3 to 5% of the vat volume) at 33°C. Gradually, the curds were cooked by

193 increasing the temperature to 40°C over a 15-min period, and then to 46°C over a 10-min period
194 by adding steam to the jacket of the vat, with continuous curd stirring. Warm water (~10 % of
195 the vat volume) was added at 44°C to facilitate the rise in temperature of the cheese to
196 46°C(±1°C), at which point the curds were held for 42 min (postwork). Whey was removed
197 after postwork at a target pH of 6.4.
198
199 Approximately 4 h from the time culture was added to the milk, pressing began. Cheese curds
200 were collected into perforated stainless steel Longhorn hoops and pressed, in the whey, by using
201 a 7 kg weight (0.02 kg/cm^2), on each hoop follower for 15 min. The whey was drained
202 completely and the cheese block was pressed for 1 hr with 11 kg (0.04 kg/cm^2), 1 hr with 23 kg
203 (0.07 kg/cm^2, and an additional 3 h with 35 kg of weights (0.11 kg/cm^2). The weights were
204 removed, and cheese was ripened in an empty basin for an additional 5 to 8 h at 22°C $\pm$ 3°C.
205 The ripening time was based on the time required for the pH of the cheese to drop to 5.25
206 (±0.05) (Accumet® Basic AB15, Fisher Scientific Inc, Pittsburgh, PA). According to
207 Kosikowski and Mistry (1997), the optimal pH at the time of salting for Swiss-style cheeses is
208 between 5.2 and 5.4. Cheese rounds were removed from the hoops, cut in half, and placed in
209 saturated brine containing 23% NaCl and 0.38% $CaCl_2$, for up to 7 to 9 hr (depending on block
210 weight, which averaged 5.3 kg; approximately 30 min/kg cheese).
211
212 Cheese surfaces were allowed to dry for 1 h before rounds were vacuum-packed in clear vacuum
213 seal bags (12 X 16 3-mil; UltraSource® LLC, Kansas City, MO) with a Koch vacuum packing
214 machine (Koch Equipment LLC©, Kansas City, MO). Cheeses were stored at 10 ± 1°C for 7 d

(pre-cool), 22 ± 3°C for 21 d (warm room), and 4 ± 1°C (cold room) for 60 d (Kosikowski and Mistry, 1997). Cheeses were flipped weekly.

Cheese Analyses

Proximate analysis was conducted by using standard methodology, at South Dakota State University. Fat was analyzed by Mojonnier method (Atherton and Newlander, 1977), moisture content by using a forced-draft oven (model OV-490A-2; Blue M, Blue Island, IL), chloride by using a Corning Chloride Analyzer (Ciba Corning Diagnostics, Medfield, MA), based on the Volhard test (Marshall, 1992 added), and total protein by measuring total nitrogen in the cheeses using the by Kjeldahl method (Kjeltec™ 2200 Auto Distillation Unit, FOSS, Eden Prairie, MN).

Sensory Analyses

Sensory quality evaluation began after 62 ± 6 d aging. A descriptive sensory analysis panel, composed of six trained panelists, evaluated the quality of the cheeses. Procedures for use of human subjects in research were approved by the Iowa State University Institutional Review Board (Ames, IA). Training consisted of at least 5 h of initial training, followed by an additional hour of refresher training between the first and second official tasting periods (separated by a month). The expectations for "ideal" characteristics of Swiss and baby Swiss cheese were explained to panelists (Cakir and Clark, 2009). For the appearance attributes, photographs of ideal Swiss and baby Swiss cheeses, as well as those exhibiting varying degrees of specific defects, were used to assist with initial training. To instill specific appearance, aroma, flavor, and body and texture defects in the minds of the panelists, products exhibiting varying levels of defects were utilized, which served as references on a 15-cm unstructured line scale during

