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Production Performance and Profiles of Milk Fatty Acids of Lactating Dairy Cows Fed Whole Safflower Seed Containing High Fat and Low Fiber

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PRODUCTION PERFORMANCE AND PROFILES OF MILK FATTY ACIDS OF LACTATING DAIRY COWS FED WHOLE SAFFLOWER SEED CONTAINING HIGH FAT AND LOW FIBER

by

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ABSTRACT

Production Performance and Profiles of Milk Fatty Acids of Lactating Dairy Cows Fed Whole Safflower Seed Containing High Fat and Low Fiber

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Oil seeds are natural sources of fat and protein in diets for lactating cows, and are usually fed whole or crushed. A recently released variety of safflower seed, "Nutrasaff," contains high fat (47% crude fat) and low fiber (26% NDF), and has a potential to be effectively used as a fat supplement for lactating dairy cows. Therefore, a lactating dairy cow trial was conducted to assess production performance of dairy cows when fed graded levels of whole Nutrasaff safflower seed (NSS), to determine the optimum level of NSS supplementation in the diet and to identify its impact on milk fat content and milk fatty acid (FA) profiles. Fifteen Holstein dairy cows in midlactation (118 ± 39 days in milk) were assigned into 5 groups of 3 cows each according to previous milk yield. The experimental design was a triple 5 × 5 Latin square with each period lasting 21 d (14 d of treatment adaptation and 7 d of data collection). The animals were fed a basal diet containing 56% forage (69% alfalfa hay and 31% corn silage) and 44% concentrate mix.

The diet was supplemented with 0 (control), 1, 2, 3, or 4% (DM basis) whole NSS. The NSS was added to the diet by replacing whole linted-cottonseed. Intake of DM ranged from 26.4 to 27.5 kg/d across all treatments, and did not differ due to NSS inclusion. Yield of milk and ECM averaged 33.7 and 31.6 kg/d, respectively, and they were similar in response to NSS inclusion. Milk fat percentage decreased with increasing NSS inclusion, while milk protein and lactose concentrations did not differ among treatment diets. Milk fat concentration was reduced by 11% when NSS was included at 4% of the dietary DM. Feeding NSS at 1, 2, or 3% resulted in a similar milk fat concentration, and these diets also had similar milk fat percentage compared with the control diet. Concentration of milk urea N decreased by NSS inclusion regardless of level of NSS inclusion, implying that NSS supplementation improved dietary N use for milk production. Digestibilities of DM ($P = 0.12$) tended to increase when NSS was supplemented at 1, 2, or 3%. *Cis*-9, *trans*-11 conjugated linoleic acid (CLA) linearly increased as the NSS inclusion increased. Total concentration of n-3 FA increased by feeding NSS at 1 and 2%, whereas total concentration of n-6 FA linearly increased with increasing inclusion level of NSS. This study clearly demonstrates that it is highly possible to use NSS as a means of fat supplementation to lactating dairy cows without negative impact on lactational performance if added less than 3% of dietary DM. The enhanced milk quality with increased *cis*-9, *trans*-11 CLA concentration due to the addition of NSS could have positive implications to human health.

(63 pages)

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LIST OF ABBREVIATIONS

ADF = acid detergent fiber

AIA = acid insoluble ash

BH = biohydrogenation

BW = body weight

CLA = conjugated linoleic acid

CP = crude protein

CTL = control diet without Nutrasaff safflower seed addition

DM = dry matter

DIM = days in milk

DMI = dry matter intake

ECM = energy corrected milk

FA = fatty acid

NDF = neutral detergent fiber

NE_L = net energy for lactation

NSS = Nutrasaff safflower seed

PUFA = polyunsaturated fatty acid

RDP = rumen degraded protein

RUP = rumen undegraded protein

SEM = standard error of least square means

SF1 = 1% Nutrasaff safflower seed diet

SF2 = 2% Nutrasaff safflower seed diet

SF3 = 3% Nutrasaff safflower seed diet

SF4 = 4% Nutrasaff safflower seed diet

SS = safflower seed

TMR = total mixed ration

INTRODUCTION

With increasing milk yield and genetic potential for milk production of dairy cows, fat supplementation has increased because its energy density is higher than for other nutrients. Safflower is grown widely in the western and central United States, and is well adapted to hot and dry climates due to its higher resistance to drought than small grains (Dajue and Mundel, 1996). Safflower seed (SS) contains 18.7 ± 1.0% CP, 41.4 ± 0.2% ether extract, 42.5 ± 8.2% NDF, and 28.9 ± 5.0% ADF (Stegeman et al., 1992; Bottger et al., 2002; Godfrey, 2006), so the composition of SS is roughly comparable to cottonseed, being higher in fat and lower in CP. The high oil content of SS makes it an attractive energy-dense feed for animals with high energy requirements, such as lactating dairy cattle. Recently, a new variety of SS (Nutrasaff^{TM}, Safflower Technologies International, Sidney, MT) has been developed, and it contains higher oil and lower fiber contents than traditional varieties. Therefore, the new variety has a potential to be used as a source of fat for lactating dairy cows especially in early lactation, when cows typically experience a negative energy balance. Recent research from our laboratory suggested that the optimum method of processing regular SS was to mix whole SS with corn in a 50:50 ratio, and then coarse grind the mixture using a hammer mill equipped with a 0.64 mm screen (Godfrey, 2006). Feeding unprocessed SS resulted in 50% seeds excreted in the manure, whereas feeding coarse ground SS at 2% of diet DM to dairy cows improved feed efficiency (ECM/DMI) by 11% compared to feeding same amounts of whole linted-cottonseed (Godfrey, 2006). In the same experiment feeding ground SS at 4% of diet DM did not enhance animal productivity (Godfrey, 2006). However, optimum level of SS and

the animal responses may vary when the new variety of SS (NSS) is fed as a whole SS due to its higher oil and lower fiber contents.

Feeding oilseeds to lactating dairy cows is one method to change the proportion of unsaturated fatty acids (FA) in milk fat with increases as high as 40% (Casper et al., 1990; Stegeman et al., 1992; Kim et al., 1993), although extensive biohydrogenation (BH) occurs normally in the rumen (Palmquist and Jenkins, 1980). Safflower seed would have a beneficial effect from a consumer viewpoint, as SS is rich in polyunsaturated fatty acids (PUFA) being a source of linoleic acid (76% of the total FA). Bell et al. (2006) reported that the addition of safflower oil at 6% of diet DM increased *cis*-9, *trans*-11 conjugated linoleic acid (CLA) in milk which has been suggested as the best natural source of CLA in the human diet due to its anticarcinogenic properties (Pariza and Hargraves, 1985). Diet-induced changes in ruminal BH with enhanced levels of CLA in milk fat are also associated with the decrease in milk fat percentage. The BH theory of milk fat depression as proposed by Bauman and Griinari (2001) is based on a concept that, under certain dietary conditions, the pathways of ruminal BH are altered to produce unique FA intermediates, some of which are potent inhibitors of milk fat synthesis such as *trans*-10, *cis*-12 CLA (Baumgard et al., 2000). Feeding safflower oil in lactating dairy diet increased levels of *trans*-10, *cis*-12 CLA in milk fat (Bell et al., 2006), similar to studies involving diet-induced milk fat depression (Piperova et al., 2000; Peterson et al., 2003). Therefore, it is likely that milk fat production would be compromised when a certain level of whole SS is fed to lactating dairy cows.

Our objective was to determine lactational performance of dairy cows when fed varying levels of whole NSS and to determine the optimum level of NSS inclusion in the

diet. In addition, we were interested in milk FA profiles in response to the NSS inclusion at different concentrations and their impact on milk fat yield.

REVIEW OF LITERATURE

Current focus in the dairy industry has been placed on means of optimizing feed and production efficiency as well as the energy balance of dairy cows during early lactation. Supplementing fat in rations is not a new idea (Palmquist and Jenkins, 1980). Research as early as 1907 found that fat supplementation was not beneficial for milk and fat yields (Palmquist and Jenkins, 1980), whereas research conducted from the 1920's to the 1940's reported a higher milk production response from cows supplemented with fat (Loosli et al., 1944). Nutritionists and researchers have, for many years now, stressed the importance of supplementing rations with fat sources.

The feeding of high-quality forages can be used to help maintain a healthy rumen environment and high milk production (Waldo and Jorgensen, 1981). Adding fats and oils to high forage diets may improve milk production and feed efficiency (Klusmeyer and Clark, 1991). Fat supplementation to high forage diets allows the ration to be high in energy density without the negative side effects of a starchy high-grain diet. Adding fat supplements to high forage diets may supply the animals with enough energy to maintain high levels of milk production and milk component yield with minimal use of cereal grains.

A recent study by Harvatine and Allen (2006a) showed that the addition of fats to the diet allows for the maintenance of energy density while increasing fiber intake, which stabilizes rumen fermentation. A fat supplement that maximizes DM intake and ruminal fiber digestion increases milk production and milk component yield, and improves health and reproduction of dairy cows. The need for various fat sources that are digestible in the

small intestine, easy to use, and cost effective has drawn a lot of attention with the increasing costs of ration ingredients.

