EFFECTS OF FEEDING RAW AND ROASTED SUNFLOWER SEEDS ON RUMINAL FERMENTATION, NUTRIENT UTILIZATION AND MILK PRODUCTION OF DAIRY COWS

By

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Abstract

Master of Science — Animal Science (Nutrition)

Pascale Sarrazin

EFFECTS OF FEEDING RAW AND ROASTED SUNFLOWER SEEDS ON RUMINAL FERMENTATION, NUTRIENT UTILIZATION AND MILK PRODUCTION OF DAIRY COWS

Three studies were conducted to determine the effects of roasting on ruminal degradability of sunflower seeds and the effects of feeding roasted sunflower seeds on ruminal fermentation, nutrient digestibility and milk yield and composition of dairy cows. Experimental treatments were a control diet with no added sunflower seed, a raw sunflower seed diet and a roasted sunflower seed diet. Sunflower seed diets contained 6% fat whereas the control diet contained 3% fat. In study one, two ruminally fistulated cows were used in a randomized complete block design to determine the effects of roasting on ruminal degradation of sunflower seeds. In the second study, three ruminally cannulated lactating Holstein cows were used in a 3x3 Latin square experiment to determine the effects of dietary treatments on ruminal fermentation and total tract nutrient utilization. In the last study, three primiparous and six multiparous Holstein cows were used in three 3x3 Latin squares to determine the effects of dietary treatments on milk yield and composition. Results showed that roasting decreased ($P < 0.05$) ruminal effective degradability of sunflower seed crude protein by 12%. Dietary treatments had no effect on ruminal pH, and concentrations of ammonia N and total volatile fatty acids. Total tract nutrient digestibilities were also unaffected by dietary treatments. Inclusion of sunflower seeds in dairy cow diets reduced ($P < 0.05$) dry matter intake as well as milk fat yield and concentration of multiparous cows. Feeding sunflower seeds reduced ($P < 0.05$) the milk concentrations of short and medium chain fatty acids (SCFA and MCFA) and increased ($P < 0.05$) the milk concentrations of long chain fatty acids (LCFA) and conjugated linoleic acid (CLA) for both parities. It was concluded that roasting reduced ruminal degradability of sunflower seeds without increasing milk yield, and that feeding sunflower seed increased CLA and LCFA concentrations in milk, but reduced milk fat yield.

Résumé

Maîtrise en science Science animale (Nutrition)

Pascale Sarrazin

LES EFFETS DE L'AJOUT ALIMENTAIRE DE GRAINES DE TOURNESOL CRUES ET RÔTIES SUR LA FERMENTATION RUMINALE, L'UTILISATION DES NUTRIMENTS ET LA PRODUCTION DE LAIT DES VACHES LAITIÈRES

Nos objectifs étaient de déterminer les effets du rôtissage sur la dégradation ruminale des graines de tournesol et les effets de l'ajout alimentaire de graines de tournesol rôties sur la fermentation ruminale, l'utilisation des nutriments ainsi que sur la production et la composition du lait des vaches laitières. Les traitements étaient un contrôle sans ajout de graine de tournesol, une diète avec graines de tournesol crues et une diète avec graines de tournesol rôties. Les rations contenant des graines de tournesol contenaient 6% de gras alors que le contrôle contenait 3%. Deux vaches avec une fistule ruminale ont été utilisées dans un plan en blocs aléatoires complets pour déterminer les effets du rôtissage sur la dégradation ruminale des graines de tournesol. Trois vaches fistulées au niveau du rumen ont été utilisées dans un carré latin pour déterminer les effets des rations sur la fermentation ruminale et l'utilisation des nutriments. Trois vaches primipares et six vaches multipares ont été utilisées dans un plan en carrés latins pour déterminer les effets des rations sur la production et la composition du lait. Les résultats ont démontré que le rôtissage réduit ($P < 0.05$) de 12% la dégradation ruminale de la protéine contenue dans la graine de tournesol. Les rations n'ont eu aucun effet sur le pH, les concentrations d'azote ammoniacal et d'acides gras volatiles dans le rumen ainsi que sur la digestion totale des nutriments. L'ajout alimentaire de graines de tournesol chez les vaches laitières a réduit ($P < 0.05$) la consommation de matière sèche et la production de gras dans le lait des vaches multipares. L'ajout alimentaire de graines de tournesol a réduit ($P < 0.05$) la teneur en acides gras courts (AGC) et moyens (AGM) et augmenté ($P < 0.05$) la teneur en acides gras longs (AGL) et d'acide linoléique conjugué (ALC) dans le lait. Il a été conclu que le rôtissage réduit la dégradation ruminale des graines de tournesol sans toutefois augmenter la production de lait. Il a aussi été conclu que l'ajout alimentaire de graines de tournesol augmente la teneur en ALC et des AGL du lait, mais réduit la production de gras.

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Contribution of Authors

In accordance to McGill thesis submission guidelines, this is a manuscript-based thesis and includes a table of contents, a brief abstract in both English and French, an introduction, a comprehensive review of literature, a final conclusion and summary, a thorough bibliography and appendices where appropriate.

Part of this thesis has already been published in the Journal of the Science of Food and Agriculture (P. Sarrazin, A.F. Mustafa, P.Y. Chouinard, G.S.V. Raghavan and S.A. Sotocinal. 2003. Effects of roasting on ruminal nutrient degradability of sunflower seed. J. Sci. Food Agric. 83:1219-1224) and was reproduced with permission. The permission is granted by John Wiley & Sons Ltd on behalf of the Society of Chemical Industry. This manuscript was co-authored by Dr. A. F. Mustafa who contributed to experimental design of the study and proof-reading of the manuscript, by Dr. P.Y. Chouinard who contributed to the analysis of fatty acids and Dr. G.S.V. Raghavan and Dr. S. A. Sotocinal who contributed in the roasting of sunflower seeds.

À mon père, qui, à travers sa courageuse lutte, m'a appris à ne jamais baisser les bras.

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1. Introduction

Recently, nutritionists have been interested in dietary manipulations to modify the profile of milk fatty acids. This is based on the fact that 50% of the fatty acids found in milk fat are taken up directly from blood stream by the mammary gland. This leaves a large room to manipulate milk fatty acids by alteration of the fatty acids being absorbed in the small intestine (Kennelly, 1996). Supplementing dairy cows with lipids rich in polyunsaturated fatty acids is one of the possible dietary manipulations available to alter milk fatty acid profile. Such modifications are of public interest considering the health benefits that have been associated with specific fatty acids (Garnsworthy, 1997).

Oilseeds are rich in polyunsaturated fatty acids, particularly oleic, linoleic and linolenic acids. Due to rapid biohydrogenation of polyunsaturated fatty acids in the rumen, the profile of lipids leaving the rumen is very different from that found in the diet (Harfoot and Hazelwood, 1997). However, feeding fat to dairy cows in a form that is protected from ruminal biohydrogenation will consequently modify the profile of fatty acids leaving the rumen and reaching the mammary gland. Feeding whole oilseeds as well as heat-treated oilseeds might partially protect fatty acids from complete ruminal biohydrogenation by reducing the release of the oil in the rumen (Byers and Schelling, 1988; Harfoot and Hazelwood, 1997).

Conjugated linoleic acid (CLA) is an intermediary product of the ruminal biohydrogenation of linoleic acid (Harfoot and Hazelwood, 1997). It can also be formed at the mammary gland level by desaturation of trans-11 $C_{18:1}$, which is also an intermediate product of the biohydrogenation of linoleic acid. Increasing the quantity of linoleic acid in the diet of dairy cows is one possible way of increasing milk CLA content (Chouinard et al., 2001). Such increase is desired considering the anticarcinogenic and other health beneficial effects that have been attributed to CLA (Garnsworthy, 1997). Sunflower seeds, which are rich in linoleic acid, would then represent an interesting option to increase CLA content of milk.

Oilseeds have other advantages than only being rich in polyunsaturated fatty acids. They are also a good source of energy that can increase the energy density of dairy cow diets. This is particularly interesting for dairy cows in early lactation, which often face negative energy balance due to reduced dry matter intake. Moreover, oilseeds have a high content of protein, which could improve milk yield by providing increased quantity of limiting amino acids to the mammary gland. In order to achieve such improvement, heat treatment can be applied to the oilseeds to increase their rumen undegraded protein value and thus increase the quantity of amino acids being available for absorption in the small intestine (Van Soest, 1994).

The objectives of this research are:

- To determine the effects of roasting on the chemical composition and the ruminal degradability of sunflower seeds.
- To determine the effects of feeding roasted sunflower seeds on ruminal fermentation parameters and total tract nutrient utilization of dairy cows.
- To determine the effects of feeding roasted sunflower seeds on dry matter intake, milk yield and milk composition and milk fatty acid composition of dairy cows.

The hypotheses of this research are:

- Roasting sunflower seeds with a particulate medium thermal processor in which the salt is heated at 250° C will reduce the ruminal degradability of sunflower seeds and therefore increase the concentrations of amino acids and fatty acids reaching the small intestine.
- Feeding whole roasted sunflower seeds will not have detrimental impacts on ruminal fermentation and total tract nutrient utilization.
- Feeding whole roasted sunflower seeds will improve milk yield and will not reduce milk fat concentration.
- Feeding whole sunflower seeds will increase the conjugated linoleic acid concentration of dairy cows' milk and roasted sunflower seeds will be more efficient in increasing conjugated linoleic acid concentration than raw sunflower seeds.

2. Literature Review

2.1. Dietary Fats

Inclusion of fat in the diet is typically done to increase the energy density of the ration. This is particularly interesting for early lactation diets because during this period it is difficult to achieve a positive energy balance due to the limiting dry matter intake. Addition of fat can also bring other potential advantages such as increasing the absorption of fat-soluble nutrients, reducing the dustiness of feed and improving reproductive performances (NRC, 2001). More recently, fat supplementation has been used to modify the fatty acid profile of milk in order to increase heath-promoting compounds such as conjugated linoleic acid and polyunsaturated fatty acids. In order to obtain benefits from supplemental fat, it must not be detrimental to ruminal fermentation, nutrient digestibility and milk production while providing the desired effect(s) on milk constituents. Fat in the rations of lactating dairy cows might have various origins. These include forages and concentrates such as animal and vegetal by-products, soaps of long chain fatty acids and oilseeds. Supplemental fats vary greatly in their fatty acid composition (Table 2-1) and other intrinsic characteristics such as their degree of rumen inertness, their digestibility, their transfer into milk and their effects on mammary gland lipogenesis (Jensen, 2002).

2.1.1. Forages

Lipids in forages are mainly in the form of structural lipids found in the leaves of the plants (Byers and Schelling, 1988). They usually represent approximately 6 to 7% of the leaf dry matter and consist mainly of glycolipids and phospholipids (Harfoot and Hazlewood, 1997). Fatty acids represent 43% of the ether extract from forages while the rest consists of waxes, chlorophyll, galactose and other unsaponifiable compounds (Palmquist and Jenkins, 1980). Linolenic acid ($C_{18:3}$) is the predominant fatty acid of forages followed by linoleic acid ($C_{18:2}$).

Table 2-1. Fatty acid composition of dietary fats and oils (% of fatty acids).

Type of fat	Fatty acids						
	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Granular fats							
Calcium salt palm oil	1.3	48.6	1.1	4.1	36.5	7.8	0.3
Hydrolyzed tallow	2.4	39.7	0.7	42.7	10.9	1.0	-
Animal / animal-vegetable blends							
Tallow	3.0	24.5	3.7	19.3	40.9	3.2	0.7
Choice white grease	1.9	23.4	4.3	13.3	43.4	10.9	1.3
Fish oil	8.0	15.1	10.5	3.8	14.5	2.2	1.5
Vegetable oils							
Canola	-	4.8	0.5	1.6	53.8	22.1	11.1
Corn	0.0	10.9	-	1.8	24.2	58.0	0.7
Cottonseed	0.8	22.7	0.8	2.3	17.0	51.5	0.2
Linseed	-	5.3	-	4.1	20.2	12.7	53.3
Soybean	0.1	10.3	0.2	3.8	22.8	51.0	6.8
Sunflower	-	6.0	-	4.0	18.5	69.5	1.0

Adapted from Kennelly (1996), Mustafa et al. (2003b) and NRC (2001)

2.1.2. Animal By-Products

Tallow and animal-vegetal blends are examples of animal fats fed to dairy cattle. Fat from animal sources is composed up to 95% of triglycerides with a large proportion of their fatty acids being saturated (Table 2-1; Enser, 1984).

2.1.3. Oilseeds

Storage lipids found in oilseeds are primarily in the form of triglycerides (Byers and Schelling, 1988). Fatty acids constitute from 65 % to 80% of the ether extract of cereals and up to 90% of the ether extract of oilseeds (Buyers and Schelling, 1988). The fatty acid pattern of those triglycerides is different for each oilseed; however, the main fatty acid being found in oilseeds is linoleic acid with the exception of linseed and safflower seeds, which are rich in linolenic acid (Table 2-1; Kennelly, 1996; Palmquist and Jenkins, 1980). Oilseeds and oilseed meals are extensively used in the dairy feed industry as sources of protein and most of the research on ruminal undegradable protein has focus on those products (NRC, 2001).

2.1.4. Sunflower Seeds

Sunflower (***Helianthus annuus***) is an important oil crop in Canada. In 2001, 63,000 hectares were used for sunflower seed production in Canada, which yielded 98,000 tons (Statistics Canada, 2001). The main producing provinces are Manitoba (89%) followed by Saskatchewan (Statistics Canada, 2001). A black hull and high oil content characterize the oil-type sunflower seed, which is recognized for its high linoleic acid concentration (Table 2-1). Sunflower seeds, as other oilseeds, are not only rich in energy, but are also a good source of protein (Table 2-2). However oilseeds, particularly sunflower seeds, are characterized by a high ruminal degradability of their protein and are thus of little value for ruminal escape protein when fed in their original form (Mustafa et al., 2002; Mustafa et al., 2003b). In order to improve the ruminal undegradable protein content of sunflower seeds, various chemical and physical treatments could be applied.

Table 2-2. Chemical composition of whole sunflower seeds (dry matter basis)

DM	CP	NDICP	ADICP	Ether Extract	NDF	ADF	Lignin	Ash
%	%	%	%	%	%	%	%	%
91.8	18.8 - 19.2	1.1 - 2.9	0.9 - 1.9	41.9 – 45.4	24.0 – 26.1	16.7 – 21.1	6.0	3.6 - 5.1

Adapted from NRC (2001) and Mustafa et al. (2003b).

2.2. Metabolism of Polyunsaturated Fatty Acids in the Rumen

Dietary lipids are rapidly and extensively transformed by microorganisms after they reached the rumen so that very little lipids escape the rumen in their original form under normal circumstances (Harfoot and Hazelwood, 1997). The major steps in lipid metabolism by rumen microbes are hydrolysis and biohydrogenation.

2.2.1. Hydrolysis

Dietary lipids enter the rumen principally in their esterified form. Post-ruminal digesta when compared with the diet is usually enriched in steric acid while content of both linoleic and linolenic acid is reduced (Harfoot and Hazlewood, 1997). Biohydrogenation of unsaturated fatty acids cannot be performed on esterified fatty acids due to the requirement for a free carboxyl group. Hydrolysis of triglycerides into free fatty acids and glycerol is therefore a mandatory first step in the modification of fatty acids in the rumen (Byers and Schelling, 1988). Lipolysis is usually rapidly and extensively performed by microbial lipolytic enzymes, however, this step could be a rate-limiting step to the modifications accomplished by rumen microbes on dietary fats (Byers and Schelling, 1988). Rumen protozoa do not seem to play a major role in the hydrolysis process of fatty acids since rapid lipolysis occurred in defaunated sheep (Harfoot and Hazlewood, 1997). Lipolytic activity in the rumen thus seems to be mainly attributed to bacteria. Such bacteria have been isolated from rumen content of sheep and the most active seemed to be a non-cellulolytic strain of *Butyrivibro fibrisolvens* (Harfoot and Hazlewood, 1997). *Butyrivibro fibrisolvens* was also found to possess the capacity of performing biohydrogenation (Harfoot and Hazlewood, 1997).

2.2.2. Biohydrogenation

The role of biohydrogenation has not been clearly established. It has been suggested that biohydrogenation produces intermediate fatty acids required for incorporation into microbial membranes or that the process could act as a disposal of reducing power (Harfoot and Hazlewood, 1997). However, the major hypothesis is that biohydrogenation acts as a detoxification mechanism (Harfoot and Hazlewood, 1997). There are some evidences that suggest that biohydrogenation is more extensively performed when food particles are present. Free fatty acids formed during the hydrolysis process have been found to be associated to rumen particulate matter by non-ionic bonds (Byers and Schelling, 1988). Surface adhering bacteria would then perform biohydrogenation. As for lipolysis, protozoa are of minor importance in the biohydrogenation process and bacteria are mainly responsible for this process (Harfoot and Hazlewood, 1997). Biohydrogenation is a multi-step process involving more than one species of bacteria. Many species of bacteria other than *Butyrivibro fibrisolvens* have been found to perform biohydrogenation and it is believed that many more remain to be discovered (Harfoot and Hazlewood, 1997). Biohydrogenating bacteria can be divided into two different groups, A and B, based on their end products and the isomerizations carried out (Table 2-3).

