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Impact of nutrition of food animals on quality of animal products

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Impact of nutrition of food animals on quality of animal products

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The student author and the program of study committee are solely responsible for the content of this dissertation. The Graduate College will ensure that this dissertation is globally accessible and will not permit alterations after a degree is conferred.

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ABSTRACT

It is an often overlooked, but vital, research question to understand how what is fed to food-producing animals ultimately affects quality and consumer acceptability of food products produced by those animals. Of interest in recent years, particularly in the Midwest, has been the effect of inclusion of dried distillers grains with solubles (DDGS) in the rations of lactating dairy cows on milk and cheese quality. Because of the high unsaturated oil content of DDGS, feeding DDGS to dairy cows could potentially lead to decreased oxidative stability of milk and cheese and also contribute to the late blowing of eyes in Swiss type cheeses. Therefore, it was of interest to investigate if feeding full-fat DDGS (~13 % fat) would result in increased development of off-flavors in milk and could result in milk that was unsuitable for use in baby Swiss cheese production. Additionally, the recent development of reduced-fat DDGS (RF-DDGS) (~6% fat) offers the possibility of feeding increased amounts of DDGS, compared to feeding full-fat DDGS, without adversely affecting milk and milk fat production. Because of the possibility of higher rates of inclusion of RF- DDGS in the rations of dairy cows than was possible with full-fat DDGS, it was necessary to investigate the effects of feeding RF-DDGS on milk production efficiency and the quality of milk for the production of baby Swiss cheese. Finally, feedstuffs containing high concentrations of unsaturated fatty acids, in particular DDGS, can adversely affect the quality of pork fat, potentially resulting in pork that is unacceptable to the producer and consumer. Specific project summaries are described in the subsequent pages.

Quality of milk from lactating dairy cattle fed dried distillers grains with solubles

The objective of this study was to examine the effect of feeding DDGS to healthy mid-lactation Holstein dairy cows (n=24) on production parameters and flavor and oxidative stability of milk. Cows were assigned to two groups and fed one of three treatment diets (0% DDGS, 10% DDGS, 25% DDGS by dry matter (DM)) as an isocaloric total mixed ration. Each group was fed all three diets after a wash-out period of 7 days. Milk yield was unaffected by both the 0% and 10% DDGS diets but decreased significantly when fed the 25% DDGS diet. The quality of milk from cows fed DDGS was characterized through chemical analyses profiling fatty acids and analyzing milk composition. Rumen volatile fatty acids were unaffected by treatment. Milk protein and solids-not-fat (SNF) increased with increasing inclusion of DDGS, but milk fat decreased concomitantly. Milk fatty acid composition, on a weight percent basis, was significantly affected; cows fed higher concentrations of DDGS produced milk with higher concentrations of unsaturated fatty acids. An assessment of milk quality by a trained sensory panel showed no effect of dietary treatment on milk oxidative stability or milk flavor. The results of this study indicate that feeding of DDGS to lactating dairy cows, under controlled conditions, does not negatively affect milk oxidative stability or flavor; feeding 25% DDGS, however, did negatively impact milk production and changed the milk fatty acid profile.

Lactational performance of lactating Holstein dairy cows fed full-fat and reduced-fat dried distillers grains with solubles

Our objective was to evaluate production performance of lactating Holstein dairy cows fed two different dietary concentrations of full-fat DDGS (13.6% fat). Thirty cows were fed 0, 10, and 20% DDGS (DM basis) as a total mixed ration (TMR) in a 3 × 3 crossover. Cows were stratified into groups of 10 by parity and days in milk and fed each of three diets in three 28-day periods. Based on previous research, we hypothesized that feeding 20% DDGS (DM basis) would negatively influence production and feed efficiency of dairy cattle. Milk yield was decreased significantly when fed 20% DDGS, and feeding DDGS caused milk fat depression and decreased daily fat yield, resulting in significant decreases in 3.5% fat-corrected milk (FCM) yield and energy-corrected milk (ECM) yield. Both protein and lactose percentages increased significantly when cows were fed 20% DDGS; neither protein nor lactose yield, however, was significantly affected. Protein efficiency, a measure of the utilization of dietary protein for milk protein synthesis, decreased significantly for cows fed 20% DDGS, likely resulting from heat-damaged protein, as indicated by proximate analyses. All three measures of energetic efficiency (ECM/DMI, kg ECM/net energy for lactation (NE_L) intake (Mcal) and gross energy of milk produced (Mcal)/NE_L caloric intake (Mcal)) were significantly decreased when cows were fed 20% DDGS but not when cows were fed 10% DDGS. These results indicate that, with the exception of an approximate loss of milk fat of 0.5% percentage points, full-fat DDGS used in this study can be effectively fed at 10% without

a loss in production performance when compared with a traditional TMR. Feeding the full-fat DDGS at 20%, however, is not advisable.

In contrast with results from feeding FF-DDGS, negative effects of feeding RF-DDGS to lactating dairy cows did not occur when 36 multiparous and mid lactation Holstein dairy cows were fed either 0 or 20% reduced-fat DDGS (RF-DDGS) in a 2 × 2 crossover design. Cows were assigned randomly to treatment groups and were fed individually to allow for collection of feed intake data. Feeding RF-DDGS as 20% DM of a TMR supplemented with rumen-protected lysine did not negatively influence production parameters related to milk composition or nutritional physiology of the cow. Milk urea nitrogen (MUN) was, however, decreased, and milk protein percentage was increased. Total milk solids concentration were not influenced by inclusion of RF-DDGS. Additionally, RF-DDGS did cause a decrease in FCM efficiency as a result of an increase in DMI. When ECM efficiency was calculated (accounting for fat, protein, and lactose concentration in milk), no difference in feed efficiency resulted.

These data indicate that RF-DDGS can be included effectively in rations of multiparous lactating dairy cows, at least when supplemented with lysine. Additionally, decreased MUN and increased milk protein percentage indicate that dietary protein utilization may be improved by including RF-DDGS as a protein source in the ration, presumably because DDGS are generally considered to be a good source of rumen undegradable protein. Taken together, these results indicate that RF-DDGS may be an attractive feed ingredient for 20% inclusion in lactating ruminant diet.

Reduced-fat dried distillers grains with solubles did not reduce quality of baby Swiss cheese

Thirty-six multiparous and mid lactation Holstein dairy cows were fed either 0 or 20% reduced-fat DDGS (RF-DDGS) in a 2 × 2 crossover design. Cheeses were produced a total of six times from each treatment. In this experiment, feeding RF-DDGS as 20% DM of a total mixed ration (TMR) supplemented with lysine did not negatively influence flavor attributes of baby Swiss cheese. Eye appearance in all cheeses was atypical but was not related to diet. Any defects in appearance of Baby Swiss cheese appeared in both control and RF-DDGS fed cows. The results indicate that lactating Holstein dairy cows can be fed RF-DDGS as 20% DM of a TMR without negatively affecting usability of milk, when compared with control, for production of Baby Swiss cheese.

Relationship of fat quality and meat quality traits of fresh pork

Feeding distillers grains to pigs can lead to undesirable traits in meat quality that adversely affect both consumer acceptability and the ability of the processor to produce high quality pork products. Additionally, interest has been expressed by both processors and the research community about how fat quality varies among anatomical locations.

Barrows and gilts (n=347) of five purebred lines and one commercial crossbred line were fed commercial swine diets with FF-DDGS inclusion at 30% of DM. For the final 30 days of feeding, DDGS were removed from the diet. Pigs were harvested at a minimal weight of 111.4 kg. At harvest, fat was collected from the back, belly, and jowl, and meat samples were taken from the longissimus muscle for evaluation of fat and meat quality characteristics. Jowl fat iodine values were significant predictors of back and belly fat iodine values, and increases in iodine value of the fat of the pork chop were

moderately and negatively correlated with several measures of meat quality. This study demonstrates that iodine value of fat from one anatomical location (i.e., back and belly) is related to iodine value of a less valuable anatomical location (i.e., jowl); additionally, increases in iodine value correlate negatively with predictors of meat quality.

CHAPTER 1

INTRODUCTION

Overall summary and justification of research

Feeds containing high concentrations of unsaturated fatty acids (i.e., corn oil in distillers grains) negatively influences production parameters of lactating dairy cattle through inhibition of ruminal fiber digestion (Brown et al., 1962; Beitz and Davis, 1964; Ensor et al., 1959; Garner and Sanders, 1938; Maynard et al., 1936; Moore et al., 1945; Ramirez Ramirez et al., 2015; Zinn et al., 1989) and inhibition of *de novo* lipogenesis in the mammary gland, particularly through the action of *trans-10 cis-12* conjugated linoleic acid (Baumgard et al., 2001). Additionally, diets with a concentration of fat over 5% can negatively influence milk production, regardless of degree of unsaturation (Zinn et al., 1989). We, therefore, hypothesized that feeding two different concentrations (10 and 20% inclusion DM basis) of full fat dried distillers grains with solubles (FF-DDGS) (~13% fat) would negatively influence production parameters and feed efficiency of lactating Holstein dairy cattle, but that feeding 20% DM reduced-fat DDGS (RF-DDGS) (~6% fat) would not.

Furthermore, incomplete biohydrogenation of unsaturated fatty acids in the rumen results in increased dietary unsaturated fatty acids being incorporated into milk (Kelly et al., 1998). Unsaturated fatty acids are particularly susceptible to oxidation, especially in the presence of riboflavin and light-induced lipid oxidation that can lead to the development of off-flavors (Minn & Boff, 2002). Light and riboflavin are both factors for milk in lighted dairy cases at grocery stores. Therefore, when concerns in the Midwest were raised over milk "going bad spontaneously", we hypothesized that the

increased unsaturated fatty acids in milk produced from dairy cattle fed high oil feedstuffs, such as FF-DDGS (~10-13% fat), could decrease oxidative stability and thus decrease shelf life and consumer acceptability of this milk.

In addition, distillers grains have been implicated in contaminating milk with *Clostridium tyrobutyricum* (Houck et al., 2007), which are spore-forming thermoduric bacteria that have been associated with "late blowing" in Swiss type cheeses resulting in the formation of imperfect eyes, decreases in consumer acceptability, and decreased value of product for the producer. *C. tyrobutyricum* was found not to be a cause of late blowing in baby Swiss cheese (Sankarlal et al., 2015).

Moreover, anecdotal evidence was being circulated amongst the dairy community that feeding distillers grains to cows was producing milk that was of unacceptable quality for production of Swiss cheese (personal conversation with Swiss Valley personnel). With distillers grains being a major co-product and potentially valuable feedstuff in the Midwest and because evidence of the relationship between feeding DDGS and usability of milk has been anecdotal to date, scientific investigation into the relationship of feeding distillers grains and milk on usability for Swiss cheese production is warranted. Because *C. tyrobutyricum* was not found in the distillers grains we fed and because other causative relationships between the feeding of DDGS and poor quality Swiss cheese seemed unlikely (e.g., increased lactose production), we hypothesized that feeding FF-DDGS (Sankarlal et al., 2015) and RF-DDGS (~4-6% fat) would not negatively influence quality of baby Swiss cheese and, eye formation particularly.

Finally, feeding diets rich in unsaturated fatty acids to pigs, such as those that incorporate DDGS, can increase incorporation of unsaturated fatty acids into the adipose

tissue of pigs and thereby result in "soft bellies" (Cromwell et al., 2014), potentially decreasing pork quality. If pork lipids become too highly unsaturated, like in milk, they can become susceptible to oxidation, particularly in the meat case, that results in decreased shelf life and consumer acceptability. Unlike the case with milk fat however, high concentrations of unsaturated fatty acids in pork also can affect the ability of the processor to successfully manipulate the products being produced (e.g., bacon is less sliceable) (Cromwell et al., 2014). Nevertheless, we hypothesized that: 1) fat quality, as represented by iodine values, varies amongst anatomical location, 2) FF-DDGS could effectively be fed to pigs at an inclusion rate of 30% as fed without producing pork fat with unacceptably high iodine values when a 30-d withdrawal period is employed pre-harvest, and 3) "cheaper" cuts (i.e., jowl) could be used as a marker of fat quality of more expensive cuts (i.e., belly and loin) because a relationship between iodine values of different anatomical locations exists.

Research objectives

The overall objective of the research contained herein was to 1) investigate the impact of feeding both FF-DDGS and RF-DDGS on the feed efficiency of dairy cows, 2) investigate the impact of feeding FF-DDGS to lactating dairy cows on the quality of milk as it relates to oxidative stability of milk, 3) investigate the impact of feeding RF-DDGS to lactating dairy cows on the quality of milk as it relates to production of baby Swiss cheese, and 4) investigate the impact of feeding DDGS with a 30-d withdrawal period to pigs on the quality of pork fat, its relationship to meat quality, and how iodine value varies amongst anatomical locations of adipose tissue of pigs.

Dissertation organization

Chapter two is a review of literature on the topics covered in chapters three through eight. Chapters three (submitted to *Professional Animal Scientist*) and four (in preparation for submission to *Journal of Dairy Science*) address the effect of feeding both FF- and RF-DDGS on feed efficiency and production parameters of lactation Holstein Dairy cows. Chapters five (published in *Journal of Dairy Science*) and six (submitted to *Journal of Dairy Science*) report results on the impacts of feeding FF-DDGS to lactating Holstein dairy cows on oxidative stability of milk and RF-DDGS on the usability of milk for production of baby Swiss cheese. Chapter seven (submitted to *Adipocyte*) reports the effects of feeding FF-DDGS to pigs on fat quality and how fat quality varies amongst adipose depots. Finally, chapter eight is a summary of results and conclusions from chapters three through seven and suggestions for future research emphases.

Explanation of appendix

The appendix on the topics of modeling adipocyte physiology (Published in *Adipocyte*) was completed as a valuable portion of my training for specialization in Biochemical and Molecular Nutrition and to provide experience and expertise for my post-graduate work.

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CHAPTER 2

REVIEW OF LITERATURE

Distillers grains and livestock production

Ethanol production has undergone tremendous expansion with the shift from fossil fuels and towards the utilization of bio-renewable fuel sources. In the United States, and particularly in the Midwest, ethanol is produced primarily from corn (Fig. 1), with roughly 30% of total corn produced being used to make ethanol (~ five billion bushels). This shift to bio-renewable fuel sources has resulted in a production increase from 314 million liters in 1981 to more than 56 billion liters in 2015 (U.S. Energy Information Administration, 2017), which, in turn, produces approximately 1.7 billion bushels of distillers grains (DG) (Liu, 2011). Because the fermentation of the carbohydrate component of the corn kernel removes about two-thirds of the total mass of the kernel, the protein and non-fermentable portion of the corn remains and is concentrated roughly three times (Liu, 2011). The fermentation process and concomitant concentration of unfermentable substrate, particularly protein, makes DG an often economical protein source for livestock. Utilization of DG in the rations of livestock has not been without risk because nutrient composition and quality have tended to vary widely depending on source (Belyea et al., 2004; Belyea et al., 2006; Rosentrater et al., 2007; Liu et al., 2008). The increased perceived value of DG as an animal feed will, however, likely continue to drive ethanol production plants to improve consistency of nutrient composition.

Depending on the type of livestock, incorporation of DG into rations can be challenging for a variety of reasons. For instance, the relatively high fat content of

traditional distillers grains (11-13%, DM basis) limits inclusion rates for ruminants because fiber fermentation is inhibited when rations contain greater than 5% fat (Zinn, 1989). Additionally, the fat (corn oil) is a highly unsaturated fat (92% unsaturated; Ramos et al., 2009), which presents problems as well. In pigs, excessive feeding of lipids rich in unsaturated fatty acids can lead to the development of soft bellies, decreased ability of the processor to produce high quality pork, and decreased oxidative stability, which can lead to decreased consumer acceptability (Stein and Shurson, 2009). In ruminants, unsaturated fatty acids are toxic to rumen microbes (Maia et al., 2007), likely because of a combination of disruption of metabolism of butyrate-producing bacteria (e.g., *Butyrivibrio fibrisolvens*; Maia et al., 2010) and disruption of cellular integrity (Cartron et al., 2014). Disrupting butyrate production would result in a decrease in substrate for *de novo* lipogenesis. In ruminants, unsaturated fatty acids can also lead to production of the bioactive *trans*-10 *cis*-12 conjugated linoleic acid, which inhibits *de novo* lipogenesis in the mammary gland (Baumgard et al., 2001), in part through inhibition of stearoyl-COA desaturase (SCD)-1 production by decrease in mRNA concentrations of liver X receptor (LXR)α and sterol regulatory element binding protein (SREBP)-1c, which are both transcription factors that regulate production of SCD-1 (Bauman et al., 2008; Obsen et al., 2012). Another concern, primarily in beef feeding, is the relatively high concentration of sulfates in DG. The rumen is a highly reducing environment and will readily reduce sulfates to hydrogen sulfide, which, when eructated, can lead to polioencephalomacia (PEM) (Schingoethe et al., 2009).

Improvements in production practices that lead to increased nutritional consistency will, however, help facilitate successful incorporation of DG into livestock

rations. Additionally, the relatively new emergence of reduced-fat DG (RFDG) in the market offers a solution that could lead to increased incorporation rates into the rations of livestock because of the decreased concentration of unsaturated fatty acids in RFDG.

There are a number of different forms of distillers products, including both wet and dry DG and full- and reduced-fat (DG). The typical dry-grind production process, including the co-products generated, is outlined in Figure 2. For brevity, and because they are the most commonly utilized forms, only DDGS and RF-DDGS will be reviewed further. Compiled compositions of RF-DDGS are shown in Table 1, along with medium- and high-fat DDGS (traditional). Medium-fat DDGS (MF-DDGS) is typical from an ethanol production plant that is not as effectively removing oil from DDGS compared with those producing RF-DDGS. With the high value of corn oil, FF-DDGS are scarcely available currently, and will likely become completely unavailable soon.

Feeding DDGS to ruminants is not a new topic. In fact, there are reports of feeding the by-products of ethanol fermentation, which were referred to at the time as "distillers slop", to ruminants dating back well over 100 years (Henry, 1900; Morrison, 1939; Garrigus and Good, 1942). Because of the recent rapid expansion of ethanol production and increased availability of DDGS, feeding DDGS has been extensively researched recently. Because DDGS are often an economical protein source (~30% protein on a DM basis), they are an attractive feedstuff for ruminant animals, but inclusion of DDGS in the ration of ruminant animals must be done with certain considerations. The fermentation process removes a major portion of the corn kernel, and the remainder of the components (e.g., protein, fat, and minerals) are concentrated approximately three-fold (Liu, 2011). This concentration of nutrients can be good, in the

case of protein, or problematic in the case of certain minerals (e.g., sulfates) or fat. Ruminants, in general, require diets that contain no more than 5% fat on a DM basis to not hinder optimal ruminal fiber fermentation (Zinn, 1989). Additionally, some sources of DDGS can contain nearly 1% sulfur on a DM basis (Schingoethe et al., 2009), which can be problematic for ruminants when high inclusion rates of DDGS are utilized because the rumen will readily convert dietary sulfates to hydrogen sulfide. The conversion of sulfate to hydrogen sulfide volatilizes as a toxic gas, which then can be eructated and inhaled. Hydrogen sulfide inhalation can lead to neurological disorders, particularly in beef cattle that are routinely fed up to 40% DDGS on a DM basis, such as polioenchephalomacia (PEM) (Schingoethe, 2009). Additionally, the primary protein in corn is zein, which is limiting in both lysine and methionine; so, utilization of DDGS must also account for providing adequate limiting amino acids. However, zein is a rumen undegradable protein (~40% digested in the rumen; McDonald, 1945), and the results of many experiments evaluating the comparative value of feeding DDGS or soybean meal showed that DDG protein is 2.4 times the economic value of soybean meal (Aines et al., 1987). Dried distillers grains with solubles are also a "good" source of NDF as determined by proximate analysis, and the NDF is highly digestible (62 – 71% digestible; Birkelo et al., 2004; Vander Pol et al., 2009). However, because of the small particle size, DDGS are not a particularly good source of physically effective NDF (Kleinschmit et al., 2007; 3.4 to 19.8% of NDF is physically effective). When managed properly, rations including DDGS, however, can be utilized effectively to decrease DMI, increase milk fat and protein production, and improve average daily gain (ADG), which will be reviewed further below.

Effects of feeding distillers grains to ruminant animals: production parameters and feed efficiency

Utilization of DDGS in the beef cattle industry

Although none of the chapters in this dissertation involve feeding DDGS to beef cattle, extensive research has investigated the effect of feeding DDGS to beef cattle; so, review is warranted. Klopfenstein and colleagues (2008) did an extensive review of literature on this topic. The research was summarized as a meta-analysis and concluded that DDGS could be effectively fed to beef cows at inclusion rates of up to 40% of dietary dry matter. When DDGS were included in the diet, DMI increased for every inclusion rate (i.e., 10, 20, 30, and 40%) as was ADG; however, the response was quadratic. So when gain:feed was analyzed, there were no differences between control and 40% DDGS, making the feeding value of DDGS equivalent between control and 40% DDGS. However, it would likely be economically favorable to feed the 40% DDGS ration over the control ration. They concluded that the optimal inclusion rate that resulted in the greatest feeding value was 20%. Yield grade also increased as inclusion rates increased; marbling score, however, tended to decrease, with the lowest score occurring when cows were fed 40% DDGS.

It is important to note that since the review by Klopfenstein and colleagues, the composition of distillers grains has changed dramatically, with FF-DDGS being the primary type of DDGS available at that time to the DDGS that are currently available, which are more like the MF-DDGS or RF-DDGS. Therefore, further review and research is warranted.

A meta-analysis using data from 20 forage-based growing systems (including both pasture and confinement systems) that utilized supplemental DDGS indicated that, in all cases, when DDGS was supplemented, ADG and final body weight (BW) increased when compared with cows fed a control diet (Griffin et al., 2012a). Additionally, when mature Angus-cross beef cows were fed a diet containing DDGS during late-gestation, they gained more weight than did cows fed control, but this weight gain was not accompanied by a difference in body condition score (BCS). Also, plasma glucose concentration between diets was not different, but cows fed DDGS diet did have greater plasma blood urea nitrogen (BUN), indicating that protein supply may have been excessive, but no changes in conception rate were observed, accompanied by no difference in occurrence of post-partal disease or milk yield and composition. However, plasma metabolites and hormones did change during gestation, indicating that cows fed DDGS during gestation were more efficient at partitioning energy to support fetal growth, as evidenced by the heavier birth weight of calves born to those cows (Radnuz et al., 2010). When investigating winter feeding strategies, lactating beef cows fed DDGS lost less weight, but there was no difference in milk production or in calf ADG; however, the control diet was estimated to be nearly a dollar more expensive per cow per day (Braungardt et al., 2010). Griffin and colleagues (2012b) performed two replicated summer experiments in which steers were fed the same inclusion rates DDGS; however, results were mixed. In the first experiment, ending body weight was not different, but in the second experiment, ADG and ending BW increased linearly but quality grade and yield grade were not affected. Feeding DDGS to beef heifers also increased organic matter, crude protein, NDF, and ether extract digestibility and, as a result, increased

digestible energy. The increase of digestible energy, however, was accompanied by increased nitrogen and phosphorus excretion, which could indicate excess supply of protein and have implications for waste management strategies (Walter, 2011).

Finally, in 2010, when 240 Angus crossbred steers were fed either 20 or 40% DDGS (DM basis), carcasses from steers fed DDGS had greater fat thickness and yield grade, and less percentage of carcasses graded one or two when compared with control. Additionally, ground beef from steers fed DDGS had greater α-tocopherol concentrations but also had greater concentrations of polyunsaturated fatty acids (PUFA), which resulted in greater concentrations of thiobarbituric acid reactive substances (TBARS) on day 2 in the retail display case. This increased concentration of TBARS is likely a result of decreased oxidative stability from increased PUFA concentrations (Koger et al., 2010). Aldai and colleagues (2010) also saw increased PUFA concentrations in back fat when steers were fed either 20 or 40% DDGS. Results regarding beef quality, however, are mixed, with improved palatability and tenderness of beef from cows fed either 20 or 40% DDGS but decreased beef flavor when compared with control (Aldai, 2010).

Utilization of DDGS in the dairy cattle industry

Utilization of DDGS in the dairy industry is also not a new topic, as DG have been fed for over 100 years (reviewed in Loosli et al., 1952) and have been reviewed extensively in Schingoethe et al. (2009). Schingoethe and colleagues (2009) concluded that DDGS are a good source of protein (~30% protein), high in RUP, and is a good source of net-energy for lactation (NE_L). The reviewed body of research, however, was focused on DDGS that contained approximately 10% fat, which they concluded could be utilized at a 20% inclusion rate as long as sufficient forage was provided (50% of DM).

Increasing DDGS inclusion rates beyond 20% could be done effectively; however, this resulted in overfeeding of crude protein (CP) and phosphorus such that the increased performance was offset by the loss of the effective utilization of protein and mineral. Finally, they concluded that DDGS may contain high concentrations of NDF, but particle size limits the effectiveness of the NDF (Schingoethe et al., 2009). However, as with the beef industry and as previously mentioned, the body of research reviewed was DDGS that contained approximately 10% fat, and as discussed, with the value of corn oil, those types of DDGS are not widely available and could likely be completely unavailable in the future. The change in composition of available DDGS resulting from improved oil extraction (Majoni et al., 2011) shifting from FF-DDGS to RF-DDGS has re-invigorated DG research with lactating dairy cows. The following review will, therefore, be focused on the feeding of the presently available RF-DDGS when compared with FF-DDGS and control diets.

When 36 early lactation Holstein cows were fed one of three diets formulated to be similar in fat, NDF, and to be isonitrogenous and isoenergetic (control, 22% DDGS; 10.8% ether extract), and 20% RF-DDGS (3.5% ether extract), DMI and milk fat and lactose percentages did not differ; milk protein percentage and yield, however, was increased when cows were fed either form of DDGS (Mjoun et al., 2010a). Additionally, plasma non-esterified fatty acids (NEFA) and glucose were unaffected by treatment for cows fed either form of DG. These results indicate that either RF-DDGS or DDGS could be included in properly balanced rations with no negative effect on measured performance parameters and with an increased milk protein yield (Mjoun et al., 2010a). Mid-lactation multiparous and primiparous Holstein cows fed either 0, 10, 20, or 30%

RF-DDGS (DM basis) in place of soybean meal produced milk that contained linearly increasing percentages of milk fat (3.18 to 3.72%) but had no influence on DMI or milk yield. Additionally, milk urea nitrogen decreased linearly as RF-DDGS inclusion increased (15.8 to 13.1 mg/dL). Interestingly, milk protein percentage responded quadratically, with control and 30% RF-DDGS not differing and peak protein percentage occurring when inclusion rates were 20%. In addition, feed efficiency (energy-corrected milk/DMI) tended to increase as inclusion rates increased as did plasma glucose concentration (Mjoun et al., 2010b). Taken together, these two studies indicate that inclusion of RF-DDGS in place of soybean meal can be included up to 30% (although 20% seems to be optimal) on a DM basis without adversely affecting production parameters and can increase milk protein percentage. This increase in milk protein percentage is likely the result of RF-DDGS containing approximately 60.4% rumen undegradable protein (RUP) that is approximately 92.4% degradable in the intestine (Mjoun et al., 2010c). Consistent with the studies by Mjoun et al. (2010a,b,c), feeding RF-DDGS did not influence DMI in the study by Foth and colleagues (2015). Inconsistent with that of Mjoun and colleagues, milk protein percentage was significantly decreased and milk fat percentage did not vary between treatments (Foth et al., 2015). When 20 Holstein dairy cows were fed one of four diets (control, 30% FF-DDGS, 30% RF-DDDS, or 30% RF-DDGS supplemented with 1.9% rumen inert fat), DMI was not affected for any of the DDGS-containing diets but was decreased with cows fed the control diet. There was also a tendency for cows fed any of the DDGS-containing diets to have increased milk production a greater protein percentage. Interestingly, only cows fed FF-DDGS had a decrease in milk fat percentage, indicating that RF-DDGS was

protective against milk fat depression associated with feeding of FF-DDGS, which is supported by the observation that *trans*-10, *cis*-12 CLA was only detected in milk from cows fed FF-DDGS (Ramirez-Ramirez et al., 2016). As mentioned previously, *trans*-10, *cis*-12 CLA can induce milk fat depression by inhibiting *de novo* lipogenesis (Baumgard et al., 2001). Finally, when cows were fed 0, 10, 20, or 30% of dietary dry matter as RF-DDGS, milk yield was unaffected by treatment in each of two replicated experiments (both treatments utilized the same treatment diets; Castillo-Lopez et al., 2014). Protein percentage tended to increase in the first experiment but was unaffected in the second experiment. Additionally, milk fat percentage was unaffected in either treatment, as was total rumen VFA concentration. On the other hand, digestibility of DM, organic matter (OM), neutral detergent fiber (NDF), and non-fiber carbohydrate (NFC) tended to increase linearly with inclusion of RF-DDGS.

Recently, limited investigations into the use of RF-DDGS in the rations of replacement heifers has been done (Anderson et al., 2015; Schroer et al., 2014). No treatment differences were observed, indicating that RF-DDGS can be utilized as 20% of the dry matter in the rations of growing heifers when compared with tradition FF-DDGS. Clearly, inclusion of RF-DDGS in the rations of lactating dairy cows can be done effectively but, because the product is relatively new, limited research has been done. Further research is warranted to evaluate the effects on production parameters of lactating dairy cows.

Feed-related off-flavors in milk

Feed-related off-flavors in milk are significant problems for the dairy industry because of the associated decreased consumer acceptability of dairy-derived products.

Understanding the causes of off-flavors is very important to enable understanding of factors contributing to the shelf-life of products, preventing loss of nutritional value associated with the oxidation of lipids and proteins of milk and improving consumption of dairy products. The adoption of dairy products in the diets of American consumers is already an issue faced by the dairy industry because of the perceived negative health effects (Haug et al., 2007) or because of availability of alternative beverages that are prefered by consumers (e.g., sugar-sweetened drinks; Gills, 2003; Forshee and Storey, 2009).

One source of oxidized-off flavors in milk is light-induced oxidation, which occurs when light oxidizes unsaturated fatty acids, producing free radicals, especially in the presence of riboflavin, which acts as a photosensitizer of milk to yield light-induced oxidation (Chapman et al., 2002). Unsaturated fatty acids are particularly susceptible to oxidation because of the relative ease of extraction of allylic hydrogens from double bonds by pro-oxidants, which can lead to free-radical-catalyzed chain-reaction lipid autoxidation unless the lipid source is depleted or the radical species are quenched by an antioxidant (Frankel, 1996). Oxidized off-flavors in milk are characterized by a "cardboard" or "burnt feather" flavor (Costello and Clark, 2009). This process occurs very rapidly in a commercial setting as one half of all milk products remain in a lighted dairy case for at least eight hours, and off-flavors can be detected in as little as 15 minutes by trained evaluators and 52 minutes by the normal consumer (Chapman et al., 2002).