238 training sessions (Table 3). The facilitator and panelists created a "Cheat Sheet" to augment
239 training and tasting sessions. The descriptors and references (Table 3) were anchored on the 15-
240 cm unstructured line scale to remind panelists of intensity references. Additionally, to assist with
241 eye size evaluation, panelists were provided a plastic square with a standard hole punched in it to
242 indicate ideal ("small") eyes and a penny to indicate "large" eyes.
243
244 To representatively sample cheeses for evaluation by panelists, every cheese was systematically
245 cut manually with a sanitized butcher knife on a sanitized cutting board, into at least 20 pieces of
246 approximately 1 cm thickness. The cutter began by making a 1 cm slice at the outside round of
247 the cheese and proceeded to make subsequent slices around the round, gradually forming a
248 smaller and smaller square out of the cheese (Figure 1). Slices were laid out sequentially on
249 sanitized, dry trays for photographing (Figures 2 – 5). When the length, width, and height of the
250 cheese were nearly equal the center-most piece of cheese was flipped vertically and the
251 remaining pieces were cut, resulting in three to four horizontal slices representing the top and
252 bottom "faces" and one to two inner-most slices. Based upon their sequential placement on
253 trays, cheese slices were selected randomly for bagging and presentation to panelists by using a
254 random number generator. A plastic index card-sized template (8.5 cm × 5 cm × 1 cm) was
255 placed on each randomly selected master slice to make a consistent "principal display" for
256 panelist evaluation. Selected slices were placed into individual re-sealable snack bags, labeled
257 with random 3-digit codes corresponding to the original cheese from which they were cut, and
258 stored at 4°C until the sensory panel.
259

During actual cheese sensory evaluation sessions, panelists were provided one blank score sheet per sample which was the same as the Cheat Sheet minus the anchored reference words. Panelists were trained to identify appearance, aroma, flavor, and body and texture attributes, but trigger words on the Cheat Sheets served to remind them where on the score sheet to mark the intensity of each cheese attribute on the 15-cm line scale. While in individual booths, panelists were provided individual bagged samples, along with a plastic knife for cutting cheese for body and texture and taste evaluation, as well as water and green grapes for cleansing the palate. Panelists were instructed to first evaluate visual attributes only on the principal display presented to them in the bag. Panelists then evaluated body and texture and finally aroma and flavor.

Statistical Analyses

Milk components, yield, and performance metrics were analyzed as a 2 × 2 crossover design. Data were analyzed by using the MIXED procedure of SAS version 9.4 (Cary, NC). The model included the fixed-effects of treatment, treatment sequence, and period, and the random-effect was cow nested within group. Means with significant treatment effects were separated by using LSMEANS with the PDIFF option. Feed fatty acids and feed proximate analysis results were analyzed by using the MIXED procedure of SAS with the fixed-effect of treatment. Sensory attributes of cheese were analyzed by using the MIXED procedure of SAS with the model including the fixed-effect of treatment. Means were separated by using LSMEANS with the PDIFF option. Differences were considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

As designed, diets were isoenergetic and isonitrogenous (Table 2) as evidenced by the similar NE_L, protein, and total digestible nutrients. Significant differences in phosphorus, sulfur, and calcium resulted in a significantly lower dietary cation anion difference (**DCAD**) in RF-DDGS rations (Table 2). Feed fatty acid compositions are reported in Table 3, and, as expected, the RF-DDGS diet contained slightly greater concentrations of total unsaturated fatty acids because corn oil is 92% unsaturated fatty acids (Ramos et al., 2009).

Dry matter intake (**DMI**) did not vary significantly between treatment groups, nor did milk yield, fat-corrected milk yield (**FCM**), or energy-corrected milk yield (**ECM**) (Table 4), which is consistent with results of Paz and Kononoff (2014) and Ramirez-Ramirez et al. (2016). Additionally, milk fat percentage (consistent with Ramirez-Ramirez et al., 2016), total milk fat production, total milk protein production, lactose percentage, and total lactose production were unaffected by treatment, which is consistent with results of Paz and Kononoff (2014) and Mjoun et al. (2010). Previous studies have indicated that increased DCAD values can increase milk fat percentage (Harrison et al., 2012; Hu et al., 2007; Wildman et al., 2007); this result, however, was not seen in our study (Table 4), which is consistent with results reported by Erdman et al. (2011). No differences were found in composition of milk from cows fed either diet, with the exception of milk protein percentage, which increased for cows fed RF-DDGS without a concomitant change in total milk protein production. Together, a decrease in milk urea nitrogen (**MUN**) and increased milk protein percentage for cows fed RF-DDGS indicate an increased efficiency of protein extraction from feed (Table 5). These results are consistent with those of