The biohydrogenation process of linoleic acid ($C_{18:2}$) proposed by Harfoot and Hazelwood (1997) starts by the isomerization of the 12-cis double bound of the cis-9, cis-12 $C_{18:2}$ into a 11-trans isomer (Figure 2-1). This reaction is mediated by a Δ-cis-12, Δ-trans-11 isomerase and results in the formation of cis-9, trans-11 $C_{18:2}$, which is one the isomers of conjugated linoleic acid (CLA). The following steps are the hydrogenation of the cis-9 followed by the trans-11 double bonds accomplished by reductases to obtain a saturated fatty acid; sterate. In the case of linolenic acid ($C_{18:3}$), an additional step for the hydrogenation of the cis-15 double bond would be required (Figure 2-1). The biohydrogenation process of a polyunsaturated fatty acid to a completely saturated fatty acid is most probably not accomplished by a single microorganism (Harfoot and Hazlewood, 1997). Depending on the extent of the biohydrogenation process, a variety of fatty acids is leaving the rumen going from completely saturated fatty acids to undisturbed fatty acids and their intermediate products.

Lipolysis and biohydrogenation can be reduced to various extents by different means such as reducing the population of lipolytic and biohydrogenating bacteria, feeding a diet low in roughages, increasing concentration of dietary lipids and providing large quantities of free fatty acids (Byers and Schelling, 1988; Harfoot and Hazlewood, 1997). Other alternatives include providing fat in a protected form such as in formaldehyde-treated casein coating, calcium-soaps, whole oilseeds, or by heat treatments (Byers and Schelling, 1988; Harfoot and Hazlewood, 1997; NRC, 2001).

Table 2-3. Rumen bacteria groups and their biohydrogenation ability.

Groups	Bacterium	End-products of hydrogenation of		
		Linoleic acid	Linoleic acid	Oleic acid
A	B. fibrisolvens	-	18:1	-
A	B. fibrisolvens A38	18:3cis-9,trans-11,cis-15 18:2 trans-11, cis-15	18:2 cis-9, trans-11 18:1 trans-9 and trans-11	NH[1]
A	B. fibrisolvens S2	18:1 trans-11	18:1 trans-11	-
A	Treponema (Borrelia)	Isomerized,then hydrogenated	18:2 cis-9, trans-11 18:1 trans-11	-
A	Micrococcus	Isomerized,then hydrogenated	18:1 trans-11	-
A	Ruminococcus albus F2/6	18:1 trans 18:1 cis 18:3cis-9,trans-11,cis-15 18:2 trans-11,cis-15	18:2 cis-9, trans-11 18:1 trans 18:1 cis	NH[1]
A	Eubacterium F2/2	18:3 cis-9,trans-11,cis-15 18:2 trans-11,cis-15 18:1 trans	18:2 cis-9, trans-11 18:1 trans-11	NH[1]

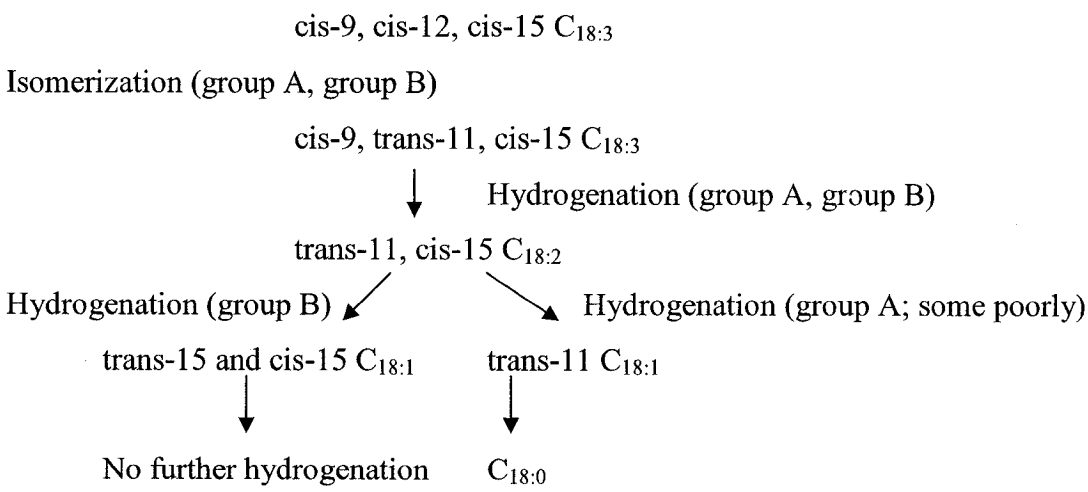
Table 2-3. Continued

Groups	Bacterium	End-products of hydrogenation of		
		Linoleic acid	Linoleic acid	Oleic acid
A	Eubacterium W461	18:3 cis-9,trans-11,cis-15 18:2 trans-11,cis-15 18:1 trans 18:1 cis	18:2 cis-9, trans-11 18:1 trans	NH[1]
A	R8/3 Gram-negative rod	18:2 trans-11,cis-15	18:1 trans-11	NH[1]
A	LM8/1A; LM8/1B Gram-negative rod	18:2 trans-11,cis-15	18:1 trans-11	NH[1]
A	2/7/2	18:2 trans-11,cis-15	18:1 trans-11 18:1 cis-9 and cis-12	-
A	EC7/2 Gram-negative rod	18:2 trans-11,cis-15 18:1 trans-11	18:1 trans-11	NH[1]
A	R7/5 Gram-negative rod	18:1 trans-11, trans-10, trans-12, cis-11 and cis-12	18:1 trans-11, trans-10, trans-12, cis-11 and cis-12	NH[1]
A	2/9/1 Gram-negative vibro	18:1 trans-11, trans-10, trans-12, cis-11 and cis-12	18:1 trans-11, trans-10, trans-12, cis-11 and cis-12	NH[1]
B	Fusocillus babrahamensis P2/2	18:3 cis-9,trans-11,cis-15 18:2 trans-11,cis-15 18:1 cis-15	18:2 cis-9,trans-11 18:1 trans-11 18:0	18:0 18:0 hydroxy
B	Fusocillus T344	18:3 cis-9,trans-11,cis-15 18:2 trans-11,cis-15 18:1 cis-15	18:2 cis-9,trans-11 18:1 trans-11 18:0	18:1 trans-11 18:1 cis-9 18:0
B	R8/5 Gram negative rod	18:2 trans-11,cis-15 18:1 cis-15 18:1 trans-15	18:1 trans-11 18:0	18:1 cis-9 18:0 hydroxy

Adapted from Harfoot and Hazelwood (1997)

[1] Not hydrogenated

Linolenic Acid



Linoleic Acid

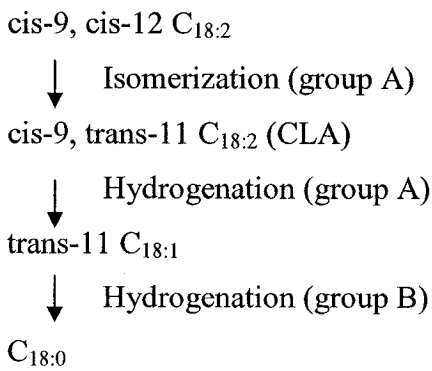


Figure 2-1. Scheme for the biohydrogenation of linolenic and linoleic acid
Adapted from Harfoot and Hazelwood (1997)

2.3. Heat Treatment of Oilseeds

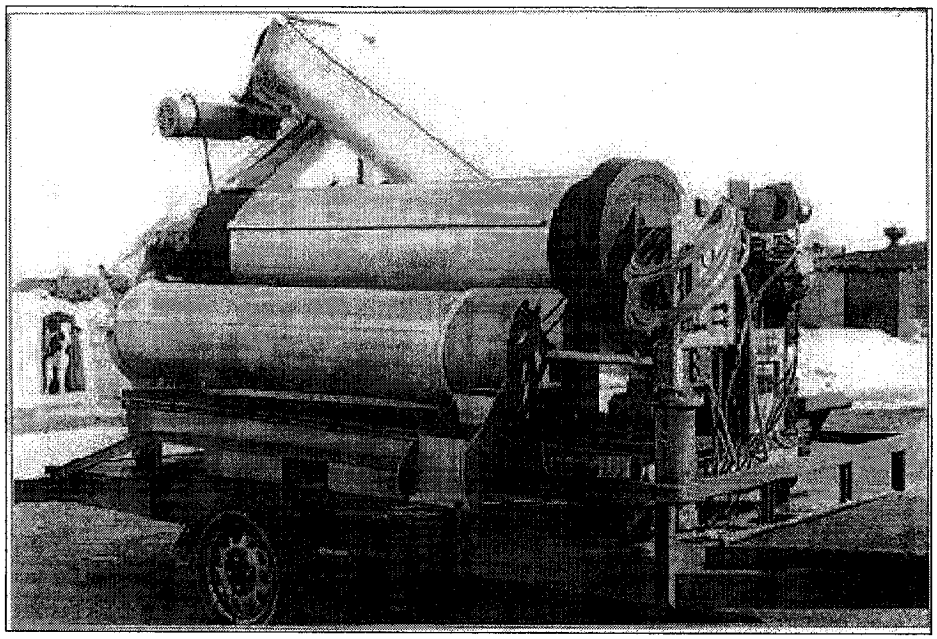
Heat is the most commonly used treatment to protect dietary proteins from ruminal degradation in North America (NRC, 2001). Heat treatments reduce ruminal degradation of protein by denaturation of protein, creation of Maillard reaction and formation of protein to protein cross-links (NRC, 2001). This consequently increases the concentration of amino acids available for digestion in the small intestine (Stern et al., 1985; NRC, 2001; Wang et al., 1999). Kennelly (1996) suggested that the application of heat treatment to oilseeds, such as sunflower seeds, might reduce ruminal biohydrogenation of dietary fatty acids by denaturing the protein matrix surrounding the

fat droplets. Heat treatments can also increase the concentration of polyunsaturated fatty acids reaching the small intestine thus increasing the secretion of polyunsaturated fatty acids into the milk.

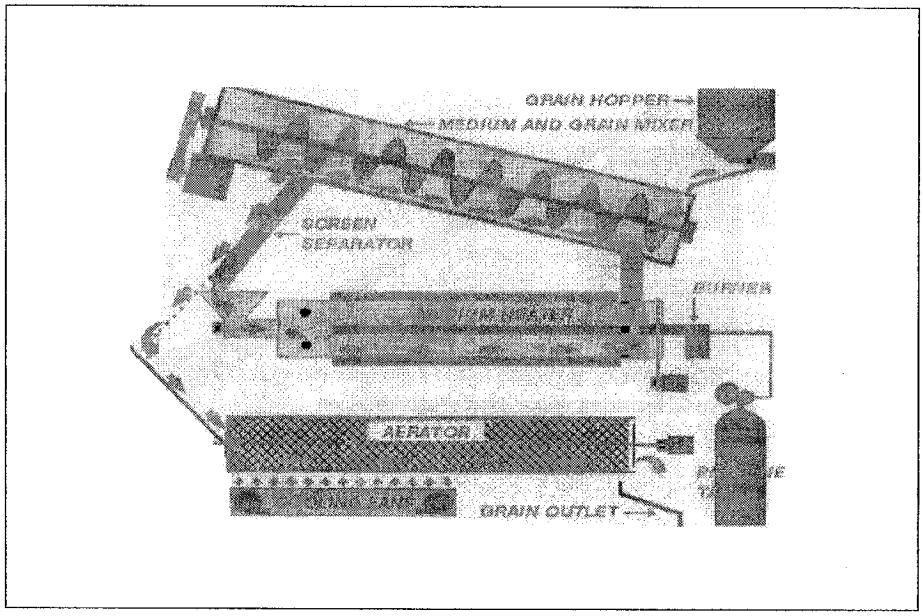
Several types of heat treatments have been used to protect oilseeds from ruminal degradation. These include moist heat treatment (autoclaving), micronization, jet-sploding and roasting. Moist heat treatment or autoclaving involves the heating of the material with steam under high pressure (Mustafa et al., 2003b). Micronization is a dry heat treatment in which infrared gas generators heat the feedstuff to temperature varying between 110 to 180° C for 30 to 60 seconds (Mustafa et al., 2002; Wang et al., 1999). A major advantage of micronization over other heat treatment methods is the shorter heating time. Moreover, micronization heats the seeds directly from inside avoiding shell damages. Jet-sploding involves rapid steam heat treatment under high pressure for a short period of time utilizing the moisture within the seed (Deacon et al., 1988). Particulate medium thermal processing is another heating method that can be used to roast various oilseeds (Sotocinal, 1997).

2.3.1. Particulate Medium Thermal Processing

Particulate medium thermal processing (roasting) is characterized by the transfer of heat by conduction from a pre-heated medium to the material to be processed (Sotocinal, 1997). A sequence of five steps is involved during the roasting process (Figure 2-2). The first step is the heating of the granular medium such as salt or sand. The medium, once heated at the desired initial temperature, is then mixed with the material to be processed in a set medium-to-material ratio. After a specified contact time between the material and the medium, the two are separated. The separated medium is then reheated and recycled back into the process while the processed material is cooled down to room temperature. During the cooling process, further heating of the material is achieved due to the large temperature gradients between processed material and ambient temperature (Sotocinal, 1997).



a



b

Figure 2-2. Example of a particulate thermal medium processor (a) and its schematic diagram (b) (Sotocinal, 1997 and Raghavan, 1998, personal communication)

Particulate medium thermal treatment is recognized to be energy efficient due to the recirculation of the heated medium. It also provides a uniform heating in a very short time without product contamination (Sotocinal, 1997). Sotocinal (1997) showed that the overall thermal efficiency of the particulate medium thermal processing (conduction) was 28% higher than conventional convection heat transfer. The advantage of particulate medium heat transfer results from the higher heat capacity of solids over air. The medium heat transfer was also found to be faster (5 times) than the convection heating to achieve maximal grain temperature (Sotocinal, 1997).

2.3.2. Impact of Heat Treatment on Chemical Composition of Oilseeds

Heat treatment modifies the chemical composition of oilseeds and oilseed meals. Changes have been found to occur in the different fiber and protein fractions as well as in the individual amino acid concentrations.

2.3.2.1. Impact of Heat Treatment on Fiber Fractions

Heat treatment of oilseeds and oilseed meals usually increases the neutral and acid detergent fiber fractions (Demjanec et al., 1995; McKinnon et al., 1995; Mustafa, 2002). The increase in those two fiber fractions is mainly due to the increase in the concentration of neutral and acid detergent insoluble nitrogen. Moshtaghi and Ingalls (1992) have noted that the changes observed in neutral and acid detergent fiber content followed a similar pattern to the changes occurring in the neutral and acid detergent insoluble protein content, thus reflecting heat-induced changes in the protein fractions of the treated material.

2.3.2.2. Impact of Heat Treatment on Protein Fractions

Protein fractions are the chemical constituents mostly affected by heat treatments. The effects of heat treatment on protein fractions are based on the fact that dietary protein consists of different fractions, which respond differently to various heat inputs (a function of both temperature and heating time). According to Sniffen et al. (1992), five protein fractions can be identified namely non-protein nitrogen (A), rapidly degradable true protein (B_1), intermediately degradable true protein (B_2), slowly degradable true protein

(B_3) and unavailable protein (C). The non-protein nitrogen and the rapidly degradable true protein fractions denature at lower heat inputs and become intermediately or slowly degradable fractions depending on the level of heat input. The slowly degradable protein fraction responds at higher heat inputs and usually becomes unavailable (heat-damaged) protein via the Maillard reaction (Figure 2-3; NRC, 2001; Van Soest et al., 1994).

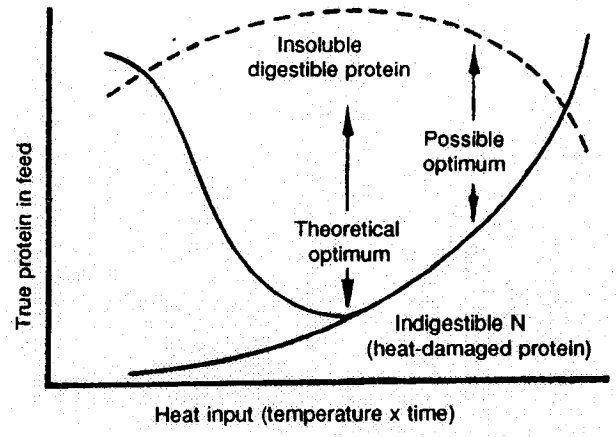


Figure 2-3. The theoretical relation between protein solubility and heat (Van Soest, 1994)

An optimum heat input varies from one dietary protein source to another. The objectives are to minimize the soluble protein fraction and maximize the slowly degradable protein fraction with little or no increase in the heat-damaged protein. For a given protein source, the optimum heat input will depend on several factors including moisture content, pH, carbohydrate content and composition, and protein content (Van Soest, 1994).