Milk fat from cows fed DG, particularly wet distillers grains (WDG), contains higher concentrations of unsaturated fatty acids and, in particular, concentrations of *cis*-9

trans-11 conjugated linoleic acid, especially when fed at concentrations higher than 20% (Anderson et al. 2006). In 2009, Nelson and Martini showed that an increase in eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and conjugated linoleic acid (CLA) in milk produced from cows fed fish-oil supplemented rations has little impact on flavor of milk as determined by a panel of trained sensory evaluators. This increased concentration of CLA is particularly interesting because of the numerous health benefits associated with increased dietary intake of CLA (Benjamin and Spener, 2009).

Increases of unsaturated fatty acids and omega-3 fatty acids, however, are not without negative effects. High concentrations of CLA tend to be associated with milk that has a "grassy" off flavor (Campbell et al., 2003). Higher concentrations of unsaturated fatty acids are also characterized by an "oily" flavor (Kolanowski et al., 2007). Increased CLA increases the susceptibility of milk to oxidation (Liu et al., 2010). Supplementation of fish oil (a source of highly unsaturated fatty acids) to the rations of dairy cows also contributes to oxidative susceptibility of milk, presumably because of the increased concentration of unsaturated fatty acids in the milk (Shingfield et al., 2006). Although increased bioactive fatty acids, such as *cis*-9 *trans*-11 CLA, EPA, DHA, and other omega-3 fatty acids are desirable from a nutritional standpoint, it is still important to consider its impact on consumer acceptability of milk because if the consumer does not consume the product they cannot benefit from healthfulness of milk, regardless of composition.

Another important factor with regards to oxidation of milk is the nutritional loss that accompanies lipid oxidation. Measurable losses of vitamin A accompanied with light-oxidized flavor can occur as soon as after two-hours of light exposure (Whited et

al., 2002), which can be particularly harmful as vitamin A deficiency is estimated to affect between 45 and 122 countries worldwide (World Health Organization, 2009). In addition to loss of nutritive components of milk through lipid oxidation, anti-health components (e.g., 4-hydroxynonenal and malonaldehyde) can be generated that can cause *in vivo* modification of lipoproteins that make them more atherogenic and lead to foam-cell formation (Esterbauer, 1993).

Attempts to improve oxidative stability of milk through supplementation of antioxidants either to the cow or directly to the milk, however, have had mixed results. Selenium, a component of the glutathione antioxidant system (i.e., glutathione peroxidase), supplemented at 25 mg day^{-1} of organic selenium to cows has little impact on oxidative stability of milk (Clausen et al., 2010). The lack of benefit of selenium supplementation is perhaps because glutathione peroxidase is hydrophilic and only able to act at the surface of the milk fat droplet whereas lipid autoxidation would predominantly occur within the lipid droplet. Tocopherols are very important to the oxidative stability of milk (Slots et al., 2007) because they are lipophilic and able to act within the milk lipid droplet, but supplementation of α-tocopherol to the diet or milk does not ensure oxidative stability of milk (Slots et al., 2007; Testroet et al., 2015). Van Aardt and colleagues (2005) demonstrated that both vitamins E and C were needed to protect milk from light-induced oxidation for 10 hours, presumably because vitamin C (hydrophilic) and vitamin E (lipophilic) provide supplemental antioxidants to both the milk serum and the milk fat. Perhaps supplementation of vitamins E and C with concomitant supplementation of sodium selenite would further improve oxidative stability of milk when compared with the results shown by Van Aardt and colleages

(2005). These investigations have led to the understanding that, although oxidation is correlated to the composition of milk, including fatty acid profile, vitamin E, vitamin C, and carotenoids, none of these can solely explain lipid oxidation (Barrefors et al., 1995; Granelli et al., 1998; Clausen et al., 2010).

Another source of off-flavors, unrelated to oxidation, but equally important to flavor, are volatile sulfur compounds. These compounds included hydrogen sulfide, methanethiol, carbonyl sulfide, dimethyl sulfide, carbon disulfide, dimethyl disulfide, dimethyl trisulfide, dimethyl sulfoxide, and dimethyl sulfone (Burbank and Qian, 2005). Because the rumen is a highly reducing environment, ruminants produce hydrogen sulfide from dietary sulfates. Distillers grains typically contain a relatively high concentration of sulfate (Neville et al., 2010) because sulfuric acid is used in the cleaning process in ethanol production plants (Klopfenstein et al., 2007). It has been shown that dietary supplementation of thiamine (150 mg/animal per day) and DG in ruminant animals, in this case sheep, increases hydrogen sulfide concentrations in the carcass of the animal (Neville et al. 2010). Increased hydrogen sulfide production in the rumen could, therefore, lead to increased incorporation of hydrogen sulfide in milk, resulting in off-flavors because of the high-volatility and low-concentration required for detection by humans (0.5-10 ppb; Ruth, 1986) and because flavor is primarily determined through olfaction (Spence, 2015).

The numerous sources of off-flavors in milk, both from lipid oxidation and transference of off-flavor compounds originating from ruminal reduction of minerals, in particular sulfates, leads to an area of investigation into milk quality that has been mostly overlooked. Extensive research has been done investigating the effects of feeding several

forms of DG to lactating dairy cows on fatty acid composition of milk and the efficiency of milk production (Schingoethe et al. (2009). Minimal research, however, has been done to investigate the effects on flavor, oxidative stability, and general consumer acceptability of milk from cows fed rations containing DGs. Distillers grains, as a dietary component, have been shown to have definite beneficial impact on the fatty acid composition of milk, but the impact of this alteration in fatty acid profile on the flavor and general consumer acceptability of milk hasn't been thoroughly investigated The need for investigation into the impact of how feeding practices affect oxidative stability and flavor of milk, and as a result consumer acceptability of milk, became apparent as early as spring of 2009 and 2010 when recurring customer complaints regarding milk from Hy-Vee stores in Iowa motivated farmers, educators, and Roberts Dairy in Des Moines, IA to investigate this problem (personal conversation). Upon investigation of the milk that was being rejected by consumers as "bad", experienced milk evaluators attributed the off-flavors to oxidized products in milk. Those off-flavors, however, were not derived from light-induced oxidation but rather were originating at the farm, occurring in raw milk that was initially good, but within three to five days became oxidized at the plant or grocery.

Because of the many potential sources of lipid oxidation and off-flavor compounds that could be introduced to milk, a thorough understanding of the effects of feeding practices of dairy cows and their influence on quality of milk produced by those cows is of great importance to investigate.

Utilization of DDGS in Swiss cheese production

High quality Swiss-type cheeses are defined by four characteristics: 1) natural, attractive, uniform ivory to light yellow color, 2) mild, pleasing, characteristic sweet

hazelnut-like flavor, 3) round or slightly oval-shaped eyes that are relatively uniform in shape, and 4) uniform, firm, smooth texture, and slightly elastic bodies (Cakir and Clark, 2009). Many defects in Swiss-type cheeses can occur, but one such defect of particular interest and relevance to the DG industry is the late-blowing defect, which can be characterized by appearance of undesired slits, cracks, splits, or blown areas in the body of the cheese. This defect is undesirable to consumers who expect uniform and even eye distribution. Additionally, this defect is undesirable to high speed slicing operations because these defective cheeses cannot be sold at full-price, resulting in economic losses for the cheese producer (White et al., 2003).

Typical eye formation in Swiss cheese occurs when fermentation of lactose to carbon dioxide and lactate takes place by the starter culture *Propionobacterium freudenrichii* spp. *shermanii.* Secondary fermentation then occurs where the lactate is further metabolized to produce propionate, which depending on the organism can occur by the acrylate pathway or the succinate pathway. Excessive gas formation, however, can occur and lead to late-blowing if the concentration of the primary substrate for fermentation, lactose, is increased in cheese milk because, if not all lactose is fermented by starter cultures, secondary fermentation of residual lactose can occur (Farkye, 2014). Another potential cause of late-blowing is contamination of cheese milk by unwanted cultures, such as *Clostridium tyrobutyricum*, which can utilize lactate to produce not only carbon dioxide but also hydrogen sulfide, butyric acid, butanoic acid, and hydrogen gas (Fox et al., 2000; Fröhlich-Wyder and Bachmann, 2007). These gasses are not as soluble in the cheese body as carbon dioxide and can lead to structural failure of the cheese as well as development of undesirable off-flavors.

The above potential causes of late blowing in Swiss-type cheeses has been linked to feeding of DDGS where it has been implicated that the feeding of low quality feed contaminated with Clostridial spores could result in the late-blowing defect (Houck et al., 2007). This hypothesis, however, is not likely unless there is contamination of the cheese milk by the environment (e.g., manure) rather than direct contamination of the milk by the feed (i.e., in a healthy animal, the bacteria would not be able to pass the gut barrier and end up in the milk). Indeed, these were the results published by Sankarlal et al. (2015) where cows were fed one of three concentrations of FF-DDGS (0, 10, and 20%), and no treatment effect on quality of baby Swiss cheese was observed. In addition, no gas-producing spores were found in the DDGS but were found in the manure, all the treatment diets, and the cheeses produced. The milk produced from those cows, however, had increased PUFA and protein and a tendency for increased lactose concentration. The increased concentrations of lactose from cows fed DDGS also has been shown previously (Tanaka et al., 2011). Because lactose is the primary substrate utilized for fermentation in cheese production, increased lactose concentration in milk could also result in excessive gas formation. The potential of lactose to increase gas formation could possibly explain the findings of gas-producing spore-forming bacteria and an atypical appearance in all cheeses regardless of treatment reported by Manimanna Sankarlal et al. (2015).

Because DDGS are becoming a prevalent feed source in the U.S. with the increased production of ethanol, it is inevitable that DDGS will be included in the rations of dairy cows. Research investigating the suitability of milk from cows fed DDGS and

more recently RF-DDGS for production of high quality dairy products, such as Swiss-type cheeses, is lacking, and further investigation is warranted.

Utilization of DDGS in the pork production industry: pork quality

Distillers grains have been utilized in swine feeding practices for more than 50 years (Fairbanks et al., 1944; Fairbanks et al., 1945) and have been reviewed extensively by Stein and Shurson (2009). Stein and Shurson (2009) concluded that DDGS are an excellent source of energy and digestible protein for swine. Inclusion rates, however, are limited by the high concentration of linoleic acid in corn oil that can result in pork fat becoming softer. The problems associated with feeding DDGS (e.g., soft belly) that are unacceptable to the producer and negatively influences consumer acceptability of pork could potentially be limited by feeding maximal inclusion rates of 20% as fed or by employing a 30-day withdrawal period pre-harvest. The effectiveness of the 30-day withdrawal period on producing pork with acceptable iodine values from pigs fed up to 30% DDGS (as fed basis) was demonstrated in 2010 by Xu et al. They also concluded that DDGS can be fed in gestational diets effectively and may even improve litter size. Since the time of review by Stein and Shurson (2010), composition of DDGS has been altered dramatically to contain less fat on a dry matter basis. Earlier work was mainly focused on grow-finish performance, whereas more recent research has been focused on the influence of feeding DDGS on pork and pork fat quality.

Inclusion of DDGS in the rations of growing pigs traditionally has been limited by the highly unsaturated nature of corn oil. Because pigs, like nearly all animals, cannot produce specific PUFA, these fatty acids can only be derived from dietary sources (Wood, 2008). Feeding rations to pigs that are rich in unsaturated fatty acids is

particularly problematic because pork adipose then becomes enriched in unsaturated fatty acids and can attain greater than 60% unsaturated fatty acids (Lawrence and Fowler 2002). If unsaturated fatty acid content of adipose is raised above 60%, oxidative stability can be negatively affected, which can result in the meat being more prone to development of off-flavors and odors (Wood et al., 2008). Dried distillers grains with solubles, especially FF-DDGS, are a particularly rich source mono- and polyunsaturated fatty acids because corn oil is approximately 92% unsaturated. Feeding FF-DDGS can, therefore, lead to the fatty acid composition of the adipose tissue in nonruminants becoming highly unsaturated in nature. It is well known that feeding DDGS can increase iodine value (IV) of pork fat (Benz et al., 2010, Kellner et al., 2014). Several problems can arise if pork fat becomes too rich in unsaturated fatty acids (e.g., soft-bellies, poor sliceability of pork, decreased oxidative stability, Stein and Shurson, 2009).

One way to determine quality of pork fat is to measure IV, either directly or indirectly (Kyriakidis and Katsiloulis, 2000), which is a metric of degree of unsaturation of pork lipids. Iodine value is the current indicator used by industry for determination of pork fat quality (Benz et al., 2011). In general, pork fat in North America is considered unacceptable if IV is greater than 74 (Boyd et al., 1997). However, it has been shown that IV varies by anatomical location (Kellner et al., 2014; Sørensen et al., 2013); so, further research is warranted to investigate how IV varies amongst anatomical location, between gender, and between breeds because of the potential economic loss to producers (i.e., does one fat quality of one sampling location accurately predict fat quality of other adipose depots). Recently, a method for predicting IV in pigs fed RF-DDGS with NIR spectroscopy has been developed, which could help producers ensure high quality pork

production (Prieto et al., 2014) by allowing quick and reliable testing of pork fat from multiple adipose depots.

As mentioned previously, when pigs are fed DG, the potential for development of off-flavors is increased. When crossbred pigs were fed DDGS, there was no difference in oxidation of intramuscular fat in the longissimus muscle and taste tests revealed no treatment effects from diet on flavor, off-flavor, tenderness, juiciness, and overall consumer acceptability. Additionally, no effects were found on the sensory attributes, with the exception of tenderness and fattiness, which decreased linearly, even though IV of back, belly, and longissimus intramuscular fat increased when DDGS were fed (Xu et al. 2009). With the recent advent of RF-DDGS, the potential to avoid negative effects of high inclusion rates of FF-DDGS is possible. When pigs were fed 20 or 40% RF-DDGS or 20 or 40% FF-DDGS, IV increased linearly, regardless of source. Additionally, the decreased energy content of RF-DDGS resulted in a linear decrease in gain to feed ratio (G:F), whereas feeding FF-DDGS did not affect G:F (Graham et al., 2014). Clearly, the results that can be expected with inclusion of DDGS in the rations of swine depend on inclusion rate and fat concentration of the diet. With the relative scarcity of FF-DDGS resulting from the shift to RF-DDGS occurring relatively recently, sensory evaluation data of pork and pork fat produced from pigs fed RF-DDGS is currently lacking and future research will need to be performed to determine the impact of utilization of this relatively new feedstuff in the rations of growing pigs

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TABLES AND FIGURES

Table 1. Nutrient compositions of distillers grains with different concentrations of fat compared with soybean meal (dry-matter basis).

Nutrient	RF-DDGS[1]	MF-DDGS[2]	FF-DDGS[3]	Soybean meal
Dry matter, %	88.83	89.26	89.63	90.0
Crude protein, % DM	32.69	31.90	28.76	38.0
Fat, % DM	5.47	8.05	12.96	18.0
ADF, % DM	11.93	16.48	15.74	
Calcium, % DM	0.12	0.11	0.13	0.25
Phosphorus, % DM	1.00	0.83	0.83	0.59
Sulfur, % DM	1.07	0.48	0.52	0.30

Adapted from Anderson and Engel, 2014
[1]Reduced-fat dried distillers grains with solubles
[2]Medium-fat dried distillers grains with solubles
[3]Full-fat dried distillers grains with solubles

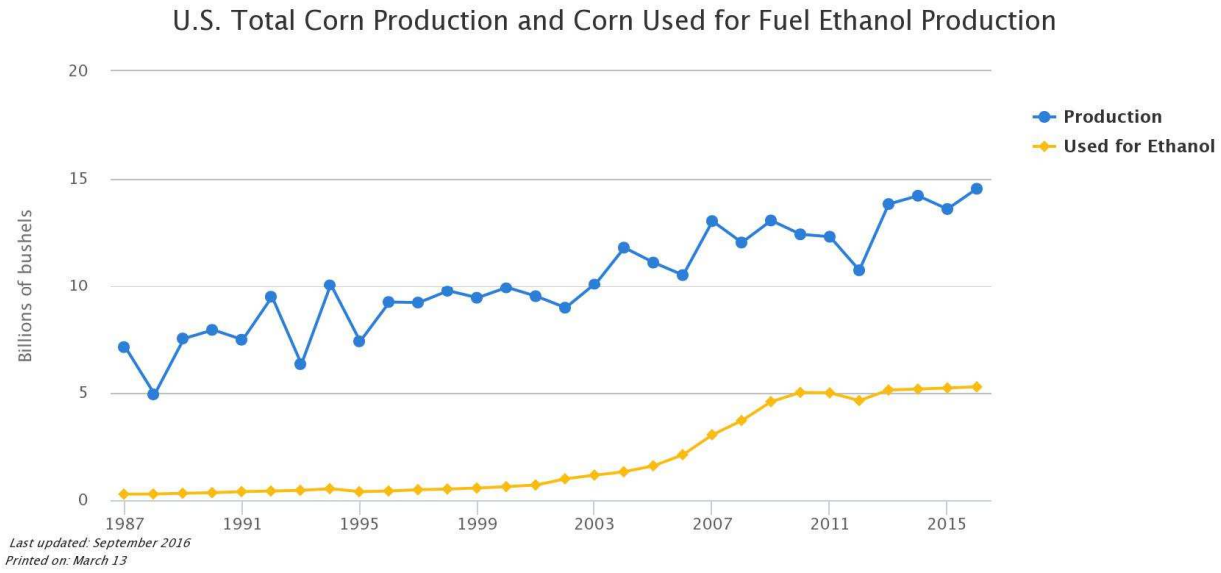

Figure 1. Schematic representation of total corn produced in the United States and the percentage of that corn that is utilized for production of ethanol.
Source: U.S. Domestic Corn Use Graph Data. Calculated by USDA Economic Research Service, 1980-2015, Last Accessed: 08/03/2016 and U.S. Corn Acreage, Production, Yield and Price data. Calculated by USDA Economic Research Service, 1926-2015, Last Accessed: 08/03/2016

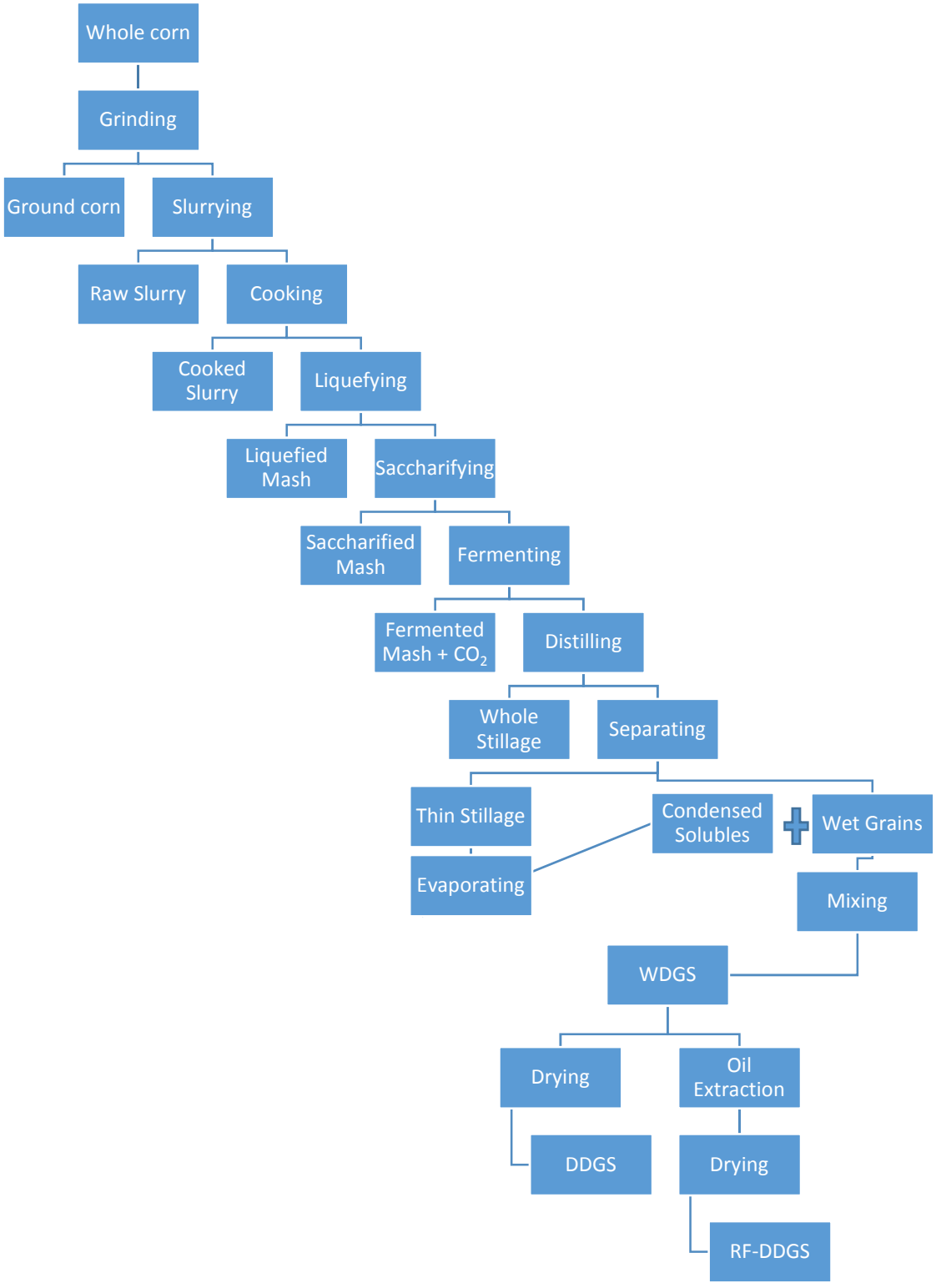

Figure 2. Schematic diagram of conventional ethanol and distillers grains from corn. WDGS = Wet distillers grains with solubles, DDGS = Dried distillers grains with solubles, RF-DDGS = Reduced-fat dried distillers grains with solubles. (Adapted from Liu, 2011)

CHAPTER 3

LACTATIONAL PERFORMANCE OF HOLSTEIN DAIRY COWS FED TWO CONCENTRATIONS OF FULL-FAT DRIED DISTILLERS GRAINS WITH SOLUBLES

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Abstract

The objective was to evaluate production performance of lactating Holstein dairy cows fed two different dietary concentrations of full-fat dried distillers grains with solubles (DDGS; 13.6% fat). Thirty cows were fed 0, 10, and 20% DDGS dry matter (DM) as a total mixed ration (TMR) in a 3 × 3 Latin square. Cows were stratified into groups of 10 by parity and days in milk and fed each of three diets in three 28-day periods. Our hypothesis was that feeding 20% DDGS (DM basis) would negatively influence production and feed efficiency of dairy cattle. Milk yield was decreased significantly when fed 20% DDGS, and feeding DDGS caused milk fat depression and decreased daily fat yield, resulting in significant decreases in 3.5% fat-corrected milk (FCM) and energy-corrected milk (ECM) yield. Both protein and lactose percentages increased significantly when cows were fed 20% DDGS; neither protein nor lactose yield, however, was affected. Protein efficiency, a measure of the utilization of dietary protein for milk protein synthesis, decreased significantly for cows fed 20% DDGS. All three measures of energetic efficiency (ECM/DMI, kg ECM/net energy for lactation (NE_l) intake (Mcal), and gross energy of milk produced (Mcal)/NE_l caloric intake (Mcal)) were significantly

decreased when cows were fed 20% DDGS. Feeding 10% FF-DDGS resulted in a 0.5% percentage decrease in milk fat concentration and feeding 20% resulted in a marked decrease in production when compared control.

Introduction

Dried distillers grains with solubles (**DDGS**) are an often economical protein source for animal production and are a good source of rumen undegradable protein (**RUP**) (Firkins et al., 1984; Powers et al., 1995) in the ration of a lactating dairy cow. Prior research has indicated that DDGS can effectively be fed to lactating dairy cows without changing dry matter intake (DMI) or milk fat percentage while either not affecting, or in some cases increasing yield of milk, energy-corrected milk **(ECM)** yield, fat percentage, protein percentage, and feed efficiency when compared with a traditional total mixed ration (**TMR**) (Anderson et al., 2006; Kleinschmidt et al., 2006; Havlin et al., 2015). Conversely, our prior research showed decreased milk fat percentage and milk yield when cows were fed 25% DDGS containing12.1% fat (Testroet et al., 2015). It was the objective of this study to investigate the effects of feeding full-fat DDGS on the feed efficiency and production performance of lactating Holstein dairy cows. In addition, based upon ours and others prior research, we hypothesized that feeding full-fat DDGS at 20% of dietary DM to lactating dairy cattle would negatively influence the production and efficiency of dairy cows when compared with a traditional TMR.

Materials and methods

Animals and diets

All experimental protocols were approved by the Iowa State University Institutional Animal Care and Use Committee prior to commencement of the study.

Thirty mid-lactation (164.4±16.2 DIM) multiparous Holstein cows were fed diets containing 0, 10, or 20% corn DDGS (Heartland Cooperative, Prairie City, IA). Cows were assigned randomly to one of three treatment sequences in 3 × 3 Latin square design. Diets were formulated (Table 1) to be isonitrogenous and isoenergetic (Table 2). Diets, however, were 0.5% crude protein greater than formulated (Table 2). Feed proximate analyses done by wet chemistry (Dairylands Lab, Arcadia, WI). Acid Detergent Fiber was determined by AOAC Official Method 973.18(1996) and Lignin by AOAC Official Method 973.18, ether extract was determined by 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 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, and dry matter was determined by using NFTA Method 2.1.4. Feed composition is represented as the mean of six samplings and presented in Table 2 showing that, as designed, no significant differences in crude protein and estimated net energy for lactation concentrations exist.

Cows were housed at the Iowa State University Dairy Farm (Ames, IA) together in a 48-cow, free-stall pen and individually fed twice daily (0700 h and 1700 h) with a Calan Data Ranger (American Calan Inc., Northwood, NH) to allow for approximately 15% refusal. Feed ingredients in a TMR were mixed prior to being loaded onto the Data Ranger by using a Patz V615 mixer (Patz Corporation, Pound, WI). Cows were allowed

ad libitum access to food and water, except for three times daily during which they were being milked (8 h apart). Initially, cows were allowed seven days to adapt to using the Calan gates (American Calan Inc, Northwood, NH). For each 28-day experimental period, the first 13 days were used as a washout and excluded from the analysis to limit carryover effects. Samples of TMR were collected and pooled over a period of three days from both daily feedings during weeks three and four of each experimental period. Samples of the otal mixed rations then were combined, randomly sampled, and sent to Dairylands Laboratory (Arcadia, WI) for proximate analyses by wet chemistry methods.

Milk yield and composition

Total milk yield was recorded daily using an automatic weighing milking system (Boumatic, Madison, WI). During each period, on days 14, 21, and 28, individual milk samples were collected automatically from each milking to represent one complete milking by the Boumatic milking system (Boumatic, Madison, WI). Milk samples (30 to 40 mL at 22± 2°C) were then immediately transported to a laboratory for proximate analyses in duplicate by using a Lacticheck-01 RapiRead Milk Analyzer (Page & Pedersen Intl. Ltd., Hopkinton, MA).

Statistical analyses

Statistical analyses of milk composition, yield, and feed efficiency were performed by using SAS version 9.3 (Cary, NC) and Proc MIXED. Data were analyzed as a 3 × 3 crossover design. The model included three fixed effects (sequence, period, and treatment) and cow(sequence) as a random effect**.** For variables with significant treatment effects, means were separated by using Tukey's multiple comparisons tests. Crude protein and calculated net energy for lactation concentrations of feed were

analyzed by using the Student's t-test. Feed fatty acid composition was analyzed by using Proc MIXED with a model that included treatment and sampling date. Statistical significance was declared at $P < 0.05$.

Results and discussion

Five cows were removed from the trial because of illness (e.g., mastitis) and were not included in data analyses. Dry matter intake (Table 3) was affected significantly by dietary treatments, but no diet-dependent trend is evident because the control is intermediate in amount to the 10 and 20% DDGS treatments. Similar previous studies have reported no change in DMI for cows fed 10 and 20% DDGS DM (Anderson et al., 2006; Kleinschmit et al., 2006). To maintain isoenergetic and isonitrogenous diets, the ingredient composition of the control and 10% DDGS rations had to be altered significantly (Table 1). It is possible that the changes in ingredient composition between the 10 and 20% DDGS diets can explain the decreased DMI observed for cows fed 10% DDGS and the increased intake observed for cows fed 20% DDGS.

Milk yield (Table 3) was significantly decreased by 20% DDGS inclusion, but feeding DDGS at 10% inclusion did not differ from control or 20% DDGS. However, both 3.5% FCM and ECM (Table 3) decreased as dietary DDGS inclusion increased. These results are supported by findings reported by Testroet et al. (2015), who also showed milk fat depression when cows were fed 10 and 25% DDGS (DM basis). Dried distillers grains with solubles used in that study contained similar concentrations of fat as those in this study. The decrease in FCM and ECM could be related to (1) inhibition of fiber digestion by increasing dietary fat (fat percentages: 0% DDGS was 5.6% fat, 10% DDGS was 6.9% fat, and 20% DDGS was 7.6% fat (Table 2)) or (2) inhibition of fat

synthesis in the mammary gland, or both. Dietary fat linearly decreases fiber digestion in ruminants, with fat concentrations of 8% decreasing fiber digestibility by over 10% (Zinn, 1989). Additionally, incomplete biohydrogenation of unsaturated fatty acids by rumen microbes leads to production of *trans*-10, *cis*-12 conjugated linoleic acid, which decreases *de novo* milk fat synthesis in the mammary gland (Baumgard et al., 2001). Moreover, feeding of unsaturated fatty acids to ruminants has long been known to induce milk fat depression (Brown et al., 1962; Beitz and Davis, 1964; Ensor et al., 1959; Garner and Sanders, 1938; Maynard et al., 1936; Moore et al., 1945; Ramirez Ramirez et al., 2015). Both the 10 and 20% DDGS treatment diets had significantly less SFA, more PUFA, and more unsaturated fatty acids **(UFA)** (Table 4). In addition, as DDGS inclusion increased, the UFA/SFA concentration ratio of fatty acids significantly increased (Table 4). Taken together, the increased UFA content, specifically the increased PUFA intake of cows fed the TMR containing DDGS diets and the inhibition of fiber digestion by the fat content of the TMR containing DDGS diets help explain the observed decrease in milk fat percentage and daily milk fat production observed in this study (Table 3).

