EVALUATION OF NONENZYMATICALLY BROWNED SUNFLOWER SEEDS FOR LACTATING DAIRY COWS

by

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EVALUATION OF NONENZYMATICALLY BROWNED SUNFLOWER SEEDS FOR LACTATING DAIRY COWS

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Initial *in vitro* and in situ trials evaluated the effect of amount of sulfite liquor on ruminally undegradable lipid (RUL) and protein for a range of oilseeds including canola, soybeans, linseed, and sunflower seeds. We selected sunflower seeds because of the desirable fatty acid profile relative to altering milk fatty acid composition, and because they could potentially deliver as much RUL to the lower tract as soybeans (previous research, summarized in Appendix).

Two forms of nonenzymatically browned sunflower seeds were evaluated in two lactating dairy cow trials to determine their effect on animal performance and ruminal fermentation. Trial 1 evaluated nonenzymatically browned, ground sunflowers with hulls, and Trial 2 evaluated nonenzymatically browned sunflower seeds that had been chipped and dehulled. In the first experiment, eight lactating Holstein cows were used in a replicated 4 x 4 Latin square with 3-wk periods. Four treatments were used: 1) control diet with no added lipid, 2) sunflower oil (SFO), 3) untreated, ground sunflower seeds (GSF), and 4) nonenzymatically browned, ground sunflower seed (NEBS). The control diet contained 50% forage (DM basis) and the remaining diets contained 50 % forage and 4% added lipid. The objective of this study was to determine the effect of NEBS on ruminal fermentation and lactational performance. Sunflower oil diet resulted in the lowest DMI, whereas DMI for cows fed GSF was intermediate. DMI for the control and

NEBS diets were highest of the diets in this study. Cows fed the SFO diet had the lowest production of 4% fat-corrected milk, whereas 4% fat-corrected milk production was intermediate for cows fed the control and GSF diets, and highest for cows fed the NEBS diet. Cows fed SFO, GSF, or NEBS had a 56% reduction of C16:0 in milk fat compared with cows fed the control diet. The proportion of C18:3, C18:2, and C18:1 *cis* fatty acids in milk fat was greater for cows fed the NEBS diet than the other diets. In contrast, the SFO and GSF diets resulted in elevated C18:1 *trans* fatty acids.

In the second trial, thirty cows were assigned to one of three diets for a period of 6 wk. Diets were fed as total mixed rations that contained 40% of a 1:1 mixture of alfalfa and corn silage and 1) wet corn milling product (CMP) with no added lipid, 2) CMP + nonenzymatically browned sunflowers (NEBS), and 3) CMP + tallow. A companion study indicated that source of RUP in the CMP had no effect on lactation performance. Diet 1 contained 3% lipid, whereas diet 2 and 3 contained 6% lipid. Our objective was to investigate the effect of supplementing a diet containing high amounts of wet corn milling product with RUP and RUL from nonenzymatically browned sunflowers on efficiency of fat-corrected milk production and composition of milk fatty acids. The DMI averaged 28.1 kg/d or 4.6% of body weight for cows fed all diets. The 4% fat-corrected milk production averaged 33.3 kg/d with no differences among the three diets. Milk fat output was similar among all diets. Milk fatty acid profile from cows fed NEBS showed higher amounts of C18:1 (*trans*-vaccenic acid), C18:2, and C18:2 (*cis*-9, *trans*-11) (conjugated linoleic acid, CLA) than cows fed the control or tallow diets. However, C14:0 and C16:0 were significantly lower in milk from cows fed NEBS diet compared with the other diets.

The results of these studies indicate that the oil in NEBS was partially protected from ruminal biohydrogenation and successfully elevated the desirable poly- and monounsaturated fatty acid content of milk fat, reduced saturated fatty acids, and increased CLA content in milk fat. The experiments also demonstrated that NEBS accomplish the positive alterations in milk fatty acid composition without reducing the output of milk fat.