Castillo-Lopez et al. (2014) who found that feeding cows RF-DDGS had no effect on milk yield and tended to increase milk protein percentage. Additionally, Ramirez-Ramirez et al. (2016) found that cows fed RF-DDGS produced milk with greater protein percentage and no effect on milk fat percentage, which is consistent with our study. They, however, observed increased milk yield for those cows fed either DDGS or RF-DDGS which was not seen in this study. In this study, milk protein percentage was significantly increased and MUN was decreased when cows were fed RF-DDGS, suggesting that protein utilization may be improved when cows are fed RF-DDGS when supplemented with rumen-protected lysine, which has been reported by Mjoun et al. (2010). Increased milk protein percentage when cows were fed RF-DDGS has been previously reported (Castillo-Lopez et al., 2014; Mjoun et al., 2010; Ramirez-Ramirez et al., 2016). Further research, however, would need to be done to confirm if the improved protein utilization reported in this present study are explained by the observations of Mjoun et al. (2010). Additionally, all metrics of feed efficiency stated in Table 4 were unaffected, which is consistent with prior research that saw no change in feed efficiency when cows were fed RF-DDGS in place of a soy-based protein (Mjoun et al., 2010). Finally, our results mirror those reported by Mjoun et al. (2010), who saw no effect on milk yield, milk fat percentage, and lactose percentage but saw increased protein percentage, and decreased milk urea nitrogen.

As expected, no difference in body weight change was seen for either treatment group (Table 5) which was reported by Mjoun et al. (2010) as well. In addition, no difference in rumen pH was observed which is consistent with results from trials with similar diets reported by Castillo-Lopez et al. (2014). Furthermore, acetate-to-propionate ratios were decreased and valerate concentration was increased (Table 6), which is consistent with results of Ramirez-Ramirez et al.

(2016) who fed similar diets. The increased propionate concentration could help to explain the lack of treatment difference in both lactose concentration, lactose yield, and milk yield. In contrast with the results related to NEFA concentrations of Mjoun et al (2010), we saw no difference in NEFA concentration, which is likely because the cows used in that study were in early lactation, whereas ours were in mid-lactation. Finally, consistent with Mjoun et al. (2010), blood glucose concentrations were not different between treatments. Combined, our results are a confirmation of prior research that reports the effectiveness of including RF-DDGS in the rations of lactating dairy cows (Castilo-Lopez et al., 2014; Mjoun et al., 2010; Ramirez-Ramirez et al., 2016). Furthermore, these results demonstrate that currently commercially available RF-DDGS can be fed at a 20% (DM-basis) inclusion rate without negatively influencing cow performance. Feeding RF-DDGS is especially promising because in this experiment cows fed RF-DDGS performed equally well with those fed the more expensive protein source (SoyPlus®) which offers an economic advantage for the inclusion of RF-DDGS in the rations of lactating dairy cows.

Swiss cheese and baby Swiss cheese standards for quality evaluation were followed (Cakir and Clark, 2009). Baby Swiss cheese eyes should be glossy, completely round, from 0.3 to 0.8 cm in diameter, and evenly distributed throughout the body of the cheese; the cheese should have a mild nutty (roasted hazelnut) and propionic acid aroma and flavor character with little to no apparent sour/lactic-acid taste. The body of baby Swiss should firm as indicated by being somewhat resistant to initial compression between the thumb, forefinger, and middle finger, but should break apart between fingers without crumbling or seeming too rubbery or dry (corky). Upon mastication, the texture should be smooth rather than grainy or rough. Other than a slight

bitter aftertaste, baby Swiss cheese should "clean up", leaving no fruity, fermented, rancid, yeasty, or other foreign flavors on the palate (Cakir and Clark, 2009).

Regarding flavor and body and texture attributes of baby Swiss cheese, "ideal" or "typical" mean scores for the attributes of importance would be close to zero (0) for acid, flat, unclean, mealy/grainy, pasty, and weak; the ideal mean score for bitter would be up to 5 and for curdy would be close to 7.5 (Table 6). Mean scores for RF-DDGS and control cheeses were close to the ideal, and not significantly different, for all attributes (Table 7).

In the case of the present work, ideal mean scores for the attributes of importance would be close to zero (0) for eye distribution, eye shape, and gas formation (defects) and close to the mid-point on the 15-cm line scale (7.5) for eye amount, gloss, and size. The visual appearance of Swiss-type cheeses is very important, and producing cheeses with a typical number, size, and distribution of eyes is difficult (Guggisberg et al. 2015). The baby Swiss cheeses from the control and RF-DDGS treatments in the present study (Table 7 and Figures 2 to 5) had glossy eyes (7.10 and 7.14, respectively), with slightly uneven distribution (3.65 and 3.33, respectively) and a propensity for irregular (somewhat flattened or oval) shape (8.39 and 8.19, respectively). In general, the cheeses looked more like Lorraine® or Lacey Swiss than baby Swiss because the small to very small eyes (5.92 and 5.19, respectively) were overset (7.74 and 7.83, respectively). The RF-DDGS cheeses did not differ from control for any attribute except size of eyes (Table 7). Although mean score for eye size of control cheeses were closer to ideal than that of DDGS cheeses ($P < 0.05$), the difference was not great enough to be practical or obvious to the average consumer. After completion of our research, O'Sullivan et al. (2016) reported on the impact of