Changes in the relative proportions of the protein fractions as a result of heat treatment can be detected by analyzing for soluble protein, neutral detergent insoluble protein and acid detergent insoluble protein (Van Soest, 1994). As heat input increases, the concentration of soluble protein decreases and that of neutral detergent insoluble protein increases. Moderate heat input has little effect on acid detergent insoluble protein (McKinnon et al., 1995; Mustafa et al., 2002; Mustafa et al., 2003b). However, heat-damaged protein generated by excessive heat input is usually characterized by elevated concentrations of acid detergent insoluble protein (Demjanec et al., 1995; McKinnon et al., 1995; Moshtaghi Nia and Ingalls, 1992; Reid, 1994).

2.3.2.3. Impact of Heat Treatment on Amino Acids Composition

Overheating can result in protein damage as well as changes in individual amino acid concentrations, particularly lysine, cystine and arginine (Mauron, 1981; NRC, 2001; Van Soest, 1982). Excessive heat treatment can result in the formation of Maillard products, which have properties similar to lignin. Maillard products being resistant to acid hydrolysis during amino acid analysis might be responsible for the reduction in arginine and lysine concentrations. The free amino groups, present in the side chain of those two amino acids, make them susceptible to Maillard product formation during heating process (Mauron, 1981; Van Soest, 1982). Following moist heat treatment applied for different length of time, the amino acid composition of sunflower seeds was not affected (Mustafa et al., 2003b). However, following moist heat treatment, concentrations of arginine and lysine in mustard meal were reduced when compared to unheated meal (Mustafa et al., 1999). Similar results were reported by Demjanec et al. (1995) after roasting soybean meal.

2.3.3. Impact of Heat Treatment on Ruminal Degradation of Oilseeds

Oilseeds are considered a poor source of ruminal undegraded protein (Deacon et al., 1988; Mustafa et al., 2002; Mustafa et al., 2003b). In situ nylon bag technique and in vitro enzymatic digestion are the most commonly used methods to study the effects of heat treatment on ruminal degradation of oilseeds and oilseed meals (Van Soest, 1994). Effective heat treatment increases the ruminal undegraded protein content by reducing the in situ soluble protein fraction and increasing the in situ slowly degradable protein fraction (Van Soest, 1994).

Dry heat treatment was one of the first methods utilized to protect oilseeds from ruminal degradation. Lindberg et al. (1982) studied the effects of different heat inputs (heating to 100, 150 and 200° C for 1 hour) on ruminal degradability of rapeseed. The authors found that heating to 100° C had no effect on ruminal protein degradability. However, heating to 150 and 200° C reduced ruminal protein degradability from 85.7% to 64.8% and 34.5%, respectively. Mustafa et al. (2003a) determined the effects of moist heat treatment on ruminal degradability of sunflower seeds. They showed that autoclaving at 127° C with a

steam pressure of 117 kPa for 30 minutes reduced ruminal dry matter and crude protein degradability of sunflower seeds by 11 and 17%, respectively. The results also showed significant increases in ruminal undegraded amino acids and polyunsaturated fatty acids as a result of the heat treatment. Micronization has been recently used to reduce ruminal degradability of canola seed and flaxseed (Wang et al., 1997; Wang et al., 1999; Mustafa et al., 2002). In these studies, micronization increased post-ruminal digestibility of amino acids from both oilseeds. Deacon et al. (1988) compared ruminal degradability and intestinal digestibility of jet-sploded canola seed heated to an internal seed temperature of 121° C with other protein sources. The authors found that jet-sploding reduced effective ruminal crude protein and dry matter degradability of canola seed. However, the heat treatment also resulted in reduced total tract digestibilities of canola seed protein and dry matter.

2.3.4. Impact of Heat Treatment on Total Tract Nutrient Utilization

In order to be effective a heat treatment must reduce ruminal degradability without impairing total tract nutrient utilization (Van Soest, 1994). However, overheating of oilseeds and other protein sources can reduce total tract nutrient utilization as a result of the formation of Maillard products (Van Soest, 1994).

Using the mobile nylon bag technique, McKinnon et al. (1995) found that heating canola meal to 125° C for 30 minutes increased the amount of dietary protein available for digestion in the small intestine without affecting total tract protein digestibility. However, heating canola meal to 145° C resulted in heat-damaged protein with lower intestinal and total tract protein digestibilities. On the other hand, Mustafa et al. (2002) found that micronization of flaxseed increased intestinal digestibility of ruminal undegraded nutrients with no effect total tract nutrient digestibility. Similarly, Wang et al. (1997) reported that micronization had no adverse effect on intestinal digestibility of ruminal undegraded protein of canola seed.

2.3.5. Impact of Heat Treatment on Milk Yield and Dry Matter Intake

Dairy cows in early lactation have high requirements and potential milk production might not be achieved due to limiting factors such as limited dry matter intake or limited quantity of essential amino acids reaching the mammary gland. Heat treatment of oilseeds increases ruminal undegraded protein and increases availability of essential amino acids in the small intestine. This also increases the potential for essential amino acids to reach the mammary gland and to be available for milk synthesis. It is therefore expected that feeding heat-treated oilseeds or oilseed meals will increase milk yield of dairy cows in early lactation. Many studies have been conducted to determine the ideal ruminal undegraded protein level that will optimize amino acid flow to the small intestine and consequently the performance of dairy cows. The response of dairy cows to high levels of ruminal undegraded protein has been inconsistent. Santos et al. (1998) published a comprehensive review on the effects of ruminal undegraded protein on dairy cow performance. The authors found that providing a source of ruminal undegraded protein resulted in increased milk yield in 17% of the studies and that heated soybean meal or fishmeal were the two ruminal undegraded protein sources that were the most likely to increase milk yield. A decrease in microbial protein synthesis in the rumen, poor essential amino acid profile of the ruminal undegraded protein, low intestinal digestibility of the ruminal undegraded protein and sufficient amount of ruminal undegraded protein in the diet before supplementation could potentially explain why positive response in milk yield are not always observed following rumen undegradable protein supplementation (Santos et al., 1998; NRC, 2001).

Chouinard et al. (1997a) examined the effects of various heat treatments (extrusion, micronization and roasting) of soybean (17.5 % of the diet dry matter) on performance of dairy cows. The authors found no difference in milk yield of cows fed diets containing heated soybeans compare with cows fed a raw soybean diet. However, in another study, Chouinard et al. (1997b) reported higher milk yield for cows fed extruded soybeans than for cows fed raw soybeans. A similar positive response of milk yield as a result of feeding extruded flaxseeds has also been reported by Dhiman et al. (1997). Mustafa et al. (2003a) found that total milk yield was not affected by heat treatment of flaxseeds,

however energy corrected milk yield was higher for cows fed micronized versus raw flaxseeds (Mustafa et al., 2003a).

It was also found that feeding heat-treated soybeans reduced the dry matter intake of dairy cows (Chouinard, 1997a), which was not the case for micronized flaxseeds (Mustafa et al., 2003a). On the other hand, others found that feeding heat-treated (roasted and extruded) soybeans improved milk yield over feeding raw soybeans while unaffecting dry matter intake (Chouinard et al., 1997b; Dhiman et al., 1997).

2.3.6. Impact of Heat Treatment on Milk Fatty Acid Composition

Heat treatment of oilseeds has the potential to change milk fatty acid composition by protecting polyunsaturated fatty acids from complete biohydrogenation in the rumen. Roasting soybeans have resulted in a slower release of free fatty acids and reduced biohydrogenation in the rumen when compared with extruded and raw soybeans (Reddy et al., 1994). The authors suggested (1994) suggested various factors that could explain the slow release of fatty acids from raw and roasted soybean. These include the intracellular location of the oil, the chemical reaction occurring during the roasting treatment and the reduction in release rate of oil into the rumen due to reduced dry matter digestion after roasting. This protection could also originate from the denaturation of the protein matrix surrounding the fat droplets, which may increase quantity of polyunsaturated fatty acids reaching the mammary gland (Kennelly, 1996).

Chouinard et al. (1997a and 1997b) found that milk fat of cows fed heat-treated soybeans contained lower levels of saturated fatty acids than milk fat of cows fed raw soybeans. The concentrations of short and medium chain fatty acids were lower for cows fed heat-treated oilseeds compare with cows fed unheated oilseeds (Chouinard et al., 1997a; Mustafa et al., 2003a).

2.4. Impact of Polyunsaturated Fatty Acids on Ruminal Fermentation

Several parameters of ruminal fermentation, such as volatile fatty acid production, pH, ammonia-nitrogen and microbial population, can be affected by dietary fat

supplementation. The degree by which fat supplementation affects ruminal fermentation is related to several factors including the nature of the fat (degree of saturation, chain length), the form in which fat is fed (protection, whole seed), the level of fat supplementation and the forage to concentrate ratio (Ferlay and Doreau, 1992).

2.4.1. Effects of Fat Supplementation on Ruminal Volatile Fatty Acids

Feeding high levels of unsaturated fatty acids can result not only in a reduced total volatile fatty acid production, but also in substantial modifications of the relative proportions of those volatile fatty acids. Broudiscou et al. (1994) found that feeding linseed oil (65 g day^{-1}) to sheep decreased total volatile fatty acid concentration in ruminal fluid. Similar findings have been reported by Drackley and Schingoethe (1986) and Tackett et al. (1996). On the other hand, addition of rapeseed oil (5.3% or 9.4% of the diet dry matter) to the diet of dairy cows did not alter total volatile fatty acid concentration (Doreau et al., 1991).

Molar proportions of volatile fatty acids can also be modified when supplemental fat is fed. Linseed oil supplementation reduced the molar proportion of acetate combined with an increase in the molar proportion of propionate resulting in a reduction of the acetate to propionate ratio (Broudiscou et al., 1994). This was also observed by Casper et al. (1988) and Doreau et al. (1991). Tackett et al. (1996) reported similar changes in the relative proportion of volatile fatty acids with supplemental fat but the extent of those changes was reduced when fat was fed with higher acid detergent fiber content. Drackley and Schingoethe (1986) observed the opposite trend for both acetate and propionate when feeding extruded sunflower seeds (9.5 % in the diet dry matter). They suggested that the high fiber content of the sunflower seed hull might explain this trend. Volatile fatty acid concentrations and relative proportions were not always modified when unsaturated fat was added to the diet (Knapp et al., 1991)

Changes in molar proportions of acetate and propionate, as a result of dietary lipid supplementation, can be due to an inhibition of both cellulolytic and methanogenic bacteria (Broudiscou et al., 1994). This is an indication that ruminal fiber digestion has

been negatively affected. Since acetate is one of the precursors for de novo synthesis of milk fatty acids in the mammary gland, milk fat composition and yield could potentially be affected when rumen fiber fermentation is modified.

2.4.2. Effects of Fat Supplementation on Ruminal pH

Maintenance of stable ruminal pH is important in order to maximize microbial growth. Several studies have shown that fat supplementation can increase ruminal pH (Broudiscou et al., 1994; Drackley and Schingoethe, 1986), while others showed no effect (Knapp et al., 1991; Pantoja et al., 1994). An increase in ruminal pH can be attributed to the decrease in total ruminal volatile fatty acid concentration.

2.4.3. Effects of Fat Supplementation on Ruminal Ammonia-Nitrogen

Addition of linseed oil to sheep diet tended to reduce ammonia-nitrogen concentration (Broudiscou et al., 1994). Those results are in agreement with the results obtained by Doreau et al. (1991) who found that supplementation of rapeseed oil to dairy cows decreased ammonia-nitrogen. Feeding extruded sunflower seeds also resulted in lower ammonia-nitrogen concentration (Drackley and Schingoethe, 1986). The reduction in ammonia-nitrogen has been attributed to the reduction in protozoal number and reduced recycling of bacterial nitrogen (Jenkins, 1993).

2.4.4. Effects of Fat Supplementation on Ruminal Microbial Population

Addition of linseed oil to sheep diet has been found to be detrimental to protozoal population (Broudiscou et al., 1994). Mohamed et al. (1988) found that feeding free oil of soybean or cottonseed as well as the whole oilseeds reduced protozoal numbers, however, feeding the same quantity of oil as roasted oilseeds did not affect the protozoal count. Emanuelson et al. (1991) found that feeding heat-treated and untreated full-fat rapeseeds (7% fatty acids in the diet dry matter) did not affect protozoal number over a control diet lower in fat (2.5 % fatty acids in the diet dry matter).

As mentioned, results obtained from fat supplementation on ruminal fermentation parameters, such as total volatile fatty acid production, pH, ammonia-nitrogen and

microbial population, have been highly variable. Devendra and Lewis (1974) have suggested four different mechanisms by which dietary polyunsaturated fatty acids could alter ruminal fermentation: (1) physical coating of the fiber by oil; (2) toxic effects modifying microbial population; (3) inhibition of microbial activity; (4) reduced cation availability due to the formation of insoluble soaps. Not only the degree of saturation determines if the supplemental fat will affect ruminal fermentation but also the quantity given and the rate at which the fat is released from the feed and exposed to rumen microorganisms. Feeding supplemental fat in the form of whole oilseeds has been suggested as a way to reduce the impacts of unsaturated fatty acids by reducing rate at which fatty acids are released from the feed and their exposure to rumen microbes (Knapp et al., 1991; Mohamed et al., 1988; NRC, 2001).

2.5. Effects of Fat Supplementation on Dry Matter Intake

Not only ruminal fermentation can be negatively affected by supplementing dairy cows with unsaturated fat, but also nutrient intake and digestibility. Reduced dry matter intake as a result of fat supplementation has been reported by several researchers (DeLuca and Jenkins, 2000; Pantoja et al., 1994). Various mechanisms by which unsaturated fat supplementation might reduce dry matter intake have been suggested. These include increased gut fill due to reduced fiber digestion, reduced gut motility, reduced acceptability of fat-containing diets, release of gut hormones, and increased intestinal absorption of fatty acids and liver oxidation of fatty acids (Allen, 2000).

In reviewing various studies, Allen (2000) found that fat supplementation in the form of oilseeds resulted in a quadratic drop on dry matter intake with the minimal effect found at approximately 2% of added fatty acids. The hypophagic effect of fat supplementation on dry matter intake increases with increased degree of unsaturation (Allen, 2000; Pantoja et al., 1994). It has been suggested that the addition of fat in the form of oilseeds to ruminant diets will have less detrimental effects on dry matter intake than if a similar amount was fed as free oil (Knapp et al., 1991; Mohamed et al., 1988; Kennelly, 1996). Dry matter intake by dairy cows was not affected by supplementing their diets with heated canola seeds at 5% added fat (Khorasani et al., 1994). However, Anderson et al.

(1984) found supplementing whole cottonseeds (10% of diet dry matter) and whole sunflower seeds (12% of the diet dry matter) decreased dry matter intake when compare with extruded soybeans (5% of the diet dry matter). Finn et al. (1985) also found that whole rolled sunflower seeds (9.7% of the diet dry matter) decreased dry matter intake.

2.6. Effects of Fat Supplementation on Nutrient Digestibility

Data on the effects of fat supplementation on nutrient digestibility are inconsistent. It seems that the nature of the supplemental fat, the form in which it fed as well as the quantity given might be important factors influencing the effect of fat supplementation on nutrient digestibility.

Doreau et al. (1993) showed that supplementation with lipid from rapeseeds (calcium salts or free oil) had no effect on ruminal and total tract digestion of neutral and acid detergent fiber. Results from Hussein et al. (1995) found that fat supplementation from crushed canola seeds (5% of the diet dry matter) did not adversely affect ruminal, post-ruminal or total tract digestibilities of neutral detergent fiber. However, total tract digestibility of organic matter was reduced. In contrast, feeding canola oil resulted in a reduction in total tract digestibility of neutral and acid detergent fiber (Ferlay and Doreau, 1992; Doreau, 1991). A shift in fiber digestion from the rumen to the hind-gut was observed as a result of lipid supplementation (Pantoja et al., 1994). This may help to explain the lack of adverse effects of fat supplementation on total tract digestibility reported in those studies even if ruminal degradation of fiber was reduced.

2.7. Impact of Polyunsaturated Fatty Acids on Milk Yield and Milk Composition

In the past years, milk and milk products have been criticized for their potential detrimental effects on human health. This perception can be partly attributed to the high content of short chain and saturated fatty acids as well as cholesterol in milk fat. Approximately 50% of the milk fatty acids are composed of 16 carbons or less (Table 2-4) where palmitic acid ($C_{16:0}$), recognized for its hypercholestrolaemic properties, is one of the most abundant fatty acid in milk (German et al., 1997). Fatty acids of less than 16 unit chain length and a portion of the C_{16} are synthesized de novo in the mammary gland

from acetate and β-hydroxybutyrate, while the reminder of C_{16} and those of more than 16-unit chain are derived from blood (Kennelly, 1996).

Table 2-4. Composition of the major fatty acids in milk fat

Fatty acid	Average range (weight percentage)
$C_{4:0}$	2-5
$C_{6:0}$	1-5
$C_{8:0}$	1-3
$C_{10:0}$	2-4
$C_{12:0}$	2-5
$C_{14:0}$	8-14
$C_{15:0}$	1-2
$C_{16:0}$	22-35
$C_{16:1}$	1-3
$C_{17:0}$	0.5-1.5
$C_{18:0}$	9-14
$C_{18:1}$	20-30
$C_{18:2}$	1-3
$C_{18:3}$	0.5-2

Adapted from Jensen (2002)

Based on the fact that about 50% of milk fatty acids are derived from blood plasma, it is easier to manipulate milk fatty acid profile than any other milk constituent. Moreover, the balance of de novo synthesis and uptake of blood fatty acids can also be altered by dietary manipulations (Kennelly, 1996). Providing supplemental oilseeds or other sources of long chain polyunsaturated fatty acids in the diet is an example of such dietary modifications. However, providing such fat sources to modify milk fatty acid composition is challenging due to the negative impacts that were described previously as well as the ones often observed on milk yield.