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INTRODUCTION

Milk fat is the most complex of all common fat sources and the milk constituent with the greatest variability in its percentage. Typical milk fat is composed of 95 to 98% triglycerides. Saturated fatty acids represent the majority of milk fatty acids (about 66%), whereas monounsaturated fatty acids represent only about 30%, with 4% polyunsaturated fatty acids. Early lactation dairy cows require a diet that contains high energy density to attain their full potential for milk production. Lipid supplementation has the ability to provide these cows with energy and simultaneously lower the risk of ruminal acidosis because less starch and other rapidly fermentable carbohydrates need to be fed. Studies have indicated that 5 to 7% fat may be incorporated into dairy rations with minimal impact on fiber digestibility if fed properly. The upper limit for feeding fat is 8 to 10% of total ration dry matter, particularly for lipid sources containing high proportions of polyunsaturated fatty acids.

Lipids also have been included in dairy rations to alter milk fatty acid composition. In recent years, there has been increased interest and research in the area of "designer milk" products. The idea has been to feed cows diets that selectively enhance the content of mono- and polyunsaturated fatty acids, while reducing the content of saturated fatty acids. Most recently, there has been interest in exploring feeding strategies to boost the content of conjugated linoleic acid in milk fat because of its putative benefits as an anticarcinogen.

One potential risk of feeding lipids to dairy cows is a negative impact on ruminal microflora and reduced fiber digestibility (Chalupa et al., 1984). When lipids are fed at high concentrations in the ration, particularly polyunsaturated fatty acids, they may cause

a reduction in ruminal fermentation, dry matter intake, milk yield and milk fat production. Polyunsaturated fatty acids are most likely to reduce ruminal fermentation, and diets need to be formulated to achieve a proper ratio of saturated and unsaturated fatty acids.

A method for protecting soybean meal protein by nonenzymatic browning was developed at the University of Nebraska to reduce ruminal degradation of protein. Subsequently, Abel-Caines (1998) demonstrated that nonenzymatic browning also indirectly protected a portion of the lipid in full-fat soybean seeds as well. This is important because of the partial protection the process would confer on the fatty acids from ruminal hydrogenation. In the rumen, dietary lipids undergo hydrogenation by ruminal microflora that results in fatty acid composition of milk fat that differs markedly from dietary fatty acid composition.

The primary purpose of this dissertation was to evaluate the effectiveness of nonenzymatically browned oilseeds, particularly sunflower seeds, on milk fatty acid composition and milk production of dairy cows fed diets high in nonforage fiber sources such as wet corn gluten feed. A diet containing large amounts (40% of ration DM) of wet corn gluten feed (referred to as wet corn milling product in this dissertation) combined with supplemental fat from nonenzymatically browned oilseeds would be a very novel feeding strategy for lactating cows. This feeding approach would potentially have the advantages of reduced risk of acidosis (due to greater energy from digestible fiber) and enhanced milk fatty acid profiles from the oilseeds. Additionally, if energy is limiting milk yield in these high-fiber diets, then the added lipid from the oilseed should improve lactational performance.

LITERATURE REVIEW

Physical structure of milk fat

The position of fatty acids on the glycerol molecule is responsible for many physical features of milk fat. These include high digestibility of saturated fatty acids, the separable nature of butter, and perhaps for the hypercholesterolemic potential of milk fat (German et al., 1997). Fatty acids attached to the glycerol molecule are usually designated as sn-1, sn-2, or sn-3. Two thirds of the fatty acids in milk fat are medium and long-chain saturated fatty acids. Long chain fatty acids are most predominant in the sn-1 position. The sn-3 position is always occupied by 4:0, 6:0, and 18:1 fatty acids. However, almost all of butyric and caproic acids are found in the sn-3 position. These are hydrolyzed rapidly by gastric lipases. The lipases release these water-soluble fatty acids to aid in emulsification of the bulk lipid in the stomach for easier digestion in the intestine. However, these short chain fatty acids are rapidly absorbed directly into the blood, followed by rapid clearance and oxidation by the liver. Therefore, these fatty acids are very important for newborn animals because they provide them with a rapidly available energy source.