intentionally adding or not adding *L. helveticus* and *L. casei* to a Swiss-type cheese. In the absence of *L. helveticus*, excess lactose, galactose, and citrate, available during the initial ripening stages, provided substrate to the facultative heterofermentative *L. casei* to produce excessive gas during ripening (overset). Although the presence of individual microorganisms was not enumerated in the present study and sanitation practices were attended to in order to minimize nonstarter bacteria introduction, the absence of *L. helveticus* in the present work may partially explain the overset cheeses, providing support for the work of O'Sullivan et al. (2016).

Written-in comments by trained panelists and enumeration of appearance defects on 3-digit coded trays of sliced cheeses (representative images included in Figures 2 – 5) support the results compiled in Table 7. When all slices were considered (in addition to the randomly selected samples presented to panelists), uneven eye distribution (84 and 63% of slices from control and RF-DDGS cheeses, respectively), irregular eye shape (76 and 68%, respectively), and very small eyes (59 and 60%, respectively) predominated slices. Blind areas (20 and 17%, respectively), wet/whey (10 and 14%, respectively), and cabbage defects (12 and 8%, respectively) were occasionally seen. Collapsed eyes (20 and 44%, respectively) and slits (18 and 28%, respectively) appeared with higher frequency in RF-DDGS cheeses, but the more serious defect, blown, was more prevalent in control cheeses (33 and 13%, respectively). None of the cheeses were split. Splits and late-blowing defects have a negative impact for both processors (sliceability is affected negatively) and consumers (who expect round glossy eyes); therefore, bad eyes are an economic issue (White et al. 2003; Manimanna Sankarlal et al. 2015). ~~White et al. (2003) demonstrated that the propensity for split defects in Swiss cheese was higher in summer than winter months and the number of splits in cheese increased with cheese storage~~

~~time. The findings by White et al. (2003) contradicted those of Fröhlich-Wyder et al. (2002), who reported that milk produced during the winter hay feeding season was more prone to late fermentation defects than summer feeding.~~ To limit late fermentation defects in Swiss-type cheese, Fröhlich-Wyder et al. (2002) recommended propionibacteria strains of low aspartase activity, addition of *L. casei* strains, and omission of *L. helveticus*. To minimize splits White et al. (2003) concluded that careful selection of *L. helveticus* and *P. shermanii* cultures is important particularly if the cheese target moisture content is high (e.g., over 37%). ~~Our work was conducted in the winter months,~~ We did not inoculate with *L. helveticus* and cheeses were ripened 78 days (+/- 5) total from brine to knife before evaluation but we did not see any splits. Though we did witness some defects associated with late fermentation, including slits and blow holes, when looked at as a whole, feeding RF-DDGS did not increase the propensity for defects. The findings in the present study are consistent with our previous research (Manimanna Sankarlal et al., 2015) where feeding full-fat DDGS did not affect the quality of baby Swiss cheese produced when compared with feeding a conventional dairy ration without DDGS to lactating Holstein dairy cows.

CONCLUSION

These results indicate that 20% RF-DDGS on a dry-matter basis can be effectively included in the rations of lactating dairy cows without any adverse effects on feed efficiency, milk composition, or measured blood markers of energy balance. These findings indicate promising prospects for the utilization of RF-DDGS, which are often more economical than traditional protein sources. Finally, consistent with previous research (Mjoun et al., 2010), our results indicate that feeding RF-DDGS may lead to improved dietary protein utilization when compared

418 with soybean-based protein. Furthermore, RF-DDGS can be fed without compromising the
419 composition of milk with the exception of slightly increased protein percentage and decreased
420 milk urea nitrogen. Additionally, there were no differences in suitability of milk used for baby
421 Swiss cheese because there was practically no difference in quality of cheese produced from
422 milk from cows conventionally-fed cows and fed RF-DDGS.
423
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TABLES AND FIGURES

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Table 1. Formulations of control and reduced-fat dried distillers grains with solubles (RF-DDGS) rations

	Treatment	
Ingredient, % dry matter	Control	20% RF-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
SoyPlus®[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

547 [1] Dairy Nutrition Plus, Des Moines, IA.

548 [2] Quality Liquid Feeds, Dunlap, IA. Custom vitamin and mineral supplement.