2.7.1. Effects of Fat Supplementation on Milk Production

Milk response to supplemental fat is inconsistent and varies considerably depending on factors such as the stage of lactation, diet composition, energy balance, type, and level of supplemental fat (NRC, 2001).

Providing supplemental fat normally aims at increasing the energy density of the ration and may result in an increased milk production. However, milk production does not always positively respond to fat supplementation. This is because fat supplementation may reduce dry matter intake, interfere with the digestion of other nutrients or may not be digested and absorbed as well as other nutrients (Coppock and Wilks, 1991). To eliminate the negative effects associated with fat supplementation, it is recommended to limit added fat level to 3 to 4% of the diet dry matter (NRC, 2001). However, fat supplements which are relatively inert within the rumen such as calcium salts of long chain fatty acids can be fed at rates above the recommended levels (Scott et al., 1995).

Cows fed supplemental fat in the form of extruded soybeans or extruded cottonseeds (12% of diet dry matter) consumed more dry matter and produced more fat-corrected milk than cows fed a control diet (Dhiman et al., 1999b). The addition of 1.7% fat from whole cottonseeds and ground canola seeds increased milk yield of dairy cows through the entire lactation by 17% with no adverse effect on dry matter intake (Johnson et al., 2002). However, a further addition of 1.6% fat from this oilseed mix had no effect on milk yield. Schingoethe et al. (1996) successfully increased milk yield when supplementing fat (6.1 % ether extract in the diet) as extruded soybeans and sunflower seeds to dairy cow with no adverse effect on dry matter intake. However the energy content of the two experimental diets (1.79 Mcal kg^{-1}) was higher than for the control diet (1.68 Mcal kg^{-1}). Improved milk yield was also reported by Knapp et al. (1991) with increasing levels of whole roasted soybeans (up to 24% of the diet dry matter). Similarly, dry matter intake was not modified by treatments, but in this case energy intake was not different between cows on control and experimental diets (Knapp et al., 1991). The authors reported that ruminal fermentation parameters were not affected by soybean

supplementation (Knapp et al., 1991). Fat supplementation in the form of heat-treated and untreated canola presscake had no effect on milk yield or dry matter intake of primiparous and multiparous cows (Jones et al., 2001). Other studies in which fat was supplemented in the form of rolled flaxseeds (Khorasani and Kennelly, 1994), micronized ground flaxseeds (Mustafa et al., 2003a) and heat-treated soybeans (Chouinard et al., 2001) also showed no adverse effect on milk yield. In other studies, ground canola seeds and whole flaxseeds resulted in reduced milk yields (Kennelly, 1996; Khorasani and Kennelly, 1994).

2.7.2. Effects of Fat Supplementation on Milk Composition

As mentioned, supplementation of oilseeds can result in either beneficial or detrimental effects on milk yields. Detrimental effects on milk composition have been observed often when supplemental fat was provided as whole seeds. Price received by dairy producers is based on the concentration of components in the milk, due to their high value for the dairy industry. Consequently, a reduction of milk protein and fat concentration and/or yield are not desired, but they have been regularly observed when supplemental oilseeds where given to dairy cows.

2.7.2.1. Effects of Fat Supplementation on Milk Protein

The response of protein concentration in milk in relation to oilseed supplementation varies considerably. Increase in milk protein content has been observed in some studies (Middaugh et al., 1988; Petit et al., 2001), while other studies showed no effect of fat supplementation on milk protein content (Dhiman et al., 2000; Mustafa et al., 2003a; Schingoethe et al., 1996). Anderson et al. (1984) found that feeding sunflower seeds increased milk protein concentration over whole cottonseeds and extruded soybeans; however the total protein yield was lower for sunflower seed diet due to reduced overall milk yield.

Milk protein percentage is often depressed as a result of dietary fat. This has been observed following full fat flaxseed (Kennelly, 1996), whole roasted soybean (Knapp et al., 1991) and sunflower seed supplementation (Finn et al., 1985). The reasons behind

this decline are not clear; however, Schingoethe (1996) suggested that there is a lag time in the appearance of the decline in milk protein meaning that this phenomenon could be undetected when experiments are conducted for short periods of time. Even with decreased in milk protein concentration, protein yield might not be reduced if proportional increase in milk yield are accomplished following lipid supplementation.

2.7.2.2. Effects of Fat Supplementation on Milk Fat

Protein is not the only milk constituent, which is negatively affected by supplemental dietary lipids. Milk fat percentage is often reduced when dairy cows are supplemented with dietary lipids. Providing dietary lipids, such as oilseeds, can result in profound changes on milk fat content and composition. Milk fat depression is regularly observed in dairy cows under high dietary intake of unsaturated fats (Ashes et al., 1997; Griinari et al., 1998; NRC, 2001). The extent to which milk fat content is reduced depends on many factors such as diet preparation, presence of other dietary components, frequency and level of feeding and individual animal. (Bauman and Griinari, 2001). Dietary supplementation of polyunsaturated fatty acids as free oil tends to depress milk fat percentage while feeding the same amount in the form of oilseeds tends to maintain or even increase milk fat content (Dhiman et al., 2000, Mohamed et al., 1988).

Cows fed micronized and raw ground flaxseeds (7% of diet dry matter) produced milk with a decreased milk fat percentage (Mustafa et al., 2003a) while feeding intact full fat flaxseeds (10% of diet dry matter) did not affect fat percentage (Kennelly, 1996). Feeding heat-treated and raw soybeans at 17.5% of the diet did not affect milk fat percentage (Chouinard et al., 2001). Milk fat depression was reported for cows fed sunflower seeds in a study conducted by Casper et al. (1988), which was not the case for McGuffey and Schingoethe (1981). Increased milk fat percentage was reported by Knapp et al. (1991) when cows were fed whole roasted soybeans (up to 24% of diet dry matter).

Milk fat depression is characterized by a reduction in fat percentage and fat yield. However, the most important reduction occurs in the fatty acids that are de novo synthesized in the mammary gland so that the proportion of long chain fatty acids

increases at the expense of short and medium chain ones (Bauman and Griinari, 2001; Chouinard et al., 1999a). Along with those changes, a substantial increase in trans-$C_{18:1}$ fatty acids is observed during milk fat depression. Different theories were proposed to elucidate the phenomenon of milk fat depression. Some researchers attributed the reduction in milk fat yield to a shortage of lipid precursors (acetate and β-hydroxybutyrate), used in the mammary gland for synthesis of milk fat, caused by a shift in ruminal volatile fatty acid pattern (Bauman and Griinari, 2001). However, others have suggested that inadequate supply of lipid precursors could not explain diet-induced milk fat depression (Bauman and Griinari, 2001). Another category of theories relates to direct inhibition of milk fat synthesis by the mammary gland. In a review on milk fat depression, Bauman and Griinari (2001) suggested that modification of regular ruminal microbial processes is a prerequisite for the development of milk fat depression. Those modifications in ruminal fermentation can be quite subtle and might be undetected. Modified microbial processes would alter the normal biohydrogenation processes resulting in the increased concentration of trans-$C_{18:1}$.

Milk fat depression would not be correlated to the total increase in trans-$C_{18:1}$ fatty acids, but to a specific increase in trans-10 $C_{18:1}$ that has been observed in several cases of milk fat depression (Figure 2-4; Bauman and Griinari, 2001). Bauman and Griinari (2001) also reported a linear relationship between the increases in trans-10 $C_{18:1}$ concentration and trans-10, cis-12 conjugated linoleic acid (CLA) concentration. Moreover, abomassal infusion of a commercial CLA preparation, which contains various isomers of CLA including trans-10, cis-12 $C_{18:2}$ caused a 50% reduction in milk fat yield (Chouinard et al., 1999b). Similar results were reported by Bell and Kennelly (2003) after infusing lactating dairy cows with different levels of synthetic CLA in the abomasum. Commercial mixtures of synthetic CLA contain a much higher concentration of trans-10, cis-12 CLA than CLA of ruminant origin, which is principally composed of the cis-9, trans-11 isomer. Baumgard et al. (2000) suggested that trans-10, cis-12 $C_{18:2}$ was the isomer responsible for milk fat depression after infusing in the abomasum supplements of pure cis-9, trans-11 and trans-10, cis-12 isomers. Only the trans-10, cis-12 resulted in reduction in milk fat percentage and yield.

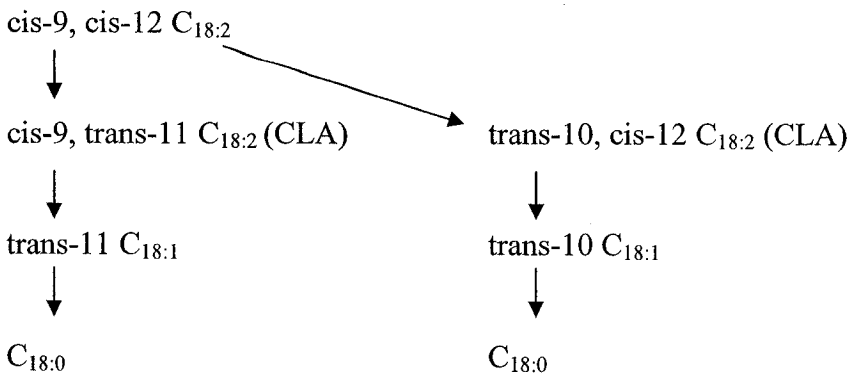


Figure 2-4. Pathway for the formation of trans-10 fatty acids from the biohydrogenation of linoleic acid (Bauman and Griinari, 2001)

2.7.2.3. Effects of Fat Supplementation on Milk Fatty Acids

Ashes et al. (1997) reported that the effect of dietary ingredients on milk fat composition is related to a number of interacting factors such as dietary fiber, concentrate-to-forage ratio, starch degradability, fatty acid composition of the diet, rumen inertness and digestibility of fat supplements. Providing oilseeds or other sources of polyunsaturated fatty acids protected from complete ruminal biohydrogenation generally results in a reduction of the proportions of short chain fatty acids and $C_{16:0}$ while the proportion of 18-carbon fatty acids increases (Chouinard et al., 2001; Kennelly, 1996; Mustafa et al, 2003a; Schingoethe et al., 1996). Reduction in the production of acetate and β-hydroxybutyrate in the rumen, dilution effect of long chain fatty acids or inhibition of de novo synthesis of biohydrogenation intermediates of long chain fatty acids have been proposed to explain the reduction in short chain fatty acid concentration in milk fat (Grummer, 1991).

2.8. Conjugated Linoleic Acid

Protecting polyunsaturated fatty acids from complete biohydrogenation has the potential to increase the concentration of intermediates of the biohydrogenation process in the milk. A lot of the recent researches have focus on increasing the concentration of conjugated linoleic acid (CLA) in milk by feeding supplemental oilseeds (Chouinard et al., 1997a; Dhiman et al., 1999b; Dhiman et al., 2000; Ward et al., 2002).

2.8.1. Health Benefits of Conjugated Linoleic Acid

In recent years, growing interests have been expressed in increasing the content of CLA in human food such as milk and milk products. This interest in CLA is related to its various health properties. CLA has been found to be a powerful anticarcinogen as well as having antiatherogenic, immunomodulating, growth promoting, lean body mass-enhancing and antidiabetic properties (Garnsworthy, 1997). However, these beneficial effects were obtained from researches in which synthetic mixtures of CLA were used. It seems that different isomers of CLA might possess different biological properties. The effects of CLA on body composition have been attributed to the trans-10, cis-12 isomer; the effects on feed/growth efficiency have been attributed to the cis-9, trans-11 isomer while the inhibition of carcinogenesis has been related to both isomers (Pariza et al., 2001). Increasing milk content of health promoting products such as CLA would be interesting in order to improve the nutritional value and to promote consumption of dairy products. However, due to the negative impacts of the trans-10, cis-12 isomer on milk fat secretion it would be impractical to increase the concentration of that particular isomer. Consequently, focus should be put on increasing milk concentration of the cis-9, trans-11 isomer.

2.8.2. Formation of Conjugated Linoleic Acid

"Conjugated linoleic acid refers to a group of positional and geometric isomers of linoleic acid (cis-9, cis-12-octadecadienoic acid) for which the double bonds have a conjugated arrangement instead of methylene interruption" (Parodi, 1999). CLA is predominantly of ruminant origin; however, it can also be synthetically produced in laboratory. In ruminant animals, there are two routes through which CLA can be produced. The first one is through ruminal biohydrogenation of linoleic acid of which process CLA is an intermediate product (Figure 2-1). CLA escaping complete ruminal biohydrogenation can be absorbed by the mammary gland and then be secreted in the milk. The second and major route of CLA formation is by desaturation of trans $C_{18:1}$ such as trans-11 $C_{18:1}$, which is an intermediate product of ruminal biohydrogenation of linoleic and linolenic acids (Harfoot and Hazelwood, 1997). Trans-11 $C_{18:1}$ is transformed into CLA by the enzyme delta-9-desaturase located within mammalian cells (Jensen, 2002).

The CLA from ruminant origin contains predominantly cis-9, trans-11 isomer, while CLA of synthetic origin is characterized by high concentration of trans-10, cis-12 isomer as well as the cis-9, trans-11 isomer with smaller quantities of other isomers (Pariza et al., 2001). Thus feeding rations rich in linoleic acid to dairy cows could provide a way to increase CLA content of milk. However, the dietary source of linoleic acid needs to be protected from complete ruminal biohydrogenation to permit ruminal escape of intermediate products such as CLA and trans-11 $C_{18:1}$.

2.8.3. Concentration of Conjugated Linoleic Acid in Milk

Normal CLA content was reported to be between 0.3 and 0.6% of total milk fatty acids (Kelly et al., 1998). However, several factors are known to modify the CLA content of dairy cows milk. Variation between individual cows is one of them (Kelly et al., 1998; Lawless et al., 1998). Peterson et al. (2002) observed two- to three-fold variation in the concentration of CLA in the milk of cows within the same treatment groups. They also observed that the hierarchy in CLA concentrations was maintained even when cows were switch back and forth between experimental diets (Peterson et al., 2002).

Another factor influencing the CLA concentration is the diet (table 2-5). Pasture intake (Dhiman et al., 1999a; Kelly et al., 1998), forage to concentrate ratio (Dhiman et al., 1999a) and intake of different types of dietary fats have been shown to induce modification in the milk CLA content (Chouinard et al., 2001; Dhiman et al., 2000; Ward et al., 2002). Dietary fat rich in polyunsaturated fatty acids, such as linoleic and linolenic acids, have potential to increase milk CLA content. Feeding animal fat by-products, fish oil, calcium salts of fatty acids of different oilseeds, high oil corn hybrid silage and oilseeds at different levels have resulted in increased CLA content to different extent (Chouinard et al., 2001). Chouinard et al. (1997a) treated soybeans with extrusion, micronization and roasting and included the different treatments at 17.5% of the diet dry matter. The heat treatments resulted in a two- to threefold increase in CLA content when compared with untreated soybeans, with the greatest increase observed with the extrusion treatment (Chouinard et al., 2001). Similar results were also reported by Dhiman et al. (2000).

Table 2-5. Dietary factors affecting the conjugated linoleic concentration (CLA) in milk

Dietary factors	Effects on milk CLA content
Lipid Substrate	
Unsaturated vs. Saturated Fat	Increased by unsaturated fat
Type of plant oils	Increased with oils rich in PUFA[1]
Level of plant oils	Dose-dependent increase
Ca salts of plant oils	Increased
High-oil plant seeds	
Raw seeds	No effect
Processed seeds	Increased
Animal fat by-products	Minimal effect
High-oil corn grain and silage	Minimal effect
Modifiers of rumen environment	
Forage:concentrate ratio	Variable effect
Nonstructural carbohydrate level	Minor effect
Fish oil/fish meal	Increased
Ionophores	Variable effect
Dietary buffers	Little effect with sufficient fiber
Forages	
Pasture	Higher than on conserved forages
Growth stage of forage	Increased with less mature forages
CLA supplement	Dose-dependent increased

[1] Polyunsaturated fatty acids.

Adapted from Jensen (2002).

In an experiment conducted by Ward et al. (2002), feeding solin, canola and flax at 8.32% of the diet dry matter did not increase the CLA content above the control diet, which resulted in a milk CLA content of 1.4% of total fatty acids. This value is however higher than the normal range reported by Kelly et al. (1998) even if the linoleic and linolenic content of the control diet was lower than for the experimental diets containing oilseeds (Ward et al., 2002).