The intake of energy is the primary limitation on milk yield (Allen, 2000), and limited by DMI capacity (Schauff et al., 1992). Without sufficient dietary energy, cows will produce milk less than their potential milk production (Harvatine and Allen, 2006b). Cows supplemented with dietary fat are able to consume higher levels of energy with less DMI, and as a result produce more milk. Feeding fat improves feed utilization for milk and milk component production (Schauff et al., 1992). Supplementing with fat helps the cow to maintain a positive energy balance.

Currently whole linted cottonseed is a common feed ingredient in dairy cattle rations because of the fat content (19.3% DM) in the seed (NRC, 2001). Farmers have also ivestigated feed ingredients that they can produce themselves to supplement in their rations and feed to their high producing cows without sacrificing intake and milk yield and milk component yield. In the western and central United States, safflower seeds have been grown because of tolerance to hot and dry climates. Safflower typically contains more fat than cottonseed but slightly less protein. Safflower is rich in unsaturated fatty acids (FA), mainly in the form of linoleic acid. Oils rich in unsaturated FA are relatively more digestible in the small intestine than saturated fats (Doreau and Ferley, 1994), but when fed unprotected and at high concentrations can interfere with rumen fermentation and metabolic processes such as milk fat synthesis in the mammary gland. This chapter reviews the research on the use of fat in lactating dairy cow diets, animal responses to fat supplements, and the mechanism by which fat supplements affect ruminal fermentation.

Production of Safflower

Safflower is one of humanity's oldest crops. It originally grew wild in Europe, Asia, and perhaps Egypt and was used as a source of cooking oil, food coloring, and cloth dye. Safflower is a broadleaf, annual oilseed crop primarily adapted to grow in the small-grain production areas of the western Great Plains (Lartey et al., 2005). It is a minor crop today, with about 800,000 metric tonnes of seed being produced annually worldwide (Gyulai, 1996). India, United States, and Mexico are the leading producers, with Ethiopia, Kazakhstan, China, Argentina, and Australia accounting for most of the remainder (Table 1). California, which exports much of its oil to Japan, grows approximately 50% of the U.S. safflower production, while the remaining domestic production comes from North Dakota, Montana, South Dakota, Idaho, Colorado, and Arizona (Berglund et al., 2007).

Historically, safflower was grown for its flowers, with the florets being used for coloring and flavoring foods, for making dyes, and as medicines (Mundel et al., 1992). Today, safflower provides three main products: oil, meal, and birdseed. Safflower oil is of two types coming from different safflower varieties: those high in monounsaturated FA (oleic acid) and those high in polyunsaturated FA (linoleic acid). Currently, the predominant oil market is for those varieties that produce seeds high in oleic acid and very low in saturated FA. High oleic acid safflower oil is higher in oleic acid and lower in saturated fatty acids than olive oil. High oleic acid oil is a beneficial agent in the prevention of coronary artery disease in humans. Also, monounsaturated fatty acids such

Table 1. World production of Safflower in 2005[1]

Country	Production, metric tonne
Mexico	212,765
India	210,000
United States	91,000
Australia	60,000
Argentina	51,000
Kazakhstan	40,000
Ethiopia	38,000
China	32,000
Kyrgyzstan	20,000
Uzbekistan	10,000
Tanzania	5,000
Tajikistan	3,000
Canada	2,000
Hungary	650
Iran	500

[1]Adapted from the Food and Agricultural Organization of the United Nations (2005) (http://www.fao.org/es/ess/top/commodity.html).

as oleic acid safflower oil tend to lower blood levels of low-density lipoprotein without affecting high-density lipoprotein. Polyunsaturated FA, such as linoleic acid, is associated with lowering blood cholesterol. Both types of oil are considered “high-quality” edible oil, and public awareness about this health topic has made safflower an important crop for vegetable oil. Oil from this type of safflower is used as heat-stable cooking oil, and is also used in cosmetics, food coatings, and infant food formulations. The oil from high-linoleic acid safflower contains nearly 75% linoleic acid, and is used primarily for edible oil products such as salad oils and soft margarines. Edible safflower oil cultivars have the highest quantity of polyunsaturated FA than other established oil crops (Knowles, 1955; Ashri, 1973). High-linoleic acid safflower oil is also used in human nutrition, but in recent years market demand has drastically shifted from the traditional high-linoleic oils to high-oleic oil due to the shift away from unsaturated fat in

human diets. Safflower meal has about 29% CP and 54% NDF (NRC, 2001), and is used as a protein supplement for ruminants.

Safflower is in the same plant family as the sunflower and is a thistle-like annual herbaceous plant with long, sharp thorns (Johnston et al., 2002). Each branch usually has one to five flower heads, and each of those heads contains 15 to 20 seeds. Safflower has a deep taproot system that can penetrate to depths of 2.2 (Dajue and Mundel, 1996) to 4.0m (Knowles, 1989). The deep taproot and xerophytic spine attributes contribute to good drought and heat tolerance (Dajue and Mundel, 1996). The seed oil content ranges from 30 to 50% (Berglund et al., 2007).

Safflower is typically sown in April or early May. Early planting allows the crop to take full advantage of the entire growing season. Seedlings generally emerge in one to three weeks. Cool soil temperatures (below 4.4°C) prevent germination and encourage seedling blight (Berglund et al., 2007).

Safflower is very susceptible to frost, but has the potential to be used as an alternative forage in the event of an early killing fall frost before crop maturity. Relative forage value peaks at or just after the bloom stage and decreases as the safflower reaches maturity. The crop usually needs 110 to 140 d to mature (Berglund et al., 2007).

Safflower grows best on deep, fertile, and well-drained loam soils with good water-holding capacity. It also can thrive in coarser-textured soils of lower water-holding capacity when rainfall amount and moisture distribution are adequate. The higher soil water use by safflower is consistent with its longer growing season and deeper root growth compared to the other crops (Merrill et al., 2002). Safflower is considered to be moderately salt-tolerant (Maas, 1986) and similar to barley in tolerance to saline soils

(Berglund et al., 2007). Bassil and Kaffka (2002) reported that safflower root growth and water use at depth were restricted in salt-affected soils, but seed yield was not affected by soil or irrigation water salinity. It is an excellent crop to grow in recharge areas because its deep taproot system uses surplus water during its long growing season. Safflower should not be planted on poorly drained land (Berglund et al., 2007).

Safflower is most often grown in rotation with small grains and annual legumes. Safflower should not follow safflower in rotation or be grown in close rotation with other crops like dry bean, field peas, sunflower, mustard, canola, and rapeseed (Berglund et al., 2007). Seed yields were suppressed when safflower was planted on its own residue (Tanaka et al., 2005; Krupinsky et al., 2006). Very little crop residue remains on the land after a safflower crop is harvested, leaving the soil susceptible to wind and water erosion (Berglund et al., 2007).

Diseases have been a problem in years of above-normal rainfall with extended periods of high humidity. The two most serious diseases under these conditions are Alternaria leaf spot and *Pseudomonus* bacterial blight (Berglund et al., 2007). The incidence of Sclerotinia head blight on safflower is low (Krupinsky et al., 2006).

Safflower has relatively few insect pests that cause economic damage. Safflower usually is directly harvested with a small-grain combine. Safflower is physiologically mature about one month after flowering and ready to harvest when most of the leaves have turned yellow. For safe long-term storage, threshed seed should not exceed 8% moisture (Berglund et al., 2007).

Among safflower varieties, Finch, Montola 2000, Montola 2003, Montola 2004, Cardinal, and Mondak are the preferred varieties for the birdseed market because they

have a pure white seed (normal hull) without any striping. Oleic and linoleic safflower varieties should not be mixed or grown within 1.6 km of each other (Berglund et al., 2007).

Feeding Fat Supplements to Ruminants

Feeding fat supplements, of any type, to lactating dairy cows has been of interest since 1907 (Palmquist and Jenkins, 1980). Common fat supplements include oilseeds, animal or animal/vegetable blends, dry fats, and rumen protected fats. Fat supplements have a higher energy density about 2.25 times that of carbohydrates, than other feed ingredients they replace. Fiber intake can be maintained while increasing the energy density and long-chain FA can be transferred directly to the milk (Coppock and Wilks, 1991). Chilliard (1993) states that fat supplements increase the absorption of fat-soluble nutrients, such as fat-soluble vitamins, and tend to increase reproductive efficiency. Staples (2006) reported that supplementing cows with fat can have several beneficial effects; usually increasing the energy density of the diet resulting in increased milk production and feed efficiency, which usually translates into more profit. Less heat may be produced in the rumen during digestion of fat supplemented diets, as FA are not digested in the rumen. Less heat produced during digestion would help cows during heat stress conditions. Because of its energy density and no contribution to heat increment, feeding fat is common during the summer months when DM intake will likely be depressed. As a result, fat inclusion can be a good choice for diet formulation.

During early lactation, high-producing cows cannot consume enough feed to meet their energy needs, thus feeding fats to dairy cows has become popular. Fats contain 2.25

times more energy than the starches and digestible fiber found in grains and forages. At the same time, adequate amounts of forage and fiber can be fed to maintain a healthy rumen. Harvatine and Allen (2005) explained that adding fat to the diet increases energy density without increasing rumen acid production, thus stabilizing ruminal pH relative to addition of grain.