Our results, however, conflict with previously reported performance data where no change in fat percentage (Anderson et al., 2006; Kleinschmit et al., 2006) and increased FCM (Kleinschmit et al., 2006) and ECM were observed with inclusion of DDGS (Anderson et al., 2006; Kleinschmit et al., 2006). One likely explanation for the differences in milk fat percentage and consequently ECM and FCM is that the fat content, and therefore the corn oil content (in particular PUFA intake, Table 3), of the DDGS in these other studies ranged from 9.7% up to 10.8%, whereas our DDGS

contained 13.5% fat. A second possible explanation, or partial contributor, to the decrease in milk fat percentage is the forage being slightly less than 50% of the DM in the diets of the 10 and 20% DDGS treatments (Table 1), which has been suggested by Kalscheur et al. (2005) to contribute to milk fat depression. Despite the high neutral detergent fiber (NDF) of DDGS, the particle size of DDGS makes them an ineffective source of fiber (Schingoethe et al., 2009). Although the more effective source of forage (i.e., hay) remained constant in the three treatment diets, corn silage content of the 10 and 20% DDGS treatments was decreased to accommodate DDGS inclusion (Table 1). Lack of forage, however, cannot solely explain the decrease in milk fat percentage because even the 10% DDGS diet that contained 47.5% forage (Table 1) resulted in a loss of about 0.5% percentage points of milk fat (Table 3). In addition, our prior research (Testroet et al., 2015) resulted in cows experiencing milk fat depression even when they were fed a 10% DDGS diet containing 57.1% forage DM and a 25% DDGS diet containing 54.3% forage, exceeding the 50% forage DM recommendation for effectively feeding DDGS at up to 20% DM (Kalscheur et al., 2005), thereby likely exceeding the 22% forage NDF requirement. In addition to our previous research, Benchaar et al. (2013) observed an increase in milk yield without a change in milk fat yield resulting from a decreased milk fat percentage when cows were fed diets containing 20 and 30% DDGS (16.3% fat) even when rations were formulated to contain 60.1% forage. Another possible contributing factor to milk fat depression is dietary cation anion difference (DCAD). Results of previous research have shown that increasing the DCAD value of the diet can improve milk fat percentage (Wildman et al., 2007; Hu et al., 2007; Harrison et al., 2012); however, Erdman et al. (2011) found no effect on milk fat percentage when

increasing DCAD. In this experiment, low DCAD (Table 2) is unlikely to have contributed to milk fat depression because the DCAD of all three diets was very similar (<2.5 meq/100 g difference), and the cows fed 0% DDGS did not experience milk fat depression. The most likely reason for the cows experiencing milk fat depression and for the inconsistent results from what has been reported previously by Kleinschmidt et al. (2006) and Anderson et al. (2006) is the amount of corn oil that was fed to the cows. In the experiment by Kleinschmidt et al. (2006), the DDGS contained 7.21% fat, resulting in the 20% DDGS DM diet containing 0.41 kg of corn oil and 4.60% fat. Anderson et al. (2006) fed DDGS that contained 9.67% fat, resulting in their 20% DDGS diet containing 0.58 kg corn oil and 4.47% fat. In this experiment, however, the DDGS contained 13.5% fat, resulting in our 10% DDGS diet containing nearly as much corn oil as 20% DDGS diets (in this experiment the corn oil content is as follows: 10% DDGS 0.47 kg corn oil, 6.9% fat; 20% DDGS 0.85 kg corn oil, 7.6%) fat in the previously two mentioned experiments. In addition, the unusually high fat content of the DDGS resulted in diets containing greater than optimal fat concentrations could have inhibited fiber digestibility and the greater amount of PUFA intake (Table 3) could have inhibited milk fat synthesis.

Milk protein percentage was greatest in the 20% DDGS treatment group with a 0.04 percentage point increase when compared with control (Table 3). It is possible that the alteration of protein source (i.e., DDGS vs. soybean meal/blood meal), to maintain isonitrogenous diets, could explain some of the protein-related results. Total daily milk protein yield was, however, unaffected by treatment (Table 3). Again, these results are inconsistent with prior research with dairy cows fed 20% DDGS that indicated a decrease in protein percentage with no change in protein yield (Anderson et al., 2006, Kleinschmit

et al., 2006). The differences can be explained by the increases in milk yield observed by both Anderson et al. (2006) and Kleinschmit et al. (2006) that diluted the total protein and decreased the protein percentage. In this study, however, we did not see increases in milk yield but, rather, a decrease in milk yield and no change in protein production when cows were fed 20% DDGS was observed.

Lactose percentage was increased for both cows fed 10 and 20% DDGS, but, as with total daily protein, total daily lactose production was unaffected by treatment (Table 3). The lactose percentage and lactose yield are not consistent with prior work that showed no change in lactose percentage but an increase in total daily lactose production (Anderson et al., Kleinschmit et al., 2006), as would be expected if the increase in lactose production offset the dilution of lactose by increased milk volume. As with the protein percentage and protein yield, the increase in lactose percentage and lack of change of total daily production of lactose could be explained by the decreased total milk volume produced when cows were fed 20% DDGS. Also, both the increases in protein and lactose percentage with cows fed DDGS are in agreement with our prior research (Testroet et al., 2015).

Protein efficiency, a measurement of utilization of dietary protein for milk protein synthesis, decreased significantly when cows were fed 20% DDGS when compared with cows fed 10 and 20% DDGS (Table 3). The most likely explanation for the decreased protein efficiency of cows fed 20% DDGS is that those diets (Table 2) contained the greatest amount of acid-detergent insoluble crude protein (a measure of heat damage), which is less available to the animal for utilization (Goering et al., 1972; Yu and Thomas, 1976). Kleinschmit et al. (2006) observed an improvement in protein efficiency in cows

fed DDGS, but the acid-detergent insoluble crude protein was much lower in their study than in ours, indicating that the decreased protein efficiency in this study may have resulted from a greater amount of heat-damaged protein. A second possible explanatory factor is the different protein sources that were utilized to maintain isonitrogenous diets. However, because DDGS are a good source of RUP (Firkins et al., 1984, Powers et al., 1995), it would be expected that protein efficiency should increase with increased inclusion of DDGS.

One measure of feed efficiency is the ratio of energy-corrected milk to dry matter intake (**ECM/DMI**). The ECM/DMI decreased significantly for cows fed 20% DDGS when compared with cows fed 0% and 10% DDGS (Table 3). The decrease in ECM/DMI is likely directly related to the decrease in milk fat production. These results are not consistent with previous research that showed an improvement in ECM/DMI for cows fed 20% DDGS (Kleinschmit et al., 2006; Anderson et al., 2006). Neither of these studies, however, observed milk fat depression, which is in contrast with our study. When compared with research published on cows fed a traditional TMR, previously reported ECM/DMI of Holstein cows ranged from 1.5 to 1.8 (Hart et al., 2014; Schingoethe et al., 2004; Wall et al., 2014). Our cows performed similarly (1.68 and 1.67 for cows fed 0 and 10% DDGS, respectively), but they performed significantly worse when fed 20% DDGS (1.41). The results can be explained because there was no change in milk or protein yield for any treatment, but there was a significant depression in milk fat yield for cows fed 20% DDGS accompanied with the greatest DMI of any treatment.

In addition to ECM/DMI, 3.5% FCM/DMI **(FCM/DMI)** can be used as a measure of feed efficiency. In this study, FCM/DMI was only significantly decreased when cows

were fed 20% DDGS (Table 3) compared with control. Even though cows fed 10% DDGS had decreased milk fat, DMI concomitantly decreased, resulting in no significant difference in FCM/DMI between the control (0%) and 10% treatments. Prior researchers reported FCM/DMI ranging from 1.5 to 1.78 when cows were fed a traditional TMR (Devries et al., 2011; Hart et al., 2014; Maiga et al., 2011; Martinez et al., 2009; Wall et al., 2014), whereas in this study cows fed 0, 10, and 20% DDGS had a FCM/DMI of 1.49, 1.45, and 1.23, respectively. In this study, cows fed 0 and 10% DDGS performed similarly to previously published data, but cows fed 20% DDGS had a considerably lower FCM/DMI, likely because of the significant milk fat depression and increased DMI of those cows when compared with cows fed the control diet.

Another measure of feed efficiency is the amount of caloric intake (represented by the net energy for lactation (NE_L)) that is needed to produce one kg of ECM (**ECM/NE_L**). Feeding cows 20% DDGS resulted in the poorest utilization of available NE_L, which was a direct result of the decreased milk fat production (Table 3).

Finally, energetic efficiency (a ratio of the estimated caloric value of milk to the NE_L of the feed) decreased significantly for cows fed 20% DDGS, meaning that more calories must be fed to produce one calorie of milk when cows are fed 20% DDGS than when they are fed 0 and 10% DDGS (Table 3). As with ECM/NE_L, the authors are not aware of a study to which a comparison can be made.

Implications

These results indicate that feeding DDGS at different concentrations had an inconsistent effect on DMI. These results, however, do support our hypothesis that, under these experimental conditions, feeding 20% DDGS diets, but not 10% DDGS diets,

significantly decreases feed efficiency of lactating Holstein dairy cows in all metrics considered. In addition, under these experimental conditions, feeding full-fat DDGS (13% fat) at both 10 and 20% concentrations resulted in significant milk fat depression, decreased 3.5% FCM and ECM yield, and increased lactose percentage. Also, feeding of 10% DDGS did not result in a loss of feed or protein efficiency but did result in a decrease of ECM and FCM because of significant decreases in milk fat production and percentage. Feeding 20% DDGS with full-fat resulted in decreased performance in nearly every measure employed in this study. Therefore, these data indicate that the limitations of feeding DDGS without inducing milk fat depression are complex and multifaceted, requiring consideration of oil content in addition to particle size and forage content when formulating rations.

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Table 1. Feed formulations of three treatment diets, fed as a total mixed ration, containing different concentrations of dried distillers grains with solubles (DDGS)

	Treatment		
Ingredient, % DM	0% DDGS	10% DDGS	20% DDGS
Corn silage	31.4	27.9	22.7
Alfalfa hay	19.5	19.5	19.5
Soybean meal (48%)	1.8	0.0	0.0
Soy Plus*	4.2	1.3	1.4
Cottonseed (whole)	8.6	8.6	8.6
Finely ground corn	28.1	26.3	23.0
DDGS	0.0	10.0	20.0
Supercharger II®†	0.9	0.9	-
Limestone	0.8	0.8	1.5
Blood meal	0.6	0.6	-
Sodium bicarbonate	0.6	0.6	0.6
Rock salt	0.5	0.5	0.5
Animal fat	-	-	0.4
Urea	0.4	0.4	-
Pork meat and bone meal	0.3	0.3	-
Monocalcium phosphate 21%	0.2	0.2	-
Magnesium oxide	0.2	0.2	0.2
Metasmart®‡	0.1	0.1	0.1
Dynamate®§	0.1	0.1	-
Dairy balancer II®#	0.1	0.1	0.1
Monensin 90®	0.01	0.01	0.01
Forages	50.9	47.5	42.3
Concentrates	49.1	52.5	57.7

*West Central Cooperative, Ralston, IA.
†Fat supplement (Origo, New Ulm, MN)
‡Methionine supplement (Adisseo USA Incorporated, Anpharetta, GA)
§Vitamin and trace mineral premix (Consumer's Supply Distributing Company, Sioux City, IA)
#Vitamin and trace mineral premix (Nutritional Professionals Incorporated, Hortonville, WI))

Table 2. Proximate analyses of dried distillers grains with solubles (DDGS) and pooled total mixed rations

		Total mixed rations*		
Component	DDGS	0%	10%	20%
Moisture, %	9.56	43.8	41.4	37.1
Dry matter (DM), %	90.44	56.2	58.7	62.9
Crude protein (CP), %	32.55	16.6	17.1	16.9
ADF, %†	17.1	20.4	23.7	21.8
aNDF w/Na_2SO_3, %‡	N/A	30.6	32.4	28.8
Lignin (sulfuric acid), %	4.4%	4.1	5.6	5.6
Lignin % of NDF, %	19.5	13.3	17.4	19.6
AD-ICP§ % of CP, %	19.0	10.9	14.0	18.9
AD-ICP % of DM, %	6.1	1.8	2.4	3.2
ND-ICP# % of CP est. w/o Na_2SO_3, %	N/A	16.0	16.0	16.0
ND-ICP % of DM est. w/o Na_2SO_3, %	N/A	2.7	2.8	2.8
Fat, %‖	13.5	5.6	6.9	7.6
Ash, %	5.1	6.9	6.7	6.9
Calcium, %	0.1	1.0	0.9	1.0
Phosphorus, %	1.0	0.4	0.4	0.4
Magnesium, %	0.4	0.3	0.3	0.3
Potassium, %	1.1	1.2	1.2	1.1
Sulfur, %	0.9	0.2	0.3	0.4
Sodium, %	0.2	0.4	0.4	0.5
Chloride, %	0.2	0.6	0.6	0.6
T. D. N** – OARDC††,%	85.2	71.5	69.1	69.8
N. F. C. ‡‡	26.3	41.0	37.8	40.7
NE_L §§ - OARDC, Mcal/kg	2.0	1.6	1.6	1.6
DCAD##, mEq/100 g	-5.3	3.9	2.8	1.8

*Data are expressed as the means of 6 composite samples as a percentage of dry matter.

†Acid detergent fiber.

‡Amylase-treated neutral detergent fiber with sodium sulfite.

§Acid-detergent insoluble crude protein.

#Neutral-detergent insoluble crude protein.

‖Total mixed rations determined by ether extract and DDGS determined by petroleum ether extract.

**Total digestible nutrients.

††A summative calculation based on an Ohio Agricultural Research and Development Center method (an approach for energy evaluation).

‡‡Non-fiber carbohydrate.

§§Net energy for lactation.

##Dietary cation anion difference (DCAD) = $(Na^+ + K^+) - (Cl^- + S^{2-})$.

Table 3. Effects of feeding dried distillers grains with solubles (DDGS) at 0, 10, and 20% dietary inclusion (DM) on dry matter intake (DMI), milk production and composition, and feed efficiency expressed as grand mean ± the standard error of the mean

Item	Treatment			SEM	*P* - Value
	0% DDGS	10% DDGS	20% DDGS		
DMI (kg/d)	25.33[b]	24.14[a]	26.30[c]	0.47	<0.0001
PUFA intake (kg/d)	0.70[a]	0.85[b]	1.08[c]	0.02	<0.0001
Milk yield (kg/d)	39.78[b]	39.24[ab]	38.98[a]	1.51	0.0125
3.5% FCM yield (kg/d)*	36.87[c]	33.67[b]	31.72[a]	1.28	<0.0001
ECM yield (kg/d)†	41.54[c]	38.75[b]	37.08[a]	1.40	<0.0001
Fat (%)	3.58[c]	3.09[b]	2.80[a]	0.13	<0.0001
Fat yield (kg/d)	1.40[c]	1.20[b]	1.08[a]	0.06	<0.0001
Protein (%)	3.68[a]	3.70[b]	3.72[c]	0.02	<0.0001
Protein yield (kg/d)	1.45	1.45	1.45	0.05	0.8251
Lactose (%)	5.12[a]	5.20[b]	5.24[c]	0.03	<0.0001
Lactose yield (kg/d)	2.03	2.03	2.03	0.07	0.9176
Protein efficiency (%)‡	35.42[b]	36.31[b]	32.93[a]	0.01	<0.0001
3.5% FCM/DMI	1.49[b]	1.45[b]	1.23[a]	0.05	<0.0001
ECM/DMI	1.68[b]	1.67[b]	1.43[a]	0.06	<0.0001
kg ECM per NE_L§ intake (Mcal)	1.03[b]	1.05[b]	0.90[a]	0.04	<0.0001
Energetic efficiency (%)#	0.66[b]	0.67[b]	0.57[a]	0.02	<0.0001

[a, b, c] Items within a row with differing superscripts differ ($P < 0.05$).

*3.5% Fat corrected milk yield = [0.4 × Milk yield (kg/d)] + [15 × milkfat yield (kg/d)].

†Energy corrected milk = [0.327 × milk yield (kg/day)] + [12.95 × milkfat yield (kg/d)] + [7.2 × protein yield (kg/d)].

‡Protein efficiency = [crude protein in milk (kg/day)]/[crude protein intake (kg/day)].

§Net energy for lactation (NE_L) calculation performed by using the summative equation described in NRC Nutritional Requirements of Dairy Cattle (2001).

#Energetic efficiency = [estimated gross energy in milk (mcal)]/[NEL caloric intake (Mcal)], estimated gross energy of milk (Mcal) = [4 × milk protein (kg/day)] + [4 × milk lactose (kg/day)] + [9 × milk fat (kg/day)].

Table 4. Fatty acid composition of three treatment pooled total mixed rations containing differing concentrations of dried distillers grains with solubles (DDGS)

	Treatment*				
Fatty acid, wt %†	0% DDGS	10% DDGS	20% DDGS	SEM	*P* - Value
C12:0 + C14:0	0.39[b]	0.36[ab]	0.31[a]	0.02	0.0274
C14:1	1.28[b]	0.90[ab]	0.57[a]	0.11	0.0036
C16:0	20.10[b]	19.09[b]	17.61[a]	0.33	0.0010
C16:1	0.66	0.64	0.45	0.07	0.1336
C17:0	0.15	0.15	0.16	0.04	0.9908
C18:0	3.03[c]	2.49[b]	2.08[a]	0.07	<0.0001
C18:1	25.00	24.52	24.36	0.29	0.3163
C18:2	44.76[a]	47.48[b]	50.64[c]	0.32	<0.0001
C18:3	4.04[b]	3.68[b]	3.13[a]	0.11	0.0006
C19:0	0.00	0.00	0.00	ND	ND
C20:0	0.08	0.19	0.16	0.03	0.1461
C20:1	0.22	0.24	0.22	0.03	0.9239
C20:2	0.04	0.02	0.07	0.20	0.4019
C20:3	0.08	0.10	0.08	0.01	0.4019
C20:4	0.00	0.00	0.00	ND	ND
C22:1	0.02	0.02	0.01	0.003	0.4019
C24:0	0.04	0.04	0.04	0.003	0.4019
C24:1	0.00	0.00	0.00	ND	ND
Other	0.01	0.01	0.07	0.01	0.0414
SFA	23.79[c]	22.29[b]	20.32[a]	0.28	<0.0001
MUFA	27.17[b]	26.31[ab]	25.62[a]	0.34	0.0292
PUFA	48.92[a]	51.26[b]	53.89[c]	0.25	<0.0001
UFA‡	76.08[a]	77.57[b]	79.51[c]	0.28	<0.0001
UFA/SFA	3.20[a]	3.49[b]	3.93[c]	0.06	<0.0001

[a,b,c]Means within a row with different superscripts differ ($P < 0.05$).
*Expressed as mean of six samples.
†Expressed as number of carbons: number of double bonds.
‡Unsaturated fatty acids.

CHAPTER 4

FEEDING TWENTY PERCENT REDUCED-FAT DRIED DISTILLERS GRAINS WITH SOLUBLES TO LACTATING HOLSTEIN DAIRY COWS DOES NOT AFFECT FEED EFFICIENCY BUT MAY IMPROVE PROTEIN UTILIZATION

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Abstract

Thirty-five multiparous lactating Holstein dairy cows (body weight 680 ± 11 kg, 52.25 ± 27.34 DIM) were fed one of two dietary treatments in a 2 × 2 crossover design. Cows were assigned randomly to treatment sequence one or two and were housed in a 48-cow free stall pen equipped with Calan gates to record individual feed intake. Treatment diet one (**control**) was a standard corn/corn silage/hay diet supplemented with Soy-Plus. 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 Soy Plus. The RF-DDGS diet was additionally supplemented with ruminally-protected lysine to ensure diets contained similar concentrations of limiting amino acids. Diets were formulated to be isoenergetic and isonitrogenous and were fed to allow for approximately 10% refusal rate. Cows were allowed *ad libitum* access to feed and water, fed twice daily, and milked three times daily.

There were no significant treatment effects on milk composition with the exception of increased protein percentage (3.11 vs 3.01 %, RF-DDGS vs control, respectively) and

decreased milk urea nitrogen (12.99 vs 14.18 mg/dL, RF-DDGS vs control, respectively). Additionally, DMI and milk yield (including ECM and FCM yields) were unaffected by treatment. Consequently, there was no treatment effect on any measures of feed efficiency. Finally, there were no significant effects on body weight change, rumen fluid pH, or blood NEFA and glucose.

These results indicate that RF-DDGS can be utilized in the rations of lactating Holstein dairy cows at a 20% inclusion rate (DM basis) without negatively influencing production parameters or the physiology of the cow when ruminally protected lysine is supplemented. Additionally, cows fed RF-DDGS produced milk with greater protein percentage and decreased milk urea nitrogen, indicating that protein utilization may be improved when cows are fed RF-DDGS with lysine.

Key words: milk fat, protein utilization, co-product

Introduction

Feeding of traditional, or full-fat, corn dried and wet distillers grains with solubles (**DGs**) to ruminant animals has been studied exhaustively and have been reviewed thoroughly (Schingoethe et al., 2009; Klopfenstein et al., 2008). 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, either positively or negatively, or yield when DGs are fed (Kleinschmit et al., 2006; Abdelqader et al., 2009). One problem with feeding traditional DG is that they contain approximately 13% fat, which is composed of mainly unsaturated fatty acids. This problem is two-fold

because inclusion of DGs can result in diets that contain concentrations of fat that exceed five percent, which can inhibit fiber digestion (Zinn, 1989). The second problem that can arise relates to the high 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 is a highly reducing environment. Incomplete biohydrogenation, however, can result in the production of bioactive forms of conjugated linoleic acid (*trans*-10 *cis*-12 CLA) which act to inhibit *de novo* lipogenesis in the mammary (Baumgard et al., 2001). Both aforementioned problems can result in milk fat depression, characterized by decreased milk fat without concomitant alteration in concentrations of other milk components (Bauman and Griinari, 2001). However, with the relatively recent improvements in oil extraction from DGs (Majoni et al., 2011) and the economic value of the corn oil extracted, it is highly unlikely that traditional DGs will be 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 reduced 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 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). With the ever-increasing prevalence in the market place of RF-DDGS and the relatively recent emergence of RF-DDGS as a commonly available feedstuff, further research is warranted. We therefore hypothesized that lactating Holstein dairy cows could effectively

be fed RF-DDGS at a 20% (DM) inclusion rate in place of Soy Plus without negatively influencing feed efficiency or milk composition; Therefore, our objectives were to test the effects of feeding RF-DDGS to lactating Holstein dairy cows on both milk composition and feed efficiency

Materials and methods

Animals and diets

All procedures were approved by the Iowa State University Animal Care and Use Committee (IACUC). Thirty-five multiparous mid-lactation Holstein dairy cows were assigned to one of two dietary treatment groups in a 2 × 2 crossover design. Each experimental period lasted 35 days with the first 14-days 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 standard corn/corn silage/hay based ration supplemented with soybean meal 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 a RF-DDGS (Poet Biorefining, Jewell, IA) containing approximately 6.0% fat in place of SoyPlus (Dairy Nutrition Plus, Des Moines, IA) (Table 1). The RF-DDGS ration was supplemented with ruminally-protected lysine to make diets similar in available limiting amino acids (Table 1). Cows were fed individually by using Calan gates (American Calan, Northwood, New Hampshire), allowing for measurement of individual feed intake. Cows were housed at the Iowa State University Dairy Farm (Ames, IA) together in a 48-cow, free-stall pen and individually fed twice daily (0800 h and 1600 h) to allow for approximately 10% refusal. Feed ingredients in a 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 (American Calan Inc, Northwood, NH) 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-days of each experimental period were an acclimation period those data were excluded from analyses. Feed samples were collected three times per experimental 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, and finally dry matter was determined by using NFTA Method 2.1.4. After the 14-day acclimation period, individual milk samples were collected weekly for all three milkings to represent a 24-hour lactation period. Individual milk samples (three from each test day) then were sent for proximate analyses and assay of milk urea nitrogen and somatic cell count (Performed by using official methods at 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 then was assayed by following the manufacturer's protocols (Wako Autokit Glucose, Wako Diagnostics, Richmond, VA). Blood NEFA were quantified following manufacture protocol (Wako HR Series NEFA-HR, Wako Diagnostics, Richmond, VA). Rumen fluid was collected via esophageal tube approximately four-hours post feeding when maximal VFA production was most likely to occur. The first few hundred milliliters of rumen fluid were discarded to limit salivary contamination. The next volume of rumen fluid then was strained through cheese cloth and frozen at -20°C. Rumen pH was measured immediately on-farm after collection.

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(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.

Results and discussion

Ration formulations are reported in Table 1. As designed, diets were isoenergetic and isonitrogenous (Table 2). Additionally, total digestible nutrients (**TDN**) did not vary between diets. Feed fatty acids are reported in Table 3, and, as expected, the RF-DDGS diet contained greater concentrations of total unsaturated fatty acids because corn oil is 92% unsaturated fatty acids (Ramos et al., 2009). However, significant differences in phosphorus, sulfur, and calcium resulted in a significantly lower dietary cation anion difference (**DCAD**) in RF-DDGS rations. Previous studies have indicated that increased DCAD values can increase milk fat percentage (Wildman et al., 2007; Hu et al., 2007; Harrison et al., 2012); this result, however, was not seen in our study which is consistent with results reported by Erdman et al. (2011) (Table 4).

Dry matter intake (**DMI**) did not significantly vary between treatment groups, nor did milk yield, fat corrected milk yield (**FCM**), or energy-corrected milk yield (**ECM**) differ between treatment (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 (Table 4) which is consistent with results of Paz and Kononoff (2014) and Mjoun et al. (2010). Milk protein percentage was, however, significantly increased, and milk urea nitrogen **(MUN)** was significantly decreased when cows were fed RF-DDGS (Table 4; consistent with Mjoun et al., 2010), indicating 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 (Ramirez-Ramirez et al., 2016; Castillo-Lopez et al., 2014; and Mjoun et al., 2010). Additionally, all metrics of feed efficiency were unaffected, which is consistent with prior research that saw no change or improved feed efficiency when cows were fed RF-DDGS (Mjoun et al., 2010).

No difference in body weight change was seen for either treatment group which was reported by Mjoun et al. (2010) as well (Table 5). In addition, no difference in rumen pH was observed, which is consistent with results reported by Castillo-Lopez et al. (2014). In contrast with the results of Mjoun et al (2010), we saw no difference in NEFA concentration, which is likely because the cows used in that study were early lactation whereas ours were in mid-lactation. Finally, consistent with Mjoun et al. (2010), blood glucose concentrations were not different between treatments.

Conclusions

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 milk composition, feed efficiency, or measured blood markers of energy balance. These results indicate promising prospects for the utilization of RF-DDGS, which are often more economical than more expensive 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 with soybean-based protein.

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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
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. 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	0.1246
Dry Matter, %	56.90	59.17	0.956	0.1246
Crude Protein, % DM	18.09	17.86	0.213	0.4464
ADF, % DM	21.37	20.52	0.678	0.3973
aNDF, % DM	28.78	29.35	0.757	0.6019
aNDF, % OM	27.93	28.20	0.776	0.8071
Lignin, % DM	4.01	4.01	0.181	0.9848
Lignin, % NDF	14.34	14.22	0.387	0.8356
AD-ICP, % DM	0.93	1.02	0.070	0.2275
ND-ICP, % DM	2.90	2.90	0.033	0.4344
Fat, % DM	4.80	5.21	0.130	0.0520
Ash, % DM	8.60	8.20	0.170	0.1171
Calcium, % DM	1.16	0.94	0.052	0.0144
Phosphorus, % DM	0.34	0.44	0.013	0.0003
Magnesium, % DM	0.26	0.24	0.016	0.5672
Potassium, % DM	1.96	1.81	0.051	0.0685
Sulfur, %DM	0.25	0.36	0.010	<0.0001
Sodium, % DM	0.56	0.51	0.016	0.0704
Chloride, % DM	0.63	0.63	0.022	0.8733
DCAD, mEq/100g	40.81	27.89	1.766	0.0004
TDN, 1 × %DM	71.56	72.09	0.505	0.4808
NE_L, Mcal/kg	1.64	1.65	0.012	0.4788

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	0.6107
C14:1	0.00	0.00	NA	NA
C16:0	20.27	19.20	0.299	0.0293
C16:1	0.37	0.28	0.017	0.0058
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	0.0037
C18:3	5.71	4.21	0.147	<0.0001
C19:0, C20:0	0.58	0.60	0.012	0.2215
C20:1	0.27	0.30	0.008	0.0071
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	0.0034
MUFA	19.31	21.14	0.200	<0.0001
PUFA	55.73	55.63	0.252	0.7985

[1]Reduced-fat dried distillers grains with solubles
[2]Total unsaturated fatty acids

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, 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
Somatic cell count	232.57	287.22	168.84	0.718
Milk urea nitrogen, mg/dL	14.18	12.99	0.285	<0.0001
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)

Table 5. Effects of feeding RF-DDGS to dairy cows on body weight, rumen fluid pH, and blood components.

	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

CHAPTER 5

DRIED DISTILLERS GRAINS WITH SOLUBLES AFFECTS COMPOSITION BUT NOT OXIDATIVE STABILITY OF MILK

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Interpretive summary:

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Feeding dried distillers grains with solubles (DDGS) to dairy cows increases the concentration of unsaturated fatty acids (UFA) in milk, potentially making milk more susceptible to oxidation. Despite increased UFA, decreased milk fat, and increased protein and SNF, feeding DDGS did not result in practically significant development of off-flavors in milk stored at 4 degrees Celsius for up to seven days. Feeding DDGS resulted in production of milk with off-flavor characteristics that were low and likely of no concern to consumers.

Abstract

Feeding lactating dairy cows dried distillers grains with solubles (DDGS) increases the concentration of unsaturated fatty acids in the milk from those cows, potentially leading to increased susceptibility to development of off-flavors. Feeding DDGS has been loosely implicated to be a cause of development of spontaneous oxidative off-flavor in milk. We hypothesized that increased feeding of DDGS would accelerate development of off-flavors and that fortification with vitamin E (0.06% w/w) or vitamin C (0.06% w/w) would prevent spontaneous oxidative off-flavors. The objective of this research was to determine the effects of feeding DDGS to lactating dairy cows on several parameters of milk quality as determined by both chemical and sensory evaluations. Twenty-four healthy mid-lactation Holstein dairy cows were fed total mixed rations containing DDGS (0%, 10%, or 25% dry matter). Cows were blocked by parity and randomly assigned to one of two groups (12 cows each). Each group received all three treatments in a three-period Youden square design so that each cow served as her own control. Samples of milk from individual cows for proximate analysis and pooled milk for pasteurization and sensory analysis were collected on days 14, 21, and 28 of each experimental period. Pooled milk was assayed for peroxides and free fatty acids and evaluated by a trained sensory panel for the presence of seven off-flavors common to milk on days 1, 3, and 7. Feeding 25% DDGS caused a significant decrease in daily milk yield ($P < 0.0001$). Increased dietary inclusion of DDGS also caused a concomitant decrease in percentage of milk fat and increase in percentages of both solids non-fat and protein ($P < 0.0001$). Milk peroxides and free-fatty acids were almost all below the detection limit, and the few exceptions were not found in replicated analyses. Sensory

analysis revealed off-flavors only in milk from cows fed 0% DDGS when that milk was stored for seven days and when milk from cows fed 25% DDGS was fortified with 0.06% w/w vitamin C ($P < 0.05$). Those few detected off-flavor scores were less than 1.5 cm on a 15-cm line scale, indicating that the differences are not practically significant. Peroxide values support the findings by the sensory panel that both feeding DDGS at 10 and 25% and vitamin E and vitamin C fortification did not practically change the oxidative stability of milk. These results, taken together, indicate that feeding DDGS under our experimental conditions modified milk composition, but did not contribute to the development of off-flavors in milk.