In the rumen, saturated fatty acids tend to be less digestible than unsaturated fatty acids. An exception occurs if the fatty acids reside in the sn-2 position of the glycerol molecule because a large proportion of these fatty acids will be absorbed as the 2-monoglycerides that are more digestible than fatty acids. Thus, because a high percentage of saturated fatty acids in milk fat are found in the sn-2 position, these fatty acids which have a potentially negative impact on human health ironically also have a higher digestibility.

Milk fat composition

Milk is composed of water, carbohydrate (lactose), fat, protein, minerals, and vitamins. There are about 400 fatty acids that have been identified in milk fat, but fewer than a dozen contribute at least 1% to the total weight of milk fatty acids (Kaylegian and Lindsay, 1995). These include the fatty acids synthesized de novo in the mammary gland from acetate and β-hydroxybuytrate and having a hydrocarbon chain length between 4 and 16 carbon atoms. All 18 carbon fatty acids and part of palmitic acid (C16:0) are derived from dietary origin. However, minor amounts of these fatty acids are derived from mobilized adipose tissue fatty acids. Ruminal microbial metabolism likely contributes to the secretion of minor fatty acids in milk fat.

Classes of lipids

Several chemical components have been identified and characterized in milk fat. The average composition of milk lipid as reported by Bitman and Wood (1990) is provided in Table 1. Triacylglycerol comprises the majority of the lipid found in milk fat. In this section, I will discuss two of the most important categories of lipids: fatty acids and triglycerols.

Fatty acids. Fatty acids are the main constituents of fats. They are comprised of carbon, hydrogen, and oxygen. Fatty acid molecules consist of carbon atoms linked to one another to form a chain of varying lengths from 4 to 26 carbon atoms. Thus, a fatty acid is composed of a long hydrocarbon chain or tail and terminal carboxyl group or head. Fatty acids occur in large amounts in biological systems, but rarely in the free, uncomplexed state. Fatty acids are typically esterified to glycerol or some other backbone structure. Most of the fatty acids found in nature have an even number of carbon atoms,

usually 14 to 24. However, certain marine organisms contain substantial amounts of fatty acids with odd number of carbon atoms.

Fatty acids are divided into two groups: saturated fatty acids which have all carbon-carbon bonding in single form or unsaturated fatty acids with one or more double bonds in the hydrocarbon chain. A fatty acid with a single double bond is called monounsaturated, whereas fatty acid with more than one double bond is termed polyunsaturated. Fatty acids can be named or described in at least three different ways. For example, a fatty acid composed of an 18-carbon chain with no double bonds can be called by its systematic name (octadecanoic acid), its common name (stearic acid), or its shorthand notation, in which the number of carbons is followed by a colon and the number of double bonds in the molecule, such as C18:0. Stearic acid (C18:0) and palmitic acid (C16:0) are the most common saturated fatty acids found in nature.

Omega-3 and omega-6 fatty acids are members of the polyunsaturated fatty acid family. The terms omega-3 and omega-6 are derived from the position of the double bond within the carbon atom chain. The carbon atom in the chain that is furthest away from the carboxyl group is labeled omega because it is at the end of the molecule. The numbers 3 and 6 denote the number of the C-atom counted from the omega-C-atom at which the first double bond occurs.

Categories of fatty acids. There are three major categories of fatty acids: saturated, polyunsaturated, and monounsaturated. These classifications are based on the number of hydrogen atoms in the chemical structure of a given molecule of fatty acid.