3. Effect of Roasting on Chemical Composition and Ruminal Degradability of Sunflower Seeds[1]

3.1 Abstract

This study was conducted to determine the effects of roasting sunflower seeds using a particulate medium thermal processor (salt heated at 250° C, salt:seed 4:1, 60 second contact between salt and seed) on chemical composition as well as ruminal degradability of the dry matter and crude protein fractions of sunflower seeds. Two ruminally fistulated cows were used in a randomized complete block design. Roasting of sunflower seeds increased ($P < 0.05$) neutral detergent insoluble protein content with little effect on the acid detergent insoluble protein content of sunflower seeds. However, roasting decreased ($P< 0.05$) lysine concentration of sunflower seeds. Results of the *in situ* nylon bag study showed that roasting decreased ($P < 0.05$) ruminal degradability of dry matter and crude protein and increased ruminal undegraded crude protein of sunflower seeds. It was concluded that roasting of sunflower seeds decrease degradability of crude protein and dry matter of sunflower seeds without negatively affecting the chemical composition of sunflower seeds except for the lysine concentration.

3.2 Introduction

Sunflower (*Helianthus annuus* L) is an important oilseed crop in western Canada particularly in Manitoba, which produces more than 89% of the total Canadian production followed by the Saskatchewan (Statistics Canada, 2001). The oil-type sunflower seeds are characterized by a black hull and a high oil content, which is recognized for its high linoleic acid concentration (Mustafa et al., 2003b). On a dry matter basis, oil-type sunflower seeds contain 40 to 45% ether extract, 18 to 20% crude protein and 32 to 36% acid detergent fiber (McGuffey and Schingoethe, 1981). Based on the most abundant fatty acid, oil-type sunflower seeds can be classified into linoleic and oleic type (McGuffey and Schingoethe, 1981). As with other oilseeds, sunflower seeds can be used as a source of protein and energy for ruminants (Finn et al., 1985; Schingoethe et al., 1996). However, oilseeds are considered a poor source of ruminal undegraded protein

[1]Results published (P. Sarrazin, A.F. Mustafa, P.Y. Chouinard, G.S.V. Raghavan and S.A. Sotocinal. 2003. Effects of roasting on ruminal nutrient degradability of sunflower seed. J. Sci. Food Agric. 83:1219-1224). Reproduced with permission. Permission is granted by John Wiley & Sons Ltd on behalf of the Society of Chemical Industry.

due to their high ruminal degradability (Deacon et al., 1988; Mustafa et al., 2002; Mustafa et al., 2003b). Heat treatment is commonly used to protect the protein of oilseeds and oilseed meals from ruminal degradation and therefore increase the concentration of amino acids available for digestion in the small intestine (NRC, 2001).

The heat treatment known as particulate medium thermal processing (roasting) involves direct heating of a medium and the transfer of heat (via conduction) between the pre-heated medium and a test material (Raghavan et al., 1974). Sotocinal (1997) described a particulate medium thermal processor in which roasting is achieved in a sequence of five steps. The first step is the heating of the granular medium such as salt or sand. The medium, once at the desired initial temperature, is then mixed with the material to be processed in a set medium-to-material ratio. After a specified contact time between the material and the medium, the two are separated. The separated medium is then reheated and recycled back into the process while the processed material is cooled down to room temperature. During the cooling process, further heating of the material is achieved due to the large temperature gradient between processed material and ambient temperature. A major advantage of particulate medium heating over other methods of heat treatment arises from the much higher heat capacity of solids than that of air. Comparative studies showed that particulate medium heating is five times faster and 28% more energy efficient than convective heating (Sotocinal, 1997).

An earlier study conducted by Raghavan et al. (1974) showed that particulate medium roasting (heating to 272° C, salt as medium, 20 second contact between salt and soybeans, and 120 second holding time) can be used effectively to improve the feeding value of soybeans for broiler chicks. The effects of particulate medium roasting on the feeding value of other oilseeds have not been determined. Therefore, the objectives of this study were to determine the effects of particulate medium thermal processing on the chemical composition and the ruminal degradability of sunflower seeds.

3.3. Materials and Methods

3.3.1. Roasting

High-oil sunflower seeds were obtained from a local producer (Ontario, Canada). Heating of the seeds was performed using a particulate medium thermal processor, described in details by Sotocinal (1997). The major components of the particulate medium thermal processor were a medium heating unit, a medium and grain mixer, a separation unit and a cooling / aeration unit. The heating unit was made of a horizontal cylinder (0.3 m diameter and 3.0 m long), which contained the medium (salt) while it made contact with a flame from a propane burner. The roasting process consisted of heating salt to 250° C in the heating unit followed by mixing the salt with the seeds (4:1 ratio). The salt and seeds were put in contact for 60 seconds in the medium and grain mixer unit. Heated sunflower seeds and medium were passed through the separation screen and the separated medium was then recycled back into the heater. The average outlet temperature of the sunflower seeds was 155° C. The roasting process was repeated four times and representative samples (500 g) of raw and roasted sunflower seeds were collected during each time for later analysis.

3.3.2. Treatments and Animals

The *in situ* incubation study was conducted at the Macdonald Campus Farm of McGill University located at Ste-Anne-de-Bellevue, Quebec. Two lactating Holstein cows (732 ± 33 kg) fitted with flexible ruminal cannulas were used during this trial. The cows were fed ad libitum 50:50 forage:concentrate total mixed rations. The total mixed rations contained (dry matter basis) 190 g kg^{-1} crude protein, 285 g kg^{-1} neutral detergent fiber, 191 g kg^{-1} acid detergent fiber and 30 g kg^{-1} ether extract.

Sub-samples of raw and roasted sunflower seeds were ground through a 2-mm screen with a Thomas-Wiley Laboratory Mill (Thomas Scientific, USA) and equal portions (200 g) of the four replicates of each seed meal were pooled to obtain a single batch for each treatment. Approximately 7 g (air dry basis) of each treatment were weighted into nylon bags (10 x 20 cm, ANKOM Technology, Fairport, NY, USA) and incubated in the rumen

(two bags per cow per incubation time) for 2, 4, 8, 12, 24 and 48 hours. At the end of the incubations, bags were removed from each cow and washed under cold tap water until the rinse water was clear. Zero time disappearances were measured by washing duplicate bags containing samples of the two treatments under cold tap water. The washed bags were then dried in a forced air oven at 55° C for 48 hours.

3.3.3. Sample Analysis and Calculations

Sub-samples of raw and roasted sunflower seeds were ground through a 1-mm screen using a Thomas-Wiley Laboratory Mill (Thomas Scientific, USA) and defatted according to the procedure of the Association of Official Analytical Chemists (method no. 920.39; AOAC, 1990).

Defatted samples were analyzed for dry matter (DM, method no. 930.15, AOAC, 1990) and ash (method no. 924.05; AOAC, 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using the Ankom System (Ankom^{200} Fiber Analyzer and F57 filter bags, Ankom Technology, Fairport, NY, USA) with heat stable alpha-amylase and without sodium sulfate. Crude protein (CP, N x 6.25) was determined using a LECO Nitrogen System FP-428 (LECO Corp., St-Joseph, MI, USA). Neutral (NDICP) and acid (ADICP) detergent insoluble crude protein were determined by analyzing NDF and ADF residues, respectively for CP.

Samples of raw and roasted sunflower seeds were also analyzed for amino acids (AA) following oxidation in perfomic acid (16 hours) and hydrolysis in 6 N HCL (24 hours) (AOAC, 1984). The oxidation step was not used for phenylalanine and histidine. All AA were determined using a Brinkmann (System 6300) High Performance Analyzer.

Samples of raw and roasted sunflower seeds were analyzed for fatty acid composition. Fat was extracted and converted to methyl esters following the procedure of Sukhija and Palmquist (1988). Fatty acid concentrations were then determined using a gas chromatograph (HP 5890 Hewlett Packard Co.) equipped with 60-m x 0.32-mm capillary column as described by Chouinard et al. (1997b). The carrier gas was He and the

temperature of the flame ionization detector was maintained at 250° C. The initial oven temperature was 150° C and increased 5° C minute^{-1} up to 200° C.

Residues from the nylon bags were analyzed for DM and CP as previously described. Ruminal DM and CP disappearance was calculated from the concentration of each nutrient in the residues and the original samples. Ruminal nutrient disappearance data were then used to estimate ruminal DM and CP degradation parameters using the equation of Dhanoa (1988):

$$\mathbf{p = a + b(1-e^{-c(t-L)})}$$

where **p** (g kg^{-1}) is ruminal disappearance at time **t** (hour), **a** is the soluble fraction (g kg^{-1}), **b** is the slowly degradable fraction (g kg^{-1}), and **c** is the rate at which the **b** fraction is degraded (% hour^{-1}), and **L** is the lag phase (hour). Ruminal effective degradability (ED) of DM and CP was estimated using the equation of Ørskov and McDonald (1979):

$$\mathbf{ED = a + bc\ (c + k)^{-1}}$$

where **k** is the rumen flow rate and is assumed to be 5 % hour^{-1}.

3.3.4. Statistical Analysis

Data of the chemical composition of sunflower seeds were analyzed as a completely randomized design (two treatments and four replicates) using the General Linear Model of the SAS Institute, Inc. (1999). Where appropriate, treatment means were separated using the Scheffe Grouping. Data of the *in situ* nylon bag study were analyzed as a randomized complete block design, using cows as blocks, using the General Linear Model of the SAS Institute, Inc. (1999). Where appropriate, treatment means were separated using the Scheffe Grouping.

3.4. Results and Discussion

3.4.1. Effects of Roasting on Chemical Composition of Sunflower Seeds

The chemical composition of sunflower seeds (Table 3-1) is similar to that reported by other researchers (Casper et al., 1988; Mustafa et al., 2003b; NRC, 2001). Acid and neutral detergent fiber, crude protein and ether extract were not affected by heating. Roasting increased ($P < 0.05$) the neutral detergent insoluble crude protein content of sunflower seeds with no effect on acid detergent insoluble crude protein (Table 3-1). These results suggest that the heat treatment applied was not severe enough to damage the sunflower seed protein. Other researchers have also found that moderate heat treatment increased neutral detergent insoluble crude protein without increasing acid detergent insoluble crude protein (McKinnon et al., 1995; Mustafa et al., 1999; Mustafa et al., 2002). Similar results were observed following autoclaving of sunflower seeds (Mustafa et al., 2003b). Ash content of roasted sunflower seeds was higher ($P < 0.05$) than that of raw sunflower seeds. Burning of organic matter during the roasting process might explain such results.

The most abundant amino acid in sunflower seed was glutamic acid, followed by aspartate and arginine (Table 3-2). The amino acid concentrations of raw sunflower seeds in this study were similar to the values reported in the literature (Canibe et al., 1999; Mustafa et al., 2003b). Amino acid composition of sunflower seeds was not affected by roasting with the exception of lysine concentration, which was reduced ($P < 0.05$) following the heat treatment (Table 3-2). Other studies have also reported similar effects of heat treatment on lysine concentration of oilseeds and oilseed meals (Demjanec et al., 1995; Mustafa et al., 1999). Demjanec et al. (1995) attributed the reduction in lysine concentration to the formation of Maillard products during the heating process, which makes lysine more resistant to acid hydrolysis during amino acid analysis.

Table 3-1. Effects of roasting on chemical composition of sunflower seeds (SFS; dry matter basis)

	Treatments		SEM[1]
	Raw SFS	Roasted SFS	
Ash (g kg $^{-1}$)	33.7[a]	36.0[b]	0.32
Ether extract (g kg $^{-1}$)	443.4	441.7	9.02
Neutral detergent fiber (g kg $^{-1}$)	274.8	297.5	12.76
Acid detergent fiber (g kg $^{-1}$)	191.4	188.2	7.79
Crude protein (g kg $^{-1}$)	189.2	190.0	2.46
NDICP[2] (g kg $^{-1}$of CP)	146.1[a]	294.7[b]	16.99
ADICP[3] (g kg $^{-1}$of CP)	61.5	83.0	7.51

[ab]Least Square means within the same row followed by different superscripts differ ($P < 0.05$).
[1]Standard error of the mean.
[2]Neutral detergent insoluble crude protein.
[3]Acid detergent insoluble crude protein.

Table 3-2. Effects of roasting on amino acid composition of sunflower seeds (SFS; g kg^{-1} of crude protein)

	Treatments		SEM[1]
	Raw SFS	Roasted SFS	
Essential			
Arginine	70.5	65.6	3.36
Histidine	19.0	17.5	0.76
Isoleucine	27.9	29.6	2.73
Leucine	53.2	55.6	2.05
Lysine	28.4[a]	21.7[b]	1.21
Phenylalanine	37.5	36.0	2.29
Threonine	32.1	30.7	1.03
Valine	35.0	34.8	1.00
Non-essential			
Alanine	38.7	44.0	2.08
Aspartatic acid	79.0	79.8	2.58
Glutamic acid	186.2	185.2	5.02
Glycine	52.9	50.2	2.18
Proline	35.6	33.0	0.97
Serine	36.4	37.0	1.19
Tyrosine	16.9	18.8	1.89

[ab] Least Square means within the same row followed by different superscripts differ ($P < 0.05$).
[1]Standard error of the mean.

Sunflower seeds contain high level of linoleic acid (Table 3-3). The fatty acid composition of sunflower seeds in this study is similar to that reported in other studies (Casper et al., 1988; Mustafa et al., 2003b) and was not altered by heat treatment. Moist heat treatment of sunflower seeds (autoclaving at 127° C up to 30 minutes) did not affect fatty acid profile (Mustafa et al., 2003b). Similar results were also reported for other heat-treated oilseeds (Emanuelson et al., 1991; Mohamed et al., 1988).

3.4.2. Effects of Roasting on *In Situ* Ruminal Degradability of Sunflower Seeds

The values of ruminal degradability of dry matter and crude protein of raw sunflower seeds in this study indicate that raw sunflower seeds are highly degradable in the rumen (Table 3-4). The high ruminal degradability of dry matter and crude protein can be attributed to the high *in situ* soluble fractions of dry matter and crude protein. Similar results have also been reported for other oilseeds such as rapeseed, flaxseed and canola seed (Emanuelson et al., 1991; Mustafa et al., 2002; Wang et al., 1997). Roasting reduced ($P < 0.05$) the *in situ* soluble and increased ($P < 0.05$) the *in situ* degradable dry matter and protein fractions of sunflower seed (Table 3-4). Degradation rate and effective degradability of sunflower seed dry matter and crude protein were also reduced ($P < 0.05$) by roasting. Similar results of heat treatment on ruminal kinetic parameters and ruminal degradability of oilseeds have been reported by several researchers (Deacon et al., 1988; Emanuelson et al., 1991; Mustafa et al., 2002; Mustafa et al., 2003b; Wang et al., 1997).

Ruminal undegraded protein (RUP; following 12 hours of incubation) value of raw sunflower seeds was low (78 g kg^{-1} of CP), indicating the high susceptibility of sunflower seed protein to ruminal degradation (Table 3-4). The RUP value of raw sunflower seeds obtained in this experiment is in accordance with that reported by the NRC (2001) and Mustafa et al. (2003b). Roasting increased ($P < 0.05$) RUP value of sunflower seed by 180%. This can be attributed to reduction in the soluble fraction, the degradation rate and the effective ruminal degradability of crude protein caused by roasting. Other researchers have also reported higher RUP values for heat-treated oilseeds than for unheated ones (Deacon et al., 1988; Mustafa et al., 2003b; Wang et al., 1997).

Table 3-3. Effects of roasting on fatty acid composition of sunflower seeds (SFS; g kg^{-1} of fatty acids)

	Treatments		SEM[1]
	Raw SFS	Roasted SFS	
$C_{16:0}$	57.9	57.6	0.48
$C_{18:0}$	43.2	42.4	0.49
$C_{18:1}$	171.1	173.0	3.88
$C_{18:2}$	726.9	726.1	3.69
$C_{18:3}$	0.9	1.0	0.10

[1]Standard error of the mean.

Table 3-4. Effects of roasting on ruminal degradability of sunflower seeds (SFS) dry matter and crude protein

	Treatments		SEM[1]
	Raw SFS	Roasted SFS	
Dry matter (DM)			
Soluble fraction (g kg^{-1})	456.1[a]	398.0[b]	8.81
Degradable fraction (g kg^{-1})	317.5[a]	385.3[b]	12.21
Degradation rate (%$hour^{-1}$)	12.7[a]	6.9[b]	0.54
Lag time (hour)	0.5[a]	2.2[b]	0.03
Effective degradability[2] (g kg^{-1})	683.2[a]	620.7[b]	4.85
Crude protein (CP)			
Soluble fraction (g kg^{-1})	576.6[a]	472.7[b]	23.01
Degradable fraction (g kg^{-1})	387.5[a]	482.8[b]	21.19
Degradation rate (%$hour^{-1}$)	15.5[a]	7.5[b]	0.62
Lag time (hour)	0.4	2.5	0.63
Effective degradability[2] (g kg^{-1})	869.1[a]	761.8[b]	11.13
RUP (g kg^{-1} of CP)[3]	77.7[a]	217.6[b]	7.37

[ab]Least Square means within the same row followed by different letters differ ($P < 0.05$).
[1]Standard error of the mean.
[2]Calculated assuming a ruminal outflow rate of 5% $hour^{-1}$.
[3]Ruminal undegraded protein determined following 12 hour of ruminal incubation.