KEY WORDS

oxidation, flavor, sensory, cow, milk fat depression

Introduction

In the early spring of 2009 and 2010, recurring consumer complaints about milk "going bad" in the Midwest drew the attention of farmers, processors, grocers, a major dairy cooperative, and educators. Experienced milk evaluators from those groups determined the primary off-flavor of the rejected milk to be "oxidized". Raw milk, initially good, "spontaneously" became oxidized within five days of milk collection. Spontaneous oxidation (**SO**) has been classified as oxidation that spontaneously happens within 48 hours of milking (Dunkley and Franke, 1967), and spontaneous oxidized flavor (**SOF**) refers to those flavors associated with SO. Investigations (i.e., grocery tours, plant tours, milk analyses, and feed analyses) led to the determination that SO and SOF originated from the cow rather than from other sources of oxidation (e.g., metal ions, light). Several factors have been implicated as the probable cause of SOF, but it is suspected that one primary source of SOF is from the diet of the cow (Government of Manitoba, Agriculture, Food, and Rural Initiatives, 2008).

Previous studies have found that feeding dried distillers grains with solubles (DDGS), a co-product of ethanol production, to dairy cows increases unsaturated fatty acids in milk (Schingoethe et al., 1999; Leonardi et al., 2005; Anderson et al., 2006). Because the corn oil in DDGS is typically more than 60% linoleic acid ($C_{18:2}$), feeding it should be expected to contribute to an increase in unsaturated fatty acids in milk (Schingoethe et al., 2009), despite the extensive biohydrogenation by rumen microbes (Jensen et al., 1991). Hence, it is reasonable to suspect that increased concentrations of unsaturated fatty acids in milk could lead to the development of oxidation in milk from cows fed DDGS (Fig. 1). In this study, we investigated how feeding lactating dairy cows

DDGS affects milk oxidative stability by testing milk with sensory and chemical tests. Much previous research has focused on the impact of feeding DDGS on production parameters (e.g., DMI, volatile ruminal short chain fatty acids, feed efficiency, milk production, milk and milk fat composition) (Schingoethe et al., 1999; Anderson et al., 2006; Schingoethe et al., 2009), but information is lacking in regards to milk sensory quality and/or susceptibility to oxidation from milk produced by cows fed DDGS. To our knowledge, this is the only study that evaluated the effect of feeding DDGS to lactating dairy cows on the milk quality as evaluated by a trained descriptive analysis panel as well as chemical analyses. We hypothesized that (1) greater DDGS inclusion in the ration of lactating dairy cows would contribute to the development of SOF in milk and that (2) vitamin E and vitamin C fortification of milk would limit development of SOF in milk.

Materials and methods

Experimental design, milk collection, and processing

All experimental protocols involving animal and human subjects were approved by the Iowa State University Institutional Animal Care and Use Committee and Institutional Review Board, respectively. Human subjects reviewed and signed an informed consent form prior to beginning the research. Animal protocols were additionally reviewed and approved by the Iowa State University Dairy Users Group prior to initiation. Two groups of 12 mid-lactation primaparous and multiparous Holstein dairy cows were fed diets containing three dietary concentrations of DDGS in a Youden square design. The three diets were formulated to be isoenergetic with 0% DDGS, 10% DDGS, and 25% DDGS as dietary DM (Table 1). Group 1 received the 0%, 10%, and

25% DDGS diet in periods 1, 2, and 3, respectively, whereas group 2 received 10%, 25%, and 0% in periods 1, 2, and 3, respectively. Thus, each cow received all three diets in different periods and served as her own control. The DDGS were produced from corn-based ethanol production by Lincolnway Energy LLC (Nevada, IA). To balance the energetic content of each diet the rations had to be altered considerably from the 0% DDGS diet to the 25% DDGS diet. Although the compositions of the treatment diets vary, a "no DDGS" diet was needed to ensure that these groups of cows did not produce milk that developed oxidized off-flavors on a typical lactation ration, thus allowing us to examine the effects of feeding DDGS. Nutritional compositions, as determined by near-infrared spectroscopy (NIR), of the rations and DDGS are presented in Table 2 (Dairyland Laboratories, Inc., Arcadia, WI). Initially, cows were allowed to acclimate to the diet for seven days. For the remainder of the experiment, the cows transitioned directly to the next treatment with the first 14 days being excluded from sampling and analysis to minimize carry-over effects. Each experimental period lasted for 28 days.

During each of three experimental periods, milk was collected from the morning milking 14, 21, and 28 days after the start of the period. On the same collection days, during the third milking of the day, milk was automatically sampled from each individual cow (Boumatic, Madison, WI) for proximate analysis, to represent one complete milking. On each collection day (first milking of the day), milk was collected from each treatment group and pooled within each treatment group. The pooled milk then was divided for three fortification treatments: no fortification (control), 0.06% (w/w) vitamin E fortification (tocopherol acetate)(Dairy House, Fenton, MO)), and 0.06% (w/w) vitamin C fortification (L-ascorbic acid, crystalline $\geq$98%) (Jianshan Pharmaceutical Co., LTD,

Jiansu, China). Vitamins were aseptically added to the raw milk. All six of the milks (three fortification milks from two groups) were high-temperature, short-time (HTST), continuous pasteurized at 74°C for 24 seconds with a UHT/HTST Lab Electric Model 25HV Hybrid pasteurizer (MicroThermics®, Raleigh, NC). Vitamin C has been shown to be stable in broccoli for at least 15 minutes at 90°C (Munyaka et al., 2010), and therefore, should remain stable through the HTST pasteurization employed in this experiment. The pasteurized milks were collected in commercial translucent plastic gallon milk jugs purchased from Anderson Erickson Dairy (Des Moines, IA). All of the gallon milk samples were labeled with a unique random 3-digit identification code and put into black opaque plastic bags to block light during transportation and storage. Milks were analyzed for the sensory off-flavors described in Table 3 and analyzed chemically (described later) for oxidation products after one, three, and seven days of storage at 3°C without light exposure.

Short-chain volatile fatty acid analysis of rumen fluid

Rumen fluid was collected prior to the start of the first experimental period as well as on the 24th day of each experimental period via an esophageal tube. The collected fluid was strained through cheesecloth to remove particulate matter. Sampling took place after the morning milking and an average of one hour after feeding. Rumen fluid was frozen at -20°C until analysis. Rumen fluids were thawed and then analyzed by using a Varian 3900 gas chromatograph (Varian Chromatography systems, Palo Alto, CA) equipped with a 30 m × 0.25 mm i.d. DB-FFAP column (Agilent Technologies, Santa Clara, CA) as described by Drewnoski et al. (2014).

Milk fatty acid composition

Total lipids were extracted from individual milk samples (5 mL) as described by Lin et al. (1995), dried under N_2 gas, and re-suspended in 5 mL chloroform (HPLC grade, Fisher Scientific, Fairlawn, NJ). Milk lipids in chloroform were stored frozen at -20°C until analysis. Lipids in chloroform were dried under N_2 gas, followed by the addition of 650 µL of re-distilled 1-butanol (ACS grade, Fisher Scientific, Fairlawn, NJ) and 100 µL of acetyl chloride (< 99% pure, Acros Organics, NJ) while vortexing**.** Samples were purged with N_2 gas and heated on a heating block at 60°C for 30 min. Samples then were cooled to room temperature, and 5 mL of 4% w/v K_2CO_3 and 1.5 mL (ACS grade, Fisher Scientific, Fairlawn, NJ**)** of hexane (HPLC grade, Fisher Scientific, Fairlawn, NJ**)** were added. Next, samples were centrifuged for 5 min at 1380 x *g* (Centrific model 228, Fisher Scientific, Fairlawn, NJ**)**. Following centrifugation, the hexane layer was transferred to a new tube and washed with 5 mL distilled H_2O while vortexing. Samples then were re-centrifuged and washed three additional times. Butyl esters were analyzed on a Hewlett-Packard 6890 (Avondale, PA) gas chromatograph equipped with a flame ionization detector, a 30-meter Supelco 2330 fused silica capillary column with a 0.25 mm i.d., 0.2 µm film thickness (Supelco, Bellefonte, PA), and a 3396A integrator. The oven and injector of the chromatograph were held at 250°C with the column at an initial temperature of 50°C with a hold time of 1.44 minutes, followed by a 5°C per minute ramp to 225°C. Commercially available external standards were used to identify peaks by retention time (GLC-74 and GLC-79, Nu-Chek Prep, Elysian, MN)**.** Pure $C_{5:0}$, $C_{11:0}$, and $C_{19:0}$ (Sigma-Aldrich, St. Louis, MO) were used as internal standards to calculate

response factors. $C_{5:0}$ was utilized to apply correction factors to fatty acids concentrations of $C_{4:0}$-$C_{8:0}$, $C_{11:0}$ for $C_{10:0}$-$C_{14:0}$, and $C_{19:0}$ for fatty acids longer than $C_{14:0}$.

Milk composition

Individual milk samples (30 to 40 mL at 22 ± 2°C) were analyzed for percent fat, SNF, and protein content by using the LactiCheck™-01 RapiRead Milk Analyzer (Page & Pedersen Intl. Ltd., Hopkinton, MA). Fat %, SNF %, and protein % were measured in duplicate.

Sensory evaluation – descriptive analysis

Fourteen milk consumers over the age of 18, from Iowa State University, served as trained sensory panelists. Ten people were selected as the official panelists, and four served as backup panelists for the study in case of absence. The panelists received a total of eight hrs of training, which consisted of two one-hour training sessions per week over a four-week period. Two additional one-hour review sessions were conducted between experimental periods. Seven common milk-related off-flavors (bitter, cooked, feed, flat, foreign, light-oxidized, and metal-oxidized) were chosen for sensory analysis. Panelists were taught both "light" and "metal" oxidized off-flavors because SOF is very similar to these two off-flavors. Attribute descriptions and training anchors are included in Table 3. The panelists were trained to score the intensity of the off-flavors on a 15-cm line scale. A "slight" off-flavor score was considered a score around 3 cm on the 15 cm line scale; a "definite" score was considered a score around 7.5 cm; a "pronounced" score was considered a score near to 13 cm.

During training, panelists were instructed to evaluate the milk with the following protocol: 1) pour milk samples into clean disposable 3 oz. plastic cups (Solo Cup

Company, Lake Forest, IL) and fill about 1/4 to 1/3 of the cup (about 30-45 mL of sample), 2) immediately cover the cup with one hand while holding the cup with the other hand to protect the milk sample from light and trap aromas inside the cup, 3) gently swirl the milk sample and use the heat of the hands to warm up the milk to release volatile compounds for about 5 seconds, 4) take a deep sniff of the milk sample when removing the hand from the cup, 5) take a generous sip of the milk, swirl it around the mouth, noting the flavors and sensations, and expectorate. Panelists were encouraged to breathe in fresh air through the mouth and then exhale through the nose to enhance the aromas in the sample retronasally. For the actual tasting sessions, the only difference in protocol was that panelists were seated in private booths, and immediately pre-poured samples were provided in 3 oz. cups labeled with randomly generated 3-digit numbers.

During training, pasteurized-homogenized fresh whole milk, in paperboard packaging (Anderson Erickson Dairy, Inc., Des Moines, IA) from a local grocery store was presented to the panelists for representation of "no defect" milk. For the actual tasting sessions the only difference involved inclusion of freshly-delivered non-homogenized whole milk (Hansen's Farm Fresh Dairy, Hudson, IA) as the "no defect" milk because the treatment milks also were not homogenized. Prior to serving, all gallon containers of milk were removed from the refrigerator, shielded from light, and left at room temperature for 30 minutes to take the chill off and enable mixing of cream layer. All gallon containers (one package per treatment per day) were inverted at least 5 times prior to pouring of individual samples into 3 oz cups for presentation to panelists. Sensory evaluations of milk samples were conducted and recorded by using Compusense® Five Release 5.4 (Compusense Inc.; Guelph, ON, CA) sensory software at

the Nutrition and Wellness Research Center Sensory Evaluation Unit at Iowa State University one day, three days and seven days after pasteurization. Each panelist had an individual booth (lit with incandescent bulb), laptop, and a unique registration code provided for evaluating milk samples. The computerized ballot had 15-cm line scales for each off-flavor. During the evaluation, panelists were provided with tap water and unsalted crackers as palate cleansers. Each panelist received samples sequentially (9 in total), in a randomized order, for each evaluation session and evaluated at his or her own pace. Panelists were not allowed to change the score once they had finished evaluating a sample.

Peroxide value and free fatty acid content of milk

Oxidative stability of the milk was determined by measuring peroxide value (**PV**). Free fatty acid (**FFA**) content was measured to indirectly evaluate milk for bacterial contamination. Peroxide and FFA contents were measured by using the SafTest™ system (MP Biomedicals, Solon, OH) with the PeroxySafe™ STD kit and FaSafe™ STD kit (MP Biomedicals, Solon, OH). Milk from the same source as those samples evaluated by the sensory panel were used for SafTest™ analyses. Milk analyses were conducted following the PeroxySafe™ STD kit protocol and FaSafe™ STD kit protocol (MP Biomedicals, Solon, OH). Peroxide value and FFA concentration were assayed in duplicate.

Statistical analysis

Twenty-four cows assigned to two groups of 12 cows each were used in the study; however, only 19 cows completed all three treatment periods because five were removed for illness (e.g., mastitis). Thus, only 19 cows were included in the final data analysis.

The experimental design was a Youden square. Groups were blocked by parity, and days in milk was made to be approximately equal. All data analysis was performed in SAS 9.3 (Cary, NC).

Fat%, SNF%, and protein% of the milk from all three dietary treatments were analyzed by using a one-way ANOVA. Sensory data analysis of the mean scores of each flavor attribute from 10 panelists (18 observations per treatment) was performed by using a MIXED model with five fixed effects (dietary treatment, collection day, fortification, storage day, experiment period, treatment × storage day, and treatment × fortification) and a random effect of group nested within period.

Milk yield and fatty acid composition data were analyzed by using a MIXED model with repeated measures. For the milk yield analysis, the fixed effects were treatment and parity, the covariate was DIM, group was included as a random effect to account for treatment sequence and pen variation, and the repeated statement included the subject cow nested within treatment × period.

Data for volatile ruminal short-chain fatty acids (**SCFA**) were analyzed by using a MIXED model that included the fixed effects parity and treatment and the covariate days in milk as well as the random effects of cow nested within period and group.

All means were separated by the least squares means (LSMEANS) command of SAS with a Tukey-Kramer multiple pairwise comparison adjustment, and statistical significance was declared at $P < 0.05$.

Results and discussion

Milk composition

Milk composition was analyzed nine times for each individual cow in the study. Results are presented as the grand mean from the nine analyses of fat, protein, and SNF percentage for all cows over the three collection days for each dietary treatment within a period (Table 4). Milk protein and SNF concentrations increased concomitantly ($P < 0.05$) with increased dietary inclusion of DDGS. Differences in milk protein percentage between control (no DDGS) and DDGS-fed cows are not always observed (Nichols et al., 1998; Leonardi et al., 2005; Anderson et al., 2006; and Janicek et al., 2008). Powers et al. (1995) observed that milk protein concentration and SNF increased when cows were fed high quality DDGS, and, consequently, it was suggested that decreased milk protein content and SNF from cows fed DDGS could be an indicator of poor quality DDGS. The results and speculation by Powers et al. (1995) could explain why we found a concomitant increase in milk SNF and protein when dietary DDGS inclusion was increased when others did not, though it is difficult to test this explanation because "good quality" is defined poorly.

Milk fat concentration of the control diet milk (3.17%) was greater than that of the 10% DDGS diet milk (2.89%; $P = 0.0275$), and feeding 25% DDGS caused even further milk fat depression (2.60%; $P < 0.0109$) (Table 4). DDGS have about 11% lipid content on a DM basis (Hoffman and Baker, 2010) and are considered a good source of energy for dairy cows (Schingoethe et al., 2009). The primary purpose of feeding fats and oils to dairy cows is to provide higher energy intake to increase milk yield; however, dietary lipid supplements also could affect the concentration of fat and the fatty acid

composition in milk (Sutton, 1989) as well as inhibit fiber digestion (Ikwuegbu and Sutton, 1982). Typically, dairy nutritionists advise limited dietary inclusion of DDGS (<20%; Anderson et al., 2006) because the increased feeding of unsaturated dietary fatty acids could cause milk fat depression. Indeed, the present study confirmed this response.

The decrease in fat content has been attributed to the effect of dietary lipids on ruminal fermentation (Kononoff, 2006), the suppression of *de novo* synthesis of fatty acids in the mammary gland (Offer et al., 1999), and insufficient forage fiber in the diet (Kalscheur et al., 2012). One possible reason for the observed milk fat depression in milk from cows fed DDGS is a result from incomplete biohydrogenation of $C_{18:2}$, resulting in production of a conjugated linoleic acid ($C_{18:2}$ *trans*-10 *cis*-12) that inhibits *de novo* milk fat synthesis in the mammary gland (Baumgard et al., 2001). A study by Leonardi et al. (2005) found milk fat concentration was decreased significantly by the dietary inclusions of up to 15% DDGS. In contrast, Anderson et al. (2006) found no differences in milk fat concentrations among all diets (0%, 10%, and 20% DDGS), but they did observe a tendency for increased feeding of DDGS to lactating dairy cows to decrease milk fat content. Similar results were found by Sasikala-Appukuttan et al. (2008). A meta-analysis done by Kalscheur (2005) observed that milk fat depression only occurred when cows were fed DDGS in rations that contained less than 22% forage NDF and 50% total forage. Our diets contained more than 32% NDF and more than 54% forage indicating that percentage forage and NDF are not enough to explain the conflicting observations of the relationship of the feeding of DDGS and milk fat depression. A second possible reason for milk fat depression in cows fed DDGS has been speculated to result from the low amount of "effective fiber" in DDGS, meaning the particles are small and therefore

are not as effective of a forage source as the forage that the DDGS replace (Kalscheur et al., 2012).

Milk fat percentage from cows fed the control diet was lower than expected for Holsteins that usually produce milk with approximately 3.5% fat (Walstra and Jenness, 1984; Overman et al., 1939). Heat stress may have caused some milk fat depression (Sasikala-Appukuttan et al, 2008) because the current study was conducted from July (mid-summer) through October (early fall).

Ruminal short chain fatty acids

In the present study, we found no differences in the concentrations of the SCFA in ruminal fluid except for concentrations of butyrate and isovalerate (Table 5). Variability in the measured concentrations of SCFA and a lack of consistent trend, however, indicate that the few differences are not related to treatment. Our results are consistent with other studies that found feeding DDGS influenced ruminal isovalerate and valerate with no apparent trend emerging (Kleinschmit et al., 2007). In another study, feeding both wet and dried distillers grains with solubles only caused changes in isovalerate concentrations (Anderson et al., 2006). On the basis of research by us and others, DDGS do not seem to affect ruminal SCFA concentrations in a meaningful way, and importantly, DDGS do not affect acetate to propionate ratios.

Milk fatty acid profile

Feeding lactating dairy cows the 25% DDGS diet significantly increased ($P < 0.05$) stearic ($C_{18:0}$), oleic ($C_{18:1}$), and linoleic ($C_{18:2}$) concentrations in milk (Table 6). These results were to be expected because they have been reported in many studies (Schingoethe et al., 1999; Leonardi et al., 2005; Anderson et al., 2006; Sasikala-

Appukuttan et al., 2008). The reason that feeding DDGS increases unsaturated fatty acid content in milk is because corn oil contains greater than 60% $C_{18:2}$, some of which escapes the rumen without any biohydrogenation or with incomplete biohydrogenation, thereby contributing to increases in the unsaturated fatty acids found in the milk (particularly $C_{18:1}$ and $C_{18:2}$). Concentrations of no other fatty acids in milk were affected by feeding DDGS ($P > 0.05$).

Sensory evaluation

We hypothesized that fortification of milk with antioxidants such as vitamin E or vitamin C is a potential way to limit development of SOF (Figure 1). Sensory properties of non-fortified (control) milk did not differ from milk fortified with tocopherol ($P > 0.4$; Table 8). In this experiment, however, vitamin C fortification resulted in the greatest metal-oxidized off-flavor score of all milks when cows were fed dietary rations containing 25% DDGS. In all other cases, antioxidant fortification had no effect on off-flavors detected by the sensory panel. As with other differences in off-flavors, all mean scores were low, suggesting no practical impact of antioxidant fortification upon milk flavor for up to seven days of storage without exposure to light.

Vitamin C fortification, unexpectedly, seemed to decrease the oxidative stability (on the basis of metal-oxidized off-flavors) of milk enough that trained panelists could notice. Under certain conditions, vitamin C can act as a pro-oxidant by regenerating the perferryl radical at the initiation step of lipid oxidation (Lindmark-Månsson and Åkesson, 2000). Haase and Dunkley (1969) reported that vitamin C has pro-oxidant properties because it is able to catalyze the oxidation of linoleic acid (Haase and Dunkley, 1969) and can convert metal ions (e.g., iron, copper, manganese) to a more reactive ionic form.

However, it was reported that abomasal infusion of iron and copper salts had no effect on the concentration of iron and copper ions in milk and did not result in differences in off-flavors detected by a trained sensory panel (Mann et al., 2013). Moreover, the 25% DDGS diet actually contained the lowest concentration of iron, with other pro-oxidant metals having an inconsistent trend (Table 2). However, because a principal role of vitamin C is to reduce the metal core of metalloproteins (e.g., oxidases) in the reduced state, and because milk from cows fed 25% DDGS had the highest protein content, it is possible that oxidases that survive pasteurization and can produce hydrogen peroxide (e.g., xanthine oxidase; Claeys et al. 2013) were in greater concentration in milk from cows fed 25% DDGS and were the mechanism through which vitamin C acted as a pro-oxidant when fortified in combination with milk from cows fed 25% DDGS. Additionally, xanthine oxidase activity has been shown to be preserved by feeding supplemental vitamins C and E in combination with L-arginine to broiler chickens (Bautista-Ortega et al., 2014), providing further evidence that xanthine oxidase, in combination with vitamin C, could promote the development of off-flavors.

Vitamin C is an antioxidant, however, Van Aardt et al. (2005) reported that vitamin C fortification in milk at 0.05% concentration can have a negative sensory impact on milk flavor. Kim (2012) reported that vitamin C showed pro-oxidant properties at 0.02% (w/v) concentration in oil-in-water emulsions, such as milk, while tocopherol had strong antioxidant capacity. Additionally, Barrefors et al. (1995) observed higher $C_{18:2}$ and $C_{18:3}$ contents, lower tocopherol contents, and higher vitamin C content in milk with SOF (Barrefors et al., 1995). Increased tocopherol content can improve the resistance of milk fat to oxidation (Charmley and Nicholson, 1993; Focant et al., 1998, Nicholson and

St-Laurent, 1991). Other studies, however, reported that increasing tocopherol concentration in milk by fortification was ineffective in controlling oxidized flavor in milk (Charmley and Nicholson, 1994; Van Aardt, 2005). The conflicting results would suggest that antioxidant fortification to prevent development of SOF is not as simple as it would seem, possibly owing to other factors in the milk composition and/or processing. Bitter and cooked off-flavors were the only off-flavors significantly affected by storage time, and those flavor defects were only detected in milk from cows that were fed the control diet (Table 7). Still, low mean scores limit the practical significance of the findings. Spontaneous oxidation was expected to manifest within seven days of storage (Timmons et al. 2001), which is why milk quality was not evaluated beyond day seven. These findings imply that these milk samples had sufficiently low-intensity off-flavor scores that they would likely be of little concern to most consumers for at least seven days. This milk was handled with great care to avoid exposure to light, and a fresh jug was opened on each day of sensory analysis.

Although significantly higher off-flavor scores were noted for milk from the 25% DDGS diet when milk was fortified with vitamin C (metal oxidized) and on the seventh day of storage for milk from cows fed the control diet (bitter), all of the off-flavor sensory scores were lower than 1.5 on a 15 cm line scale. So, practically speaking, no real apparent oxidized off-flavors were detected in any of the milk samples and DDGS did not contribute to a decrease in milk oxidative stability (Tables 7 and 8).

Peroxide value and free fatty acid content

The PV for all milk samples were lower than 0.5 meq peroxides/kg (data not shown), which was lower than the 0.7 meq/kg PV reported in a previous study (Let et al.,

2005) for non-oxidized milk. Low amounts of FFA were detected (less than 1.05% oleic acid, data not shown) in several milk samples from the first period, but those results were not reproduced in the second and third period milks because all were below the detection limits. Strong correlations between concentrations of one of the main lipid oxidation products (lipid hydroperoxides) and the oxidized flavor in sensory analyses has been reported (Hedegaard et al., 2006). In the current study, the chemical analysis results support the sensory evaluation; no primary lipid oxidation products (i.e., peroxides) were detected by quantification of PV, likely because sensory analysis is able to distinguish small differences in oxidized flavor in milk undetectable by chemical analysis of PV (Let et al., 2005). Because we did not detect peroxides in any of the milk samples, one of two explanations are possible: 1) there was no lipid oxidation or 2) all the peroxides formed had decomposed into secondary lipid oxidation products (e.g., aldehydes and ketones). If the second scenario was true, the sensory panel would have detected much higher oxidized off-flavors in the milk samples. No practically significant oxidation, however, was detected by the sensory panel. Therefore, the PV and sensory results were sufficient to support our finding that milk from cows fed DDGS up to 25% DM under these experimental conditions would likely be acceptable to the consumer.

As mentioned previously, feeding of 10 and 25% DDGS increased long-chain unsaturated fatty acids, $C_{18:1}$ and $C_{18:2}$ in milk (Table 6). These increases, however, did not cause any increase in PV (all less than 0.5 meq/kg), data not shown) in any of the milk samples, nor was any practically significant oxidized flavor detected by the trained sensory panel (Tables 7 and 8). A similar situation was observed in a previous study where greater concentrations of $C_{18:1}$ in milk did not result in a higher concentration of

lipid hydroperoxides, but the elevated concentration of PUFA did (Havemose et al., 2006). And in a second study, Liu et al. (2010) observed decreased oxidative stability in milk with increased ω-3 PUFA content. In our study, we did not detect any differences in $C_{18:3}$ content in milk from the three dietary treatments, potentially because $C_{18:3}$ and $C_{20:0}$ co-eluted. Havemose et al. (2004) reported that milk with lower $C_{18:1}$ and $C_{18:2,}$ but higher $C_{18:3}$ had significantly higher content of lipid hydroperoxides. Additionally, the development of SOF in milk has been related to the higher concentration of PUFA in milk fat (Timmons et al., 2001), suggesting that $C_{18:3}$ content could possibly be an indicator of the oxidative stability in milk. Timmons et al. (2001), however, suggested that PUFA alone is not always sufficient to promote the development of SOF in milk, which supported the results in the present study and those of Fearon et al. (2004).

Thermal treatment of milk may possibly increase the antioxidant capacity in milk because of protein unfolding and exposure of thiol groups (Taylor and Richardson, 1980; Tong et al., 2000). Heat pasteurization could be one of the factors explaining why no practically significant oxidation was found by either the trained sensory panel or by chemical analyses. Milk samples were pasteurized within 4 hours after collection from the farm, which is shorter than common industry practice. Milk lipid oxidation is a complicated process, because fatty acid composition and antioxidants are only two aspects of the many factors that could influence milk oxidative stability. Pro-oxidative factors also can affect to the oxidative stability of milk (e.g., riboflavin). Clearly, lipid oxidation in milk is a complex interplay of pro-oxidants and antioxidants (Lindmark-Månsson and Åkesson, 2000).); the balance between pro-oxidants and antioxidants is a critical factor for the oxidative stability of milk (Stapelfeldt et al., 1999; Morales et al.,

2000). Granelli et al. (1998) related the development of SOF in milk with the ratios between antioxidants and polyunsaturated fatty acids (PUFA) and found that milk samples without SOF tended to have higher antioxidant to PUFA ratios than the milk samples with SOF (Granelli et al., 1998). Genetics of cows has been mentioned as an influence on the occurrence of SOF in milk (Juhlin et al., 2010b). Additionally, Juhlin et al. (2010a) found that copper ion concentration in milk from first lactation cows (one to three weeks postpartum) was significantly linked to development of SOF. In our study, we used both primparous and multiparous mid-lactation dairy cows; so, no direct comparison can be made. Clearly, a number of factors have been implicated in the development of SOF in milk; so, the cause is likely not a simple one but rather a multifaceted problem.

The DDGS used in this study was not responsible for SOF in milk. Additionally, because our cows were mid-lactation and were primiparous and multiparous rather than fresh first-lactation cows, we cannot discount SOF that may appear if this experiment was repeated by using first-lactation transition cows, which produce milk with more copper ions (Juhlin et al., 2010a). Future studies, however, would need to be done to address the question of the occurrence of SOF in early lactation.

Conclusion

Feeding 10 and 25% DDGS (DM basis) in a total mixed ration to lactating dairy cows resulted in significant milk fat depression and concomitant increase of SNF and protein content of milk. Milk fatty acid profile was altered by dietary inclusion of DDGS. As expected, $C_{18:0}$ and long-chain unsaturated fatty acids ($C_{18:1}$ and $C_{18:2}$) increased in milk from cows fed DDGS diets. Ruminal SCFAs were not affected by treatment, with

the exception of butyrate and isovalerate. A trained sensory panel did detect statistically significant off-flavors in milk from cows fed DDGS, but the low intensity of those defects was of little practical significance. Very low peroxide values from assayed milk supported the results reported by our trained sensory panel. Because no apparent oxidation was detected in milk from cows fed DDGS, the explanation for spontaneous oxidation and SOF is still unclear. By using our experimental conditions and DDGS source, DDGS does not cause development of SOF in milk.

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Table 1. Ingredients of three treatment diets containing differing concentrations of dried distillers grains with solubles (DDGS) [1]

	Treatment		
Ingredient, % DM	0% DDGS	10% DDGS	25% DDGS
Corn silage	41.7	43.2	41.1
Alfalfa hay	13.1	13.9	13.2
Soybean meal (47%)	12.3	6.1	1.7
Cottonseed (whole)	8.5	9.0	8.5
Finely ground corn	1.6	8.6	3.3
DDGS	0.0	10.8	24.3
Lactation mix[2]	22.6	8.4	8.0

[1]All ingredients were mixed and fed as a total mixed ration.