549 [3] Kemin Industries, Des Moines, IA

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Table 2. Analysis of control and reduced-fat dried distillers grains with solubles (RF-DDGS) rations

	Treatment			
Item	Control	20% RF-DDGS	SEM	*P*-Values
Moisture, %	43.10	40.83	0.956	0.125
Dry matter (DM), %	56.90	59.17	0.956	0.125
Crude protein, % DM	18.09	17.86	0.213	0.446
ADF[1], % DM	21.37	20.52	0.678	0.397
aNDF[2], % DM	28.78	29.35	0.757	0.602
aNDF, % OM[3]	27.93	28.20	0.776	0.807
Lignin, % DM	4.01	4.01	0.181	0.985
Lignin, % NDF	14.34	14.22	0.387	0.836
AD-ICP[4], % DM	0.93	1.02	0.070	0.228
ND-ICP[5], % DM	2.90	2.90	0.033	0.434
Fat, % DM	4.80	5.21	0.130	0.052
Ash, % DM	8.60	8.20	0.170	0.117
Calcium, % DM	1.16	0.94	0.052	0.014
Phosphorus, % DM	0.34	0.44	0.013	0.0003
Magnesium, % DM	0.26	0.24	0.016	0.567
Potassium, % DM	1.96	1.81	0.051	0.069
Sulfur, % DM	0.25	0.36	0.010	<0.0001
Sodium, % DM	0.56	0.51	0.016	0.070
Chloride, % DM	0.63	0.63	0.022	0.873
DCAD, mEq/100g	40.81	27.89	1.766	0.0004
TDN[6], 1 × %DM	71.56	72.09	0.505	0.481
NE_L[7], Mcal/kg	1.64	1.65	0.012	0.479

[1]Acid-detergent fiber
[2]Neutral-detergent fiber
[3]Organic matter
[4]Acid-detergent insoluble crude protein
[5]Neutral-detergent insoluble crude protein
[6]Total digestible nutrients
[7]Net energy for lactation

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Table 3. Feed fatty acid composition

	Treatment			
Fatty acid, wt%	Control	RF-DDGS[1]	SEM	*P*-Value
C12:0, C14:0	0.30	0.23	0.085	0.611
C14:1	0.00	0.00	NA	NA
C16:0	20.27	19.20	0.299	0.029
C16:1	0.37	0.28	0.017	0.006
C17:0	0.00	0.00	NA	NA
C18:0	3.22	2.68	0.080	0.0005
C18:1	18.68	20.55	0.209	<0.0001
C18:2	50.02	51.43	0.265	0.004
C18:3	5.71	4.21	0.147	<0.0001
C19:0, C20:0	0.58	0.60	0.012	0.222
C20:1	0.27	0.30	0.008	0.007
C20:2, C20:3	0.00	0.00	NA	NA
C20:4	0.00	0.00	NA	NA
C22:1	0.00	0.00	NA	NA
C22:6	0.00	0.00	NA	NA
C24:0	0.00	0.00	NA	NA
C24:1	0.00	0.00	NA	NA
tUFA[2]	75.04	76.77	0.320	0.003
MUFA	19.31	21.14	0.200	<0.0001
PUFA	55.73	55.63	0.252	0.799

[1]Reduced-fat dried distillers grains with solubles

[2]Total unsaturated fatty acids

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Table 4. Effects of feeding RF-DDGS to lactating Holstein dairy cows on milk components and yield

	Treatment			
Item	Control	RF-DDGS	SEM	*P*-Value
Dry matter intake (DMI), kg/day	20.69	20.89	0.538	0.200
Milk yield, kg/day	35.66	35.39	0.978	0.329
FCM[1]	36.27	35.78	0.889	0.105
ECM[2]	36.43	36.30	0.887	0.663
Milk fat, kg/day	1.27	1.25	0.043	0.416
Milk fat, %	3.65	3.61	0.096	0.517
Milk protein, kg/day	1.05	1.08	0.032	0.204
Milk protein, %	3.01	3.11	0.051	0.002
Lactose, kg/day	1.63	1.62	0.057	0.884
Lactose, %	4.62	4.64	0.050	0.819
Milk total solids, %	12.19	12.28	0.167	0.478
Milk urea nitrogen, mg/dL	14.18	12.99	0.285	<0.0001
Somatic cell count/mL × 1000	232.57	287.22	168.84	0.718
Feed efficiency, kg milk/kg DMI	1.78	1.75	0.062	0.168
FCM efficiency, kg FCM/kg DMI	1.81	1.77	0.056	0.110
ECM efficiency, kg ECM/kg DMI	1.82	1.80	0.056	0.351