3.5. Conclusions

Results of this study indicated that roasting, using salt as a particulate medium, did not affect the chemical composition of sunflower seeds except for the neutral detergent insoluble protein level that was higher and the lysine concentration that was lower for roasted than for raw sunflower seeds. Roasting reduced ruminal degradability and increased RUP value of sunflower seeds. The reduction in ruminal degradability of roasted sunflower seeds is likely due to a reduction in the size of the soluble fraction as well as a reduction of the ruminal degradation rate.

4. Effects of Feeding Raw and Roasted Sunflower Seeds on Ruminal Fermentation and Total Tract Nutrient Utilization

4.1. Abstract

Three multiparous ruminally cannulated lactating Holstein cows (182 ± 35 days post partum and 750 ± 50 kg body weight) were used in a 3 x 3 Latin square experiment to determine the effects of supplementing dairy cows with raw and roasted sunflower seeds on ruminal fermentation parameters and total tract nutrient utilization. Diets were fed as total mixed rations and consisted of a control diet with no added sunflower seed, a raw sunflower seed diet and a roasted sunflower seed diet. Sunflower seed diets contained 7.8% (dry matter basis) raw or roasted sunflower seeds. The ether extract content was 3.0% (dry matter basis) for the control diet and 5.8 % (dry matter basis) for both the raw and roasted sunflower seed diets. Results showed that dietary treatments had no effect on ruminal pH, ammonia nitrogen and total volatile fatty acid concentrations. Minimal effects were also observed on ruminal concentrations of acetate, propionate and butyrate. Dry matter intake tended (P = 0.07) to be lower for cows fed the sunflower diets than for those fed the control diet. Total tract digestibilities of dry matter, organic matter, neutral and acid detergent fiber, crude protein, starch and gross energy were similar among dietary treatments. Total tract digestibility of ether extract was higher for the cows fed the sunflower seed diets than the control diet. It was concluded that inclusion of raw and roasted sunflower seeds up to 7.8% of the diet dry matter has no adverse effects on ruminal fermentation or total tract nutrient utilization of dairy cows.

4.2. Introduction

Providing supplemental fat in rations of dairy cows typically aims at increasing the energy density of the ration. This is particularly interesting for early lactation cows since energy intake is often limited by dry matter intake at this stage. However, providing supplemental lipids, particularly those rich in polyunsaturated fatty acids, can have detrimental effects on ruminal fermentation, dry matter intake and total tract nutrient utilization.

The extent to which supplemental fat will negatively affect ruminal fermentation depends on numerous factors such as the degree of saturation, chain length of fatty acids, form in which the fat is fed (free oil, whole seed, salts of oil, etc.), level of fat supplementation, basal ration and forage to concentrate ratio (Ferlay and Doreau, 1992). Reduction in total volatile fatty acid concentration, reduction in acetate to propionate ratio, increase in ruminal pH are all potential consequences of lipid supplementation that have been regularly observed (Broudiscou et al., 1994; Casper et al., 1988; Doreau et al., 1991). Those changes reflect a reduction in ruminal fiber fermentation. In some cases, even if ruminal digestion of fiber is reduced, total tract digestion of fiber is not affected due to a shift of fiber digestion toward the hind gut (Pantoja et al., 1994). Devendra and Lewis (1974) have suggested four different mechanisms by which dietary polyunsaturated fatty acids could alter ruminal fermentation: 1) physical coating of the fiber by oil, 2) toxic effects modifying microbial population, 3) inhibition of microbial activity and 4) reduced cation availability due to the formation of insoluble soaps.

Reduced dry matter intake has been reported in several studies where fat was fed to dairy cows (DeLuca and Jenkins, 2000; Pantoja et al., 1994). Reduced fiber digestion leading to increased gut fill is one potential mechanism that has been suggested to explain the hypophagic effect of lipid supplementation along with reduced gut motility, acceptability of lipid supplemented diets, release of gut hormones, and increased intestinal absorption of fatty acids and liver oxidation of fatty acids (Allen, 2000).

Feeding supplemental fat in the form of whole oilseeds might help to alleviate the impact of unsaturated fatty acids by reducing the rate at which fatty acids are released in the rumen (Knapp et al., 1991; NRC, 2001). Furthermore, Kennelly (1996) has suggested that heat treatment of oilseeds could protect dietary lipid in oilseeds and thus could reduce its exposure to rumen microbes by the denaturation of the protein matrix surrounding the fat droplets.

The objectives of this study were to determine the effects of feeding raw and roasted whole sunflower seeds to dairy cows on various ruminal fermentation parameters, on dry matter intake and on total tract nutrient utilization.

4.3. Materials and Methods

4.3.1. Treatments and Animals

The study was conducted at the Macdonald Campus Farm of McGill University located at Ste-Anne-de-Bellevue, Quebec. Three multiparous lactating Holstein cows fitted with flexible ruminal cannulae (182 ± 35 days post partum and 750 ± 50 kg BW) were used in a 3 x 3 Latin square experiment with three 21-day periods. The first 10 days were used for diet adaptation and the last 11 days for data collection. The animals were housed in tie stalls and had continuous access to water.

Diets were fed twice daily (8:00 AM and 4:00 PM) as total mixed rations (Tables 4-1 and 4-2) and consisted of a control diet with no added sunflower seed, a raw sunflower seed diet and a roasted sunflower seed diet. Diets were formulated to be isonitrogenous and similar in neutral detergent fiber content. During the first 15 days, cows were fed ad libitum twice daily to allow for 5 to 10% refusals. Cows were restricted to 90% of their voluntary intake from day 16 to 21 to ensure complete consumption. Chromic oxide (10 g twice daily) was used as an indigestible marker and was administrated intra-ruminally from day 12 to 21.

Table 4-1. Ingredients and chemical composition of diets used in the ruminal fermentation and total tract nutrient utilization study (dry matter basis)

	Treatments		
	Control	Raw SFS[1]	Roasted SFS[1]
Ingredients (g kg^{-1})			
Dry hay	72.3	73.1	73.1
Alfalfa silage	184.8	186.8	186.8
Corn silage	229.2	231.6	231.6
High moisture corn	289.1	292.2	292.2
Cracked corn	57.5	0.0	0.0
Soybean meal	61.6	56.5	56.5
Commercial concentrate 1[2]	11.5	41.5	41.5
Commercial concentrate 2[3]	84.6	31.1	31.1
Mineral supplement [4]	9.5	9.1	9.1
Sunflower seed	0.00	78.0	78.0
Chemical composition (g kg^{-1})			
Ash	71.2	65.6	67.1
Ether extract	30.1	57.9	57.5
Neutral detergent fiber	284.5	324.8	322.2
Acid detergent fiber	190.6	215.4	217.2
Lignin	29.1	32.9	31.5
Crude protein (CP)	190.3	188.1	189.9
NDICP (g kg^{-1}of CP)[5]	158.4	170.6	170.3
ADICP (g kg^{-1}of CP)[5]	55.2	55.6	59.0
Starch	206.4	152.3	165.2
Calcium	9.3	8.5	9.1
Phosphorus	5.5	5.0	5.2
Net energy of lactation (Mcal kg^{-1})[6]	1.70	1.76	1.77

[1]Sunflower seeds.
[2] Contained: 500 g crude protein, 40 g crude fat, 50 g crude fiber, 2.5 g Na, 25 g Ca, 15 g P, 8.0 g Mg, 5.0 g S, 5.0 g K, 7.4 mg I, 700 mg Fe, 125 mg Cu, 370 mg Mn, 370 mg Zn, 1.2 mg Co, 50 mg F, 160 mg vitamin E, 39,500 IU vitamin A, 11,850 IU vitamin D_3 kg^{-1}.
[3] Contained: 385 g crude protein, 20 g crude fat, 100 g crude fiber, 14 g Na, 34.5 g Ca, 22 g P, 15 g Mg, 5.0 g S, 11 g K, 11.5 mg I, 1,400 mg Fe, 200 mg Cu, 600 mg Mn, 600 mg Zn, 2 mg Co, 50 mg F, 230 IU vitamin E, 57,600 IU, vitamin A, 17,300 IU vitamin D_3 kg^{-1}.
[4] Contained: 100 g Ca, 100 g P, 78 g Na, 80 g Mg, 30 g S, 45 mg I, 3,600 mg Fe, 740 mg Cu, 2,300 mg Mn, 2,300 mg Zn, 10 mg Co, 500 mg F, 300,000 IU vitamin A, 90,000 IU vitamin D_3, 800 IU vitamin E kg^{-1}.
[5] NDICP: neutral detergent insoluble crude protein, ADICP: acid detergent insoluble crude protein.
[6] Calculated according to Weiss et al. (1992).

Table 4-2. Fatty acid composition of the diets used in the ruminal fermentation and total tract nutrient utilization study (g kg^{-1} of fatty acids)

	Treatments		
	Control	Raw SFS[1]	Roasted SFS[1]
$C_{16:0}$	184.1	117.4	121.0
$C_{16:1}$	6.2	3.8	3.9
$C_{18:0}$	33.2	43.0	43.1
$C_{18:1}$	252.0	197.6	204.8
$C_{18:2}$	436.5	589.6	581.0
$C_{18:3}$	88.0	48.6	46.1

[1]Sunflower seeds.

4.3.2. Sample Collection

Ruminal fluid samples were collected with a syringe equipped with a filtering device (ANKOM Technology, Fairport, NY, USA) on day 11 before the morning feeding and every 2 hours thereafter until 4:00 PM. The pH was immediately measured using a Metrohm Herisau E 300 B pH meter. A 20 ml aliquot of ruminal fluid was dispensed in a polyethylene snap cap container, acidified with 25% metaphosphoric acid and was frozen until volatile fatty acid analysis was performed. An additional 20 ml aliquot was acidified with 0.1 N hydrochloric acid, capped and was frozen until ammonia nitrogen analysis was performed. Grabbed fecal samples were collected four times a day during the last three days of each period. Samples were dried at 55° C in a forced-air oven for 48 hours and were then pooled by cow within each period. Feed samples were collected during the voluntary intake and on the days of ruminal fluid and feces collection. Samples were then dried at 55° C in a forced-air oven for 48 hours and then pooled by treatment within each period.

4.3.3. Sample Analysis and Calculations

Ruminal fluid samples acidified with 0.1 N hydrochloric acid were thawed and centrifuged at 12,000 x g for 10 minutes. The supernatant was removed and diluted (1:50). The ammonium of the solution was heated with salicylate and hypochlorite in an alkaline phosphate buffer. The green color was measured colorimetrically at 660 nm on a flow injection instrument (Lachat Instrument. QuickChem Method 12-107-06-2A. Lachat Instruments, 6645 West Mill Road, Milwaukee, WI 53218 USA).

Ruminal fluid samples acidified with 25% metaphosphoric acid were thawed and centrifuged for 15 min at 10,000 x g. Isocaproic acid was added as an internal standard and samples were analyzed for acetic, propionic, butyric, iso-butyric and valeric acids by gas chromatography (Varian model 3400; Varian Canada Inc., Ville St-Laurent, Quebec, Canada) equipped with a 30-m capillary column (Stabilwax-DA, 0.53 mm ID; Restek Corporation, Bellefonte, PA). Initial column temperature was set at 80° C for 30 second, then temperature was increased at the rate of 15° C $minute^{-1}$ until it reached 180° C; this temperature was maintained for 1 minute. Therefore, run time was 8.16 minute. Injector

and detector temperatures were 250 and 300° C, respectively. Gas flows were 30, 300 and 30 ml minute^{-1} for He, air and H_2, respectively. Volume of sample injected was 0.4 µl.

Fecal samples were ground through a 1 mm-screen using a Thomas-Wiley Laboratory Mill (Thomas Scientific, USA) and analyzed for dry matter (DM; method no. 934.01); ash (method no. 924.05) and ether extract (EE; method no. 920.39) according to the Association of the Official Analytic Chemists (AOAC, 1991). Crude protein (CP; N x 6.25) was determined using a LECO Nitrogen System FP-428 (LECO Corp. St-Joseph, MI, USA). Analysis of neutral (NDF) and acid (ADF) detergent fibers were determined according to the procedure of Van Soest (1991) using the Ankom System with heat stable alpha-amylase and without sodium sulfate (Ankom^{200} Fiber Analyzer and F57 filter bags Ankom Technology, Fairport, NY, USA). Starch was determined as described by McCleary et al. (1997). Gross energy (GE) was determined with an adiabatic calorimeter (Parr Instrument Company, Moline, Illinois, USA). Fecal samples were also analyzed for chromic oxide as described by Fenton and Fenton (1979).

Feed samples were analyzed for DM, ash, CP, NDF, ADF, EE, starch and GE as described above. Feed samples were also analyzed for lignin using the Ankom System (Ankom^{200} Fiber Analyzer and F57 filter bags Ankom Technology, Fairport, NY, USA). Neutral (NDICP) and acid (ADICP) detergent insoluble crude protein were determined by analyzing NDF and ADF residues, respectively for CP. Calcium concentration was determined following digestion with perchloric-nitric acid mixture (method no. 915.13 AOAC, 1990) using Perkin-Elmer Model 5000 atomic absorption spectrophotometer (Technicon GTPC auto analyzer II). Phosphorus concentration was determined colorimetrically (Pharmacia LKB ultraspec. III).

Feed fatty acids were extracted and converted to methyl esters following the procedure of Sukhija and Palmquist (1988). Fatty acid concentrations were then determined using a gas chromatograph (HP 5890 Hewlett Packard Co.) equipped with 60-m x 0.32-mm capillary column as described by Chouinard et al. (1997b). The carrier gas was He and

the temperature of the flame ionization detector was maintained at 250° C. The initial oven temperature was 150° C and increased 5° C minute^{-1} up to 200° C.

Total tract nutrient utilization values were calculated as the difference between the amount of nutrient in feed and feces and were expressed as a proportion of total nutrient intake.

4.3.4. Statistical Analysis

The Mixed procedure of the SAS® Institute, Inc. (1999) was used. Data from the ruminal fermentation trial were analyzed as a 3x3 Latin square design with the different sampling times as repeated measures using the model:

$$Y_{ijkl} = \mu + T_i + P_j + C_k + S_l + T_i{*}S_l + e_{ijkl}$$

Data from the total tract nutrient utilization trial were analyzed as a 3 x 3 Latin square design using the model:

$$Y_{ijkl} = \mu + T_i + P_j + C_k + e_{ijkl}$$

For both models, μ is the overall mean, T_i the effect of the ith treatment, P_j the effect of the jth period, C_k the effect of the kth cow, S_l the effect of the lth sampling time, $T_i{*}S_l$ is the treatment by time interaction and e_{ijkl} the residual error. For both trials, the period and cow factors were considered as random and single degree of freedom contrasts were used to compare treatment effects of fat supplementation (i.e. control versus sunflower seed supplementation) and heat treatment (i.e. raw versus roasted sunflower seeds).

4.4. Results and Discussion

4.4.1. Experimental Diets

Ether extract was almost double in sunflower seed supplemented diets (57.8 g kg^{-1}) relative to the control diet (30.1 g kg^{-1}); this resulted in increased net energy of lactation for sunflower seed diets (1.77 kcal kg^{-1}; Table 4-1). Even though dietary treatments were formulated to be similar in neutral detergent fiber, diets containing sunflower seed had 4 % more neutral detergent fiber than the control diet (Table 4-1). This can be attributed to the high fiber content of sunflower seed hull. Crude protein content was similar for all

dietary treatments. The concentration of $C_{18:2}$ was greater in the sunflower seed diets then in the control diet due to the high content of linoleic acid in sunflower seed oil (Table 4-2).

4.4.2. Ruminal Fermentation

Ruminal fermentation parameters are shown in Table 4-3. Treatment by time interaction was tested and was not statistically significant ($P > 0.05$) therefore only the mean values of the sampling times were reported. Ruminal pH, ammonia-nitrogen and total volatile fatty acids were not affected by sunflower seed supplementation. Our results were in good agreement with the results reported by Finn et al. (1985) who supplemented dairy cows with sunflower seeds (9.6% diet dry matter). Casper et al. (1988) and McGuffey and Schingoethe (1981) who supplemented dairy cows with 8% and 10% sunflower seeds, respectively also obtained similar results. On the other hand, Drackley and Schingoethe (1986) observed an increase in ruminal pH with sunflower seed supplementation (19% diet dry matter), which they attributed to reduction in total volatile fatty acid production. The authors also observed a reduction in ammonia-nitrogen concentration from 16.5 to 13.0 mg/dl.

Sunflower seed supplementation modified relative proportions of individual volatile fatty acids (Table 4-3). Feeding sunflower seed reduced ($P < 0.05$) concentration of acetate as well as butyrate and increased ($P < 0.05$) that of propionate and iso-butyrate without affecting concentration of valerate. Consequently, acetate to propionate ratio was reduced by sunflower seed supplementation. Those changes in individual volatile fatty acid concentrations indicate that ruminal population of cellulolytic bacteria might have been modified by sunflower seed supplementation. Sunflower seed supplementation did not affect ruminal volatile fatty acid pattern in various studies (Casper at al., 1988; Finn et al., 1985; McGuffey and Schingoethe, 1986). Markus et al. (1996) did not find any difference in ruminal volatile fatty acid pattern when comparing cows fed a diet supplemented with whole sunflower seeds (7.1 % diet dry matter and 4.2% ether extract) with cows fed a control diet low in fat (1.8 % ether extract) or with cows fed a diet supplemented with tallow (4.2 % ether extract).