Saturated fatty acids are found primarily in animal products, including dairy products, such as whole milk, cream and cheese, and fatty meats like beef, lamb, pork,

and ham. Some vegetable products including coconut oil, palm kernel oil, and vegetable-shortening also contain high concentrations of saturated fatty acids. The liver uses saturated fatty acids to synthesize cholesterol. Therefore, excessive dietary intake of saturated fats can significantly raise blood cholesterol levels, especially the level of low-density lipoproteins (LDL), or "bad" cholesterol. Some recommend that the daily intake of saturated fatty acids should be kept below 10% of total caloric intake (Department of Health, 1991).

Polyunsaturated fatty acids are found in greatest abundance in corn, soybean, safflower, and sunflower seeds (Drackley and Schingoethe, 1986; Finn et al., 1985; Bottger et al., 2002). Certain fish oils are also high in polyunsaturated fatty acids. Unlike saturated fats, polyunsaturated fats may actually lower total blood cholesterol levels. However, large amounts of polyunsaturated fatty acids may reduce high-density lipoproteins (HDL) in the human body (Lada and Rudel, 2003).

Monounsaturated fatty acids are found mostly in vegetable and nut oils such as olive and peanut. These fats may reduce blood levels of LDL without affecting HDL (Penny et al., 1999). Intake of monounsaturated fatty acids should be kept between 10 and 15% of total caloric intake (Penny et al., 1999).

Most food, including plant-derived foods, contains a combination of all three types of fatty acids, but one of the types usually predominates. Thus, a fat or oil is considered saturated when it is composed mostly of saturated fatty acids. These fats are usually solid at room temperature. Similarly, a fat or oil composed mostly of polyunsaturated fatty acids is called polyunsaturated, whereas a fat or oil composed mostly of monounsaturated fatty acids is termed monounsaturated.

Triglycerides. Triglycerides are the most important long-term energy reservoirs in the body of animals because they represent a highly efficient form of energy storage. Triglycerides are less oxidized than other dietary molecules due to their chemical structure. They yield significantly more energy than carbohydrates or proteins when degraded and metabolized.

Kaylegian and Lindsay (1995) defined triglyceride composition and structure of milk lipid as follows. Triglyceride composition is expressed as the total carbon number or as the triglyceride class, such as trisaturated or SSS. The triglyceride structure includes the location of a fatty acid in the three positions of the triglyceride molecule (sn-1, sn-2, and sn-3) and ultimately, the identity of the major triglyceride. Table 4 presents a brief description of the stereospecific position of the major fatty acids in triacylglycerol. The classes of triglyceride are identified by the types and amounts of unsaturated fatty acid present in the triglycerides, such as SSS, SUS, SSU, etc. where S is saturated and U is unsaturated.

The structure of the triglyceride influences the action of lipolytic enzymes, and therefore, absorption. Also, it influences the flavor of cheeses (Jensen and Newberg, 1995). Triglyceride structures found in milk are responsible for the melting points, crystallization behavior, and rheological properties of milk fat globules (Keenan and Patton, 1995; Hawke and Taylor, 1995; Walstra, 1995).

The body stores triglycerides in specialized cells called adipocytes that can be almost entirely filled with fat globules. They are located in adipose tissue that occur in particular under the skin and in the abdominal cavity. The energy reservoir in the adipose tissue is sufficient for normal humans to survive starvation for 2 to 3 months. Adipocytes

uptake triglycerides that are synthesized in the liver and transported by the blood stream. However, the adipocytes can synthesize triglycerides themselves from fatty acids and glycerol-3-phosphate when the level of nutrients in the body is very high. On the other hand, when the dairy cow is in negative energy balance, the energy reserves are mobilized by breaking down triglycerides to fatty acids and glycerol by the action of lipase enzymes. The fatty acids and glycerol are released from the adipocytes into the blood stream and transported to the liver where they can be converted directly into energy. Glycerol can be converted to glucose and fatty acids to ketone bodies that can be utilized for energy generation by other organs and tissues.