[2]Lactation mix ingredients as fed, %: 73.9% ground rolled corn; 4.5 % Supercharger II (fat supplement; Origo, New Ulm, MN); 4.3% limestone; 3.6% blood meal; 3.2% sodium bicarbonate; 2.7% fine rock salt; 2.3% urea; 1.8% pork meat and bone meal; 1.2% monocalcium phosphate 21%; 0.9% magnesium oxide; 0.7% Metasmart (methionine supplement (Adisseo USA Incorporated, Anpharetta, GA)); 0.5% Dynamate (vitamin and trace mineral premix (Consumer's Supply Distributing Company, Sioux City, IA)), ; 0.5% Dairy Balancer II (vitamin and trace mineral premix (Nutritional Professionals Incorporated, Hortonville, WI)); 0.04% Rumensin 90.

Table 2. Composition of dried distillers grains with solubles (DDGS) and total mixed rations

		Total mixed rations[1]		
Component	DDGS	0%	10%	25%
Crude protein (CP), %	29.5	17.8	16.9	18.5
AD-ICP, % of CP[2]	N/A	6.7	7.0	6.5
Protein Sol., % of CP	17.0	28.8	30.4	26.2
Fat	12.1	5.9	5.5	7.2
aNDF, %[3]	28.8	32.5	32.7	33.9
Lignin, % of NDF[4]		10.4	10.5	10.0
Starch, %	N/A	24.8	24.7	19.7
ND-ICP, % of DM (est.)[5]	5.1	2.8	2.7	3.0
Ash, %	4.6	6.5	6.9	6.4
Calcium, %	0.1	0.8	0.6	0.6
Manganese, ppm	N/A	48.0	36.5	33.0
Zinc, ppm	N/A	79.5	59.5	72.5
Copper, ppm	N/A	19.5	15.5	18.0
Iron, ppm	N/A	241.5	212.0	207.0
T.D.N.[6] (OARDC)[7], %	85.8	73.1	72.3	73.4
N.F.C.[8], %	N/A	39.0	39.0	34.9
NE_L[9] (OARDC)[7], Mcal/kg	40.8	1.67	1.65	1.68

[1] Expressed as a percentage of dry matter
[2] Acid detergent insoluble crude protein
[3] Amylase-treated neutral detergent fiber
[4] Lignin % of neutral detergent fiber
[5] Neutral-detergent insoluble crude protein as a percent of dry matter estimated
[6] Total digestible nutrients
[7] A summative calculation based on an Ohio Agricultural Research and Development Center method (anapproach for energy evaluation)
[8] Non-fiber carbohydrate
[9] Net energy of lactation

Table 3. Descriptions and recipes* for seven off-flavors of milk evaluated by trained panelists.

Off-flavor	Descriptors	Recipes for creating "definite" level anchor for defect
Bitter	Aftertaste towards the back of the throat; piercing and throbbing	Prepare 0.5% quinine solution with deionized water. Add ½ tsp to 1L of pasteurized whole milk from paperboard.
Cooked	Eggy, sulfur, custard	Organic ultrapasteurized whole milk from paperboard.
Feed	Grassy, stalky, hay	Boil 1.5 L of tap water; add ½ cup of timothy/alfalfa hay; steep 5 min. Filter through coffee filter. Add 1.5 TBS to 1 L. pasteurized whole milk from paperboard.
Flat	Watered down, thinner mouth feel, less dairy fattiness	Lowfat (1%) pasteurized milk from paperboard.
Foreign	Bleach, sanitizer, chemical, and/or other atypical flavor for milk not included in the other categories	Prepare a proper chlorine or iodine sanitizer solution. Add 2 tsp to 1 L pasteurized whole milk from paperboard.
Light oxidized	Cardboard, pasty taste, mouth-drying sensation, smells like wet brown paper towel	Whole milk in translucent plastic exposed to at least 30 min of UV or fluorescent light.
Metal oxidized	Copper penny aroma/flavor, tingling sensation on tongue; some similar mouth-drying characteristics as light oxidized	Prepare 0.25% cupric sulfate solution with deionized water. Add ¼ tsp to 1 L pasteurized whole milk from paperboard. Allow to stand for 90 min. prior to tasting.

*Modified from Costello and Clark, 2009.

Table 4. Composition and yield of milk from cows fed 0, 10, and 25% dried distillers grains with solubles (DDGS) supplemented as total mixed ration[1]

Item	Treatment			*P*-Value
	0% DDGS[2]	10% DDGS[2]	25% DDGS[2]	
Fat, %	3.17 ± 0.10[c]	2.89 ± 0.10[b]	2.60 ± 0.09[a]	<0.0001
Protein, %	3.71 ± 0.03[a]	3.77 ± 0.02[b]	3.83 ± 0.02[c]	<0.0001
SNF, %	9.90 ± 0.07[a]	10.02 ± 0.06[b]	10.19 ± 0.06[c]	<0.0001
Yield, kg/day	33.40 ± 1.37[b]	32.90 ± 1.38[b]	30.62 ± 1.38[a]	<0.0001

[a, b, c] Means within a row with different superscripts differ (P <0.05)

[1]Mean value from 19 cows

[2]Expressed as percentage of dry matter

Table 5. Ruminal SCFA from lactating dairy cows fed 0, 10, and 25% dried distillers grains with solubles (DDGS) as a total mixed ration[1]

Measurement	Day 0	Treatment			*P*-value
		0% DDGS[2]	10% DDGS[2]	25% DDGS[2]	
SCFA, mol %					
Acetate (A)	62.97±0.88	61.99±1.01	63.00±0.92	60.94±1.00	0.278
Propionate (P)	23.00±0.62	24.21±0.68	23.19±0.66	23.67±0.69	0.406
Isobutyrate	0.86±0.06	0.87±0.07	0.85±0.06	0.96±0.07	0.476
Butyrate	10.11±0.56[ab]	9.90±0.60[a]	10.10±0.58[ab]	11.29±0.60[b]	0.036
Isovalerate	1.70±0.11	1.53±0.12	1.48±0.11	1.66±0.12	0.047
Valerate	1.34±0.08	1.36±0.09	1.37±0.09	1.38±0.09	0.961
Total, m*M*	73.11±4.08	79.17±4.68	72.83±4.25	68.08±4.60	0.152
A:P	2.78±0.10	2.58±0.12	2.77±0.11	2.64±0.12	0.358

[a, b] Means within a row with different superscripts differ after Tukey HSD multiple comparisons analysis ($P < 0.05$)

[1]Least squares means plus or minus the standard error of the mean

[2]Expressed as a percentage of dry matter

Table 6. Fatty acid composition of milk from cows fed dried distillers grains with solubles (DDGS)[1]

	Treatment			
Fatty acid, wt. %	0% DDGS[2]	10% DDGS[2]	25% DDGS[2]	*P*-Value
$C_{4:0}$	1.10±0.16	1.16±0.16	1.04±0.17	0.848
$C_{6:0}$	0.45±0.08	0.33±0.08	0.45±0.08	0.479
$C_{8:0}$	0.80±0.11	0.72±0.11	0.71±0.11	0.719
$C_{10:0}$	2.59±0.32	2.00±0.33	2.13±0.34	0.389
$C_{12:0}$	3.33±0.42	2.33±0.43	2.63±0.44	0.217
$C_{14:0}$	10.79±0.94	8.73±0.97	9.05±0.99	0.248
$C_{14:1}$	2.33±0.48	1.28±0.50	1.75±0.51	0.299
$C_{15:0}$	1.64±0.30	0.89±0.31	1.20±0.31	0.101
$C_{15:1}$	0.75±0.29	0.73±0.30	0.23±0.31	0.430
$C_{16:0}$	27.70±1.03	26.45±1.06	25.58±1.09	0.374
$C_{16:1}$	1.66±0.51	1.70±0.52	2.14±0.52	0.598
$C_{17:0}$	1.11±0.40	0.33±0.41	0.76±0.42	0.375
$C_{17:1}$	0.40±0.18	0.63±0.18	0.36±0.19	0.537
$C_{18:0}$	12.17±0.56^{a}	14.00±0.57^{ab}	14.27±0.59^{b}	0.019
$C_{18:1}$	28.56±1.42^{a}	33.52±1.46^{b}	34.37±1.51^{b}	0.010
$C_{18:2}$	2.87±0.15^{a}	3.45±0.16^{b}	3.52±0.16^{b}	0.006
$C_{18:3} + C_{20:0}$	1.38±0.10	1.52±0.10	1.50±0.10	0.518
$C_{22:0}$	0.40±0.05	0.37±0.05	0.35±0.05	0.819

[a, b] Means within a row with different superscripts differ ($P < 0.05$)

[1]Least squares means plus or minus the standard error of the mean

[2]Expressed as a percentage of dry matter

Table 7. Mean sensory scores[2] of milk from cows fed 0, 10, and 25% dried distillers grains with solubles (DDGS) and stored for 1, 3, and 7 days

	Treatment										
	0% DDGS[1]			10% DDGS[1]			25% DDGS[1]				
Flavor	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7	SEM	*P*-Value
Bitter, cm	0.12[b]	0.10[ab]	0.38[b]	0.18	0.19	0.13	0.08	0.08	0.12	0.06	0.032
Cooked, cm	0.80[b]	0.45[ab]	0.36[a]	0.51	0.55	0.39	0.34	0.51	0.50	0.10	0.017
Feed, cm	0.26	0.30	0.24	0.31	0.16	0.24	0.28	0.18	0.33	0.06	0.247
Flat, cm	0.56	0.63	0.32	0.74	0.54	0.73	0.69	0.61	0.54	0.11	0.210
Foreign, cm	0.96	0.55	1.03	1.03	0.95	0.93	1.03	0.95	0.85	0.22	0.248
Light-oxidized, cm	0.64	0.88	0.84	0.77	0.83	0.73	0.77	0.64	0.80	0.11	0.367
Metal-oxidized, cm	0.66	0.35	0.47	0.50	0.56	0.51	0.91	0.71	0.74	0.12	0.608

[a,b] Means within a treatment and row with different superscripts differ ($P < 0.05$)

[1] Expressed as a percentage of dry matter

[2] Obtained from panelist ratings on 15-cm line scales

Table 8. Mean sensory scores[2] of milk from cows fed 0, 10, and 25% dried distillers grains with solubles (DDGS) and fortified with vitamin E or vitamin C

	Treatment[1]										
	0% DDGS[1]			10% DDGS[1]			25% DDGS[1]				*P*-Value
Flavor	None	Vit. E	Vit. C	None	Vit. E	Vit. C	None	Vit. E	Vit. C	SEM	Trt×Fort
Bitter, cm	0.13	0.31	0.16	0.15	0.15	0.19	0.08	0.08	0.14	0.06	0.304
Cooked, cm	0.69	0.38	0.53	0.53	0.41	0.50	0.42	0.51	0.42	0.10	0.335
Feed, cm	0.25	0.27	0.28	0.24	0.24	0.22	0.24	0.30	0.26	0.06	0.976
Flat, cm	0.41	0.66	0.44	0.67	0.80	0.54	0.78	0.70	0.36	0.11	0.240
Foreign, cm	0.55	0.98	1.01	0.94	1.08	0.90	0.97	0.73	1.14	0.22	0.052
Light-oxidized, cm	0.72	0.75	0.90	0.84	0.57	0.93	0.65	0.61	0.95	0.11	0.547
Metal-oxidized, cm	0.27	0.54	0.67	0.44	0.49	0.65	0.51[a]	0.45[a]	1.40[b]	0.11	0.001

[a,b] Means within a treatment and row with different superscripts differ ($P < 0.05$)

[1] Expressed as a percentage of dry matter. Vitamin E and Vitamin C fortification at 0.06% w/w fortification

[2] Obtained from panelist ratings on 15-cm line scales

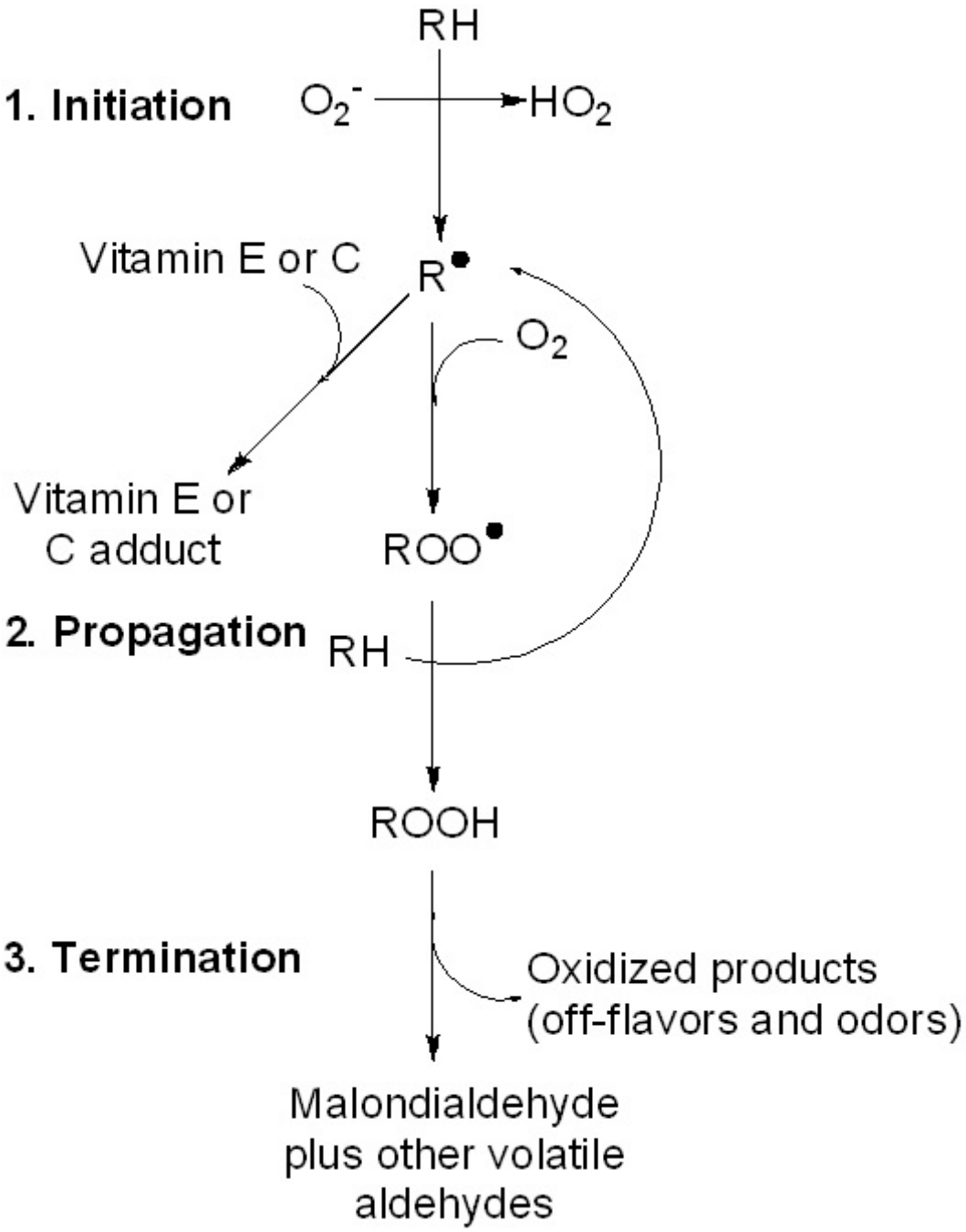

Figure 1. Illustration of oxidative off-flavor development and autoxidation of milk fat. Initiators of this chain reaction in milk include metals and UV light. Initiation of the reaction illustrated in this figure (1), followed by propagation of the reaction (2), and finally termination (3) of the chain reaction are described. The chain reaction will continue until the radical species is quenched by an antioxidant or by combination with another radical species.

CHAPTER 6: SHORT COMMUNICATION: FEEDING REDUCED-FAT DRIED DISTILLERS GRAINS WITH SOLUBLES TO LACTATING HOLSTEIN DAIRY COWS DOES NOT NEGATIVELY INFLUENCE QUALITY OF BABY SWISS CHEESE

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Abstract

Swiss type cheese quality is dependent on the formation of ideal eyes in the cheese. Late-blowing defects have a negative impact for both processors (sliceability is affected negatively) and consumers (who expect round glossy eyes). Late-blowing defects are, therefore, an economic issue. Feeding of dried distillers grains with solubles (DDGS) to lactating dairy cows has been implicated as a cause of late blowing defects but has limited support from scientific research. 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 high-quality baby Swiss cheese. Based on our previous research, we hypothesized that feeding 20% RF-DDGS to dairy cows would not result in adverse effects in milk composition or in quality of baby Swiss cheese produced from that milk. To test this hypothesis, 35 multiparous and lactating Holstein dairy cows were assigned randomly to

one of two dietary treatments in a 2 × 2 crossover design. Treatment one was a standard corn/corn silage/hay diet with Soy Plus, and treatment two was based on the same diet with the exception of 20% of RF-DDGS as DM in place of Soy Plus. Treatment diet two was supplemented with ruminally-protected lysine to ensure diets were isonitrogenous, isoenergetic, and contained similar limiting dietary amino acids. There were no treatment effects on milk yield (15.98 and 15.85 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). Milk protein percentage, however, was increased (3.01 and 3.11%) by feeding RF-DDGS 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 with ruminally-protected lysine. For cheese production, milk was collected and pooled six times for each dietary treatment. Regarding appearance, cheeses were overset, but the only significant treatment effect was in eye size, with the control cheese eyes being closer to ideal than the RF-DDGS cheese. These results indicate that RF-DDGS can effectively be fed to cows at a 20% inclusion rate (DM) without negatively influencing quality and suitability of milk for production of quality baby Swiss chesse.

Keywords: DDGS, late-blowing, eyes, sensory

Introduction

Late-blowing defects in Swiss type cheeses are unacceptable to both the producer and the consumer. Cheeses that exhibit the late-blowing defect cannot be sold at full price, cause problems for high-speed slicing operations, and, in general, result in an economic loss for the producer (White et al., 2003). 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). However, our research group investigated the effects of feeding full-fat DDGS to dairy cows on the quality of baby Swiss cheese and found no significant differences in the quality of baby Swiss cheese when cows were fed a conventional TMR or a TMR containing full-fat DDGS (~ 13% fat) (Sankarlal et al., 2015). Another concern that has been raised is the contamination of milk by *Clostridium tyributiricum* originating from DDGS. Our research team, however, found no *C. tyributiricum* in the DDGS fed to those cows whose milk was used for the production of that Swiss cheese, but our team did find gas-producing spores in non-DDGS feedstuffs, feces, milk and baby Swiss cheese (Manimanna Sankarlal et al., 2015).

Because of the economic value of corn oil and increased efficiency of the extraction of corn oil from DDGS, in the future, full-fat DDGS are not likely to be widely available. Therefore, our objective was to investigate the effects of feeding reduced-fat DDGS (RF-DDGS) (~ 6% fat) on the composition of milk produced and the quality of baby Swiss cheese produced from that milk compared with a conventional TMR. We hypothesized, based on our previous research, that feeding RF-DDGS to lactating

Holstein dairy cows would not adversely affect milk composition or quality of baby Swiss cheese made from the milk of those cows.

Materials and methods

All procedures were approved by the Iowa State University Animal Care and Use Committee (IACUC). Thirty-five multiparous lactating Holstein dairy cows were assigned to one of two dietary treatment groups. Rations were formulated to meet NRC requirements, to be isonitrogenous and isoenergetic (Chapter 4, Tables 1 and 2), 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 by using the same base ration as the control but with 20% of the dry matter being a RF-DDGS (Poet Biorefining, Jewell, IA) containing approximately 6% fat in place of SoyPlus (Dairy Nutrition Plus, Des Moines, IA) (Details reported in Testroet et al., 2017 (Chapter 4, Table 1). The RF-DDGS ration was supplemented with ruminally protected lysine to make diets similar in available limiting amino acids (Details reported in Testroet et al., 2017 (Chapter 4, Table 1). 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).

After a 14-day acclimation period, weekly individual milk samples were collected at the three milkings for proximate analyses and assay of milk urea nitrogen and somatic

cell count (Reported in Chapter 4, Table 3) (Performed by using official NIR methods at Dairy Lab Services, Dubuque, IA).

For cheese making, milk from one complete milking of each treatment group (control or RF-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. Milk was transported 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 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). 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 with gentle agitation of the milk.

Baby Swiss cheese was made by using 0.32g (± 0.03) CHOOZIT 60 (DuPont ™ Danisco®, New Century, KS) and 0.12g (± 0.02%) CHOOZIT eyes (DuPont ™ Danisco®) per 100 kg milk. 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 one minute. 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. 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). Whey was removed after postwork at a target pH of 6.4.

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. The pressing time was based on the time required for the pH of the cheese to drop from 6.4 to 5.25 (±0.05) (Accumet® Basic AB15, Fisher Scientific Inc, Pittsburgh, PA). The weights were removed, and cheese was fermented in an empty basin for an additional 5 to 8 hr at 22°C $\pm$ 3°C. 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 flipped weekly. Proximate analysis (conducted using standard methodology, at South Dakota State University) and sensory quality evaluation began after at least 60 days aging.

A descriptive sensory analysis panel, composed of six trained panelists, evaluated the quality of the cheeses. Panelists were recruited from students of the Department of Food Science and Human Nutrition (Ames, IA). Training consisted of at least 5 hours of initial training, followed by an additional hour of refresher training between the first and second official tasting period (which were separated by a month). Panelists were trained to evaluate baby Swiss cheese in relation to set quality standards, which served as references during training sessions. For the appearance attributes, photographs of ideal and atypical baby Swiss and Swiss cheeses were initially used to assist with training. To assist with eye size evaluation, panelists were provided a plastic standard hole-punched square to indicate ideal ("small") eyes and a penny to indicated "large" eyes (Figure 1).

To cement specific aroma, flavor and body and texture defects in the minds of the panelists, various cheeses exhibiting attributes and defects were utilized. The "Cheat Sheet" (Figure 2) was used to augment training. The words at specific locations on the 15 cm unstructured line scale indicate where panelists were trained with intensity references. During actual cheese evaluation sessions, panelists were provided one blank score sheet per sample (Figure 3). Panelists were expected to remember 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.

To prepare cheeses for evaluation by panelists, every cheese was systematically cut, manually with a sanitized butcher knife on a sanitized cutting board, into at least 20 pieces of approximately 1 cm thickness. The cutter began by making a 1 cm slice at the outside round of the cheese, and proceeded to make subsequent slices around the round, gradually forming a smaller and smaller square out of the cheese (Figure 4). Slices were laid out sequentially on sanitized, dry trays for photographing (Figures 5 – 8). When the length, width, and height of the cheese were nearly equal, the center-most piece of cheese was flipped vertically and the remaining pieces were cut, resulting in three to four horizontal slices representing the top and bottom "faces", and one to two inner-most slices. Based upon their sequential placement on trays, cheese slices were randomly selected for bagging and presentation to panelists by using a random number generator. A plastic template (3 cm X 2 cm) was placed on each randomly selected master slice to make a consistent "principal display" for panelist evaluation. Selected slices were placed into individual re-sealable snack bags, labeled with random 3-digit codes corresponding

to the original cheese from which they were cut, and stored at refrigeration temperature until the sensory panel.

For the tasting sessions, 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.

Milk components were analyzed as a 2 × 2 crossover design. Data were analyzed by using the mixed procedure of SAS 9.4 (Cary, NC). The model included the fixed effects of treatment, treatment sequence, and period and the random effect was cow (group). Means were separated by using LSMEANS with the PDIFF option. Sensory attributes of cheese were analyzed by using the mixed model of SAS with the model including the fixed effect of treatment. Means were separated by using LSMEANS with the PDIFF option.

Results and discussion

No differences were found in composition of milk from cows fed either diet, with the exception of milk protein percentage that increased significantly for cows fed RF-DDGS, without a concomitant change in total milk protein production, and a decrease in milk urea nitrogen for cows fed RF-DDGS. These findings may indicate an increased efficiency of protein extraction from feed (Chapter 4, Table 3). These results are consistent with Castillo-Lopez et al. (2014) who found that feeding cows RF-DDGS at similar inclusion rates 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. 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.

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. Baby Swiss 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 be somewhat resistant to initial compression between the thumb, forefinger, and middle finger (firm), but should break apart between fingers without crumbling or seeming too rubbery or dry (corky). Upon mastication, the texture should be smooth (not grainy or rough). Other than a slight bitter aftertaste, baby Swiss should clean up, leaving no fruity, fermented, rancid, yeasty or other foreign flavors on the palate.

Regarding appearance (Figures 5 – 8), the baby Swiss cheeses were slightly atypical. Compared with the ideal, the cheeses were characterized by a high number (overset) of very small to small eyes (0.01 to 0.3 cm in diameter) many of which were irregular in shape (including frog mouth, collapsed, and rarely cabbage), and the distribution was slightly uneven. Cheeses were not significantly different from control for any attribute except size of eyes (Table 2). Mean score for eye size of control cheeses were closer to ideal than DDGS cheeses ($P < 0.05$). Eyes exhibited the typical glossy appearance, but some exhibited a wet (free whey) appearance, suggesting incomplete

pressing. Although gas formation appeared normal in most cheeses, several cheeses exhibited checks, picks, and rarely, splts or blowholes. These results are consistent with our previous research (Manimanna Sankarlal et al., 2015), where feeding full-fat DDGS did not affect quality of baby Swiss cheesed produced when compared with feeding a conventional dairy ration to lactating Holstein dairy cows.

Conclusion

These results indicate that RF-DDGS can be fed without compromising the composition of milk, with the exception of increased protein percentage and decreased milk urea nitrogen. Additionally, there were no differences in suitability of milk used for baby Swiss cheese because there was no difference in quality of cheese produced from conventionally fed cow milk and milk from cows fed RF-DDGS.

Acknowledgements

We are grateful for the funding provided by the Minnesota Corn Growers Association for supporting this research. In addition, we are thankful to the Iowa State University Dairy Research Farm for their assistance in coordination and implementation of the logistics of performing the work described herein. Proximate analysis of cheese was conducted by students at South Dakota State University. We appreciate the many undergraduate assistants who provided labor and care of the research animals, sample collection, and cheese making. We would also like to thank Amber Testroet for her assistance in preparation of this manuscript. Finally, we acknowledge the time and expertise provided by our trained panelists.

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.

TABLES AND FIGURES

List of figures

Table 1. Effects of feeding RF-DDGS to lactating Holstein dairy cows on milk components and yield

	Treatment			
Item	**Control**	**RF-DDGS**	**SEM**	***P*-Value**
Milk yield, kg/day	15.98	15.85	0.504	0.700
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
Somatic cell count	232.57	287.22	168.84	0.718
Milk urea nitrogen, mg/dL	14.18	12.99	0.285	<0.0001

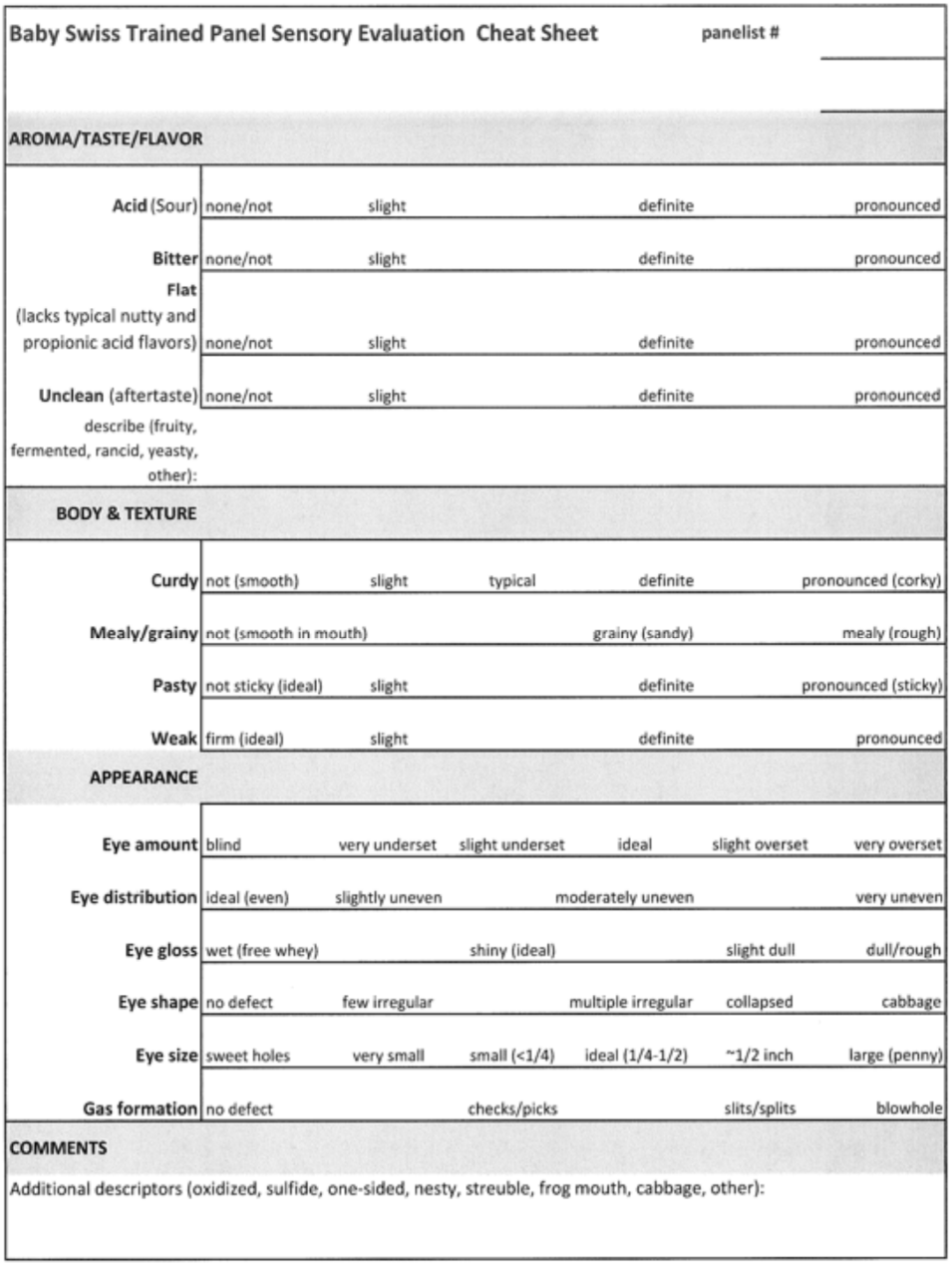
Table 2. Cheese flavor, body and texture, and appearance of eyes.[1]

	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	3.82	4.69	0.59	0.266

[1] Values on a 15-cm line scal

Figure 1. Evaluation of eye size being conducted by a trained panelist using a washable standard hole-punched plastic square.

a



Baby Swiss Trained Panel Sensory Evaluation Cheat Sheet panelist # _____

AROMA/TASTE/FLAVOR

Acid (Sour)	none/not	slight		definite	pronounced
Bitter	none/not	slight		definite	pronounced
Flat (lacks typical nutty and propionic acid flavors)	none/not	slight		definite	pronounced
Unclean (aftertaste)	none/not	slight		definite	pronounced
describe (fruity, fermented, rancid, yeasty, other):					

BODY & TEXTURE

Curdy	not (smooth)	slight	typical	definite	pronounced (corky)
Mealy/grainy	not (smooth in mouth)			grainy (sandy)	mealy (rough)
Pasty	not sticky (ideal)	slight		definite	pronounced (sticky)
Weak	firm (ideal)	slight		definite	pronounced

APPEARANCE

Eye amount	blind	very underset	slight underset	ideal	slight overset	very overset
Eye distribution	ideal (even)	slightly uneven		moderately uneven		very uneven
Eye gloss	wet (free whey)		shiny (ideal)		slight dull	dull/rough
Eye shape	no defect	few irregular		multiple irregular	collapsed	cabbage
Eye size	sweet holes	very small	small (<1/4)	ideal (1/4-1/2)	~1/2 inch	large (penny)
Gas formation	no defect		checks/picks		slits/splits	blowhole

COMMENTS

Additional descriptors (oxidized, sulfide, one-sided, nesty, streuble, frog mouth, cabbage, other):

Figure 2. Baby Swiss Trained Panel Sensory Evaluation Cheat Sheet.