Recommended feeding level for fat supplements is up to 3% of DM intake (NRC, 2001). Amaral-Phillips et al. (1997) recommended that diets for lactating cows not exceed 5% total fat from natural fat sources which include forages, cereal grains, oilseeds, and tallow. Two to 3% fat could be supplied by the forages and normal cereal grains found in the diet. Other 2 to 3% could be supplied from oilseeds or tallow. An additional 2 to 3% fat (to make a total of 8% fat in the total ration) can be added by using specialty or ruminally inert fats. Exceeding these recommendations may decrease fiber digestion and cause milk fat depression (Amaral-Phillips et al., 1997) as well as interfering with calcium and magnesium metabolism.

Results from feeding fat supplements, as a whole, may differ between studies and by sources of fat supplements and rate of supplementation (Allen, 2000). Possible explanations for the variation in response to supplementation of individual fat supplements include management practices and nutrition effects (Scott et al., 1995). Differences between fat supplements may be levels of saturation, ratios of individual FA, and the level to which the fat is protected from processing in the rumen (Coppock and Wilks, 1991; Allen, 2000). Results from individual fat supplements cannot be generalized to all supplements, and inconsistent results, both within supplement and overall supplements, are found in DMI and milk yield.

Yield of Milk and Milk Components and Dry Matter Intake

Generally, milk fat percentage has increased when the recommended amount of supplemental fat is fed to dairy cows. Feeding fat also enhances production of milk (Schneider et al., 1988). It was found that increased fat in the diet did not change milk yield, but fat-corrected milk increased without a change in milk protein concentration (West and Hill, 1990). Schauff and Clark (1989) used fat in place of corn with no increase in forage, and found that DMI and milk fat and protein concentration were not affected by feeding fat. Jenkins and Jenny (1989) reported that hydrogenated yellow grease improved milk yield. Fat-corrected milk was increased when fat was added, and later it was found that adding lipid caused no change in milk yield, but the yield of milk fat increased (Palmquist and Moser, 1981). When fat was added and dietary forage increased, milk fat percent increased (Palmquist and Conrad, 1980). Milk yields have increased when energy was added to the diet in the form lipid (Drackley et al., 2003). High grain diets had lower milk fat content than high forage diets (Palmquist and Conrad, 1980; Grummer et al., 1987). This could be attributed to milk fat depression caused by elevated levels of propionate production in the rumen causing a shift of lipid synthesis precursors away from the mammary gland and towards insulin-sensitive tissues of the body. Formation of *trans*-10 18:1 fatty acids increase in an acidic rumen environment (Lock et al., 2008).

Zheng et al. (2005) tested the effects of feeding vegetable oil high in total C18 on performance of dairy cows and found that using oils derived from cottonseed, soybean, and corn had no effect on milk production or milk protein percent, but the oil

supplementation affected milk fat percent; when the supplement was derived from cottonseed, there was no difference in milk fat percent, but when fed with oils derived from soybean or corn the milk fat percent was significantly decreased.

Responses in DMI with the feeding of fat have varied. In some of the trials where DMI decreased, the amount of energy consumed has remained constant or increased slightly to account for the increase in milk production (Amaral-Phillips et al., 1997). Decreased DMI may be found, especially when the amount of fat in the diet exceeded the amount needed for milk fat synthesis (Amaral-Phillips et al., 1997).

Elliott et al. (1996) suggested that DMI may be more influenced during early lactation than mid to late lactation. Pantoja et al. (1994) compared the effects of fat saturation on digestion and milk production. Compared to control diet (no fat supplementation), all fat supplemented diets showed no overall difference in DMI. However, analysis of individual fat supplements showed that as the level of saturation decreased, DMI also decreased. No difference in NDF digestibility was seen, so it is unlikely that DMI was decreased due to gut fill. On the other hand, digestibility of DM and fiber can be associated with the level of saturation of the fat supplements. Harvatine and Allen (2006c) investigated Holstein cows fed different fat supplements at varying levels of saturation. Four diets were fed, three of which were supplemented at a rate of 2.5% of diet DM with a saturated, intermediate, or unsaturated fat supplement. In the study, all fat supplemented diets showed a decreased DMI compared to control. Furthermore, there was a linear decrease in DMI with increasing levels of unsaturated fat. This suggests that there may be characteristics specific to each fat supplement that decreases DMI (Harvatine and Allen, 2006c). Zheng et al. (2005) tested the effects of vegetable oil high

in total C18 on dairy cow performance. They found that using oils derived from cottonseed, soybean, and corn had no effect on DMI when supplemented at 2% of dietary DM; however, the oil was supplemented in a liquid form at a low rate. Pantoja et al. (1994), comparing degrees of FA saturation, showed no differences in milk production, but reported a decrease in milk protein percent. There was also a linear trend for a decrease of milk fat concentration and milk fat production with increasing levels of unsaturation.

Feeding Unsaturated Fat Supplements to Ruminants

As the unsaturation of a rumen available fat increase, so does the negative impact it will have on rumen fermentation (NRC, 2001). If the ruminal microorganisms' capacity for hydrogenation is exceeded, unsaturated FA can accumulate in the rumen and potentially interfere with fermentation, especially fiber fermentation. Whole oil seeds release FA slowly and have minimal effects; extruded or ground oilseeds expose more of the FA to the rumen microorganisms and thus have greater impacts (NRC, 2001).

Unsaturated FA, a FA containing one or more double bond, are toxic to many rumen bacteria, so the major transformation that dietary lipids undergo in the rumen is biohydrogenation (BH) of polyunsaturated fatty acids (PUFA) (Bauman and Lock, 2006). Linoleic acid is generally the most common FA present in diets for U.S. dairy cows, and the intake varies widely; however, only a fraction of the linoleic acid consumed is actually available for absorption. For milk fat depression to occur the diet must contain unsaturated FA and the pathways of their BH in the rumen must be altered. Thus, the

induction of milk fat depression is centered on both an altered rumen environment and an alteration of PUFA pathways in rumen BH.

Unsaturated free FA have relatively short half-lives in ruminal fermentation because they are rapidly hydrogenated by microbes to more saturated end products. Although the evolutionary purpose is debated, the microbial pathway undoubtedly serves some role in protecting microbes from toxic effects of unsaturated FA (Jenkins, 1993). Ruminal digestion of structural carbohydrates can be reduced 50% or more by less than 10% added fat (Jenkins and Palmquist, 1984). When fat supplements inhibit ruminal fermentation, limited hindgut fermentation may lessen the fiber digestibility depression in the whole digestive tract (Jenkins, 1988), but increased fiber excretion in feces often still occurs (Palmquist and Jenkins, 1980). The amount of unsaturated FA appearing in the small intestine is likely influenced not only by the source of supplemental fat but also by DMI influencing passage rate of digesta, the amount of fat fed influencing ruminal fermentation, and the fiber content of diet influencing mastication of oil seeds and release rate of oil (Staples et al., 1998).

Several mechanisms have been proposed to explain how lipids interfere with ruminal fermentation. The lipid “coating” theory and the theory of direct antimicrobial effects have received the most attention (Zheng et al., 2005). The coating theory attempts to explain reduced fermentation by a lipid layer over feed particles that inhibit digestion of cellulose. This lipid covering is proposed to cause detrimental effects by inhibiting close contact of microbial cells or their hydrolytic enzymes with feed particles. Close physical attachment of microbial matter to feed particles is necessary for cellulose digestion in the rumen (Cheng et al., 1991).

Jenkins and Lundy (2001) stated that unsaturated FA act as antimicrobial agents by interfering with normal function of the ruminal microbes. As a result, fiber digestion can be depressed by added fat. The depression can be serious enough that much of the extra energy from the fat supplement can be offset by increased excretion of fiber energy in the feces. Feeding fat reduces fiber digestion by inhibiting microbial fermentation in the rumen. Depression of fiber digestion is most severe for fat sources high in unsaturated FA, which inhibit growth and function of ruminal microbes more than saturated FA (Jenkins, 1993). Staples (2006) speculated that the act of BH by bacteria is an attempt to protect themselves, as unsaturated fats can be toxic to bacteria, primarily cellulolytic bacteria. If feeding unsaturated fats reduces the numbers or activity of cellulolytic bacteria in the rumen, then DM intake, milk production, and milk fat concentration can decrease. During the process of BH of unsaturated fats in the rumen, the conversion to the saturated state may be incomplete. Excessive concentrations of unsaturated fat will interfere with fiber digestion in the rumen, and high concentrations of total fat may decrease DM intake (Eastridge, 2006).

Jenkins and McGuire (2006) stated that untreated vegetable oils high in unsaturated FA have only limited ability to alter milk FA composition. The reason for this is attributed to the microbial population located mainly in the rumen that transforms dietary unsaturated FA. Therefore, delivery of unsaturated FA to mammary tissue is limited even when their dietary concentration is high.