Kaylegian and Lindsay (1995) indicated that bovine milk lipid contains 12 fatty acids in amounts greater than 1% (Table 3). Therefore, if all fatty acids are randomly distributed then milk lipid may contain about 1,728 triglyceride species. However, bovine milk lipid contains at least 400 fatty acids, then the total possible triglyceride species would be 64,000; however, because the distribution of fatty acids in milk is not random, the number of triglycerides does not approach this figure, but several thousand triglycerides are probably found in milk.

The synthesis and breakdown of triglycerides is regulated by hormones that influence the number and activity of the lipase enzymes that break down the triglycerides to glycerol and free fatty acids. The hormones epinephrine, norepinephrine, glucagon, and ACTH (adrenocorticotropic hormone) increase the number and activity of lipase enzymes and therefore stimulate the energy generation from triglycerides. The hormone insulin, on the other hand, suppresses lipase enzymes and slows down the utilization of the body energy reservoir in the adipocytes.

Factors affecting milk fatty acid composition

Several factors have been proposed to influence fatty acid composition of milk fat. These factors include: 1) stage of lactation, 2) physical form and oilseed processing, 3) fatty acid profile of fat supplement, 4) forage:concentrate ratio, and 5) genotype.

Genotype. There is limited information on the effect of dairy cattle breed on milk fatty acid composition. Palmquist and Beaulieu (1992) showed that Jersey milk fat contains more caproic (C6:0), caprylic (C8:0), capric (C10:0), lauric (C12;0), myristic (C14:0), and stearic (C18:0) acids, and lower concentrations of oleic acid compared with milk fat from Holsteins. Most research has used Holsteins as a model which reflects this breed's dominance of the US dairy industry.

Stage of lactation. Plasma fatty acids extracted by the mammary gland for use in the synthesis of triglycerides may be derived from the diet or from fatty acids mobilized from triglyceride stores within adipose tissue. In early lactation, dairy cows are usually in negative energy balance and the most prevalent mechanism to satisfy the increased requirement for energy is by mobilization of body fat reserves. Therefore, it would be most difficult to influence milk fatty acid composition through diet in early lactation because of this effect caused by fatty acid dilution from adipose tissue mobilization.

For example, it was reported by Hawkins et al. (1985) that milk fatty acid composition in midlactation was altered by feeding cottonseed; however, no differences were observed for early lactation cows fed the same diets. Kennelly (1989) fed lactating dairy cows canola seed as a lipid supplement in early, mid, and late lactation. The researcher observed greater changes in fatty acid profiles in early lactation compared with

late or mid lactation, although the changes did not reflect the fatty acid profile of the diets.

Glascock et al. 1976 (as cited in Storry, 1981) observed that transfer of intravenously infused and labeled tryiglyceride to milk fat was much greater in early lactation (30%) compared with late lactation (5%). Milk fatty acid composition of dairy cattle fed supplemental tallow has been studied during two stages of lactation when cows were either gaining or losing body weight (Storry, 1981). Yields of C16:0 to C18:0 fatty acids were related negatively to live weight change. The researcher also observed that the transfer efficiency of plasma fatty acid to mammary tissue was reduced as lactation shifted toward mid or late lactation. This effect may reflect partitioning of nutrients toward adipose tissue as the animal reaches greater positive energy balance.

During the initial days of lactation, colostrum secreted by the cow contains lower concentrations of short-chain fatty acids, but during the next two weeks palmatic acid (C16:0) content increases (Sneft and Klobasa, 1970). It is well understood that the amount of short-chain fatty acids, with the exception of butyric acid (C4:0), increases for the first 8 to 10 weeks of lactation, while C16:0 remains unchanged. However, stearic (C18:0) and oleic (C18:1) acid content decreases. As lactation progresses to 10 weeks, changes in fatty acid composition tend to be relatively minor (Christie, 1980).