[1]Fat-corrected milk = (0.432 × milk)+(16.23 × milk fat)

[2]Energy-corrected milk = (0.327 × milk)+(12.95 × milk fat)+(7.65 × milk protein)

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Table 5. Effects of feeding RF-DDGS to dairy cows on body weight, blood components, rumen fluid pH and ruminal short-chain fatty acid concentrations

	Treatment			
Item	Control	RF-DDGS	SEM	*P*-Value
Body weight change, kg	+12.19	+17.33	2.43	0.139
Rumen fluid pH	6.55	6.50	0.057	0.365
Blood NEFA, μeq/L	164.54	159.53	6.10	0.508
Blood glucose, mg/dL	53.08	54.85	1.24	0.319
Short-chain fatty acids, mM				
Acetate	40.40	41.68	2.40	0.707
Butyrate	7.03	7.80	0.44	0.227
Isobutyrate	0.39	0.42	0.03	0.484
Isovalerate	1.70	1.57	0.11	0.415
Propionate	13.54	16.02	1.11	0.071
Valerate	0.88	1.11	0.06	0.013
Acetate:Propionate	3.16	2.75	0.10	<0.0001

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567 **Table 6.** Descriptive analysis lexicon for baby Swiss cheeses (adapted from Cakir and Clark,
568 2009)

Descriptor	Definition and ideal location on 15-cm unstructured line scale	Reference and location on 15-cm scale*
	Aroma, Taste & Flavor	
Acid	A basic taste, characteristic of lactic acid and citric acid Ideal = 0 – 2.5 cm	0 – 2.5 = Skim milk 2.5 – 5 = 1 tsp cultured buttermilk/L milk 5 – 10 = 2 TBS buttermilk/L milk 10 – 15 = cultured buttermilk
Bitter	A basic taste, generally toward the back of the tongue and/or throat, characteristic of quinine or caffeine Ideal = 0 – 5 cm	0 – 2.5 = Skim milk 2.5 – 5 = slight 7.5 – 10 = 1 tsp 0.5% quinine sulfate/L milk 10 – 15 = 1 TBS 0.5% quinine sulfate/L milk
Flat	Practically devoid of characteristic sweet roasted hazelnut flavor of Swiss cheese Ideal = 0 – 2.5 cm	0 – 2.5 = Kraft Big Slice Swiss 2.5 – 5 = slight 5 – 7.5 = definite 10 – 15 = Monterey Jack cheese
Unclean	Persistent, complex and unpleasant flavor; could smell somewhat fecal Ideal = 0 cm	0 – 3.5 = Skim milk 3.5 – 8 = slight 8 – 12 = definite 12 – 15 = Grafton Extra mature Cheddar
	Body & Texture	
Curdy	Resistant to compression between thumb and fore-fingers, slightly rubbery Ideal = 6 – 8.5 cm	0 – 2.5 = Hy-Vee sharp Cheddar 2.5 – 5 = slight 5 – 7.5 = Shullsburg Creamery baby Swiss 7.5 – 10 = definite 10 – 15 = Hy-Vee mild Cheddar
Mealy/grainy	Coarse, crumbly mass between fingers; small sandy to rough granular particulates in mouth with mastication Ideal = 0 – 5 cm	0 – 2.5 = Cream cheese 2.5 – 7.5 = slight 7.5 – 10 = Cacique Cotija cheese 10 – 15 = pronounced
Pasty	Sticky between fingers and in mouth Ideal = 0 – 2.5 cm	0 – 2.5 = not 2.5 – 7.5 = slight 7.5 – 10 = definite 10 – 15 = cream cheese
Weak	Immediately yields between fingers when worked between thumb and fore-fingers Ideal = 0 – 2.5 cm	0 – 2.5 = Shullsburg Creamery baby Swiss 2.5 – 5 = Hy-Vee Monterey Jack 5 – 10 = definite 10 – 15 = Cream cheese
	Appearance	
Eye amount	Blind = no eyes (if seen in one area of the cheese, the cheese is also faulted for uneven distribution)	0 – 2.5 = blind 2.5 – 5 = underset