Unsaturated fats have also been shown to have positive impacts on dairy cattle nutrition. Feeding unsaturated fats at a level that will not exceed the ruminal capabilities of the animal is the key to feeding along with a well balanced diet meeting the

requirements of the animal. Ideally added unsaturated fats, if used properly, to lactating dairy cattle diets can maintain DMI without reducing ruminal fermentation and digestibility of forages. Unsaturated fats have also shown the ability to enhance the energy density of the lactating dairy cow diet, thus increasing milk yield.

Sanchez and Block (2002) stated that even though DM intake may be reduced slightly, unsaturated FA increase milk production and feed efficiency of high producing dairy cows. Avila et al. (2000) showed that, limited evidence indicates that fiber digestibility is not affected, nor is changes in ruminal fermentation patterns substantially when diets include whole oilseeds.

Some of the expected results in the milk FA composition when feeding unsaturated fats include a decrease in the proportion of short- and medium-chain FA and an increase in the proportion of long-chain FA (Hermansen, 1995). Some of these long-chain FA such as conjugated linoleic acid has been shown to have positive health benefits in humans (Whigham et al., 2000; Pariza et al., 2001).

Feeding Whole Safflower Seed to Dairy Cows

Little research has been conducted on feeding whole, unprocessed safflower seed (SS) to dairy cows. Godfrey (2006) showed that feeding unprocessed SS resulted in 50% of the seeds being excreted in the manure. Feeding coarsely ground SS at 2% of diet DM to dairy cows improved feed efficiency by 11% (Godfrey, 2006). Milk fat concentration was unchanged when cows were fed rolled SS at 10% of dietary DM in diets containing at least 50% of the forage as alfalfa (Stegeman et al., 1992). The potential negative impact of unsaturated fat from oilseeds as safflower could be minimized if the oilseeds

are fed either whole or coarsely cracked rather than extruded (Faldet and Satter, 1991). Whole oilseeds help to lessen the severity of digestion problems by encapsulation of antimicrobial FA within their hard outer seed coat (Jenkins and Lundy, 2001). This would allow the oil to be released at a slower rate in the rumen, or some of the oil may escape rumen BH and be absorbed in the small intestine.

Lammoglia et al. (1999) suggested that the whole SS need to be processed to improve digestibility. The recommendations are to process (roll) the SS with enough pressure to crack about 90% of the seed hulls without extracting the oil (Lammoglia et al., 1999).

Jenkins and Lundy (2001) concluded that whole seeds were broken by the cow both in the chewing and by microbial action during the rumination process, so processing was considered unnecessary before feeding. They found that whole seeds provide some protection from BH because of the nature of their hard outer seed coat. Disruption of the seed coat exposes the oil to the microbial population, which may result in the potential for fermentation problems and BH.

Grummer (1991) suggests that oil may have been introduced into the rumen more gradually when oil is fed as part of a whole oilseed and, therefore, BH can be more extensive. Feeding free oil depressed milk fat yield, but feeding oil as part of whole oilseeds did not alter milk fat yield (Grummer, 1991). In other studies, feeding whole oilseeds maintained or increased milk fat yield (Grummer, 1991). Therefore, feeding whole oilseeds represents a means by which favorable changes in milk FA profile can be obtained without reduced milk fat percentage.

Dairy producers, in some regions, are showing interest in using whole SS in dairy rations. Whole SS could be a used as a good source of energy for lactating dairy cows

especially in the early lactation, when the cows experience negative energy balance. Because of the high oil content in the seed, it is a high energy feed and a good source of RDP. Fats and oils are used to increase the energy density of dairy rations (Palmquist, 1984).

Another processing method of SS is to mechanically squeeze the oil from the seed, and feed the extracted oil to the dairy cows. Oilseeds are commonly extruded to enhance their handling, intake, or digestibility, which can significantly reduce their resistance to BH (Jenkins and Lundy, 2001). Extrusion of the oilseeds appears to consistently depress milk fat concentration across a number of oilseed sources. The extrusion process of oil seeds likely results in a faster and greater availability of oil in the rumen than when whole oil seeds are fed (Staples, 2006).

Wu et al. (1994) reported that adding 2.2% safflower oil to the diet resulted in increased milk yield as well as C18:1, C18:2, and C18:3 FA in the milk. Rindsig and Schultz (1974) showed that adding 250 mL of safflower oil daily to the ration decreased milk fat percentage. The milk had higher concentrations of C18:1 and C18:2. Bell et al. (2006) reported a decrease in yield and percentage of fat when diets were supplemented with safflower oil. Conjugated linoleic acid (CLA) concentrations were significantly higher in the milk of cow supplemented with the safflower oil (Bell et al., 2006).

Nutrasaff Safflower Seed as a Source of Unsaturated Fat

Recently, Nutrasaff^{TM} safflower developed at the Eastern Agricultural Research Center (Sidney, MT) in cooperation with the Williston Research Extension Center of North Dakota Agricultural Experiment Station (Williston, ND) has been released in 2004.

The new variety of SS contains 52% crude fat, 22% CP, and 26% NDF on DM basis (Table 2). The Nutrasaff oil is high in linoleic acid, accounting for 75% of total FA, and therefore is a major source of PUFA.

Table 2. Chemical composition of safflower seed

Item	Traditional safflower[1]	Nutrasaff™ safflower[2]
DM, %	96.3	93.8
CP, % DM	18.2	21.7
ADF, % DM	24.8	26.4
NDF, % DM	37.6	19.2
Crude fat, % DM	41.2	51.7
TDN, % DM	122	137
NE_l, Mcal/kg	0.70	0.66
NE_m, Mcal/kg	0.76	0.73
NE_g, Mcal/kg	0.56	0.46
Ca, % DM	0.22	0.23
P, % DM	0.39	0.53
Mg, % DM	0.17	0.29
K, % DM	0.51	0.83
Na, % DM	0.003	0.04
S, % DM	0.35	0.21
Fe, ppm	82	117
Zn, ppm	27	74
Cu, ppm	11	24
Mn, ppm	12	29
Mo, ppm	0.4	-

[1]Results obtained from Dairy One Forage Testing Laboratory (Ithaca, NY).

[2]A new variety of safflower seed developed by Safflower Technologies International (Sidney, MT). Results obtained from Midwest Laboratories (Omaha, NE).

Biohydrogenation of Fatty Acids in the Rumen and its Impact on Milk Fat Depression

When dietary material enters the rumen, it enters a large fermentation vat, where it undergoes a wide range of chemical changes performed by the microbial population (Harfoot, 1978). The rumen microbial population consists mainly of ciliate protozoa, anaerobic bacteria, and anaerobic fungi (Jenkins et al., 2008). Lipids are extensively

altered in the rumen, resulting in marked differences between the FA profile of lipids in the diet (mostly unsaturated FA) and lipids leaving the rumen (mostly saturated FA). Ruminal microbes transform lipids entering the rumen via 2 major processes, lipolysis and BH (Jenkins et al., 2008). Lipids entering the rumen are first transformed by microbial lipases in a process called lipolysis. After lipolysis, unsaturated FA undergo BH by ruminal microbes. This process (Figure 1) converts the unsaturated FA to saturated FA via isomerization to *trans* FA intermediates, followed by hydrogenation of the double bonds (Harfoot and Hazlewood, 1988).

Under certain dietary situations the rumen environment is altered and a portion of BH occurs via a pathway that produces *trans*-10, *cis*-12 CLA and *trans*-10 18:1 FA (Figure 1). Therefore, dietary situations causing milk fat depression alter the pathways of rumen BH resulting in changes in the specific *trans*-18:1 FA and CLA isomers. As shown in Figure 1, this '*trans*-10 FA shift' in BH pathways, and the associated increase in the *trans*-10 18:1 FA content of milk fat, is indicative of the complex changes in ruminal BH pathways, which is a characteristic of milk fat depression. Although *trans*-10 18:1 FA does not directly inhibit mammary synthesis of milk fat (Lock et al., 2007), it is relatively easy to analyze compared to *trans*-10, *cis*-12 CLA and other CLA isomers. Therefore, in general, this FA can serve as a surrogate marker for the type of alterations in rumen BH that characterize diet-induced milk fat depression (Lock et al., 2007). This is highlighted in Figure 2, which shows the relationship between the content of *trans*-10 18:1 FA concentration in milk fat and milk fat concentration (Bauman and Griinari, 2003). Also shown in Figure 1 are the three predominant ways in which dietary components can impact the risk of milk fat depression: 1) through increasing substrate supply of 18-

carbon unsaturated FA, 2) by altering the rumen environment and BH pathways, and 3) via changes in the rate of BH at various steps in the BH process.

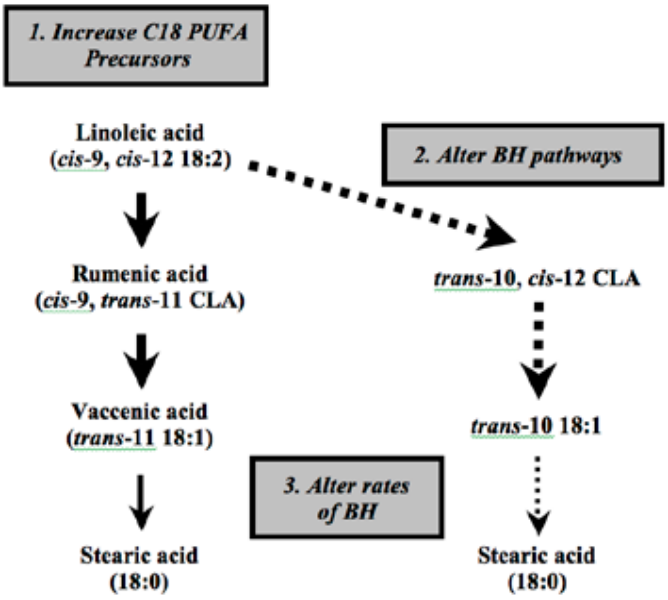

Figure 1. Biohydrogenation pathway of linoleic acid under normal conditions (left side) and during diet-induced milk fat depression (dotted lines, right side). Adapted from Griinari and Bauman (1999).