Table 4-3. Effects of feeding raw and roasted sunflower seeds (SFS) on rumen fermentation parameters

	Treatments			SEM[1]	Contrasts[2]	
	Control	Raw SFS	Roasted SFS		1	2
pH	5.9	6.0	5.9	0.1	0.28	0.03
Ammonia-nitrogen (mg dl^{-1})	26.9	25.8	25.6	3.47	0.54	0.93
Total VFA (mmol)	91.4	87.3	91.8	33.27	0.44	0.12
Individual VFA (mol 100 mol^{-1})						
Acetate (A)	65.6	65.3	63.7	1.0	0.03	<0.01
Propionate (P)	19.4	20.4	22.4	1.0	<0.01	0.03
Butyrate	10.7	10.2	10.1	0.58	<0.01	0.55
Iso-butyrate	1.0	1.1	1.0	0.05	0.01	0.16
Valerate	1.3	1.3	1.3	0.34	0.10	0.61
A:P	3.4	3.2	2.9	0.19	<0.01	<0.01

[1] Standard error of the mean.
[2] Contrasts: 1: control vs. sunflower seed diets; 2: raw sunflower seed diet vs. roasted sunflower seed diet.

Drakley and Schingoethe (1986) found that sunflower seed supplementation increased acetate concentration by 6.3% and reduced propionate level by 12% thus increasing the acetate to propionate ratio. The opposite is usually expected when feeding supplemental lipid rich in unsaturated fatty acids. The authors attributed those changes to the high fiber content of sunflower seeds. Similarly, the diets containing sunflower seeds in our study had higher concentrations of neutral detergent fiber (32.3%) than the control ration (28.5%), but individual volatile fatty acid concentrations were still affected by sunflower seed supplementation.

A statistically significant difference was observed when comparing ruminal pH of cows fed raw or roasted sunflower seed (Table 4-3). This difference being equal to 0.1 unit, it might not affect productivity of dairy cows. Providing roasted sunflower seeds accentuated the changes observed in acetate and propionate concentrations by 1.9% and 3%, respectively. Mohamed et al. (1988) observed that feeding free cottonseed oil and whole raw cottonseed increased the concentration of propionate compare with a control diet. However, providing the same amount of fat in the form of whole roasted cottonseed did not alter concentrations of propionate, which is the opposite of the trend observed in our study. The reason behind this effect is not clear.

4.4.3. Dry Matter Intake

The results of intake and total tract nutrient utilization are shown in Table 4-4. Cows fed the sunflower seed diets tended (P= 0.07) to consume less dry matter than cows fed the control diet. Reduced dry matter intake has been often associated with lipid supplementation in dairy cows diet (Allen, 2000; De Luca and Jenkins, 2000; Pantoja et al., 1994). Finn et al. (1985) observed a reduction in dry matter intake following sunflower seed supplementation (9.7% of the diet). Similarly, Anderson et al. (1984) found that feeding whole sunflower seeds (12% of the diet) decreased dry matter intake when compare with extruded soybeans (5% of the diet). These results are in contrast with Drackley and Schingoethe (1986) and Markus et al. (1996) who showed no effect of feeding sunflower seeds on dry matter intake. Several studies have shown no detrimental effect of other supplemental oilseeds, such as soybeans and flaxseeds, on dry matter

intake (Casper at al., 1988; Grummer and Luck, 1993; Knapp et al., 1991; Mustafa et al., 2003a; Petit, 2002).

In our study, heat treatment of sunflower seeds had no effect on dry matter intake (Table 4-4). This agrees with the results obtained by others who have fed heat-treated sunflower seeds (Casper et al., 1988; McGuffey and Schingoethe, 1986) and other heat-treated oilseeds (Chouinard et al., 1997b; Dhiman et al., 1997; Knapp et al., 1991). However, reduced dry matter intake has been reported in various studies where roasted oilseeds were fed to dairy cows (Chouinard et al., 1997a; Mohamed et al., 1988).

4.4.4. Total Tract Nutrient Utilization

Total tract digestibility of dry matter, organic matter, neutral and acid detergent fiber, crude protein, starch and gross energy were not affected by sunflower seed supplementation or by roasting (Table 4-4). This is in accordance with the study performed by Finn et al. (1985) where dietary supplementation with sunflower seeds did not affect total tract dry matter digestibility. Similarly, canola seed supplementation (5% diet dry matter) did not affect total tract digestibility of neutral detergent fiber, however, total tract digestibility of organic matter was reduced (Hussein et al., 1995). On the other hand, supplementation of canola oil resulted in reduced total tract digestibility of neutral and acid detergent fibers (Ferlay and Doreau, 1992; Doreau, 1991). Mohamed et al. (1988) have observed a reduction in dry matter digestibility with free soybean oil and raw whole soybean supplementation. However, the negative impacts were not observed when roasted soybeans were fed.

Table 4-4. Effects of feeding raw and roasted sunflower seeds (SFS) on intake and total tract nutrient utilization (dry matter basis)

	Treatments			SEM[1]	Contrasts[2]	
	Control	Raw SFS	Roasted SFS		1	2
Intake (kg)						
DM intake	25.1	23.8	23.6	1.34	0.07	0.78
NDF intake	5.7	6.8	6.4	0.38	0.21	0.56
Digestibility (g kg^{-1})						
Dry matter	746.5	712.2	721.2	12.43	0.15	0.61
Organic matter	700.5	690.0	682.9	13.25	0.42	0.70
Neutral detergent fiber	483.8	475.3	474.2	45.85	0.80	0.98
Acid detergent fiber	481.5	459.9	468.8	47.79	0.53	0.77
Crude protein	696.0	706.9	697.5	10.96	0.49	0.38
Ether extract	808.0	884.1	867.7	7.42	<0.01	0.11
Starch	926.5	946.2	946.2	13.73	0.15	0.81
Gross energy (cal $kcal^{-1}$)	683.8	672.9	664.4	12.97	0.41	0.66

[1] Standard error of the mean.
[2] Contrasts: 1: control vs. sunflower seed diets; 2: raw sunflower seed diet vs. roasted sunflower seed diet.

Excessive heating can result in reduced total tract utilization of protein due to the formation of Maillard products (McKinnon et al., 1995; Van Soest, 1994), which was not the case in this experiment and as well as in another experiment involving roasted soybean supplementation (Knapp et al., 1991).

Ether extract digestibility was higher for the sunflower seed diets (876 g kg^{-1}) than for the control diet (808 g kg^{-1}). This can be explained by the different nature of the ether extract in the diets. Lipids in the control diets were mainly from forage origin while a large proportion of the lipids in the sunflower seed diets is coming from sunflower seeds. The ether extract of oilseeds is mainly represented by fatty acids (90%) while the ether extract of forages contains only 43% of fatty acids (Byers and Schelling, 1988; Palmquist and Jenkins, 1980). Waxes, chlorophyll, galactose and other unsaponifiable compounds represent the rest of it, which contributes to reduced digestibility of the ether extract (Palmquist and Jenkins, 1980).

4.5. Conclusions

Inclusion of sunflower seeds in the diet of lactating dairy cows did not affect ruminal pH, ammonia nitrogen concentration and total volatile fatty acid concentration. Minimal, but statistically significant impacts of raw and roasted sunflower seed supplementation were observed on the relative proportions of the individual volatile fatty acids. Acetate was decreased by 0.5 % when feeding raw sunflower seed and by 2.9 % when feeding roasted sunflower seeds, while propionate was increased by 10.3 % when feeding raw sunflower seeds and by 15.5 % when feeding roasted sunflower seeds. Providing supplemental dietary lipid in the form of sunflower seeds tended to decrease dry matter intake, but no difference in intake was observed due to heat treatment of sunflower seeds. The total tract utilization of dietary nutrients was not negatively affected by either raw or roasted sunflower seed supplementation. In conclusion, supplementation with raw and roasted sunflower seeds did not negatively affect total tract nutrient utilization and ruminal fermentation, except for minor changes in individual volatile fatty acid proportions.

5. Effects of Feeding Raw and Roasted Sunflower Seeds on Intake, Milk Yield and Milk Composition

5.1. Abstract

A dairy production study was conducted using three primiparous (50 ± 17 days in milk) and six multiparous (63 ± 17 days in milk) Holstein cows in early lactation in three 3x3 Latin squares to determine the effects of feeding raw and roasted sunflower seeds on milk yield and milk fatty acid composition. Treatments were a control diet with no added sunflower seed, a raw sunflower seed diet and a roasted sunflower seed diet. Sunflower seed diets contained 7.8% (dry matter basis) raw or roasted sunflower seeds. Ether extract content was 3.2%, 6.2% and 6.4% for the control, raw and roasted sunflower seed diets, respectively. Results showed that sunflower seed supplementation reduced ($P < 0.01$) dry matter intake of multiparous cows, but not that of primiparous cows. Sunflower seed supplementation reduced ($P < 0.01$) milk fat yield of multiparous cows. Multiparous cows fed the control or the roasted sunflower seed diet produced more ($P < 0.01$) milk than those fed the raw sunflower seed diet. Feeding sunflower seeds resulted in a lower ($P < 0.05$) milk fat percentage while the concentrations of other milk components were not affected. Feeding roasted and raw sunflower seeds reduced ($P < 0.05$) the concentrations of short and medium chain fatty acids and increased ($P < 0.01$) the concentrations of long chain fatty acids (LCFA) for both primiparous and multiparous cows. Inclusion of raw and roasted sunflower seeds increased ($P < 0.05$) the concentration of conjugated linoleic acid (CLA) in milk of both primiparous and multiparous cows. It was concluded that feeding sunflower seeds to dairy cows increased the concentrations of LCFA and CLA in milk. Feeding roasted sunflower seeds had no effect on milk yield of dairy cows.

5.2. Introduction

Conjugated linoleic acid (CLA) represents a group of different isomers of linoleic acid for which the double bonds have a conjugated arrangement (Jensen, 2002). The CLA in dairy cow's milk has two major origins. CLA is an intermediate product of the biohydrogenation process of linoleic acid performed by ruminal bacteria. CLA can also be formed at the mammary gland level by delta-9-desaturase from trans-11 $C_{18:1}$ (Jensen,

2002). The major isomer of CLA found in the milk is the cis-9, trans-11 $C_{18:2}$, which is recognized for its anticarcinogenic properties (Pariza et al., 2001). Thus, increasing CLA concentration in milk is an interesting way to promote consumption of milk products since they have been criticized in the past years for their poor milk fatty acid profile.

Increasing quantity of CLA and trans-11 $C_{18:1}$ reaching the mammary gland can increase the concentration of CLA in milk (Bauman and Griinari, 2001; Bell and Kennelly, 2003). This can be accomplished by providing supplemental dietary linoleic acid partially protected from complete ruminal biohydrogenation. Supplementing dairy cow diets with fat in the form of whole treated oilseeds, such as roasted sunflower seeds, could be a way of protecting linoleic acid from complete ruminal biohydrogenation. Reddy et al. (1994) suggested various factors that could explain the slower release of fatty acids from roasted soybeans and the reduction in biohydrogenation. These include the intracellular location of the oil, the chemical reaction occurring during the roasting treatment and the reduction in release rate of oil into the rumen due to reduce dry matter digestion after roasting.

Feeding heat-treated sunflower seeds might also have beneficial effects on milk yield by increasing the quantity of rumen undegradable protein and thus increasing the quantity of amino acids available for milk protein synthesis.

The objectives of this study were to determine the effects of feeding raw and roasted sunflower seeds on dry matter intake, milk yield and milk fatty acid composition of primiparous and multiparous cows.

5.3. Materials and Methods

5.3.1. Treatments and Animals

The study was conducted at the Macdonald Campus Farm of McGill University located at Ste-Anne-de-Bellevue, Quebec. Dietary treatments consisted of a control diet with no added sunflower seed, a raw sunflower seed diet and a roasted sunflower seed diet (Tables 5-1 and 5-2). All diets were fed as total mixed rations with 50:50

forage:concentrate ratio and were formulated to be isonitrogenous, similar in neutral detergent fiber content and to meet the NRC (2001) requirements for dairy cows. Sunflower seed supplemented diets were formulated to contain 60 g kg^{-1} of ether extract (dry matter basis).

Nine Holstein cows in early lactation were used during this trial. Three cows were in first lactation (50 ± 17 days post partum and 541 ± 15 kg BW) and six cows were in second lactation (multiparous; 63 ± 17 days post partum and 621 ± 57 kg BW). The experiment consisted of three replicated 3x3 Latin squares with one square comprised of primiparous cows and two squares comprised of second lactation cows. Animals within squares were randomly assigned to one of the three dietary treatments. All diets were fed twice daily (8:00 AM and 4:00 PM) as total mixed rations to allow for 5 to 10% refusals. Experimental periods were 31 days in duration with the first 10 days used for diet adaptation and the last 21 days used for data collection. The animals were housed in tie stalls and had continuous access to water. Cows were milked three times daily (4:30 AM, 11:30 PM and 7:30 PM).

5.3.2. Sample and Data Collection

Milk yields were recorded from day 11 to 31. During the last 21 days, weekly milk samples were collected (in duplicate) for each milking and were pooled within each day. One duplicate was put into plastic bottles containing bronopol as preservative and was sent to the Programme d'Analyse des Troupeaux Laitiers du Quebec for milk component analysis. Additional milk samples collected during the same period were kept frozen for later fatty acid analysis.

Feed samples were collected weekly during the last 21 days of each period, were dried at 55° C in a forced-air oven for 48 hours and were pooled within each period.

Table 5-1. Ingredients and chemical composition of the control and sunflower seed (SFS) diets used in the dairy production study (dry matter basis)

	Treatments		
	Control	Raw SFS[1]	Roasted SFS[1]
Ingredients (g kg^{-1})			
Dry hay	72.3	73.1	73.1
Alfalfa silage	184.8	186.8	186.8
Corn silage	229.2	231.6	231.6
High moisture corn	289.1	292.2	292.2
Cracked corn	57.5	0.0	0.0
Soybean meal	61.6	56.5	56.5
Commercial concentrate 1[2]	11.5	41.5	41.5
Commercial concentrate 2 [3]	84.6	31.1	31.1
Mineral supplement [4]	9.5	9.1	9.1
Sunflower seed	0.00	78.0	78.0
Chemical composition (g kg^{-1})			
Ash	68.4	64.5	65.5
Ether extract	32.1	62.4	64.2
Neutral detergent fiber	275.1	320.2	303.6
Acid detergent fiber	164.2	187.6	181.2
Lignin	18.6	27.5	26.5
Crude protein (CP)	193.1	191.3	194.3
NDICP (g $kg^{-1}$of CP)[5]	133.3	159.2	150.4
ADICP (g $kg^{-1}$of CP)[5]	44.7	44.6	46.4
Starch	262.8	191.1	200.6
Calcium	9.2	8.0	8.2
Phosphorus	5.8	5.1	5.3
Net energy of lactation (Mcal kg^{-1})[6]	1.77	1.81	1.83

[1] Sunflower seeds

[2] Contained 500 g crude protein, 40 g crude fat, 50 g crude fiber, 2.5 g Na, 25 g Ca, 15 g P, 8.0 g Mg, 5.0 g S, 5.0 g K, 7.4 mg I, 700 mg Fe, 125 mg Cu, 370 mg Mn, 370 mg Zn, 1.2 mg Co, 50 mg F, 160 mg vitamin E, 39,500 IU vitamin A, 11,850 IU vitamin D_3 kg^{-1}.

[3] Contained 385 g crude protein, 20 g crude fat, 100 g crude fiber, 14 g Na, 34.5 g Ca, 22 g P, 15 g Mg, 5.0 g S, 11 g K, 11.5 mg I, 1,400 mg Fe, 200 mg Cu, 600 mg Mn, 600 mg Zn, 2 mg Co, 50 mg F, 230 IU vitamin E, 57,600 IU vitamin A, 17,300 IU vitamin D_3 kg^{-1}.

[4] Contained (g kg^{-1}) 100 Ca, 100 P, 78 Na, 80 Mg, 30 S. Supplied 45 mg I, 3,600 mg Fe, 740 mg Cu, 2,300 mg Mn, 2,300 mg Zn, 10 mg Co, 500 mg F, 300,000 IU vitamin A, 90,000 IU vitamin D_3, 800 IU vitamin E kg^{-1}.

[5] NDICP: neutral detergent insoluble crude protein, ADICP: acid detergent insoluble crude protein.

[6] Calculated according to Weiss et al. (1992).

Table 5-2. Fatty acid composition of the control and sunflower seed (SFS) diets used in the dairy production study (g kg^{-1} of total fatty acids)

	Treatments		
	Control	Raw SFS	Roasted SFS
$C_{16:0}$	170.0	114.2	117.3
$C_{16:1}$	17.1	3.3	3.4
$C_{18:0}$	29.5	40.7	41.5
$C_{18:1}$	237.1	198.1	200.7
$C_{18:2}$	460.5	599.3	594.1
$C_{18:3}$	85.9	44.4	43.2

5.3.3. Sample Analysis and Calculations

Milk samples were analyzed for fat, protein, lactose and urea in the laboratory of the PATLQ (Programme d'Analyse des Troupeaux Laitiers du Québec, Ste-Anne-de-Bellevue, Quebec, Canada) using an infrared system with an electric Milk-O-Scan 4000 (Foss-Food Technology, Hillerød, Danemark). Total solid content was determined according to the procedures of the Association of Official Analytical Chemists (method no. 925.23, AOAC, 1990).