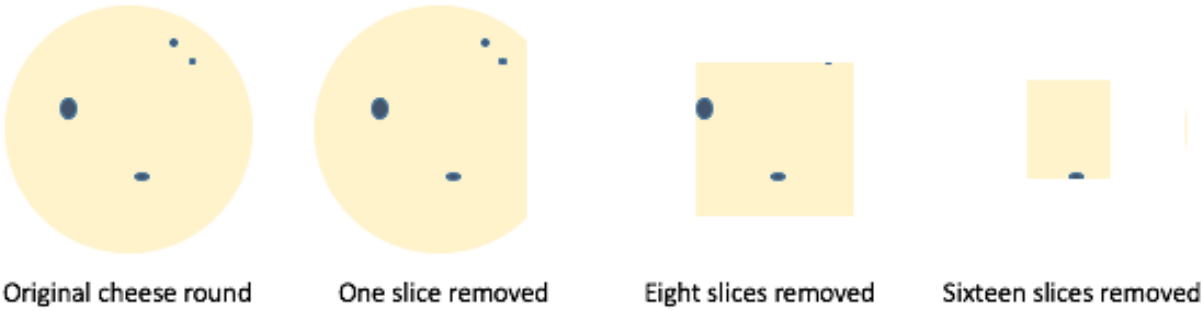
	Underset = too few eyes (more than 2 cm between eyes) Ideal = 1 to 2 cm distance between eyes Overset = less than 1 cm between eyes, overcrowding Ideal = 5 – 7.5 cm	5 – 7.5 = ideal 7.5 – 10 = slight overset 10 – 12.5 = moderate overset 12.5 – 15 = very overset
Eye distribution	Ideal = even or equal sized spacing between eyes Uneven = tendency for eyes to predominate one area of a cheese, or not be evenly distributed throughout the cheese Ideal = 0 – 2.5 cm	0 – 2.5 = even 2.5 – 6.5 = slight uneven 6.5 – 12 = moderately uneven 12 – 15 = very uneven
Eye gloss	Wet = free whey Ideal = Shiny but not wet Slightly dull = dry, with lack of luster Dull/rough = in extreme cases the inside of an eye may look like a nutshell Ideal = 5 – 7.5 cm	0 – 2.5 = wet 2.5 – 5 = slight wet 5 – 7.5 = Ideal 7.5 – 12 = slight dull 12 – 15 = dull/rough
Eye shape	Ideal = completely round, no defect Irregular = not round (oval, lenticular) Collapsed = eyes appear to have flattened after development Cabbage = multiple adjacent eyes adjoin one-another; a paper-thin layer of cheese may appear between eyes Ideal = 0 – 2.5 cm	0 – 2.5 = no defect 2.5 – 6.5 = few irregular 6.5 – 10 = multiple irregular 10 – 12.5 = collapsed 12.5 – 15 = cabbage
Eye size	Sweet holes = < 0.1 cm diameter Very small = 0.1 to 0.2 cm Small = 0.2 to 0.34 cm Ideal = 0.4 to 0.8 cm diameter; standard punch hole Medium = 0.8 to 1.8 cm diameter Large = >1.8 cm; penny Ideal = 7.5 – 10 cm	0 – 2.5 = sweet holes 2.5 – 5 = very small 5 – 7.5 = small 7.5 – 10 = ideal 10 – 12.5 = medium 12.5 – 15 = large
Gas formation (defect)	Ideal = no defect Checks/picks = tiny (picks, 1-2 mm) to small (checks <5 mm), irregular cracks or ragged openings Slits/splits = slits are cracks (0.5-1.5 cm); splits exceed 1.5 cm and can traverse entire piece of cheese Blow hole = irregular excess gas formation collapses cheese structure, leaving large, irregular gaping hole in cheese Ideal = 0 – 2.5 cm	0 – 2.5 = no defect 2.5 – 5 = picks 5 – 7.5 = checks 7.5 – 10 = slits 10 – 12.5 = splits 12.5 – 15 = blow hole

*Photographic images used for appearance references are not included in the present work.

Table 7. Mean scores* for cheese flavor, body and texture, and appearance of eyes

	Treatment			
Flavor	Control	RF-DDGS	SEM	*P* - Value
Acid	0.99	1.07	0.14	0.683
Bitter	3.74	3.48	0.34	0.558
Flat	1.33	1.60	0.23	0.398
Unclean	1.40	1.86	0.29	0.220
Body and Texture				
Curdy	7.88	7.46	0.34	0.361
Mealy/Grainy	5.60	6.44	0.42	0.132
Pasty	0.66	0.91	0.18	0.298
Weak	0.89	1.13	0.16	0.254
Appearance of Eyes				
Amount	7.74	7.83	0.31	0.834
Distribution	3.65	3.33	0.40	0.556
Gloss	7.10	7.14	0.22	0.877
Shape	8.39	8.19	0.28	0.588
Size	5.92	5.19	0.24	0.021
Gas formation (defects)	3.82	4.69	0.59	0.266