Given that the specific FA that cause milk fat depression are intermediates produced during ruminal BH of PUFA, it is logical that the amount of initial substrate (linoleic acid and perhaps linolenic acid) may be related to the amount of the key BH intermediates that are produced. Linoleic and linolenic acids represent a large percentage of the FA found in most forages and other plant-based feedstuffs fed to dairy cattle, with linoleic acid representing the predominant PUFA in corn and corn byproducts. As a result, if linoleic acid is the major dietary FA, particularly when corn silage comprises the majority of the forage base in the ration and oilseeds are the major source of added dietary fat. Estimates

of linoleic acid intake in these situations can approach and even exceed 400 to 500 g/d. Therefore, it would appear that typical rations have more than enough substrate as linoleic acid to meet the required presence of PUFA for milk fat depression to occur if rumen fermentation is altered (Lock et al., 2008).

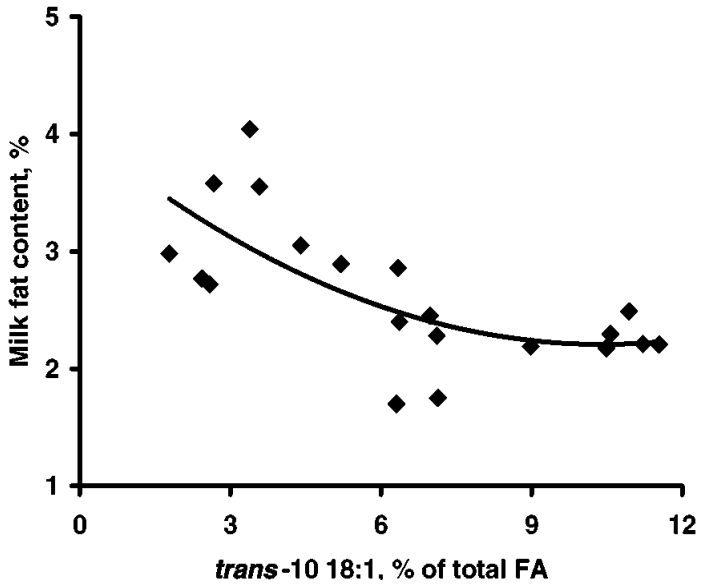

Figure 2. Relationship between the change in the fat content of milk and the *trans*-10 18:1 fatty acid content of milk fat (expressed as percent of total fatty acids). Adapted from Bauman and Griinari (2003).

MATERIALS AND METHODS

Cows and Diets

Fifteen multiparous lactating Holstein cows were used, and the cows were assigned into 5 groups of 3 cows each according to previous milk yield ranging from 39.5 to 50.5 kg. Days in milk averaged 118 ± 39 d at the start of the experiment. Average BW was 700 ± 52 kg at the beginning of the experiment and 750 ± 76 kg at the end of the experiment. The dairy cows used in this study were cared for according to the Live Animal Use in Research Guidelines of Institutional Animal Care and Use Committee at Utah State University.

The study was conducted in a triple 5 × 5 Latin square design with each period lasting 21 d (14 d of treatment adaptation and 7 d of data collection), and the 3 squares were conducted simultaneously. Within square, cows were randomly assigned to one of 5 dietary treatments: control (CTL) without whole Nutrasaff safflower seed (NSS), 1% NSS (SF1), 2% NSS (SF2), 3% NSS (SF3), and 4% NSS (SF4) on DM basis (Table 3). The diets contained 56% forage (69% alfalfa hay and 31% corn silage, Table 4) and 44% concentrate mix on average. The NSS added to the SF1, SF2, SF3, and SF4 diets replaced whole linted-cottonseed in the CTL diet (Table 3). Diets were formulated based on NRC (2001) recommendations to provide sufficient net energy and protein, vitamins, and minerals to produce 38 kg/d of milk with 3.5% fat and 3.0% true protein.

Cows were housed in individual tie stalls fitted with rubber mattresses, bedded with straw, and were fed as a TMR for ad libitum intake with at least 10% of daily feed refusal. All cows were individually fed twice daily at 0430 and 1630 h with

Table 3. Nutrient composition of the treatment diets fed to midlactating Holstein dairy cows

	Experimental diet[1]				
Item	CTL	SF1	SF2	SF3	SF4
Ingredient, % of DM					
Alfalfa hay	36.7	39.4	39.0	38.6	38.2
Corn silage	16.5	17.7	17.5	17.4	17.2
Corn grain, steam flaked	17.8	19.1	18.9	18.7	18.5
Whole linted-cottonseed	7.6	-	-	-	-
Whole Nutrasaff safflower seed[2]	-	1.0	2.0	3.0	4.0
Dried sugar beet pulp	5.7	6.1	6.0	5.9	5.9
Soybean meal, expeller	1.5	1.6	1.6	1.6	1.5
Canola meal	1.9	2.0	2.0	2.0	2.0
Molasses, sugar beet	2.4	2.5	2.5	2.5	2.4
Corn dry distiller grain	2.5	2.7	2.7	2.6	2.6
Corn hominy	4.9	5.3	5.2	5.1	5.1
Blood meal	1.0	1.1	1.1	1.1	1.0
Mineral and vitamin mix[3]	1.5	1.6	1.6	1.6	1.6
Chemical composition, % of DM					
DM, %	61.2	60.8	61.8	61.6	61.8
OM	88.9	90.3	90.3	90.1	90.5
CP	18.8	18.7	18.5	18.5	17.8
ADF	22.1	20.4	20.4	21.4	21.2
NDF	37.6	33.8	34.4	34.2	35.3
Ether extract	5.2	5.2	5.7	6.7	7.0
NE_L, Mcal/kg[4]	1.55	1.56	1.57	1.59	1.60

[1]CTL = control diet without whole Nutrasaff™ safflower seed (NSS; Safflower Technologies International, Sidney, MT); SF1 = 1% NSS; SF2 = 2% NSS; SF3 = 3% NSS; SF4 = 4% NSS on DM basis.

[2]A recently developed new variety of safflower seed by Safflower Technologies International (Sidney, MT).

[3]Contained (per kilogram of DM) a minimum 250,000 IU of vitamin A; 65,000 IU of vitamin D; 2,100 IU of vitamin E; Fe 400 mg; Cu 540 mg; Zn 2,100 mg; Mn 560 mg; Se 15 mg; I 35 mg; and Co 68 mg.

[4]Based on tabular value (NRC, 2001).

approximately 70% and 30% of total daily feed allocation at each feeding, respectively. Feed offered and refused data was recorded daily and daily samples were collected to determine DMI. Cows had free access to water.

Cows were milked twice daily at 0400 and 1600 h. Milk production was recorded daily throughout the experiment. Cows were turned outside to a dry-lot for exercise for at least 1 h daily in the morning after being milked. Milk was sampled during the a.m. and p.m. milkings on 3 consecutive days (d 15 to d 20) in each period. Milk samples were preserved with Broad Spectrum Microtabs II (D & F Control Systems Inc., San Ramon, CA), and were stored at 4°C. Individual milk samples were analyzed for fat, true protein, lactose, SNF, SCC, and MUN by the Rocky Mountain DHIA Laboratory (Logan, UT) with mid-infrared wave-bands (2 to 15 µm) procedures using an infrared instrument (Bentley 2000; Bentley Instruments, Chaska, MN) calibrated weekly using raw milk standards provided by Eastern Laboratory Services (Fairlawn, OH). An enzymatic procedure was used to determine MUN using a Chemspec 150 instrument (Bentley Instruments, Chaska, MN). Milk composition was expressed on weighted milk yield of a.m. and p.m. samples. Milk fat and protein yields were calculated by multiplying milk yield from the respective day by fat and protein content of the milk on an individual cow. Energy-corrected milk was calculated on an individual cow using milk yield, fat, and protein content (Tyrrell and Reid, 1965). Feed efficiency was calculated by dividing daily ECM by DMI on an individual cow.

Cows were weighed at approximately 0830 h at the beginning and end of each period, and these weights were used to calculate the mean BW of cows for each experimental period.

Sample Collection and Analyses

Corn silage, chopped alfalfa hay, and concentrates were sampled weekly to determine DM content. Diets were adjusted weekly to account for changes in DM content. Samples of the TMR fed and orts for individual cows were collected daily during the data collection period, dried at 60°C for 48 h, ground to pass a 1-mm screen (standard model 4; Arthur H. Thomas Co., Philadelphia, PA), and stored for subsequent analyses.