Baby Swiss Trained Panel Sensory Evaluation	panelist #	
	sample #	
AROMA/TASTE/FLAVOR		
Acid		
Bitter		
Flat		
Unclean		
(Describe unclean)		
BODY & TEXTURE		
Curdy		
Mealy/grainy		
Pasty		
Weak		
APPEARANCE		
Eye amount		
Eye distribution		
Eye gloss		
Eye shape		
Eye size		
Gas formation		
COMMENTS		

Figure 3. Baby Swiss Trained Panel Sensory Evaluation Sheet.

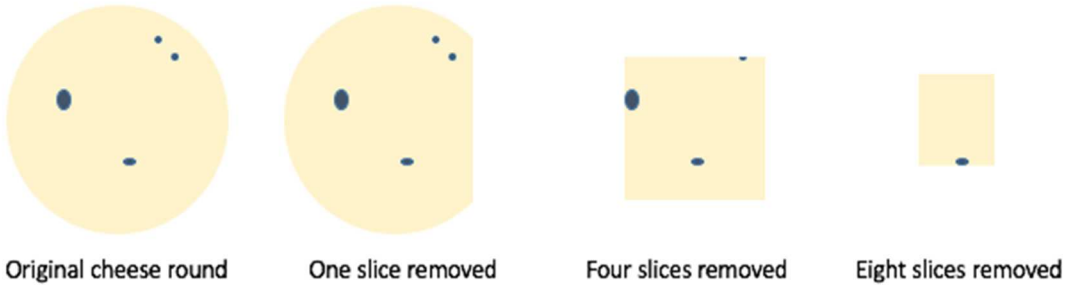


Figure 4. Visual representation of the process used to systematically slice cheeses for photographs and evaluation by trained panelists.

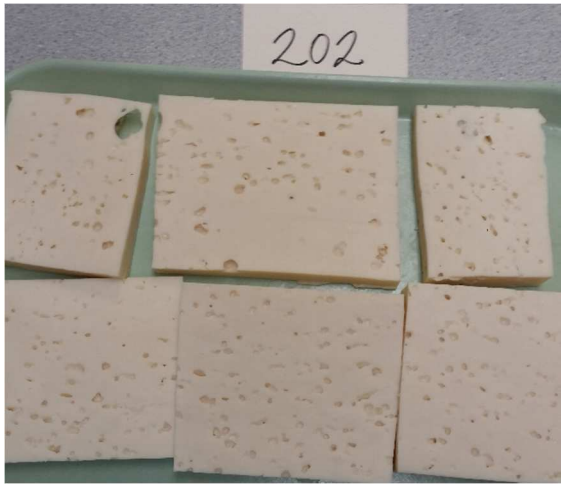


Figure 5. Representative photograph of baby Swiss cheese from milk of cows fed control diet in period 1.

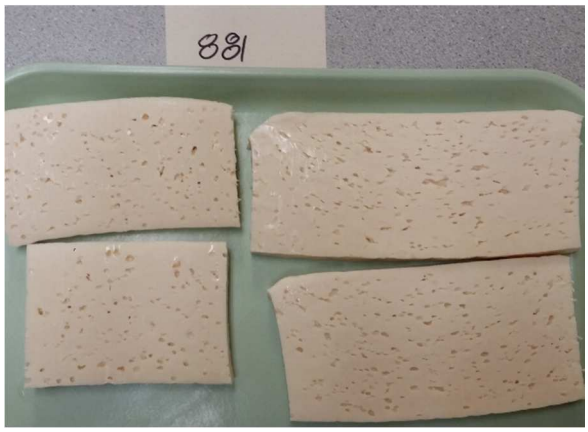


Figure 6. Representative photograph of baby Swiss cheese from milk of cows fed DDGS diet in period 1.

Figure 7. Representative photograph of baby Swiss cheese from milk of cows fed control diet in period 2.

Figure 8. Representative photograph of baby Swiss cheese from milk of cows fed DDGS diet in period 2.

CHAPTER 7

IODINE VALUES OF ADIPOSE TISSUE VARIED AMONG BREEDS OF PIGS AND WERE CORRELATED WITH PORK QUALITY

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Abstract

Our objectives were to investigate fatty acid composition variation amongst adipose tissue sites, breed effects on fat quality, and the relationship of pork fat quality to fresh pork quality. Barrows and gilts (n =347) of five purebred and one commercial crossbred line were fed commercial swine diets with DDGS inclusion at 30% (as fed) from 31.8 kg body weight until 30-d prior to harvest at 111.4 kg. Immediately after harvest, hot carcass weight was determined, adipose tissue was collected from the back, belly, and jowl, and meat samples were taken from the longissimus muscle for evaluation of pork quality. Iodine values (IV) varied between anatomical site and breed. Jowl fat IV were correlated to back and belly fat IV. Minor but significant correlations were observed between IV and meat quality characteristics. These results support our hypotheses that minor relationships exist between fat and fresh pork quality and that IV vary by anatomical location.

Keywords: Iodine Values, Fresh Pork Quality, Adipose Tissue, Fatty Acid Composition, Adipose Physiology

Abbreviations:

ADG = average daily gain
BF = backfat thickness
DDGS = dried distillers grains with solubles
FFL = fat-free lean
%FFL = percentage fat-free lean
IV = iodine value
MUFA = monounsaturated fatty acid
PUFA = polyunsaturated fatty acid
SCD = stearoyl-CoA desaturase
SFA = saturated fatty acid
TAG = triacylglyceride

Introduction

With the increased production of ethanol, dried distillers grains with solubles (DDGS) have become readily available and are often an economical feed source for inclusion in the diet of market pigs. Feeding DDGS, however, may negatively affect pork production by increasing the incidence of soft bellies[1]. Because DDGS often are incorporated into commercial diets for pigs, it is important to understand and evaluate fat quality of pigs fed diets containing DDGS. Indeed, it has been reported that as dietary unsaturated fatty acid consumption increased so did adipocyte iodine value (**IV**; a measure of fat quality, with a greater number indicating a greater concentration of unsaturated fatty acids and thus softer fat**)** of three different anatomical locations[2]. Additionally, the relationship between fatty acid composition of pork muscle and adipose tissue of different breeds of pigs fed DDGS with a 30-day withdrawal and fresh pork quality has yet to be quantified. Whole carcass near infrared spectroscopy (NIR) analysis of fat quality of pigs was performed, adipose IV (fat quality) varied amongst anatomical locations, and it was concluded that other factors (e.g, gender, breed) should be

analysed[3]. Additionally, the focus of meat quality metrics has recently shifted from fat quantity to fat quality and is considered of paramount importance to consumer acceptance of meat products[4]. Therefore, our hypotheses were that measures of pork fat and fresh pork quality vary among breeds of pigs and that a significant relationship between pork fat IV and fresh pork quality exists. To test these hypotheses, the relationship among several measures of fat and pork quality from market pigs fed diets that included DDGS at 30% on an as-fed basis with a 30-day withdrawal period was quantified. Our objectives were to: 1) quantify measures of fat quality by determining the fatty acid composition and IV of adipose tissues from the jowl fat, belly fat, and 10th rib backfat from pigs of five purebred lines and one commercial crossbred line when fed DDGS with a 30-day withdrawal period, 2) evaluate the relationship of fat quality of jowl fat to fat quality of the belly fat and 10th rib backfat, and 3) determine the relationship between IV as a measure of fat quality and fresh pork quality traits including pH, visual firmness, marbling, and muscle color.

Materials and methods

Animals and diets

All research was approved by the Iowa State University Institutional Animal Care and Use Committee (IACUC ID 5-12-7367-S and 8-14-7851-S). A total of 347 barrows and gilts of five pure breeds and one PIC commercial crossbred line (for breed and gender see footnote of Table 1) were delivered to the Iowa Swine Testing Station (Ames, IA) and housed in a slatted finishing barn with eight pigs per pen. Pigs were housed in pens consisting of only one breed or genetic line. The pen average pig weight was 31.8 kg when pigs began the performance test. All pigs were fed a six-phase commercial

corn/soy-based diet with DDGS inclusion at 30% on an as fed basis (finishing diet: 0.5% choice white grease, 57.8% corn, 7.5% soybean meal, 30% DDGS, 4.2% base mix with vitamin and mineral supplement). Dried distillers grains with solubles were removed from the ration for the final 30 days of the finishing period at the request of the meat processor (final diet: 0.5% choice white grease, 85.2% corn, 7.5% soybean meal, 6.8% base mix with vitamin and mineral supplement). Pigs were individually weighed every two weeks and completed the performance test at a minimum weight of 111.4 kg when they were transported to Hormel Foods (Austin, MN) for slaughter the following morning.

Carcass evaluation

After harvest, hot carcass weight was determined. Backfat thickness, loin muscle area, and carcass weight were obtained 24-h post-mortem after a 24-h chill at Hormel Foods (Austin, MN). Standard carcass collection procedures, as outlined in the Pork Composition and Quality Assessment Procedures[5], were followed to obtain measurements of the 10th rib backfat thickness and loin muscle area. Ultimate pH was measured on the 10th-rib of the longissimus muscle by using a pH probe (SFK Ltd; Hvidovre, Denmark). Hunter L score (a measure of light reflectance where lower values indicate darker color) was measured on the 10th rib face of the loin after allowing the sample to bloom by using a Minolta CR-310 (Minolta Camera Co., Ltd., Japan) with a 50-mm diameter aperture and D65 illuminant that was calibrated to the white calibration plate. A section of bone-in loin containing the 10-12th ribs was excised from the carcass and transported on ice to the Iowa State University Meat Laboratory (Ames, IA) for additional pork quality assays. The 11th and 12th rib sections were cut into 2.54 cm thick

chops and placed freshly cut side up for 10 min to allow the sample to bloom. Subjective measures of color (1-6), marbling (1-10), and firmness (1-3) were evaluated on the 11th rib face by the same panelist according to NPPC procedures[5].

Fat-free lean tissue (**FFL**) and percentage fat-free lean tissue (**%FFL**) of carcasses were calculated as previously described by the National Pork Producers Council[5].

FFL = 8.588-(21.896 × backfat thickness) + (3.005 × longissimus muscle area) + (0.465 × carcass weight)

Fatty acid analysis and iodine values

Following harvest, upon arrival of the split carcasses to the chillbox and while on the slaughter line, subcutaneous adipose tissue samples from all three layers of adipose (about 20 g) were excised as soon as possible from 1) the right side of the jowl where the carcass was split, 2) the back over the 10th rib, 3) and the middle of the lateral side of the belly, and placed in 0.9% NaCl solution at 37°C and transported to Iowa State University (Ames, IA) at 37°C for cellularity assays (not reported) and fatty acid analysis. Subsamples for fatty acid analysis were frozen at -20°C within six hours of animal death until assay.

To verify that storage at 37°C in 0.9% NaCl solution would not cause oxidation of fatty acids, adipose tissue (about 20 g) from the jowl, back over the 10th rib, and middle of the lateral side of the belly was excised immediately following harvest (consistent with how samples were collected at Hormel Foods) from seven crossbred pigs at the Iowa State University Swine Research Farm. Following on-farm collection, tissues were stored either in 0.9% NaCl solution for 6 hours at 37°C and then frozen (-20°C) or immediately frozen (-20°C) until analysis. Analysis of fatty acid composition of adipose

tissue confirmed that storage at 37°C up to six hours did not significantly affect any measured fatty acid of any anatomical location including unsaturated fatty acids that would be most vulnerable to oxidation (e.g., C18:1, C18:2, C18:3; data not shown). Total lipids were extracted in triplicate from 2 g of adipose tissue by using a chloroform and methanol mixture[6], quantified gravimetrically, and methylated directly with acetyl chloride and methanol[7]. Fatty acid methyl esters were quantified by a gas chromatograph (Varian 3800, Agilent Technologies, Palo Alto, CA) equipped with a Supelco SP-2380 (L × I.D., 100 m × 0.25 mm) column and a flame ionization detector. Gas chromatograph conditions were as follows: The initial column temperature was 70° C with a hold time of four min, and the temperature ramp was 13°C per min with a final column temperature of 215°C. Peaks were identified by using commercially available fatty acid methyl ester standards (Nu-Chek-Prep Inc., Elysian, MN). Fatty acid composition was expressed on a weight percentage basis. The activities of the stearoyl-CoA desaturase (Δ^9-desaturase or **SCD**) were estimated by calculating the ratio of enzyme substrate to product[8-9]. Three different indices of SCD activity were calculated with the following formulas:

Δ^9 *(16) desaturase index*= 100 x [16:1]/([16:1] + [16:0])

Δ^9 *(18) desaturase index* = 100 x [18:1]/([18:1] + [18:0])

Δ^9 *desaturase index* = 100 x ([16:1] + [18:1])/([16:1] + [16:0] + [18:1] + [18:0])

Iodine values were calculated from the fatty acid composition of fat samples isolated from each of the three adipose depots by using the following American Oil Chemists Society formula[10];

IV = [C16:1] x 0.95 + [C18:1] x 0.86 + [C18:2] x 1.732 + [C18:3] x 2.616 + [C20:1] x 0.785 + [C22:1] x 0.723.

Statistical analyses

All statistical analyses were performed by using SAS version 9.3 (SAS Inst. Inc.; Cary, NC). Correlations between jowl IV and back and belly IV, as well as relationships between 10th rib backfat IV and ultimate pH, Hunter L, visual color (1-6), visual marbling (1-10), and visual firmness (1-3) were analyzed by using the CORR procedure of SAS. In addition to a simple correlation, a simple linear regression was performed to accompany the correlation between jowl IV and back and belly IV.

Grow-finish performance data were analyzed by using the MIXED procedure of SAS with a model including breed, sex, contemporary group (sample date), and breed × sex interaction as fixed effects. Percentage FFL was used as a covariate to represent the components of the measurement that define FFL and to normalize/account for some of the variance in adiposity of the different breeds as well as to account for differences in growth rate because leaner pigs (higher FFL%) tend to grow slower than do pigs with lower FFL%.

Iodine value and fatty acid data were analyzed by using the MIXED procedure of SAS with the model including anatomical location (i.e., back, belly, jowl), breed, sex, contemporary group (sample date), and breed × sex, breed × location, and sex × location interactions as fixed effects with %FFL as a covariate.

All means were separated by using an *F*-protected least-squares means separation and reported as the least squares mean plus or minus the SEM. When significant interaction effects were found, *P*-values were determined by using the SLICE command of LSMEANS. Statistical significance was declared at a $P < 0.05$, and tendencies were declared for *P*-values greater than 0.05 and less than 0.10.

Results and discussion

Grow-finish performance data

Performance data expressed as both ADG and %FFL of the five purebred and one crossbred line are summarized in Table 1. For these experimental conditions, Crossbred, Duroc, and Yorkshire pigs had the greatest rate of gain, with Chester White being equal to all breeds. Additionally, at harvest, Berkshire had the least %FFL. Chester White had the least backfat thickness whereas Duroc had the thickest, but differences were less than a tenth of a cm. Our data were consistent with those of previous studies that have shown leaner breeds of pigs to grow faster than more obese breeds of pigs[11-13].

Sex, breed, and anatomical location effects on fatty acid composition

Because the objectives of the study were to determine how fat quality varied between anatomical locations, concentrations of individual fatty acids in adipose tissue are presented in Table 2. For additional comparison, fatty acids have been separated into SFA, MUFA, PUFA, and the calculated ratio of PUFA to SFA (P:S) (Table 2). Although there were significant differences in C14:0 concentration in these animals, they are likely not of any practical significance because the concentrations are only about one percent of the total pork fatty acid content. Gilts had a higher concentration of PUFA than did barrows. Because gilts are generally leaner than barrows and backfat thickness is inversely related to C18:2 concentration (a major PUFA), it is to be expected that gilts would have a greater concentration of PUFA[14-15]. Additionally, the P:S ratio was similar among all breeds except for Durocs that had a significantly lower concentration of PUFA than did all other breeds. The P:S ratio difference in Durocs is discussed in detail in the following section. Finally, P:S ratio varied significantly amongst anatomical locations,

with belly fat having the lowest ratio and backfat having the highest ratio, indicating belly fat would be less nutritionally desirable from a consumer perspective than backfat. Our results that show belly fat has the least P:S ratio are in agreement with previously published data that showed belly fat to have a lower P:S ratio than jowl fat when pigs were fed a traditional corn/soy diet[16].

Iodine values

Iodine value is a valuable tool for describing the degree of unsaturation of the fatty acids contained in the fat of food. The practical implication of measuring IV relates to the fact that, as IV or unsaturated fatty acids increase, fat becomes softer in texture and eventually less desirable to the consumer, producer, and the processor. Additionally, although consumers generally desire food with less SFA and more PUFA, increases in unsaturated fatty acids (**UFA**) can negatively affect pork quality in general because pork adipose is typically composed of approximately 60% UFA[17]; so, increasing the UFA content through feeding of PUFA beyond 60% can negatively affect oxidative stability and promote the generation of undesirable off-odors and -flavors[14]. The relationship of soft pork fat (soft bellies) with feeding DDGS is a result of the PUFA content of corn oil, which is typically more than 60% C18:2. Pigs are unable to synthesize linoleic C18:2$^{\Delta 9,12}$ and alpha-linolenic $^{\Delta 9,12,15}$ (C18:3) acids; therefore, these fatty acids in adipose lipids are derived entirely from the diet[18].

Iodine values at the three adipose depots varied significantly, albeit only by about a value of two, with backfat having the greatest IV and belly fat having the least ($P <$ 0.0001, Table 3). These data are slightly greater than the average IV of pork adipose tissue (64.5 mg iodine/100 g fatty acid)[19]. The discrepancy between our results and those

of Cromwell et al. (2011) is likely a result of the inclusion of DDGS in the diets of pigs in our study because feeding DDGS increases IV of pork fat[20]. Indeed, when Cromwell et al. (2011) fed pigs 30% DDGS with no withdrawal period, they observed an IV in backfat of 77.1. Likely, the lack of withdrawal period explains the difference between our IV data and those of Cromwell et al. (2011) because the IV reported in our study would have likely been higher had we not removed DDGS from the finishing diet for the last 30 days. It has been reported that up to 30% DDGS could be included in the diet of the grower-finisher pig and removed for 3 weeks prior to slaughter without adverse effects on pork fat quality[21].

A likely partial explanation for the greater IV ($P < 0.05$) of backfat can be made when considering the activity indices of SCD (Table 4). Stearoyl-CoA desaturase is responsible for the conversion of palmitic acid (C16:0) to palmitoleic acid (C16:1) and stearic acid (C18:0) to oleic acid (C18:1), and a greater calculated value for the three indices indicates greater SCD activity. When considering the Δ^9 (16) index, a measure of the conversion of C16:0 to C16:1, backfat had the lowest index of all locations for both barrows and gilts ($P < 0.05$; Table 4). Additionally, the Δ^9 (18) index, an indicator of the conversion of C18:0 to C18:1, of backfat is lower than that of all other locations for both barrows and gilts ($P < 0.05$), with the exception of belly fat of gilts. Jowl adipose tissue had the highest SCD index, an indicator of C16:0 to C16:1 and C18:0 to C18:1, (Table 4) but had an intermediate IV of the three assayed adipose depots (Table 3), indicating that SCD activity is not the sole contributor to IV.

Additionally, barrows had a lower IV for the three depots than did gilts ($P = 0.0003$; Table 3). This observation led us to hypothesize that the increased concentration

of UFA found in the adipose depots of gilts was a result of estrogenic stimulation of SCD. Estrogen increases SCD activity seven-fold when injected into roosters[22]. These data do not, however, support the hypothesis that increased concentrations of estrogen in gilts compared with that in barrows results in increased activity of SCD because of the source of the increase in IV. Increased IV in gilts is a result of increased proportions of the dietary-derived fatty acids, C18:2$\Delta^{9,12}$ and C18:3$\Delta^{9,12,15}$ in adipose tissue, rather than of desaturase-derived C16:1 $\Delta^{9,}$ and C18:1 Δ^{9}. Rather, the likely explanation is that the gilts were leaner than the barrows (backfat thickness of 2.30 cm and 2.46 cm respectively, $P < 0.0001$; loin muscle area of 45.33 and 42.28 cm^2, respectively, $P < 0.001$; hot carcass weight of 85.82 and 87.20 kg, respectively, $P = 0.003$; %FFL, Table 1) and the dietary PUFAs are "diluted" in barrows by *de novo* fatty acid synthesis. Our data agree with the hypothesis that leaner pigs have a greater IV, as supported by the negative correlation between backfat thickness and backfat IV ($r = -0.358$, $P < 0.001$) and the positive association between %FFL and IV ($r = 0.337$, $P < 0.001$).

Backfat thickness is inversely related to C18:2, a significant factor in the calculation of IV[14-15]. This negative correlation between backfat thickness and C18:2 also was found in our study ($r = -0.427$, $P < 0.001$). The phospholipids of the adipocyte membrane are richer in C18:2 than the TAG within the adipocyte. Consequently, as an adipocyte becomes larger, the phospholipid bilayer represents a smaller portion of the total lipid in the adipose tissue[14] and, thus, a smaller percentage of the total lipid is C18:2. Consequently, gilts being leaner than barrows results in a greater IV in their adipose resulting from an increased concentration of C18:2. In addition, larger adipocytes contain more products of *de novo* fatty acid synthesis (i.e., membrane to neutral lipid

ratio is smaller), further diluting the effects of dietary PUFA. The same explanation, we presumed, held true for all three adipose depots. Backfat, however, had the greatest concentration of C18:2 when compared with the jowl and belly fats ($P < 0.05$; Table 2). Furthermore, we observed that Duroc pigs also had the lowest IV (Table 3) because of the low concentration of dietary-derived PUFA in the adipose tissue (Table 2) in combination with low SCD activity (Table 4).

The results of IV data in the present study (i.e., back is greater than jowl and jowl is greater than belly) are supported by previous research that assessed IV of the back, belly, and jowl of PIC crossbred medium-weight barrows fed 40% DDGS (9.6% oil)[23]. Our results for IV of the belly may differ from those of other researchers because we sampled adipose from the middle of the lateral side of the belly for consistency, but it has been shown that the IV of the belly is highly variable depending upon sampling location (i.e., dorsal, central, and ventral belly adipose differ in IV)[24]. The variability of adipose IV amongst anatomical location is also supported by whole body NIR imaging used to determine fatty acid composition that showed different anatomical locations in the same animal had different IV[3].

The only significant difference found when comparing the adipose tissue IV of the six breeds assayed was that the adipose tissue of Durocs had a significantly lower IV than did the other five breeds ($P < 0.05$; Table 3). Likely the reason for Durocs having adipose tissue with the least IV is a genotypic difference resulting in decreased SCD activity (Table 4) that was less than other breeds except for Chester White ($P < 0.05$); a phenomenon that occurs both within breeds of a species and between breeds a species[25-26]. Several variants of the SCD gene have been identified that showed significant

association between SCD haplotypes and fatty acid composition in a population of Duroc pigs[27]. The presence of SNPs in SCD gene resulted in the identification of three different genotypes of SCD that accounted for some of the difference in fatty acid profiles that were found within this breed of cattle[28]. Also, SCD activity varied between breed which was also demonstrated in our data (Table 4).

Relationship of iodine values to fresh loin meat quality

Pork fat in North America is recommended to have an IV of 74 or less[29]. In this study, IV of all breeds and sampling locations were under the threshold values for most packing plants, even with 30% inclusion of DDGS (as fed basis) until 30 days before harvest (Table 3). Additionally, IV varied by location (back, belly, and jowl); thus, sampling site may be of consequence when evaluating pork fat quality.

Back, jowl, and belly fat IV were analyzed to determine if statistically significant correlations between IV and measurements of meat quality existed. Iodine values from all anatomical locations were significantly correlated with laboratory measures of meat quality, but jowl fat IV were the most highly correlated with the laboratory measures of meat quality shown in Table 5. Jowl IV were positively correlated to Hunter L values ($P < 0.05$); So, as IV increase, laboratory measures typically associated with a reduction in meat quality are impacted negatively. Additionally, jowl IV were correlated negatively with ultimate pH, visual color, visual marbling ($P < 0.05$) and tended to be correlated negatively with visual firmness ($P = 0.052$). These correlations, although small, support the hypothesis that a relationship between pork fat quality and pork meat quality exists, although not all of the correlations may be of practical relevance.

Relationship of iodine values of the jowl to iodine values of back and belly fat

Meat processors have expressed a desire to determine relationship of jowl fat IV with IV of backfat and belly fat. In this research, we identified that jowl fat IV is moderately and positively correlated to both back and belly fat IV ($P < 0.001$; Fig. 1). Our data, however, do not show the IV of the jowl to be as strongly correlated to IV of the belly ($r = .733$)[16], possibly owing to sampling location (midsection of the belly versus left side of the carcass), which has been shown to influence IV[24].

Conclusion

Pork from pigs with greater adiposity have a lower IV. The pork from pigs of all breeds had similar IV except that of Durocs, which were lower, suggesting that there may be genetic influences on IV. Additionally, IV, specifically jowl IV, were significantly correlated, albeit correlations were small, with several meat quality laboratory measurements, indicating that, as IV increases, fresh pork quality decreases. Because, the correlations were small, the practical significance of the relationships seem minor rather than major. It was additionally found that 30% DDGS could be fed to six breeds of pig in a six-phase commercial swine diet (excluding the last 30 days) without IV exceeding recommended values. Finally, of value to the pork industry, is the finding presented that IV of jowl is only a moderate predictor of IV of other anatomical sites.

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TABLES AND FIGURES

Table 1.
Average daily gain, fat-free lean percentage, and backfat thickness of five purebred and one crossbred line of pigs

	Breed[2]						Sex		*P*-Values		
Item	Berkshire	Chester White	Crossbred	Duroc	Landrace	Yorkshire	Barrow	Gilt	Breed	Sex	Breed ×Sex
ADG, kg	0.80[a]	0.83[ab]	0.85[b]	0.87[b]	0.79[a]	0.87[b]	0.85[b]	0.82[a]	<0.001	0.011	0.151
	(± 0.01)	(± 0.02)	(± 0.01)	(± 0.01)	(± 0.01)	(± 0.01)	(± 0.01)	(± 0.01)			
FFL, %[3]	47.94[a]	50.59[bc]	53.37[d]	52.53[cd]	51.19[b]	52.39[cd]	49.33[a]	53.34[b]	<0.001	<0.001	0.016
	(± 0.41)	(± 1.15)	(± 0.56)	(± 0.39)	(± 0.44)	(± 0.40)	(± 0.24)	(± 0.43)			
BF, cm[4]	2.346[ab]	2.366[a]	2.422[b]	2.493[c]	2.467[c]	2.415[ab]	2.455[b]	2.382[a]	<0.0001	<0.0001	0.0004
	(±0.01)	(±0.04)	(±0.02)	(±0.01)	(±0.01)	(±0.01)	(±0.01)	(±0.01)			

[a, b, c, d] Within a row and main effect, means without a common superscript differ ($P < 0.05$).
[1] Standard error in parenthesis below mean value.
[2] $n = 347$ pigs; number of pigs within each breed (number of barrows, number of gilts): Berkshire, $n = 87$ (61, 26); Chester White, $n = 22$ (20, 2); crossbred, $n = 40$ (23, 17); Duroc, $n = 76$ (40, 36); Landrace, $n = 52$ (22, 30); Yorkshire, $n = 70$ (47, 23).
[3] ADG = average daily gain.
[4]FFL = fat-free lean percentage.
[5] BF = backfat thickness in cm.