571 * Mean values of 14 cheeses, evaluated by a trained descriptive panel of six judges, in duplicate,
572 by using a 15-cm line scale.
573



574

575 **Figure 1.**

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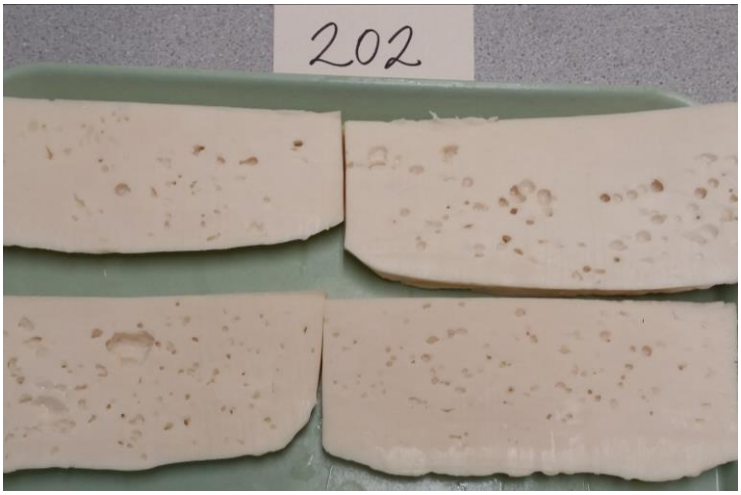

579

580 **Figure 2.**

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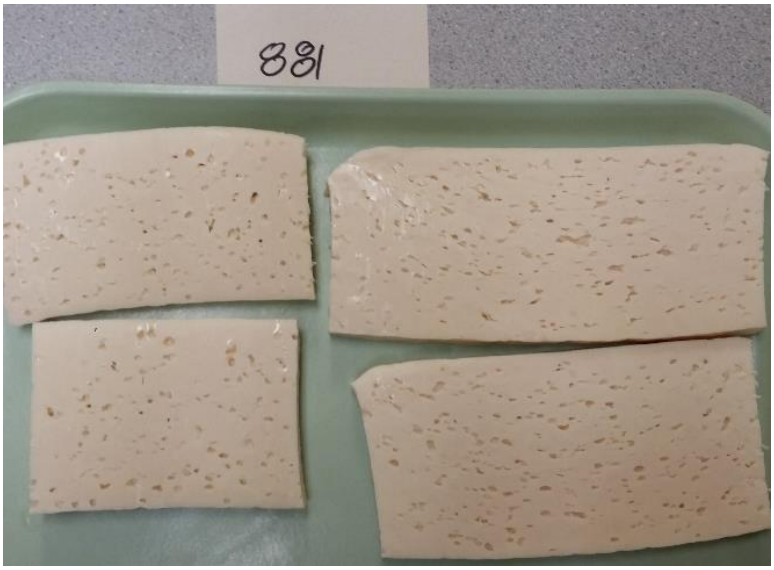
583 

584 **Figure 3.**

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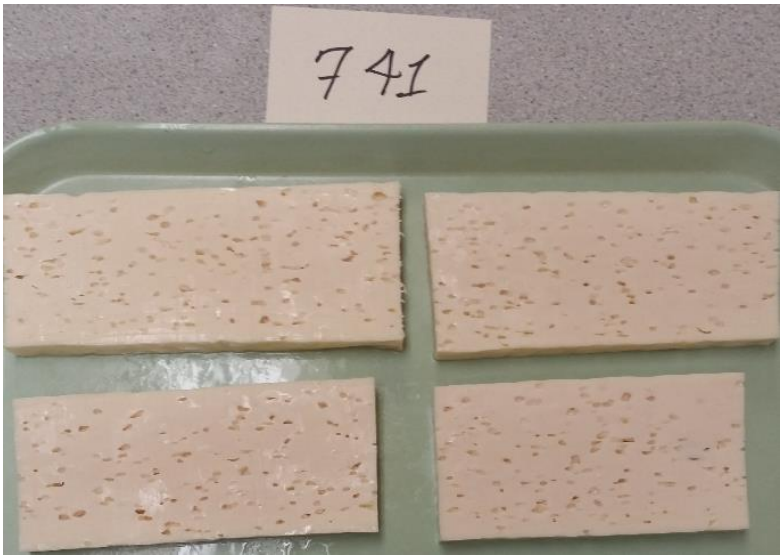
588 

589 **Figure 4.**

590

591

592 **Figure 5.**