Analytical DM content of samples was determined by oven drying at 135°C for 3 h; OM was determined by ashing, and N content was determined using an elemental analyzer (LECO TruSpec N, St. Joseph, MI) (AOAC, 2000). The NDF and ADF contents were sequentially determined using an ANKOM200/220 Fiber Analyzer (ANKOM Technology, Macedon, NY) according to the methodology supplied by the company, which is based on the methods described by Van Soest et al. (1991). Sodium sulfite was used in the procedure for NDF determination and pre-treatment with heat stable amylase (Type XI-A from *Bacillus subtilis*; Sigma-Aldrich Corporation, St. Louis, MO) was included.

Weighted composite milk samples from individual cows were analyzed for FA composition. Milk fat was extracted by boiling milk in a detergent solution. Extracted fat was derivatized to methyl esters using an alkaline methylation procedure by mixing 40 mg of fat with a sodium methoxide methylation reagent ($NaOCH_3$/MeOH) as described by Chouinard et al. (1999) with minor modifications. After FA methyl esters were formed, anhydrous calcium chloride pellets were added and allowed to stand for 1 h to remove water in the sample. Samples were then centrifuged at 2600 rpm at 5°C for 5 min.

Separation of FA was achieved by using a GLC (Model 6890 Series II; Hewlett Packard Co., Avandale, PA) fitted with a flame ionization detector. Samples containing methyl esters in hexane (1 μL) were injected through the split injection port (100:1) onto CP-Sil 88 fused silica 100 m x 0.25 mm column, 0.20 μm film (Varian CP-Sil 88 model; Varian, Inc., Palo Alto, CA). Oven temperature was set at 80°C and held for 10 min, then increased to 190°C at 12°C/min for 39 min. The temperature was then increased again to 218°C at 20°C/min and held for 21 min. Injector and detector were set at 250°C. Total run time was 70.57 min. Heptadecadenoic acid was used as a qualitative internal standard. Each peak was identified using FA and FA methyl esters (Nu-Chek Prep, Elysian, MN; Matreya, Pleasant Gap, PA; Supelco, Bellefonte, PA). Percentage of each individual FA were obtained simply by taking the individual area of each FA as a % of total FA and were reported as g/100 g of FA methyl esters. The yield of CLA was calculated by multiplying CLA content with total fat yield corrected for glycerol content (Chouinard et al., 2001) on an individual cow.

Feed DM digestibility was measured during the last week in each period using acid-insoluble ash (**AIA**) as an internal marker (Van Keulen and Young, 1977). Fecal grab samples (approximately 200 g, wet weight) were collected for all cows at 0500, 1000, 1600, and 2200 h on day 19 and at 0300, 0800, 1300, and 1900 h on day 20 of each period. Samples were composited across sampling times for each cow, dried at 60°C for 72 h, ground to pass a 1-mm screen (standard model 4), and stored for chemical analysis. Apparent total tract nutrient digestibilities were calculated from concentrations of AIA and nutrients in diets fed, orts, and feces using the following equation: apparent digestibility = $100 - [100 \times (AIA_d/AIA_f) \times (N_f/N_d)]$, where AIA_d = AIA concentration in

the diet actually consumed, AIA_f = AIA concentration in the feces, N_f = concentration of the nutrient in the feces, and N_d = concentration of the nutrient in the diet actually consumed.

During the last 2 days in each period (d 20 and d 21), fresh fecal grab samples (approximately 500 g, wet weight) from each cow were collected to determine the fecal excretion of NSS. A measured amount (approximately 400 g, wet weight) of fresh fecal sample was washed with water gently through screens (4.75, 3.35, 1.18, and 0.60 mm) to collect intact NSS. A portion of the fresh fecal sample was dried in a forced air oven at 60°C for 72 h to determine the fecal DM content. Residue retained on each screen was dried at 60°C for 24 h, and visible intact NSS were separated manually and expressed as g of NSS excreted/kg of fecal during the sampling period. The fecal output was calculated by multiplying feed DMI by 1 minus fractional feed DM digestibility on an individual cow.

Statistical Analyses

Analysis of variance was conducted using the MIXED procedure (Littell et al., 1998) of SAS (SAS Institute, 2001) for all the statistical analyses in this study. For the analysis of DMI, milk yield, milk component concentration and yield, feed efficiency, and digestibility of DM and nutrients, the model included the effects of square, dietary treatment, day, and interactions among the fixed effects, with cow within square and period within square designated as random variables. The effect of day was included as a fixed repeated measurement. Simple, autoregressive one, and compound symmetry covariance structures were used in the analysis depending on low values for the Akaike's

information criteria and Schwartz's Bayesian criterion. For the analysis of milk FA composition, the model included the effects of square, dietary treatment, and the interaction between square and dietary treatment, with the random variable being the cow within square and period within square. For all models used, degrees of freedom were estimated with the Kenward-Roger specification in the models. Means were compared using a protected ($P < 0.05$) LSD test. Orthogonal polynomial contrasts were performed to determine linear and quadratic effects of level of NSS in the diets. Cubic and quartic effects were not examined, because they could not be interpreted biologically. Least square means are reported throughout. Treatment effects were declared significant at $P < 0.05$, and differences were considered to indicate a trend toward significance at $0.05 < P < 0.15$.

RESULTS AND DISCUSSION

Nutrient Composition of Diets and Dietary Ingredients

Replacing cottonseed with SS decreased NDF concentrations of the diets (Table 3) due to lower NDF concentration of NSS (26.4%) compared to that of cottonseed (55.7%; Table 4). However, CP concentrations were similar among all the diets. Fat concentration of the diets measured as ether extract increased, as the level of SS inclusion increased.

Traditional SS varieties were reported to have 18.7 ± 1.0% CP, 42.5 ± 8.2% NDF, 28.9 ± 5.0% ADF, and 41.4 ± 0.2% ether extract (Stegeman et al., 1992; Bottger et al., 2002; Godfrey, 2006). Therefore, the NSS used in this study contained less CP (14.2 vs. 18.7%), NDF (26.4 vs. 42.5%), and ADF (16.2 vs. 28.9%), but more ether extract (47.2 vs. 41.4%) compared to traditional SS varieties.

Table 4. Nutrient composition of the major ingredients (DM basis) used in diets

Item	Alfalfa hay	Corn silage	Corn grain[1]	WLC[2]	NSS[3]
DM, %	93.6	36.4	85.9	91.3	95.7
OM, %	92.2	94.1	98.9	95.8	97.0
CP, %	23.7	6.5	8.6	20.2	14.2
NDF, %	30.7	41.6	26.4	55.7	26.4
ADF, %	26.6	23.9	3.0	40.5	19.2
Ether extract, %	2.1	4.4	1.2	14.3	47.2

[1]Steam flaked corn grain.
[2]WLC = whole linted-cottonseed.
[3]NSS = Nutrasaff safflower seed (Safflower Technologies International, Sidney, MT).

Intake, Milk Production, and Milk Composition

Intake of DM ranged from 26.4 to 27.5 kg/d across all treatments, and did not differ due to SS inclusion (Table 5). Bell et al. (2006) reported that feeding SS oil at 6% (DM basis) did not influence DMI. In contrast, a higher percentage of whole SS (20% of dietary DM) resulted in decreased DMI (Stegeman et al., 1992). The reduction in DMI reported by Stegeman et al. (1992) was due to palatability of the whole SS and the acceptability of the cows to consume the whole SS.

Yield of milk and ECM averaged 33.7 and 31.6 kg/d, respectively (Table 5), and were similar in response to NSS inclusion. Milk fat yield tended ($P = 0.10$) to decrease with linear response by increasing NSS inclusion into the diets, whereas milk protein and lactose yields were not affected by NSS inclusion. Stegeman et al. (1992) found that supplementing whole SS at 20% of DM did not affect milk yield. Bell et al. (2006) reported that milk yield was not affected by the addition of SS oil at 6%. Therefore, supplementing NSS, even up to 4% of dietary DM, had no negative effects on milk yield as well as DMI.

While milk fat concentration linearly decreased with increasing NSS inclusion, milk protein and lactose concentrations were not different among treatment diets. Milk fat concentration was greatly affected when NSS was included at the highest level with 11% reduction. However, feeding the SF1, SF2, or SF3 diet resulted in a similar milk fat concentration, and these diets had also a similar milk fat percentage compared with the CTL diet. Similar to our result, decrease in milk fat concentration was found by Bell et al. (2006) who added SS oil at 6% of diet DM.