Forage:concentrate ratio. Typical changes in milk fatty acid profile and fatty acid yields when high forage diets replace low forage diets are presented in Table 3. When lactating dairy cows are fed low roughage diets, the content of C6:0 to C16:0 of milk fat is usually reduced and the proportion of C18:1 and C18:2 are increased (Clapperton et al., 1980; Jorgensen et al., 1965; Storry et al., 1974). In contrast to lipid

supplementation, the proportion of C18:0 is reduced slightly due to feeding low fiber diets. This effect may be caused by reducing ruminal biohydrogenation of polyunsaturated fatty acids. This results in less C18:0 reaching the duodenum. Storry and Rook (1966) found that the proportion of C18:1 present as a *trans*-isomer in milk fat increased from approximately 15 to 50% when lactating cows were switched to low forage from high forage diets.

It is likely that forage:grain ratio will affect the ability of supplemental lipid to alter milk fatty acid composition. Evidence indicates that manipulation of milk fatty acid composition with fat supplementation would be easier when the level of fiber in the diet is sufficient to maintain milk fat percentage. Storry et al. (1974) showed that, when protected tallow was fed to dairy cows, a transfer rate of 20% to milk fat was observed when a low fiber diet was fed, which was almost one-half that observed in previous experiments when high-fiber diets were fed.

Physical form and oilseed processing. Feeding vegetable oil as part of the whole oilseed, particularly if heat-treated, protects oil from ruminal microbial metabolism to some extent. Mohamed et al. (1988) fed lactating dairy cows two oil supplements, soybean oil or cottonseed oil, in either free form or as part of a raw or roasted whole oilseed, at 4% of ration DM. Both fat supplements caused C18:0 and C18:1 to increase and C16:0 to decrease in milk fat, regardless of feeding method. Supplementation of free oil caused a marked decrease in the ratio of C18:0 to C18:1 in ruminal fluid. This response may indicate that the hydrogenating ability of the ruminal microbes was exceeded when large amounts of free oil were included in the diet. Feeding canola, either in ground or unground form, has resulted in increased amounts of C18:0 and C18:1 and a

decrease in C6 to C16:0 of milk fat, but feeding ground seeds elicited the greatest magnitude of change (Kennelly, 1989; Murphy and McNeill, 1988). The influence on fatty acid composition by grinding of oilseeds may be due to the release of the oil from the canola or to increased surface area available for microbial degradation of the seed, thus reducing the natural protection provided by feeding a whole oilseed.

Fatty acid profile of fat supplement. Most common lipid supplements in dairy rations contain approximately 90 to 95% fatty acids with a chain length greater than 14 carbons. The percentage of fatty acids with chain length of 18 carbons is approximately 75%. Oils from cottonseed, soybean, and sunflower seeds contain greater than 50% polyunsaturated fatty acids, whereas tallow, canola oil, and sunflower are relatively high in monounsaturated fatty acids. When proportions of saturated, polyunsaturated, and monounsaturated fatty acids in the various fat supplements were compared with ideal milk fat (Grummer, 1991), high-oleic sunflower oil was most similar to the ideal milk fatty acid profile. Feeding whole canola, high-oleic sunflower, or sunflower oil resulted in a reduction in the percentage of C4:0 to C16:0 fatty acids, while the percentage of C18:0 and C18:1 fatty acids in milk fat was increased by 55 to 80% (Middough et al., 1988; Murphy and McNeill, 1988). These results indicate the powerful influence that ruminal biohydrogenation, intestinal, and mammary desaturase enzymes have on milk fatty acid composition. Feeding lipid supplements high in C18:0 or C18:1, such as high-oleic sunflower or safflower oil may result in less *trans* C18:1 compared with *cis* C18:1 in milk fat, whereas feeding oils high in polyunsaturated fatty acids will result in relatively high proportions of *trans* C18:1 compared with *cis* C18:1 (Bank, 1980). However, feeding whole oilseeds may result in slow release of oil from the seed during