Table 2.
Fatty acids from adipose tissue as influenced by sex, breed of pig, and anatomical location[1]

Fatty acid	Sex		Breeds [2]						Location			*P* - Values		
	Barrow	Gilt	Berkshire	Chester White	Crossbred	Duroc	Landrace	Yorkshire	Back	Belly	Jowl	Sex	Breed	Location
12:0	0.04 (± 0.01)	0.04 (± 0.01)	0.04^{ab} (± 0.002)	0.06^{c} (± 0.005)	0.03^{a} (± 0.003)	0.04^{b} (± 0.002)	0.03^{a} (± 0.002)	0.04^{a} (± 0.002)	0.036^{a} (± 0.002)	0.044^{b} (± 0.002)	0.037^{a} (± 0.002)	0.279	0.001	0.001
14:0	1.09 (± 0.008)	1.08 (± 0.01)	1.03^{a} (± 0.01)	1.20^{c} (± 0.03)	1.06^{a} (± 0.02)	1.11^{b} (± 0.01)	1.05^{a} (± 0.01)	1.04^{a} (± 0.01)	1.03^{a} (± 0.01)	1.15^{c} (± 0.01)	1.07^{b} (± 0.01)	0.477	<0.001	<0.001
16:0	21.68 (± 0.06)	21.48 (± 0.11)	21.45^{a} (± 0.11)	22.08^{b} (± 0.27)	21.41^{a} (± 0.14)	21.88^{b} (± 0.10)	21.44^{a} (± 0.11)	21.22^{a} (± 0.10)	21.23^{a} (± 0.09)	22.24^{b} (± 0.09)	21.27^{a} (± 0.09)	0.130	<0.001	<0.001
16:1	2.24^{b} (± 0.02)	2.06^{a} (± 0.04)	2.27^{d} (± 0.04)	2.42^{d} (± 0.09)	1.95^{a} (± 0.05)	2.02^{ab} (± 0.03)	2.09^{bc} (± 0.04)	2.14^{c} (± 0.03)	1.88^{a} (± 0.03)	2.31^{b} (± 0.03)	2.25^{b} (± 0.03)	<0.001	<0.001	<0.001
17:0	0.32 (± 0.003)	0.31 (± 0.006)	0.30^{a} (± 0.006)	0.34^{bc} (± 0.01)	0.31^{a} (± 0.01)	0.30^{a} (± 0.01)	0.33^{c} (± 0.01)	0.31^{ab} (± 0.01)	0.34^{b} (± 0.01)	0.30^{a} (± 0.01)	0.30^{a} (± 0.01)	0.325	0.001	<0.001
17:1	0.25^{b} (± 0.002)	0.23^{a} (± 0.01)	0.24^{bc} (± 0.01)	0.26^{cd} (± 0.01)	0.23^{b} (± 0.01)	0.21^{a} (± 0.004)	0.26^{d} (± 0.01)	0.26^{cd} (± 0.01)	0.25^{b} (± 0.004)	0.23^{a} (± 0.004)	0.25^{b} (± 0.004)	0.006	<0.001	<0.001
18:0	10.62 (± 0.06)	10.63 (± 0.10)	10.07^{a} (± 0.11)	10.28^{ab} (± 0.26)	10.74^{b} (± 0.13)	11.88^{c} (± 0.09)	10.54^{b} (± 0.11)	10.25^{a} (± 0.10)	10.89^{b} (± 0.08)	10.94^{b} (± 0.08)	10.04^{a} (± 0.09)	0.966	<0.001	<0.001
18:1c9	39.33^{b} (± 0.10)	38.80^{a} (± 0.17)	39.51^{c} (± 0.17)	37.21^{a} (± 0.42)	39.54^{c} (± 0.22)	38.88^{b} (± 0.15)	39.52^{c} (± 0.17)	39.70^{c} (± 0.16)	37.92^{a} (± 0.14)	38.87^{b} (± 0.14)	40.40^{c} (± 0.14)	0.010	<0.001	<0.001
18:2n6	17.79^{a} (± 0.12)	18.90^{b} (± 0.21)	18.41^{b} (± 0.21)	19.58^{c} (± 0.51)	18.32^{b} (± 0.27)	17.26^{a} (± 0.19)	18.26^{b} (± 0.21)	18.24^{b} (± 0.19)	19.95^{b} (± 0.17)	17.58^{a} (± 0.17)	17.51^{a} (± 0.17)	<0.001	<0.001	<0.001
18:3n3	0.53^{a} (± 0.004)	0.59^{b} (± 0.01)	0.57^{c} (± 0.01)	0.64^{d} (± 0.02)	0.54^{ab} (± 0.01)	0.52^{a} (± 0.01)	0.55^{bc} (± 0.01)	0.55^{bc} (± 0.01)	0.60^{b} (± 0.01)	0.54^{a} (± 0.01)	0.54^{a} (± 0.01)	<0.001	<0.001	<0.001
20:0	0.22 (± 0.03)	0.17 (± 0.05)	0.20 (± 0.05)	0.19 (± 0.13)	0.13 (± 0.07)	0.26 (± 0.05)	0.20 (± 0.05)	0.18 (± 0.05)	0.23 (± 0.04)	0.17 (± 0.04)	0.18 (± 0.04)	0.470	0.641	0.533
20:1n9	0.73^{b} (± 0.01)	0.70^{a} (± 0.01)	0.72^{bc} (± 0.01)	0.65^{a} (± 0.03)	0.71^{b} (± 0.01)	0.74^{cd} (± 0.01)	0.72^{bc} (± 0.01)	0.76^{d} (± 0.01)	0.73^{b} (± 0.01)	0.64^{a} (± 0.01)	0.78^{c} (± 0.01)	0.001	<0.001	<0.001
20:2n6	0.81 (± 0.01)	0.81 (± 0.01)	0.82^{b} (± 0.01)	0.76^{a} (± 0.03)	0.84^{b} (± 0.01)	0.84^{b} (± 0.01)	0.77^{a} (± 0.01)	0.84^{b} (± 0.01)	0.88^{c} (± 0.01)	0.71^{a} (± 0.01)	0.85^{b} (± 0.01)	0.963	<0.001	<0.001
20:3n3	0.108^{a} (± 0.001)	0.113^{b} (± 0.002)	0.112^{b} (± 0.002)	0.117^{bc} (± 0.01)	0.107^{b} (± 0.002)	0.097^{a} (± 0.002)	0.120^{c} (± 0.002)	0.109^{b} (± 0.002)	0.113^{b} (± 0.001)	0.107^{a} (± 0.002)	0.112^{b} (± 0.002)	0.048	<0.001	0.011
20:4n6	0.27^{a} (± 0.003)	0.30^{b} (± 0.005)	0.29^{b} (± 0.004)	0.30^{bc} (± 0.01)	0.28^{b} (± 0.01)	0.24^{a} (± 0.004)	0.32^{c} (± 0.004)	0.30^{b} (± 0.004)	0.29^{b} (± 0.004)	0.30^{c} (± 0.004)	0.28^{a} (± 0.004)	<0.001	<0.001	<0.001
22:6n3	0.13 (± 0.002)	0.13 (± 0.003)	0.15^{c} (± 0.003)	0.14^{bc} (± 0.01)	0.11^{a} (± 0.003)	0.11^{a} (± 0.003)	0.13^{b} (± 0.003)	0.14^{c} (± 0.003)	0.13 (± 0.002)	0.13 (± 0.002)	0.13 (± 0.002)	0.958	<0.001	0.471
Other	3.84 (± 0.09)	3.66 (± 0.16)	3.81 (± 0.16)	3.80 (± 0.39)	3.69 (± 0.20)	3.61 (± 0.14)	3.66 (± 0.16)	3.94 (± 0.15)	3.51^{a} (± 0.13)	3.74^{ab} (± 0.13)	4.00^{b} (± 0.13)	0.336	0.674	0.013
SFA	33.09 (± 0.10)	33.71 (± 0.19)	33.09^{ab} (± 0.19)	34.14^{c} (± 0.46)	33.68^{bc} (± 0.24)	35.47^{d} (± 0.17)	33.60^{bc} (± 0.19)	33.04^{a} (± 0.17)	33.75^{b} (± 0.15)	34.85^{c} (± 0.15)	32.91^{a} (± 0.15)	0.255	<0.001	<0.001
MUFA	42.55^{b} (± 0.10)	41.79^{a} (± 0.19)	42.74^{c} (± 0.18)	40.53^{a} (± 0.46)	42.43^{c} (± 0.24)	41.85^{b} (± 0.17)	42.60^{c} (± 0.19)	42.85^{c} (± 0.17)	40.77^{a} (± 0.15)	42.005^{b} (± 0.15)	43.68^{c} (± 0.15)	0.001	<0.001	<0.001
PUFA	19.65^{a} (± 0..12)	20.85^{b} (± 0.22)	20.35^{b} (± 0.22)	21.54^{c} (± 0.55)	20.20^{b} (± 0.29)	19.07^{a} (± 0.20)	20.14^{b} (± 0.22)	20.17^{b} (± 0.21)	21.97^{b} (± 0.18)	19.36^{a} (± 0.18)	19.41^{a} (± 0.18)	<0.001	<0.001	<0.001
P:S[3]	0.59^{a} (± 0.005)	0.63^{b} (± 0.008)	0.62^{b} (± 0.01)	0.64^{b} (± 0.02)	0.61^{b} (± 0.01)	0.54^{a} (± 0.01)	0.61^{b} (± 0.01)	0.62^{b} (± 0.01)	0.66^{c} (± 0.01)	0.57^{a} (± 0.01)	0.60^{b} (± 0.01)	<0.001	0.001	<0.001

a, b, c, d Within a row and main effect, means without a common superscript differ ($P < 0.05$).

[1] Standard error in parenthesis below mean value. Values are expressed as a weight percentage.

[2] n =347 pigs, number of pigs within each breed shown in footnote 2 of Table 1.

[3] Ratio of polyunsaturated to saturated fatty acids.

Table 3.
Iodine values of adipose tissue as influenced by anatomical location, sex, and breed of pig[1]

Location		Sex		Breed		*P*-Values		
						Location	Sex	Breed
Back	71.07[c] (± 0.30)	Barrow	68.72[a] (± 0.18)	Berkshire	70.09[b] (± 0.33)	<0.001	0.003	<0.001
Belly	68.01[a] (± 0.30)	Gilt	70.15[b] (± 0.33)	Chester White	70.40[b] (± 0.80)			
Jowl	69.21[b] (± 0.30)			Crossbred	69.55[b] (± 0.42)			
				Duroc	67.19[a] (± 0.30)			
				Landrace	69.56[b] (± 0.33)			
				Yorkshire	69.81[b] (± 0.30)			

[a, b, c,] Within a column, means without a common superscript differ ($P < 0.05$).

[1] Standard error in parenthesis below mean value. Values are expressed as milligrams iodine per 100 grams of lipid

Table 4.
Indices of stearoyl-CoA desaturase activity from adipose tissue as influenced by breed of pig and anatomical location within sex[1]

	Breeds [2]						Location						*P* - Values	
							Back		Belly		Jowl			Location X
Indices	Berkshire	Chester White	Crossbred	Duroc	Landrace	Yorkshire	Barrow	Gilt	Barrow	Gilt	Barrrow	Gilt	Breed	Sex
Δ^9 (16)[3]	9.55^{d}	9.75^{cd}	8.33^{a}	8.41^{a}	8.84^{b}	9.11^{bc}	8.27^{a}	7.91^{a}	10.01^{d}	8.72^{c}	9.65^{b}	9.44^{b}	<0.001	<0.001
	(±0.13)	(±0.31)	(±0.16)	(±0.11)	(±0.13)	(±0.12)	(±0.11)	(±0.17)	(±0.11)	(±0.17)	(±0.11)	(±0.17)		
Δ^9 (18)[4]	79.68^{c}	78.33^{b}	78.60^{b}	76.56^{a}	78.91^{b}	79.50^{c}	77.66^{a}	77.76^{a}	78.61^{b}	77.41^{a}	79.91^{c}	80.25^{c}	<0.001	<0.001
	(±0.19)	(±0.48)	(±0.25)	(±0.17)	(±0.19)	(±0.18)	(±0.17)	(±0.26)	(±0.17)	(±0.26)	(±0.17)	(±0.26)		
Δ^9-desaturase[5]	57.00^{bc}	55.00^{a}	56.35^{b}	54.76^{a}	56.54^{b}	57.10^{c}	55.25^{ab}	55.47^{bc}	56.08^{c}	54.62^{a}	57.49^{d}	57.84^{d}	<0.001	<0.001
	(±0.19)	(±0.47)	(±0.24)	(±0.17)	(±0.19)	(±0.18)	(±0.17)	(±0.26)	± (0.17)	(±0.26)	(±0.17)	(±0.26)		

a, b, c, d, e Within a row and main effect, means for breeds without a common superscript differ ($P < 0.05$).

[1] Standard error in parenthesis below mean value.

[2] n =347 pigs, number of pigs within each breed shown in footnote 2 of Table 1.

[3] Δ^9 (16) = Δ^9 (16) desaturase index = 100 * [16:1]/([16:1]+[16:0]).

[4] Δ^9 (18) = Δ^9 (18) desaturase index = 100 * [18:1]/([18:1]+[18:0]).

[5] Δ^9-desaturase = Δ^9 desaturase index = 100 * ([16:1] + [18:1])/([16:1] + [16:0] + [18:1]+ [18:0]).

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Table 5.
Correlation of iodine values of jowl fat and of meat quality metrics

Item	Value[1]	Std. Dev.	*r*	*P* - Value[2]
pH	5.65	0.18	-0.18	0.001
Hunter L	47.86	3.28	0.22	< 0.001
Visual color	2.89	0.86	-0.27	< 0.001
Visual marbling	1.90	0.89	-0.24	< 0.001
Visual firmness	2.27	0.85	-0.11	0.052

[1] Mean value $n = 315$ pigs.
[2] *P*-values for difference from zero.

Figure Legends

Figure 1. Relationship of iodine values of jowl adipose tissue of six breeds of pig to iodine values of back and belly adipose tissue. Numbers are expressed in grams iodine consumed per 100 grams of lipid. *P*-value is for difference from zero. (a) Relationship of iodine value of back adipose tissue and iodine value of jowl adipose tissue. (b) Relationship of iodine value of belly adipose tissue and iodine value of jowl adipose tissue.

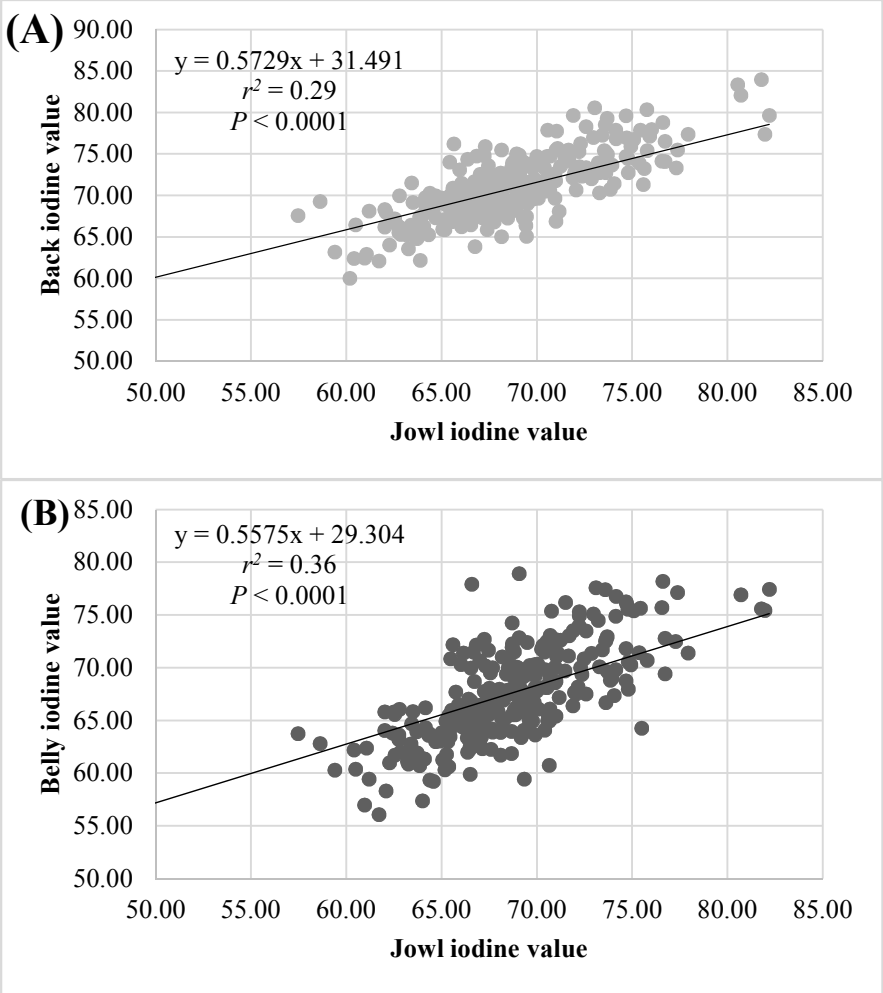

Figure 1

CHAPTER 8: SUMMARY AND CONCLUSIONS

Summary

When cows were fed FF-DDGS (~13% fat; 20% inclusion rate, DM basis), feed efficiency was markedly decreased in all metrics measured. Furthermore, milk fat depression was observed (0.5 percentage points) when cows were fed FF-DDGS at a 10% inclusion rate (DM basis), despite recommendations that distillers grains could be effectively incorporated into the diet of the lactating dairy cow at up to a 20% inclusion rate (DM basis) (Schingoethe et al., 2009). Feeding 10% FF-DDGS also decreased ECM and FCM yields because of the decreased milk-fat production despite the rations containing only 2.5 percentage points less forage than the recommended 50% forage inclusion rate to prevent milk fat depression (Kalscheur, 2005). These results are likely the result of a combination of factors resulting from the relatively high concentration of PUFA in the distillers grains, resulting in greater PUFA intake and likely inhibiting *de novo* milk fat synthesis in addition to inhibiting fiber digestion. The results of feeding 10 and 20% FF-DDGS to lactating dairy cows indicate that a 20% dry matter inclusion rate is not advisable when the source of DDGS is very high in fat concentration (i.e., greater than 13% fat), and that even a 10% inclusion rate can have detrimental effects on production parameters. However, when cows were fed 20% RF-DDGS (~6% fat) with ruminally protected lysine, no negative effects on production were observed and protein percentage was increased accompanied by a decrease in milk urea nitrogen, indicating that cows fed RF-DDGS may more effectively utilize dietary protein in agreement with previous research (Mjoun et al., 2010).

No difference in quality of milk was detected by a trained sensory panel when milk from cows fed 0, 10, and 25% FF-DDGS was evaluated over a period of seven days. As expected, concentration of milk unsaturated fatty acids increased as DDGS inclusion rate increased, but, despite this change in composition, no change in oxidative stability or development of oxidative off-flavors was detected as determined by testing peroxide value and trained sensory panel. These results indicate that, although feeding DDGS alters composition of milk, quality was unaffected.

Inclusion of both FF- and RF-DDGS in the rations of dairy cows producing milk meant to be used for production of baby Swiss cheese did not influence quality of cheeses produced. Rather, it seems that environmental contamination, not originating from DDGS, is more likely to cause introduction of microorganisms involved in secondary fermentation that lead to the late-blowing defect.

Finally, it was found that pigs fed FF-DDGS with a 30-d withdrawal period had adipose tissue that varied in fatty acid composition by anatomical location. There was a significant but moderate correlation between IV of one anatomical site and another. Additionally, IV of pork fat from leaner pigs was greater than IV of pork fat from pigs with more adiposity. Even though none of the IV exceeded the maximal threshold allowed by processors, statistically significant but moderate correlations between the IV of pork fat and measurements of meat quality occurred, indicating that as IV increases pork would likely be deemed less desirable to consumers.

Conclusions and future research recommendations

Because of the recent shift in composition of DDGS, in particular with regard to fat content, FF-DDGS are not likely to be available in the future. However, the vast

majority of research conducted to date focused on the effects of feeding FF-DDGS. There is a need for research in the beef, dairy, and pork industry to examine the effects of feeding RF-DDGS on not only production parameters, but also on feeding value, and equally important on quality of products derived from animals fed RF-DDGS (e.g., sensory attributes, consumer acceptability, quality as perceived by the processor). Future research should focus on investigating all aspects of feeding programs, from production parameters through product quality, which will require multidisciplinary collaborative research projects.

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Appendix A

A NOVEL AND ROBUST METHOD FOR TESTING BIMODALITY AND CHARACTERIZING PORCINE ADIPOCYTES OF ADIPOSE TISSUE OF FIVE PUREBRED LINES OF PIG

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Abstract

Sizes of adipocytes from adipose tissue of mature animals form a bimodal distribution; thus, reporting mean cell size is misleading. The objectives of this study were to develop a robust method for testing bimodality of porcine adipocytes, describe the size distribution with an informative metric, and statistically test hypertrophy and appearance of new small adipocytes, possibly resulting from hyperplasia or lipid filling of previously divided fibroblastic cells. Ninety-three percent of adipose samples measured were bimodal ($P < 0.0001$); therefore, we describe and propose a method of testing hyperplasia or lipid filling of previously divided fibroblastic cells based upon the probability of an adipocyte falling into two chosen competing "bins" as adiposity increases. We also conclude that increased adiposity is correlated positively with an adipocyte being found in the minor mode ($r = 0.46$) and correlated negatively with an adipocyte being found in the major mode ($r = -0.22$), providing evidence of either hyperplasia or lipid filling of previously divided fibroblastic cells. We additionally conclude that as adiposity

increases, the mode of the major distribution of cells occurs at a larger diameter of adipocyte, indicating hypertrophy.

Introduction

It is well established that populations of adipocytes from adipose tissue of adult animals have a bimodal distribution and are, thus, not normally distributed [1-9]. Even so, to date the standard reported metric of cell size is typically the mean cell size of the distribution. This reporting occurs despite recommendations by Rogers et al.[7] that the normality assumption, at least in Zucker rats, is erroneous. We also believe that it is inappropriate to use a single grand mean cell size as representative of adult adipose cell size distribution specifically because the presence of a secondary mode in the range of measured smaller cells artificially lowers the mean and may be misleading. One would expect an animal with a greater mean adipocyte cell size to have more adiposity, which may not be the case if the cells are bimodally distributed.

Many methods have been proposed to arrive at a simple hypothesis test for bimodality. A discussion of some of them is included in Jackson et al.[10]. All of those methods discussed rely on the assumption of normality under both the null and alternative hypotheses. As discussed above, however, this condition is not met in our situation. In spite of this condition, we have the good fortune of data whose empirical distribution is such that a simple and robust hypothesis can be used. This method is described in detail in the Materials and Methods section below. No model distribution is necessary using this method.

The objective of this study was to characterize populations of adipocytes of adipose tissue of five different purebred lines of pigs of varying adiposity and to develop

an appropriate statistical method of describing these populations based on the hypothesis that a single grand mean is an inappropriate metric for describing adipocyte populations. We now proceed to the details of this study.

Materials and methods

Animals and diets

All research was approved by the Iowa State University Institutional Animal Care and Use Committee (IACUC). A total of 149 barrows and gilts of five different breeds (Berkshire = 40 barrows and 25 gilts; Chester White = 15 barrows and 2 gilts; Duroc = 7 barrows and 4 gilts; Landrace = 6 barrows and 17 gilts; Yorkshire = 17 barrows and 16 gilts) were delivered to the Iowa Swine Testing Station (Ames, IA) and housed in a commercial slatted finishing barn with eight pigs randomly assigned per pen. Pigs began the performance test when the pen average pig weight was 31.8 kg. All pigs were fed a six-phase commercial corn/soy based diet with dried distillers grains with solubles (DDGS) inclusion at 30% of dietary DM. Dried distillers grains with solubles was removed from the ration for the final 30 days of the finishing period. Pigs were removed from the performance test at a minimal weight of 111.4 kg and transported to Hormel Foods (Austin, MN) for slaughter the following morning.

Adipose tissue cellularity

Following harvest and upon arrival of the split carcasses to the chillbox, adipose tissue samples (about 20 g) were excised while still warm from the jowl, the back over the 10^{th} rib, and the midsection of the belly, placed in 0.9% NaCl solution at 37°C, and transported to Iowa State University (Ames, IA) at 37°C for cellularity assays. An approximately 200 mg section of fresh adipose tissue from the back (outer and middle

subcutaneous fat), belly, and jowl were fixed in 5 mL of 2% osmium tetroxide in 50 mM collidine hydrochloride buffer at pH 7.6 as described by Hirsch and Gallian[11] approximately 6 hours post-slaughter. Connective tissue debris was solubilized with 8 M urea as described by Etherton et al.[12]. Adipocytes were freed by washing with a 0.9% NaCl solution containing 0.01% Triton X-100, pH 10, through a 250-µm nylon mesh filter and collected onto a 20-µm nylon mesh filter. Mean and mode adipocyte cell size and cellularity (cells per gram of tissue) were determined by using a Coulter Counter (Beckman-Coulter, Brea, CA). The sample diluent was 0.9% NaCl containing 0.01% Triton X-100, pH 10, and the Kd (diameter calibration) of the machine was calibrated to 631.48. Samples were sized and counted by using a 400 µm aperture into 300 size bins ranging from 20 to 240 µm and counted in triplicate. The mean of the three measurements was used. The coefficient of variation between replicates was less than five percent.

Robust hypothesis testing of adipose bimodality and hyperplasia or lipid filling of previously divided fibroblastic cells

Formal testing of bimodality of each distribution of adipocytes was performed by identifying two(essentially) equal-width bins (Fig. 2A; i.e., coarsening the data), with bin 1 containing the with the suspected minor mode and bin 2 containing an area assumed to separate the major and minor modes to be used for comparison. Bin 1 contained measured adipocytes with a diameter between 25.0125 and 40.1052 µm. Bin 2 contained measured adipocytes with a diameter between 50.1565 and 65.3792 µm. For the k^{th} cell, we defined the event $[X_k = 1]$ with probability $p = \Pr[X_k = 1]$ if that cell's diameter falls

in bin 1 and the event $[X_k = 0]$ with probability $1 - p = \Pr[X_k = 0]$ if it falls in bin 2. The following natural *estimator* of (p) was used:

$$\hat{p} = \bar{X} = \frac{1}{N}\sum_{k=1}^{N} X_k \qquad \textbf{(1)}$$

As mentioned in the introduction, the data we are analyzing has the fortuitous property of having the structure shown in Fig. 1A. Specifically, there are two regions that, together, suggest bimodality (bins 1 and 2). Furthermore, because they have approximately equal width, under the unimodal assumption, it must be that the probability associated with bin 1 is no greater than that of bin 2. By focusing on only these two bins, the size of our data set is reduced to only data included in these bins. This sacrifice in the amount of available data is, however, offset by the simplicity of the associated random variables defined in relation to (1) above. These are simple 0/1 Bernoulli random variables. The *P*-value estimator given by (1) is simply a scaled binomial random variable that, for sufficiently large N, can be approximated as a normal random variable by invoking the Central Limit Theorem. No assumption of normality, or for that matter any population distribution, is needed.

The hypothesis test for distribution bimodality is simply:

H_0: $p = 0.5$ versus H_1: $p > 0.5$.

Because the sample size, N, associated with the number of cells falling in either of the two bins was on average on the order of 6,600 adipocytes counted for a given test, the Central Limit Theorem clearly applies and results in the following standard normal test statistic (Z):

$$Z = \frac{\hat{p} - 0.5}{0.5/\sqrt{N}} \qquad (2)$$

There are a number of tests for multimodality. One is the likelihood ratio test[13] that requires specification of the form of the distributions. Another is the dip test[14] which does not require *a priori* specification and, instead, uses the uniform distribution as the least favorable prior. Both tests are of a general nature in the sense that there is no *a priori* knowledge of any specifics associated with potential multimodality. Our data however, strongly support specific information. Specifically, our data show that the potential minor mode is well separated from the major mode and that the locations of the minor mode and saddle point are known. In this case, the aforementioned tests can be replaced by the much simpler test we used in this research.

Other statistical analyses

All statistical analyses other than the test for bimodality were performed by using SAS version 9.3 (SAS Inst. Inc., Cary, NC). Correlations were analyzed by using the CORR procedure of SAS.

Adipocyte cellularity (mean cell size, mode cell size, and adipocytes per gram of adipose tissue) data were analyzed by using the MIXED procedure of SAS with sampling location, breed, sex, sample date (contemporary group), and the interactions of breed × location and sex × location as fixed effects with percentage fat-free lean tissue (%FFL) as a covariate.

All means were separated by using an F-protected least-square means separation and reported as the mean plus or minus the SEM. When significant interaction effects were found, *P*-values were determined by using the SLICE command of LSMEANS. Statistical significance was declared at a $P < 0.05$.

Results and discussion

Bimodality of the adipocyte size distribution

For equal representation of lean and fat animals, these data were generated from breeds of growing pigs of varying adiposity as shown in Table 1. Evidence that sample mean adipocyte diameter is an inappropriate metric and that the data are bimodally distributed is shown in Fig. 3. Clearly, the most probable cell diameter, which one would expect to be the sample mean in a unimodal distribution, does not occur in the region of the grand mean (~91 μm averaged across all breeds and locations) but rather in the location of the mode of the major distribution of adipocytes (~112 μm averaged across all breeds and locations; Fig. 1D). The maximal probability of a particular adipocyte diameter that is located in the minor mode occurs quite near where we would expect to see it based on the minor mode data shown in Table 2 and Fig. 1D.

The cells that contribute to the cellularity of the minor mode could be of three distinct origins: 1) Nondifferentiated stem cells become differentiated to preadipocytes that begin to accumulate lipid and come to be found in the minor mode, 2) Proliferative pre-adipocytes from embryonic development may begin to add lipid and come to be found in the minor mode (~8 μg to 34 μg of lipid), and 3) Mature cells may proliferate and appear in either the major or minor mode [15]. The cells found in the major mode are of two types: 1) Mature adipocytes that have accumulated enough lipid (approximately 144 μg or more of lipid) to be found in the major mode and 2) Mature adipocytes that have proliferated but are still large enough to be found in the major mode. It is, however, beyond the scope of this methods paper to investigate the origin of the small adipocytes that appear in the minor mode.

Additionally, reporting sample mean cell size is only appropriate if cells are unimodally distributed. In carrying out the hypothesis test for bimodality described in the Materials and Methods section, we observed that, indeed, 93.18% of the 455 measured adipose tissue samples had a bimodal distribution of adipocytes at a P-value < 0.05. The additional 6.82% of samples that did not test positive for being bimodal were visually inspected to verify that no secondary mode existed. These samples were, however, not a normal distribution. So, sample mean adipocyte diameter is not the appropriate metric to use for describing distributions of adipocyte diameters. Thus, we propose using adipocyte diameter of the mode of the minor (Table 2) and major (Table 3, Fig. 1D) distributions of cells as a metric for reporting cell distributions for future work. For sake of comparison to traditional metrics of adipocyte size, we also have included grand mean adipocyte cell sizes in Table 4.

Adipose tissue cellularity and mean and mode cell size

Cellularity, defined as the number of cells (adipocytes) per gram of adipose tissue, of backfat was significantly and negatively correlated to backfat thickness (r = -0.515; Table 5). Adipose tissue cellularity (Table 6) was consistent with, but slightly lower than, cellularity data reported for 114 kg pigs sampled from dorsal subcutaneous neck adipose tissue by Mersmann and Macneil[5] (1.81×10^6 cells x gram^{-1}). A possible explanation for our data being slightly lower is that the adipose of the dorsal neck may differ from the adipose of the ventral neck. Significant breed effects in cellularity were found when comparing back, belly, and jowl adipose depots (P = 0.0004, P < 0.0001, and P = 0.0209, respectively) across breeds (Table 6). Differences also were found when comparing anatomical locations within a breed, with the belly always having the

numerically greatest cellularity and back always having the numerically least cellularity. It is also worth noting that most of the measured depots had a fairly large standard error of mean (SEM) indicating that there is considerable variability in the cellularity of these measured depots both within and across breeds.

Significant differences for mean adipocyte cell size were only found in belly fat ($P = 0.0007$) across breeds, with Duroc pigs having the smallest mean adipocyte size and Berkshire pigs having the largest. Within Berkshire pigs, the mean cell size of jowl fat adipocytes was significantly smaller in diameter those that in belly and backfat ($P < 0.05$; Table 4).

The adipocyte diameter of the mode of the minor distribution of cells (Table 2; modes described in Fig. 1B) did not vary significantly across breeds but varied significantly within a breed. The general trend of adipocyte diameter of the minor mode was for backfat adipocytes in the minor distribution of cells to be of the smallest diameter and belly fat adipocytes of the minor mode always to be of the largest diameter. It is, however, unclear if the differences in adipocyte diameter of the mode of the minor distribution is of practical significance because the differences were 4 μm or less and because the number of cells that fall into the minor mode is very small in comparison to the cells found in the major mode. In addition, adipocyte diameter of the minor mode is not significantly correlated to adiposity as measured by backfat thickness (data not shown). Therefore, the differences in adipocyte diameter of the minor mode are not likely of any physiological consequence. Adipocyte diameter of the mode of the minor distribution of cells was also significantly different for barrows and gilts (approximately 1.6 μm) which, again, is likely not of any physiological significance.