Table 5. Intake of DM, milk production and composition, and efficiencies of DM and N use for milk production of midlactating Holstein dairy cows fed varying levels of Nutrasaff safflower seed

	Dietary treatment[1]						Significance of effect[2]		
Item	CTL	SF1	SF2	SF3	SF4	SE	NSS	L	Q
DMI, kg/d	27.5	26.4	27.4	26.8	27.3	0.85	0.27	0.98	0.28
Milk yield, kg/d									
Milk, kg/d	33.6	34.0	33.6	33.7	33.5	1.88	1.00	0.87	0.84
ECM[3]	31.9	32.6	31.5	31.5	30.4	1.59	0.54	0.16	0.45
Fat	1.05	1.11	1.03	1.02	0.94	0.06	0.10	0.03	0.16
Protein	1.03	1.03	1.04	1.03	1.02	0.05	0.97	0.74	0.61
Lactose	1.60	1.58	1.60	1.61	1.60	0.10	1.00	0.86	0.91
Milk composition, %									
Fat	3.25^{ab}	3.38^{a}	3.19^{ab}	3.09^{bc}	2.88^{c}	0.14	<0.01	<0.01	0.10
Protein	3.17	3.14	3.14	3.13	3.10	0.06	0.72	0.19	0.89
Lactose	4.81	4.79	4.81	4.83	4.82	0.05	0.95	0.62	0.76
Efficiency									
ECM/DMI	1.15	1.25	1.15	1.21	1.17	0.05	0.23	0.90	0.43
MUN, mg/dL	14.1^{a}	12.3^{b}	13.0^{b}	12.7^{b}	12.8^{b}	0.62	<0.01	0.04	0.02

^{a,b,c}Means within a row that do not have a common superscript differ at $P < 0.05$.

[1]CTL = control diet without whole Nutrasaff™ safflower seed (NSS; Safflower Technologies International, Sidney, MT); SF1 = 1% NSS; SF2 = 2% NSS; SF3 = 3% NSS; SF4 = 4% NSS on DM basis.

[2]NSS = effect of level of whole Nutrasaff™ safflower seed in diet; L = linear effect of NSS; Q = quadratic effect of NSS.

[3]ECM = Energy-corrected milk.

Dairy efficiency, calculated as ECM divided by DMI, averaged 1.19 and was not influenced by NSS inclusion (Table 5). However, MUN concentration decreased by NSS inclusion regardless of level of NSS inclusion, implying that SS inclusion from 1 to 4% of dietary DM in the diets improved dietary N use for milk production, but the inclusion level of NSS was not critical for the N efficiency. Milk urea N is used as a management tool to improve dairy herd nutrition and monitor the nutritional status of lactating dairy cows. Urinary N excretion has been shown to have a positive linear relationship with MUN (Ciszuk and Gebregziabher, 1994; Jonker et al., 1998). Elevated MUN indicates excess protein has been fed to the dairy cow for her given level of production (Broderick

and Clayton, 1997; Jonker et al., 1998). Broderick (1995) reported that MUN more clearly reflected dietary CP intake than ruminal ammonia concentration. In our case, however, it is likely that increased energy availability from NSS in the diets may improve microbial conversion of feed N by reducing ammonia N production.

Total tract digestibility of DM ($P = 0.12$) tended to increase by feeding the SF1, the SF2, and the SF3 diets compared with the CTL diet, but the highest NSS inclusion in the diet decreased the digestibilities similar to those of the CTL diet, resulting in quadratic responses (Table 6). Feeding the SF1 diet tended ($P = 0.12$) to increase DM digestibility with 8.7% improvement compared with the CTL diet. But, further increases in NSS inclusion at 2, 3, and 4% DM resulted in similar DM digestibility observed by feeding the CTL diet. Excretion of NSS into feces increased (linear and quadratic effects) with increasing NSS inclusion.

Table 6. Digestibility and whole safflower seed (SS) excretion into feces of midlactating Holstein dairy cows fed varying levels of Nutrasaff safflower seed

	Dietary treatment[1]						Significance of effect[2]		
Item	CTL	SF1	SF2	SF3	SF4	SE	NSS	L	Q
DMD[3]	61.8	67.2	63.6	63.5	61.8	2.36	0.12	0.49	0.08
SS excretion[4]	-	11.0[d]	24.9[c]	33.5[b]	44.3[a]	2.25	<0.01	<0.01	0.49

[a,b,c]Means within a row that do not have a common superscript differ at $P < 0.05$.

[1]CTL = control diet without whole Nutrasaff™ safflower seed (NSS; Safflower Technologies International, Sidney, MT); SF1 = 1% NSS; SF2 = 2% NSS; SF3 = 3% NSS; SF4 = 4% NSS on DM basis.

[2]NSS = effect of level of whole Nutrasaff™ safflower seed in diet; L = linear effect of NSS; Q = quadratic effect of NSS.

[3]DMD = DM digestibility, %.

[4]Excretion of SS (Nutrasaff™ safflower seed) into feces, g/kg fecal DM.

Milk FA Composition

In general, the concentrations of the short- to medium-chain FA (6:0 to 17:0) in milk were elevated by feeding the SF1 diet, but they were decreased by the diets with higher inclusion level of SS (SF3 and SF4 diets; Table 7). The concentration of the long-chain FA (≥ 18:0) had an opposite pattern to the short- to medium-chain FA, with the lowest concentration by the SF1 diet and the highest concentration by the SF3 and the SF4 diets. Proportion of 14:0 FA was higher with the SF1 and the SF2 diets than the other diets (quadratic effect), whereas proportion of 16:0 FA linearly decreased with increasing NSS inclusion level. Similar result of the short- and medium-chain FA were reported by Stegeman et al. (1992), and Bell et al. (2006). Chilliard et al. (2000) reported that when fats and oils were supplemented, concentrations of the short- and medium-chain FA were typically reduced. A decreased availability of acetate and butyrate due to changes in the rumen bacterial population and changes in rumen VFA production could contribute to the large decrease in mammary short- and medium-chain FA synthesis (Chilliard et al., 2000), which depresses milk fat concentration in the current study.

The high level of fat in the SF4 diet (7.0% ether extract) may induce changes in the rumen BH leading to the accumulation of intermediate metabolites of altered ruminal BH. In this study, inclusion of NSS raised the levels of 18:1 *trans*-10, 18:1 *trans*-11, and total 18:1 *trans* FA with linear and quadratic responses, and its effects were much more pronounced for the SF3 and the SF4 diets compared to the CTL and the lower level of NSS diets. Bell et al. (2006) reported that addition of SS oil resulted in increases in all 18:1 *trans* FA isomers in milk with the most pronounced increase in 18:1 *trans*-11.

Table 7. Fatty acid (FA) composition in the milk of midlactating Holstein dairy cows fed varying levels of Nutrasaff safflower seed

	Dietary treatment[2]						Significance of effect[3]		
FA[1]	CTL	SF1	SF2	SF3	SF4	SE	NSS	L	Q
6:0	1.62^{abc}	1.69^{a}	1.65^{ab}	1.59^{bc}	1.55^{c}	0.050	0.01	<0.01	0.03
8:0	1.07^{bc}	1.16^{a}	1.13^{ab}	1.08^{bc}	1.03^{c}	0.036	<0.01	0.01	<0.01
10:0	2.69^{b}	2.99^{a}	2.88^{a}	2.69^{b}	2.55^{b}	0.110	<0.01	<0.01	<0.01
11:0	0.33^{c}	0.39^{a}	0.36^{ab}	0.33^{bc}	0.32^{c}	0.018	<0.01	0.03	<0.01
12:0	3.27^{b}	3.73^{a}	3.56^{a}	3.32^{b}	3.18^{b}	0.133	<0.01	0.02	<0.01
13:0	0.14	0.13	0.13	0.12	0.11	0.010	0.17	0.01	0.51
14:0	11.5^{b}	12.4^{a}	12.0^{a}	11.6^{b}	11.3^{b}	0.24	<0.01	0.05	<0.01
16:0	30.1^{a}	30.3^{a}	28.0^{b}	26.7^{c}	25.8^{c}	0.63	<0.01	<0.01	0.29
16:1 *cis*-9	1.38	1.47	1.33	1.31	1.37	0.123	0.46	0.42	0.77
17:0	0.54	0.58	0.49	0.47	0.50	0.037	0.18	0.11	0.64
17:1 *cis*-10	0.19^{bc}	0.20^{a}	0.19^{bc}	0.19^{ab}	0.17^{c}	0.009	0.02	0.05	0.05
18:0	12.0^{a}	10.3^{b}	11.5^{a}	12.4^{a}	12.1^{a}	0.55	<0.01	0.04	0.04
18:1 *trans*-10	0.43^{cd}	0.36^{d}	0.48^{bc}	0.54^{b}	0.67^{a}	0.054	<0.01	<0.01	0.01
18:1 *trans*-11	1.62^{c}	1.68^{c}	1.80^{c}	2.17^{b}	2.44^{a}	0.086	<0.01	<0.01	0.01
18:1 *trans*, total	4.02^{d}	3.79^{d}	4.51^{c}	5.42^{b}	6.21^{a}	0.145	<0.01	<0.01	<0.01
CLA *cis*-9, *trans*-11	0.49^{d}	0.55^{cd}	0.60^{c}	0.72^{b}	0.82^{a}	0.04	<0.01	<0.01	0.20
CLA *trans*-10, *cis*-12	0.04	0.04	0.03	0.05	0.04	0.007	0.26	0.25	0.97
18:2 n-6	2.81^{b}	2.80^{b}	2.92^{ab}	3.08^{a}	3.04^{a}	0.103	<0.01	<0.01	0.96
18:3 n-3	0.56^{c}	0.61^{a}	0.61^{a}	0.59^{ab}	0.56^{bc}	0.022	<0.01	0.78	<0.01
18:3 n-6	0.05	0.05	0.05	0.05	0.05	0.005	0.18	0.75	0.04
20:3 n-6	0.15	0.14	0.14	0.14	0.14	0.010	0.23	0.21	0.11
20:4 n-6	0.17^{ab}	0.18^{a}	0.17^{b}	0.17^{b}	0.16^{b}	0.007	0.02	0.01	0.08
20:5 n-3	0.05^{a}	0.05^{a}	0.05^{a}	0.05^{a}	0.03^{b}	0.003	<0.01	<0.01	<0.01
22:4 n-6	0.03	0.03	0.03	0.03	0.03	0.002	0.21	0.04	0.85
22:5 n-3	0.08^{b}	0.08^{a}	0.07^{b}	0.08^{ab}	0.07^{b}	0.004	0.02	0.04	0.17
22:6 n-3	0.10	0.12	0.11	0.09	0.10	0.011	0.12	0.48	0.37
MUFA	28.4^{d}	27.9^{d}	29.8^{c}	31.1^{b}	32.8^{a}	0.56	<0.01	<0.01	<0.01
PUFA	4.55^{c}	4.70^{bc}	4.79^{b}	5.08^{a}	5.10^{a}	0.154	<0.01	<0.01	0.81
SFA	67.0^{a}	67.4^{a}	65.4^{b}	63.8^{c}	62.0^{d}	0.66	<0.01	<0.01	0.02
PUFA/SFA	0.07^{c}	0.07^{bc}	0.07^{b}	0.08^{a}	0.08^{a}	0.003	<0.01	<0.01	0.42
SCFA	7.71^{abc}	8.03^{a}	7.87^{ab}	7.54^{bc}	7.32^{c}	0.227	<0.01	<0.01	0.02
MCFA	50.2^{b}	52.3^{a}	49.0^{b}	46.8^{c}	45.7^{c}	0.80	<0.01	<0.01	<0.01
LCFA	42.1^{b}	39.7^{c}	43.1^{b}	45.6^{a}	47.0^{a}	0.82	<0.01	<0.01	<0.01
n-3	0.78^{c}	0.87^{a}	0.83^{ab}	0.80^{bc}	0.77^{c}	0.028	<0.01	0.13	<0.01
n-6	3.22^{b}	3.21^{b}	3.31^{ab}	3.47^{a}	3.43^{a}	0.113	0.02	<0.01	0.99
n-6:n-3	4.19^{ab}	3.73^{c}	3.98^{bc}	4.37^{a}	4.50^{a}	0.129	<0.01	<0.01	<0.01

[a,b,c,d] Means within a row that do not have a common superscript differ at $P < 0.05$.

[1]18:1 *trans*, total = 18:1 t-4,5 + 18:1 t-6,8 + 18: t-9 + 18:1 t-10 + 18:1 t-11 + 18: t-12 + 18:1 t-13,14 ;CLA = conjugated linoleic acid; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; SFA = saturated fatty acids; SCFA = short-chain

fatty acids; MCFA = medium-chain fatty acids; LCFA = long-chain fatty acids; n-3 = 18:3 n-3 + 20:5 n-3 + 22:5 n-3 + 22:6 n-3; n-6 = 18:2 n-6 + 18:3 n-6 + 20:3 n-6 + 20:4 n-6 + 22:4 n-6.

[2]CTL = control diet without whole Nutrasaff™ safflower seed (NSS; Safflower Technologies International, Sidney, MT); SF1 = 1% NSS; SF2 = 2% NSS; SF3 = 3% NSS; SF4 = 4% NSS on DM basis.

[3]NSS = effect of level of whole Nutrasaff™ safflower seed in diet; L = linear effect of NSS; Q = quadratic effect of NSS.

Typically, unsaturated FA undergo partial BH in the rumen, resulting in the production of 18:1 *trans*-10 FA. Because NSS contains 75% linoleic acid on its lipid composition (Bergman et al., 2007), and linoleic acid is one of the main substrates for BH (Harfoot and Hazlewood 1997), a sizable shift in the BH pathway was evidenced with increased 18:1 *trans*-10 FA when NSS was fed at higher levels.

Increasing level of NSS inclusion linearly increased *cis*-9, *trans*-11 CLA (Table 7). Bell et al. (2006) also reported cows fed SS oil produced milk fat with 7.5 times more *cis*-9, *trans*-11 CLA than the control diet. Relatively lower increase of *cis*-9, *trans*-11 CLA by supplementing NSS in this study compared to the increase reported by Bell et al. (2006) may be due to different types of SS (whole vs. oil) and actual fat contents in the supplementations between the studies. Conjugated linoleic acids have been shown to have a wide array of health benefits in studies with animal disease and cancer models (Whigham et al., 2000; Pariza et al., 2001). Dairy products accounted for approximately 60% of the CLA intake in US diets (Ritzenhaler et al., 2001), and detailed analysis of milk fat has identified 19 different isomers of CLA (Sehat et al., 1998). However, the predominant CLA isomer is *cis*-9, *trans*-11, and it generally accounts for 75 to 90% of the total CLA present in milk fat (Sehat et al., 1998; Bauman et al., 2000). Diet of the cow has a major influence on the milk fat content of CLA, so feeding NSS to dairy diet

would enhance milk quality with increased CLA for potential health benefits from human consumption of milk and dairy products.

It is unclear whether any intermediate metabolite in the altered ruminal BH directly inhibited the milk fat synthesis in the mammary gland of cows fed higher levels of NSS. While *trans*-10, *cis*-12 CLA has been identified as a potent inhibitor of milk fat synthesis (Bauman and Griinari, 2001), *trans*-10 18:1 FA does not directly inhibit mammary synthesis of milk fat (Lock et al., 2007). In the current study, *trans*-10, *cis*-12 CLA did not differ among treatment diets, whereas *trans*-10 18:1 FA was markedly increased when NSS was fed at 2, 3, and 4% DM with 12, 26, and 56% increase compared to the CTL diet, respectively. The increase in the *trans*-10 18:1 content of milk fat is indicative of the complex changes in ruminal BH pathways, so this fatty acid has been suggested as an alternative marker for the type of alterations in rumen BH that characterize diet-induced milk fat depression (Lock et al., 2007). Further research is needed to identify if other CLA isomers or 18:1 *trans* FA are involved in the milk fat depression when NSS is fed to lactating dairy diets.

Milk monounsaturated FA and PUFA concentrations linearly increased, but saturated FA linearly decreased as the inclusion level of NSS increased in the diets, resulting in increased PUFA to saturated FA ratio with higher levels of NSS inclusion (SF3 and SF4 diets). Stegeman et al. (1992) found similar results with an increase of unsaturated FA and a decrease in saturated FA compared to control diets, when supplemented their diets with whole SS and SS oil, respectively.

While the proportion of 18:2n-6 linearly increased with increasing inclusion level of NSS, the proportion of 18:3n-3 increased with feeding the SF1 and the SF2 diets but

decreased with further increases in NSS inclusion. Total concentration of n-3 FA increased by feeding the SF1 and the SF2 diets, whereas total concentration of n-6 FA linearly increased with increasing inclusion level of NSS. The n-6 to n-3 FA ratio in milk was significantly reduced by feeding the SF1 diet compared with the CTL diet, which would improve the nutritive value of milk from a human health point of view. According to Sim (1998), the current high ratio should be decreased to less than 4 to 1 to reduce the potential risk of coronary heart diseases; feeding whole NSS to dairy cows at low level could contribute in improving human health by a greater intake of n-3 FA in enriched dairy products.

CONCLUSIONS

This study assessed supplementation of whole NSS to determine whether it improved lactational performance and milk FA profile of dairy cows. The present study demonstrated that supplementation of NSS into the lactating dairy diet had no effect on DMI and milk yield up to 4% of inclusion rate. However, higher inclusion levels of NSS resulted in decreased total tract DM digestibility due to increased fecal excretion of SS. Therefore, it would be beneficial to supplement NSS with maximum of 3% of inclusion rate in view of lactational performance. Inclusion of NSS from 1 to 4% of dietary DM in the diet improved dietary N use for milk production as indicated by the decrease in the MUN concentration, implying that NSS in lactating dairy diet would improve efficiency of N utilization in the rumen. Further research is needed on the effects of NSS on ruminal fermentation.

We also demonstrated that supplementing NSS at higher levels decreased milk fat percentage. However, increasing the level of NSS addition in the diets increased *cis*-9, *trans*-11 CLA, raising the potential health benefits in milk for humans. Many dietary treatments producing high levels of CLA also induce a shift in the major BH pathways characterized by increased accumulation of *trans*-10 18:1. The remarkable changes in the ruminal BH would directly affect the reduced milk fat concentration when NSS was added at higher levels.

The current study clearly demonstrates that it is highly possible to use NSS as a means of fat supplementation to lactating dairy cows without negative impact on lactational performance if added less than 3% of dietary DM. The enhanced milk quality

with increased *cis*-9, *trans*-11 CLA concentration with the addition of NSS is an additional benefit on human health issue. However, reduced milk fat concentration with increasing NSS supplementation is an obvious challenge and future research goal for the safe use of NSS to lactating dairy cows.

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