The adipocyte diameter of the mode of cells found within the major distribution (Table 3) varied significantly within both a breed and anatomical location within sex. As expected, Durocs, one of the leanest breeds of pigs (Table 1), had the smallest adipocyte diameter for the mode of the major distribution of cells (Table 3). Interestingly, Yorkshire and Chester White pigs that were equally as lean as Duroc pigs had an intermediate adipocyte diameter for the mode of the major distribution of cells, indicating that adiposity is not the only factor influencing the adipocyte diameter of the mode of the major distribution of adipocytes in Duroc pigs (Table 3). The largest adipocyte diameter of the mode of the primary distribution of cells was found in Berkshire pigs, the breed with greatest adiposity in this experiment. It seems that Duroc pigs may be somewhat of an anomaly as based on this cellularity-related data, likely as a result of genetic differences. When Duroc pigs are ignored, the range between the smallest and largest major mode cell size is only about 5 μm, which might be of little physiological consequence (Table 3).

Adipose tissue hypertrophy and appearance of small adipocytes in the minor mode

Understanding how adipose tissue development and growth is regulated is of great clinical importance[16]. Adipose tissue growth occurs by hypertrophy, hyperplasia, or both hypertrophy and hyperplasia[15]. Understanding the mechanism of adipose tissue growth is important to basic science and relevant to animal and human health. Adipose growth by hypertrophy is linked with inflammation, fibrosis, and insulin resistance[17], whereas adipose hyperplasia is linked with increased insulin sensitivity[17-18]. Because the adipose samples we studied were distributed bimodally, we investigated if the nature of this bimodal distribution was evidence of hypertrophy, hyperplasia, or both hypertrophy and

hyperplasia occurring in pigs of market weight. Because backfat thickness was the indicator of adiposity, it would be inappropriate to correlate attributes of backfat adipose to any depot other than the back (Table 5). As would be expected, mean and mode cell size of the back adipose tissue is correlated negatively to the cellularity of backfat adipose, and adipocyte cell size is positively correlated with backfat thickness, indicating that, as an animal becomes larger, its adipose tissue is filled with larger adipocytes and fewer adipocytes per gram of tissue (Table 5). When comparing the adipocyte diameter of the minor mode to backfat thickness (Table 5), we found no significant correlation between the two measurements, indicating that the adipocyte diameter of the minor mode is not dependent on the adiposity of the pig. The ratio of the adipocyte diameter of the minor mode to the adipocyte diameter of the major mode (r_d) was significantly and negatively correlated to backfat thickness (Table 5) and, because the adipocyte diameter of the minor mode is not dependent on the adiposity of the pig, this negative correlation indicates that as the backfat thickness increases the adipocyte diameter of the major mode becomes larger and the minor distribution of cells becomes more well defined (i.e., more adipocytes represented by the minor mode; Fig. 1C).

Next, we computed an estimate of the ratio of probabilities (r_p), the probability of an adipocyte being in the minor mode divided by probability of an adipocyte being in the major mode. A significant and positive correlation (Table 5) between r_p and backfat thickness indicates that as adiposity increases the probability of an adipocyte being found in the minor mode is greater, which is consistent with adipose hyperplasia or differentiation of pre-adipocytes into nascent adipocytes. The correlation of r_d and r_p to backfat thickness supports the idea that in the finishing phase these pigs were undergoing

adipose growth via both hypertrophy and hyperplasia, differentiation of pre-adipocytes to nascent adipocytes, or lipid filling of previously divided fibroblastic cells. The histogram of probabilities associated with adipocyte diameters (Fig. 3a) indicates that the maximal adipocyte diameter is limited to approximately 240 μm because the probability of finding an adipocyte of that size or greater drops to nearly zero. Thus, the limit of hypertrophy seems to occur when porcine adipocytes reach a diameter of around 240 μm.

More evidence of hyperplasia or differentiation of pre-adipocytes into nascent adipocytes was indicated when comparing r_p to backfat thickness. As backfat thickness increases, r_p increases (Table 5), suggesting that as adiposity increases appearance of small adipocytes in the minor mode increases (i.e., differentiation of preadipocytes to adipocyte, both hyperplasia and differentiation of preadipocytes, or lipid filling of previously divided fibroblastic cells). The notion that these data represent an increase in number of small adipocytes in adipose tissue is supported even further when comparing r_p to r_d (Fig. 2). Figure 2 demonstrates this notion, because these two ratios are negatively correlated. As the adipocyte diameter of the major mode becomes larger (or the adipocyte diameter of the minor mode becomes smaller), the probability of finding an adipocyte in the distribution of adipocytes containing the minor mode becomes larger (or the probability of finding an adipocyte in the distribution of adipocytes containing the major mode becomes smaller). The results that indicate hyperplasia, differentiation of pre-adipocytes to nascent adipocytes, or lipid filling of previously divided fibroblastic cells is occurring in these pigs at slaughter weight are not consistent with previous research that observed no further hyperplastic growth and only hypertrophic growth was observed in extramuscular fat of Hampshire × Yorkshire and Minnesota 3 × 1 pigs after

83 kg live weight[20]. However, both adipocyte hypertrophy and hyperplasia were observed in both Meishan (~52 kg) and Landrace pigs (~83 kg) at 5 months of age[20]. Traditionally, including the work done by Nakajima and colleagues[21], researchers have relied on an observed bimodal/biphasic distribution to be evidence of hyperplasia. We have taken that one step further and applied rigorous statistical analyses to describe the bimodal distribution evidence of an increase in the number of small adipocytes gleaned from these distributions by quantitative rather than by qualitative methods. It is possible that in a younger pig you would not see such a prominent minor mode, and the probability of finding an adipocyte in the major mode or minor mode may be reversed. It is worth taking into consideration that these pigs were adolescent in terms of human growth at the time of slaughter and that hyperplasia has been demonstrated in humans to occur through adolescence[22]. So, observing adipose growth by both hypertrophy and an increase in number of small adipocytes at this age of the pig is consistent with data previously reported. Additionally, high fat diets, such as those for the pig, cause hyperplasia in rats[23]. It also has been demonstrated that in mice hypertrophy and hyperplasia occur as a result of fat pad mass increases rather than as a function of age[6]. Finally, variations between individuals occur and that some New Zealand rabbits at one year of age have normally distributed adipocytes and some have bimodally distributed adipocytes[24]. The authors speculated that the bimodal distribution was a result of recruitment of new small adipocytes. These data reported in our study assume that small cells (located in the minor distribution) are new cells and also rely on only one time point in the life of the animal. However, differentiating between new cells and old small cells is not possible. Future confirmation of in vivo cellular division by BrdU/PCNA[25-26]

studies should be done to differentiate between the many ways an adipocyte may be found in the minor mode. These ways include both hyperplasia of adipocytes, differentiation of pre-adipocytes to nascent adipocytes, or lipid filling of previously divided fibroblastic cells and cannot be distinguished by using this indirect method of measuring hyperplasia. To fully understand how adipose growth progresses, a longitudinal animal study examining regulation and development of adipose tissue over time, and going well into adulthood for the animal, needs to be done to determine if hyperplasia occurs in adulthood.

It was determined that sample mean adipocyte cell size is an inappropriate descriptor of adipocyte cell size because of the bimodal distribution of adipocytes found in the vast majority of adipose depots assayed. We recommend that future adipose tissue research include the analysis proposed in this study to quantitatively identify bimodality of adipocyte cell distributions.

Finally, convincing evidence of hypertrophy and increased numbers of small adipocytes (either by hyperplasia or by differentiation of pre-adipocytes into nascent adipocytes) both being a factor in expansion of adipose tissue of pigs at market weight was shown through quantitative methods and should be applied to future adipose tissue growth and development research. Increased numbers of small adipocytes in adipose tissue that is still occurring in pigs of market weight begs the question whether or not hyperplasia of adipose tissue continues on into adult life, particularly in high fat feeding programs. More research will need to be done to determine whether or not hyperplasia of adipose tissue is a factor in adult-life, to identify mechanisms controlling hyperplasia of

adipose tissue, and to develop the potential implications to better understand the growth and development of adipose tissue of food-producing animals and of humans.

Disclosure of potential conflicts of interest

The authors have no potential conflicts of interest to disclose.

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Table 1.
Average daily gain, fat-free lean percentage, and backfat thickness of five purebred and one crossbred line of pigs

	Breed[2]					Sex		*P*-Values		
Item	Berkshire	Chester White	Duroc	Landrace	Yorkshire	Barrow	Gilt	Breed	Sex	Breed ×Sex
ADG, kg	0.80[a] (± 0.01)	0.83[ab] (± 0.02)	0.87[b] (± 0.01)	0.79[a] (± 0.01)	0.87[b] (± 0.01)	0.85[b] (± 0.01)	0.82[a] (± 0.01)	<0.001	0.011	0.151
FFL, %[3]	47.94[a] (± 0.41)	50.59[bc] (± 1.15)	52.53[cd] (± 0.39)	51.19[b] (± 0.44)	52.39[cd] (± 0.40)	49.33[a] (± 0.24)	53.34[b] (± 0.43)	<0.001	<0.001	0.016
BF, cm[4]	2.346[ab] (±0.01)	2.366[a] (±0.04)	2.493[c] (±0.01)	2.467[c] (±0.01)	2.415[ab] (±0.01)	2.455[b] (±0.01)	2.382[a] (±0.01)	<0.0001	<0.0001	0.0004

[a, b, c, d] Within a row and main effect, means without a common superscript differ ($P < 0.05$).

[1] Standard error in parenthesis below mean value.

[2] n = 149 pigs; number of pigs within each breed (number of barrows, number of gilts): Berkshire = 65 (40, 25); Chester White = 17 (15, 2);
Duroc = 11 (7, 4); Landrace = 23 (6, 17); Yorkshire = 33 (17, 16).

[3] FFL = fat-free lean.

[a, b, c, d] Within a row and main effect, means without a common superscript differ ($P < 0.05$).

[4] BF = backfat thickness in cm.

Table 2. Minor mode adipocyte diameter from five breeds of pigs as affected by breed, anatomical location, and sex (μm)[1, 2]

	Breed					Sex[3]		*P* – Value	
Depot	Berkshire	Chester White	Duroc	Landrace	Yorkshire	B	G	Breed × Location	Sex
Back	30.67^{X}	31.95^{X}	29.12^{X}	31.17^{X}	30.06^{X}	32.85	31.17	0.1960	0.0002
	(±0.45)	(±0.84)	(±1.03)	(±0.75)	(±0.64)	(±0.27)	(±0.35)		
Belly	33.00^{Y}	34.81^{Y}	33.67^{Y}	34.68^{Y}	32.66^{Y}			0.1166	
	(±0.44)	(±0.92)	(±1.28)	(±0.81)	(±0.64)				
Jowl	32.93^{Y}	32.01^{Y}	30.70^{XY}	30.50^{X}	32.29^{Y}			0.0900	
	(±0.45)	(±0.88)	(±1.13)	(±0.83)	(±0.63)				

[1] *n* =149 pigs; number of pigs within each breed (number of barrows, number of gilts): Berkshire = 65 (40, 25); Chester White = 17 (15, 2); Duroc = 11 (7, 4); Landrace = 23 (6, 17); Yorkshire = 33 (17, 16).
[2] Standard error in parenthesis below mean value.
[3] B = Barrow, G = Gilt.
[X, Y, Z] Within a column, means without a common superscript differ ($P < 0.05$).

Table 3. Major mode adipocyte diameter from five breeds of pigs as affected by breed and anatomical location within sex (μm)[1, 2]

Item	Breed					Location						*P* – Values	
						Back		Belly		Jowl			
	Berkshire	Chester White	Duroc	Landrace	Yorkshire	Barrow	Gilt	Barrow	Gilt	Barrow	Gilt	Breed	Location ×Sex
Major Mode[3]	117.18[c]	112.15[b]	106.14[a]	112.80[b]	111.01[b]	117.03[c]	112.03[b]	104.43[a]	111.83[b]	111.91[b]	113.91[b,c]	<0.0001	<0.0001
	(0.85)	(1.50)	(1.89)	(1.34)	(1.12)	(1.28)	(1.54)	(1.29)	(1.55)	(1.28)	(1.60)		

[1] *n* = 149 pigs; number of pigs within each breed (number of barrows, number of gilts): Berkshire = 65 (40, 25); Chester White = 17 (15, 2); Duroc = 11 (7, 4); Landrace = 23 (6, 17); Yorkshire = 33 (17, 16).
[2] Standard error in parenthesis below mean value.
[3] Adipocyte diameter of the mode of cells within the major distribution.
[a, b, c] Within a row, means for breeds without a common superscript differ ($P < 0.05$).

Table 4. Mean adipocyte cell size from five breeds of pigs (μm)[1, 2]

Depot	Breed					*P* - Value
	Berkshire	Chester White	Duroc	Landrace	Yorkshire	Breed × Location
Back	94.26[Y] (± 0.88)	91.05 (± 1.69)	90.59 (± 2.07)	93.46 (± 1.45)	92.46 (± 1.22)	0.3019
Belly	95.41[Y, c] (± 0.88)	89.02[a,b] (± 1.74)	87.42[a] (± 2.07)	93.26[b,c] (± 1.48)	92.49[b,c] (± 1.22)	0.0007
Jowl	89.15[X] (± 0.91)	90.57 (± 1.70)	92.08 (± 2.07)	90.73 (± 1.51)	90.67 (± 1.24)	0.6873

[1] *n* =149 pigs; number of pigs within each breed (number of barrows, number of gilts): Berkshire = 65 (40, 25); Chester White = 17 (15, 2); Duroc = 11 (7, 4); Landrace = 23 (6, 17); Yorkshire = 33 (17, 16).
[2] Standard error in parenthesis below mean value.
[X, Y, Z] Within a column, means without a common superscript differ ($P < 0.05$).
[a, b, c] Within a row, means without a common superscript differ ($P < 0.05$).

Table 5. Correlations of cellularity and cell size related measures of back adipose tissue

Items	Data[1]	Data[2]	*r*	*P*- Value*3*
Cellularity × 10^6 of backfat to BF[4]	1.09 ± 0.42	2.48 ± 0.79	-0.5157	<0.0001
Cellularity × 10^6 of backfat to mean cell size of backfat (µm)	1.09 ± 0.42	93.21 ± 7.71	-0.6610	<0.0001
Cellularity × 10^6 of backfat to major mode cell size of backfat (µm)	1.09 ± 0.42	117.16 ± 13.75	-0.6491	<0.0001
BF[4] to major mode cell size of backfat (µm)	2.48 ± 0.79	117.16 ± 13.75	0.7818	<0.0001
BF[4] to secondary mode cell size (µm) of backfat	2.48 ± 0.79	30.81 ± 3.10	-0.0681	0.4226
BF[4] to probability ratio of modes of backfat[5]	2.48 ± 0.79	0.19 ± 0.04	0.4266	<0.0001
BF[4] to ratio of mode cell sizes of backfat[6]	2.48 ± 0.79	0.26 ± 0.04	-0.6021	<0.0001
BF[4] to probability of a cell being found in the minor mode of backfat	2.48 ± 0.79	0.15 ± 0.02	0.4601	<0.0001
BF[4] to probability of a cell being found in the in the major mode of backfat	2.48 ± 0.79	0.78 ± 0.03	-0.2228	0.0079

[1] Mean numerical value from first correlate in each line plus/minus standard deviation, n = 143 pigs.
[2] Mean numerical value from second correlate each line plus/minus standard deviation, n = 143 pigs.
[3] *P*-values for difference from zero.
[4] BF = backfat thickness in centimeters.
[5] Probability of finding an adipocyte in the minor mode divided by the probability of finding an adipocyte in the major mode.
[6] Adipocyte cell diameter of the minor mode divided by the adipocyte cell diameter of the major mode.

Table 6. Adipose tissue cells per gram of tissue of five breeds of pig (X 10^6) [1]

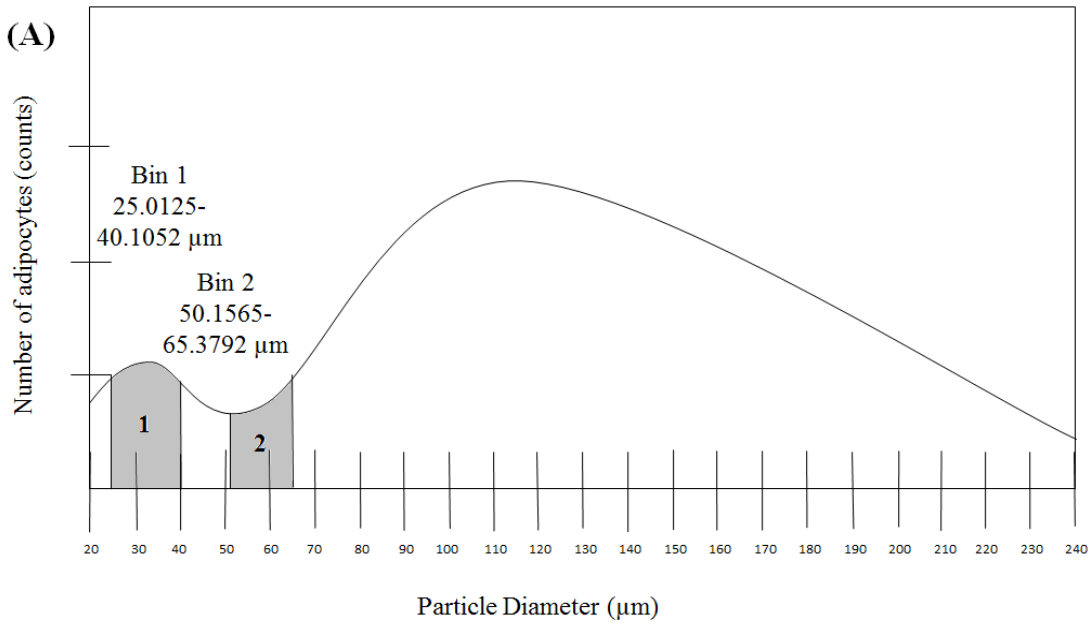
	Breed[2]					*P*-Value
Depot	Berkshire	Chester White	Duroc	Landrace	Yorkshire	Breed × Location
Back	1.01[a, X]	1.15[a, X]	1.55[b, X]	1.07[a, X]	1.04[a, X]	0.0004
	(± 0.05)	(± 0.09)	(± 0.11)	(± 0.08)	(± 0.07)	
Belly	1.35[a, Y]	1.68[b, Y]	2.24[c, Y]	1.63[b, Z]	1.51[a, b, Y]	<0.0001
	(± 0.05)	(± .10)	(± 0.11)	(± 0.08)	(± 0.07)	
Jowl	1.24[a, b, Y]	1.45[c, Y]	1.46[b, c, X]	1.31[a, b, c, Y]	1.14[a, X]	0.0209
	(± 0.05)	(± 0.09)	(± 0.11)	(± 0.08)	(± 0.07)	

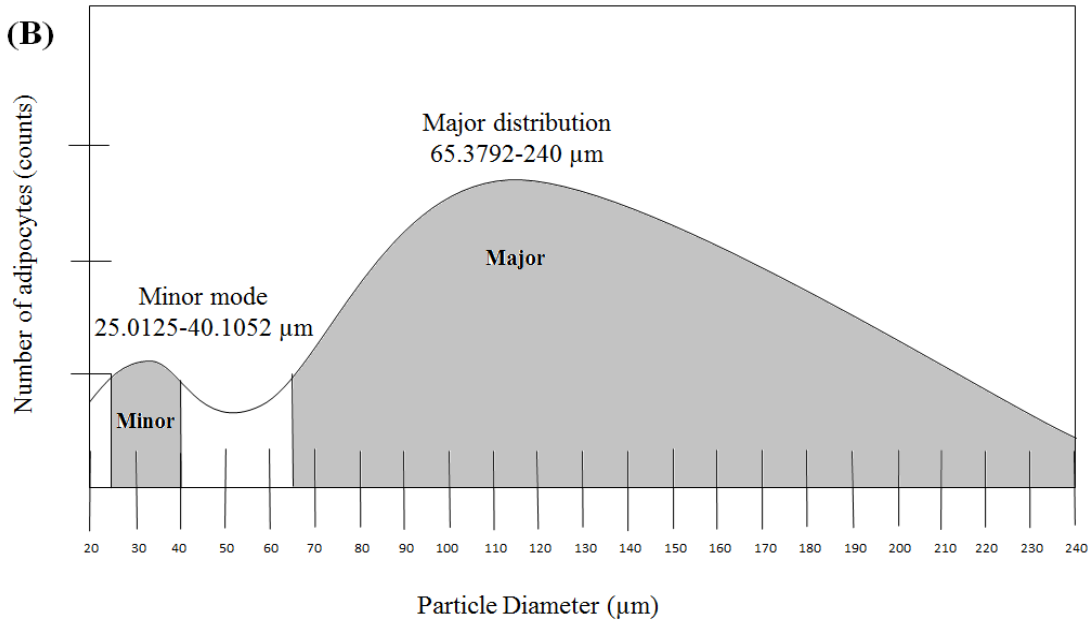
[1] Standard error in parenthesis below mean value.

[2] n = 149 pigs; number of pigs within each breed (number of barrows, number of gilts): Berkshire = 65 (40, 25); Chester White = 17 (15, 2); Duroc = 11 (7, 4); Landrace = 23 (6, 17); Yorkshire = 33 (17, 16).

[X, Y, Z] Within a column, means without a common superscript differ ($P < 0.05$).

[a, b, c] Within a row, means without a common superscript differ ($P < 0.05$).





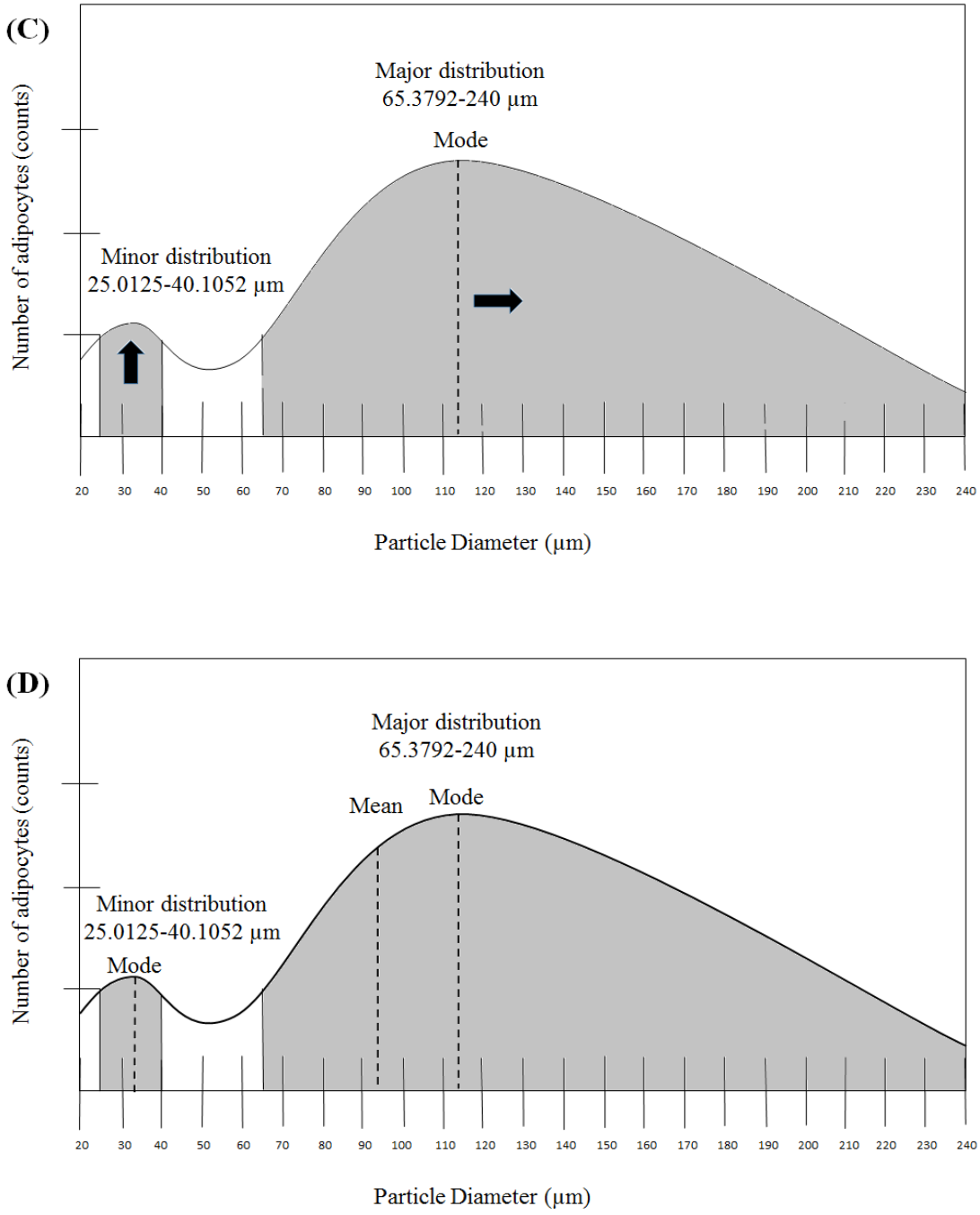


Fig. 1. Representation of typical distribution of adipocytes in porcine adipose tissue. (A) Representation of bin 1 and bin 2 (defined in Materials and Methods) used in hypothesis testing for determining unimodality or bimodality of adipocyte distribution. (B) Representation of the "minor" and "major" modes with the "dead zone." (Bin 2 in Fig. 1A; 50.1565-65.3792 µm) (C) Illustration of the behavior of the minor and major modes as an animal increases in adiposity. The minor distribution of cells becomes better defined (i.e., taller), and the mode of the major distribution of cells moves to the right.

(D) Illustration of why mean adipocyte size is an inappropriate metric for describing bimodal adipocyte cell distributions. It is clear that the mean is misleading because the presence of the minor mode lowers the value of the mean. The mode of the major distribution and the mode of the minor distribution more accurately represent the most abundant diameter of adipocyte in both distributions.

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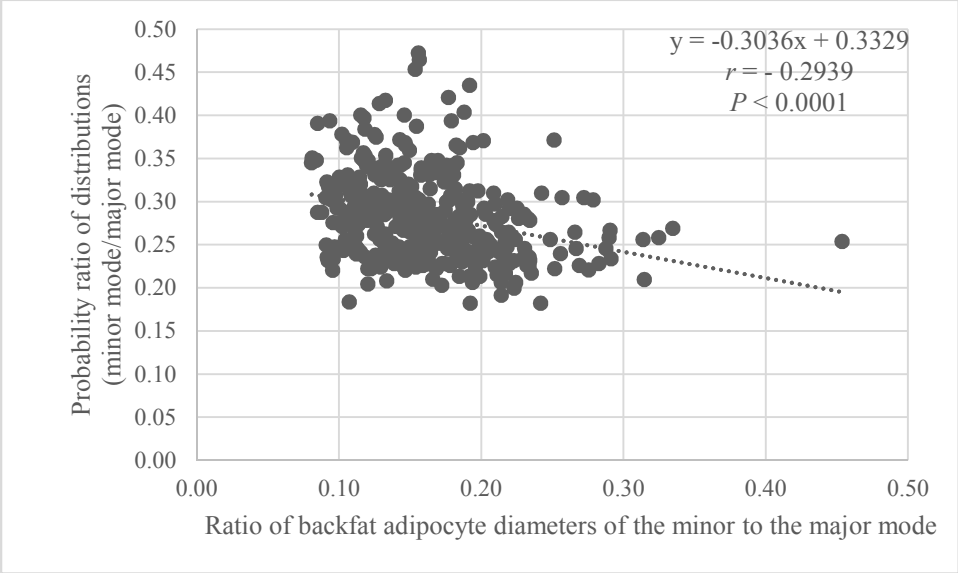

Fig. 2. Behavior of the major and minor modes in relation to probability of an adipocyte being found in either the major or minor mode**.** Relationship (probability ratio of modes) of the area under the curve of the distribution of adipocytes containing minor mode (smaller cell diameter) divided by the area under the curve of the distribution of adipocytes containing the major mode (larger cell diameter) to the ratio of the adipocyte diameter of the mode of the minor adipocyte distribution divided by the adipocyte diameter of the mode of the major adipocyte distribution (ratio of adipocyte diameters of the minor to the major mode).

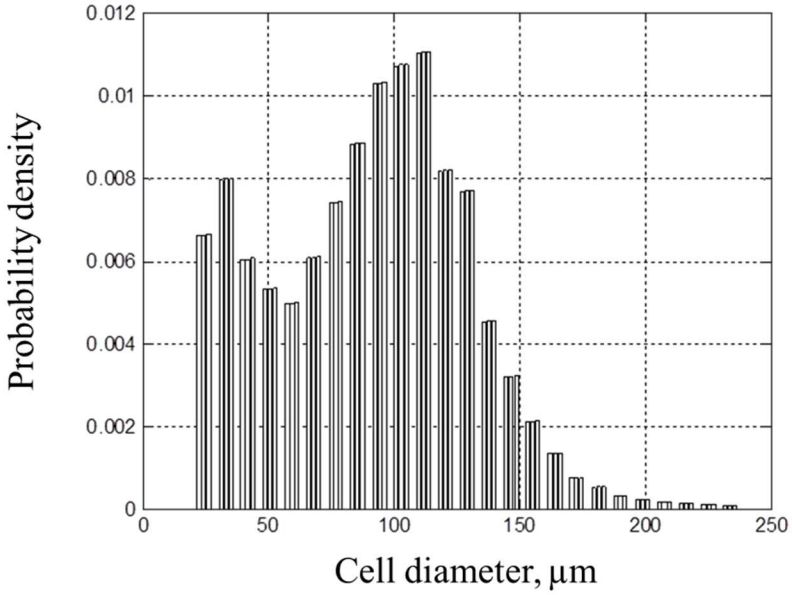

Fig. 3. Histogram-based adipocyte diameter probability distribution function. Probability distribution function was generated from 14,703,815 size bins. Probability distribution function of adipocyte diameter is from all breeds, sexes, and anatomical locations of assayed pigs (n = 149 pigs x 3 anatomical locations = 447). In each cluster of bars, the left bar is the lower standard (2-sigma) error, the middle bar is the estimated probability distribution function, and the right bar is the upper standard (2-sigma) error. As in Fig. 2C, we would expect that as an animal gains more adiposity the probability of the minor distribution of cells as a whole would increase, and maximum probability of the major distribution of adipocytes would shift to a larger adipocyte diameter. The probability of a chosen bin is the bin width (8.7141 µm) multiplied by the height of that chosen bin