Feed samples were ground through a 1 mm-screen using a Thomas-Wiley Laboratory Mill (Thomas Scientific, USA) and were analyzed for dry matter (method # 934.01); ash (method no. 924.05) and ether extract (EE, method no. 920.39) according to the Association of the Official Analytic Chemists (AOAC, 1990). Crude protein (CP, N x 6.25) was determined using a LECO Nitrogen System FP-428 (LECO Corp. St-Joseph, MI, USA). Analysis of neutral (NDF) and acid (ADF) detergent fiber were determined using the Ankom System (Ankom^{200} Fiber Analyzer and F57 filter bags Ankom Technology, Fairport, NY, USA) with heat stable alpha-amylase and without sodium sulfate. Starch was determined as described by McCleary et al. (1997). Feed samples were also analyzed for lignin using the Ankom System (Ankom^{200} Fiber Analyzer and F57 filter bags Ankom Technology, Fairport, NY, USA). Neutral (NDICP) and acid (ADICP) detergent insoluble crude protein were determined by analyzing NDF and ADF residues, respectively for CP. Calcium concentration was determined following digestion with perchloric-nitric acid mixture (method no. 915.13 AOAC, 1990) using Perkin-Elmer Model 5000 atomic absorption spectrophotometer (Technicon GTPC auto analyzer II). Phosphorus concentration was determined colorimetrically (Pharmacia LKB ultraspec. III).

Milk fatty acids were extracted and converted to methyl esters by transesterification with sodium methoxyde according to the method detailed by Chouinard et al. (1997b). Feed fatty acids were extracted and converted to methyl esters following the procedure of Sukhija and Palmquist (1988). FA concentrations were then determined using a gas chromatograph (HP 5890 Hewlett Packard Co.) equipped with 60-m x 0.32-mm capillary

column as described previously by Chouinard et al. (1997b). The carrier gas was He and the temperature of the flame ionization detector was maintained at 250° C. The initial oven temperature was 150° C and increased 5° C minute^{-1} up to 200° C.

5.3.4. Statistical Analysis

The Mixed procedure of the SAS® Institute, Inc. (1999) was used. Data from the first and second lactation cows were analyzed separately. Data were analyzed as a 3x3 Latin square design for the primiparous cows with the weeks of milk sampling as repeated measures using the model:

$$Y_{ijkl} = \mu + T_i + P_j + C_k + W_l + T_i{*}W_l + e_{ijkl}$$

The data for the multiparous cows were analyzed as a 2 concurrently replicated Latin squares using the model:

$$Y_{ijkl} = \mu + T_i + P_j + C_{mk} + W_l + S_m + T_i{*}W_l + e_{ijkl}$$

For both models, μ is the overall mean, T_i the effect of the ith treatment, P_j the effect of the jth period, C_k the effect of the kth cow, W_l the effect of the lth sampling week, S_m the effects of the mth square, $T_i{*}W_l$ is the treatment by week interaction and e_{ijkl} the residual error. For both trials, the period and cow factors were considered as random and single degree of freedom contrasts were used to compare the effects of fat supplementation (i.e. control versus sunflower seed supplementation) and heat treatment (i.e. raw versus roasted sunflower seed).

5.4. Results and Discussion

5.4.1. Experimental Diets

Ether extract (Table 5-1) was almost double in sunflower seed supplemented diets (63.2 g kg^{-1}) compare to the control diet (32.1 g kg^{-1}) resulting in increased net energy of lactation for the sunflower seed diets (1.81 and 1.83 kcal kg^{-1} for the raw and roasted sunflower seed diets, respectively). Even though dietary treatments were formulated to be similar in neutral detergent fiber, diets containing sunflower seed contained 3.7 % more neutral detergent fiber than the control diet. This can be attributed to the high content of fiber in the sunflower seed hull. Crude protein content was similar for all

dietary treatments. The concentration of $C_{18:2}$ (Table 5-2) was greater in the sunflower seed diets (589.6 and 581.0 g kg^{-1} of total fatty acids for the raw and roasted sunflower seed diets) then in the control diet (436.5 g kg^{-1} of total fatty acids) due to the high content of linoleic acid in sunflower seed oil.

5.4.2. Dry Matter Intake

Sunflower seed supplementation did not affect the dry matter intake of primiparous cows (Table 5-3), but reduced ($P < 0.01$) that of multiparous cows (Table 5-4). Primiparous and multiparous cows fed the control diet had lower ($P < 0.01$) ether extract intake than cows fed the sunflower seed diets due to lower fat content in the control diet. Reduction in dry matter intake was also observed during the study on ruminal fermentation and total tract nutrient utilization that we performed (Section 4). Several studies showed no effect of sunflower seed supplementation on dry matter intake (Drackley and Schingoethe, 1986; Markus et al., 1996). Other studies showed that inclusion of sunflower seeds in dairy cow diets reduced dry matter intake (Anderson et al., 1984 and Finn et al., 1985). Several studies have shown no detrimental effect of supplemental oilseeds on dry matter intake (Casper at al., 1988; Grummer and Luck, 1993; Knapp et al., 1991; Mustafa et al., 2003; Petit, 2002).

Neutral detergent fiber intake was not affected for both primiparous and multiparous cows, except for the third week of treatment in primiparous cows, even though the neutral detergent fiber content of the control diet was smaller than that of the sunflower seed diets.

Table 5-3. Effects of feeding raw and roasted sunflower seeds (SFS) on intake, milk yield and milk composition of primiparous cows.

	Treatments			SEM[1]	Contrasts[2]	
	Control	Raw SFS	Roasted SFS		1	2
Intake						
DM (kg day^{-1})	21.6	21.3	20.4	1.17	0.21	0.16
EE (kg day^{-1})	0.7	1.3	1.3	0.06	<0.01	0.17
NDF (kg day^{-1})	7.9	8.2	8.1	0.68	0.29	0.17
Milk Yield (kg day^{-1})						
Total milk	36.2	38.8	36.7	2.43	0.41	0.33
Energy-corrected milk [3]	36.7	39.1	35.7	3.02	0.69	0.13
Fat[4]						
Week 1	1.07	1.40	1.12	0.188	0.06	0.02
Week 2	1.26	1.18	1.00	0.188	0.10	0.12
Week 3	1.24	1.06	1.10	0.188	0.11	0.72
Protein	1.10	1.16	1.12	0.070	0.26	0.45
Lactose	1.76	1.83	1.76	0.126	0.78	0.58
Milk Composition						
Fat (g kg^{-1})[4]						
Week 1	29.5	35.8	29.1	3.78	0.20	0.02
Week 2	34.4	30.3	27.3	3.78	0.02	0.26
Week 3	33.4	27.4	30.1	3.78	0.05	0.30
Protein (g kg^{-1})	30.4	29.7	31.0	1.94	0.99	0.24
Lactose (g kg^{-1})	48.7	47.0	48.0	0.51	<0.01	0.02
Total solids (g kg^{-1})	127.3	127.8	125.6	3.07	0.86	0.56
Milk urea nitrogen (mgdl^{-1})	14.2	20.2	12.5	3.81	0.65	0.17

[1] Standard error of the mean.
[2] Contrasts: 1: control vs. sunflower seed diets; 2: raw sunflower seed diet vs. roasted sunflower seed diet.
[3] Calculated according to Tyrell and Ried (1965).
[4] Treatment x week interaction is significant ($P < 0.05$).

Table 5-4. Effects of feeding raw and roasted sunflower seeds (SFS) on intake, milk yield and milk composition of multiparous cows.

	Treatments			SEM[1]	Contrasts[2]	
	Control	Raw SFS	Roasted SFS		1	2
Intake						
DM (kg day^{-1})	25.9	23.9	23.6	0.97	<0.01	0.50
EE (kg day^{-1})	0.8	1.5	1.5	0.07	<0.01	0.46
NDF[3] (kg day^{-1})						
Week 1	9.4	9.5	9.6	0.50	0.51	0.82
Week 2	9.3	9.0	9.5	0.50	0.81	0.12
Week 3	9.8	9.1	9.3	0.50	0.02	0.60
Milk Yield (kg day^{-1})						
Total milk	47.4	45.5	47.4	3.61	0.09	<0.01
Energy-corrected milk [4]	47.1	44.3	46.4	3.26	0.10	0.09
Fat	1.61	1.39	1.48	0.124	<0.01	0.12
Protein	1.35	1.31	1.33	0.085	0.18	0.49
Lactose	2.21	2.14	2.21	0.148	0.24	0.02
Milk Composition						
Fat (g kg^{-1})	34.0	30.5	31.0	1.71	<0.01	0.70
Protein (g kg^{-1})	28.6	28.8	28.2	0.87	0.77	0.25
Lactose (g kg^{-1})	46.7	47.2	46.8	1.00	0.09	0.06
Total solids (g kg^{-1})	122.9	123.6	123.2	2.11	0.79	0.83
Milk urea nitrogen (mgdl^{-1})	14.8	13.5	14.6	1.33	0.35	0.26

[1] Standard error of the mean

[2] Contrasts: 1: control vs. sunflower seed diets; 2: raw sunflower seed diet vs. roasted sunflower seed diet.

[3] Treatment x week interaction is significant ($P < 0.05$).

[4] Calculated according to Tyrell and Ried (1965).

Heat treatment had no effects on intake of dry matter, ether extract or neutral detergent fiber of both primiparous and multiparous cows. This agrees with the results obtained by others who have fed heat-treated sunflower seeds (Casper et al., 1988; McGuffey and Schingoethe, 1986) and other heat-treated oilseeds (Chouinard et al., 1997b; Dhiman et al., 1997; Knapp et al., 1991). However, reduced dry matter intake has been reported in various studies relating to roasted oilseed supplementation (Chouinard et al., 1997a; Mohamed et al., 1988).

5.4.3. Milk Yield and Composition

Milk yield and milk composition for primiparous and multiparous cows are shown in Tables 5-3 and 5-4, respectively. Total yield of milk and yield of energy-corrected milk of primiparous and multiparous cows were not affected by sunflower seed supplementation, even though energy density of the sunflower seed diets was greater than that of the control diet. Similarly, feeding rolled or extruded sunflower seeds (10% of the diet) did not result in increased milk yield (McGuffey and Schingoethe, 1981). However increased milk yield was observed by Schingoethe et al. (1996) when supplementing diets with 7.5% sunflower seeds.

Yield and concentration of protein of both primiparous and multiparous cows were not affected by sunflower seed supplementation. These results are in accordance with those of Schingoethe et al. (1996), who reported similar protein yield and concentration following sunflower seed supplementation. Others also reported similar results (Casper et al., 1988; Markus et al., 1996; McGuffey and Schingoethe, 1981). However, reduced protein concentration has often been associated with dietary lipid supplementation from various oilseeds (Kennelly, 1996; Knapp et al., 1991; Finn et al., 1985).

Concentrations of total solids and milk urea nitrogen were not affected by sunflower seed supplementation for both parities. Lactose yield and concentration in milk of multiparous cows were not affected by sunflower seed supplementation. Lactose concentration was lower ($P < 0.01$) for primiparous cows fed the sunflower seed supplemented diets, however, it did not result in a reduction of lactose yield.

Lower ($P < 0.05$) milk fat concentration in the last two weeks of the study reduced milk fat yield for the primiparous cows, but the difference was not statistically significant. Milk fat yield and concentration were lower ($P < 0.01$) for multiparous cows fed sunflower supplemented diets than for those fed the control diet. The three primiparous cows used might not have been enough to result in a statistically significant reduction in milk fat yield even if this reduction was numerically similar to the reduction observed in multiparous cows. Other studies have reported milk fat depression as a result of sunflower seed supplementation (Casper et al., 1988; Drackley and Schingoethe, 1986; Finn et al., 1985). However, sunflower seed supplementation did not result in milk fat depression in the studies of Markus et al. (1996) and McGuffey and Schingoethe (1981). Finn et al. (1985) have noted that milk fat percentage was similar to the control diet when additional limestone was added to the sunflower seed diet (9.6% of diet dry matter). Mohamed et al. (1988) found that providing 4% soybean or cottonseed oil in the diet of lactating dairy cows depressed milk fat percentage, but this was not observed when the oil was provided as part of the whole seeds.

The actual causes of milk fat depression as a result of feeding dietary fat are unclear. Recently, it has been suggested that increased concentration of trans-10 $C_{18:1}$ and trans-10, cis-12 $C_{18:2}$ caused milk fat depression (Bauman and Griinari, 2001; Chouinard et al., 1999b; Bell and Kennelly, 2003). Moreover, trans-10, cis-12 $C_{18:2}$ seems to be one isomer responsible for milk fat depression (Baumgard et al., 2000). Modification of microbial ruminal fermentation may alter the normal biohydrogenation processes and could result in increased concentration of the trans-10, cis-12 isomer (Bauman and Griinari, 2001). In this study, only total quantities of CLA were measured (Tables 5-5 and 5-6), but it could be hypothesized that proportion of the trans-10, cis-12 $C_{18:2}$ has been increased in milk of cows fed sunflower seeds due to the milk fat depression that was observed in multiparous cows and the similar tendency observed for primiparous cows.

Total milk yield and yield of energy corrected milk of primiparous cows were not affected by heat treatment of sunflower seeds. However, total milk yield were higher ($P < 0.01$) for multiparous cows fed the roasted sunflower seed diet than for those fed the raw sunflower seed diet, but yield of energy-corrected milk was not affected. Providing extruded sunflower seeds instead of rolled sunflower seeds did not improve total milk yield or 4% fat-corrected milk yield (McGuffey and Schingoethe, 1981). Similarly, providing soybeans (17.5 % of the diet dry matter) treated with various heat treatments (extrusion, micronization and roasting) did not increase milk yield over untreated soybeans (Chouinard et al., 1997a). However, in another study, Chouinard et al. (1997b) reported higher milk yield for cows fed extruded soybeans than for cows fed raw soybeans. Dhiman et al. (1997) have also reported a similar positive response of milk yield when feeding extruded flaxseed.

Milk yield and concentration of protein of both primiparous and multiparous cows were not affected by heat treatment of sunflower seeds. Similar results were observed for concentrations of protein, total solids and milk urea nitrogen. Milk lactose concentration was lower ($P < 0.01$) for primiparous cows fed the raw sunflower seed diet than for those fed the roasted sunflower seed diet; however, this did not reduce the lactose yield in milk. Lactose yield in milk was lower ($P < 0.05$) for multiparous cows fed the raw sunflower seed diet than for cows fed the roasted sunflower seed diet. This can be attributed to the reduction in milk yield observed for multiparous cows fed the raw sunflower seed diet.

Table 5-5. Effects of feeding raw and roasted sunflower seeds (SFS) on milk fatty acids composition of primiparous cows (g kg^{-1} of fatty acids)

	Treatments			SEM[1]	Contrasts [2]	
	Control	Raw SFS	Roasted SFS		1	2
$C_{4:0}$	23.1	23.5	20.5	4.49	0.41	0.14
$C_{6:0}$	22.1	19.8	17.1	3.62	0.07	0.14
$C_{8:0}$	12.5	9.8	8.4	1.74	0.03	0.21
$C_{10:0}$	28.0	18.9	16.6	2.83	0.03	0.35
$C_{12:0}$	37.8	24.4	22.7	1.98	0.02	0.51
$C_{14:0}$	119.6	90.4	88.2	4.68	0.03	0.76
$C_{14:1}$	7.9	6.8	6.4	0.70	0.15	0.64
$C_{15:0}$	13.4	9.5	9.7	1.03	0.09	0.93
$C_{16:0}$[3]						
Week 1	298.4	236.7	230.2	9.72	<0.01	0.26
Week 2	315.4	227.2	233.3	9.72	<0.01	0.29
Week 3	335.2	243.5	237.6	9.72	<0.01	0.31
$C_{16:1}$	13.8	9.8	10.1	2.34	0.09	0.47
$C_{18:0}$	129.7	171.3	167.8	12.59	0.02	0.68
Trans $C_{18:1}$	21.9	60.6	71.6	16.73	0.08	0.55
Cis $C_{18:1}$	222.7	277.8	281.2	15.08	0.01	0.72
$C_{18:2}$	23.5	26.2	29.2	2.14	<0.01	0.01
$C_{18:3}$	3.5	2.4	2.4	0.26	0.06	0.87
CLA	4.1	12.6	14.0	1.93	<0.01	0.54
SCFA[4]	123.6	96.5	85.3	14.40	0.03	0.26
MCFA[5]	471.0	352.3	348.9	9.75	<0.01	0.78
LCFA[6]	405.4	551.2	565.8	22.5	<0.01	0.46

[1] Standard error of the mean.
[2] Contrasts: 1: control vs. sunflower seed diets; 2: raw sunflower seed diet vs. roasted sunflower seed diet.
[3] Treatment x week interaction is significant ($P < 0.05$).
[4] Short chain fatty acids correspond to $C_{4:0}$ to $C_{12:0}$.
[5] Medium chain fatty acids correspond to $C_{14:0}$ to $C_{16:1}$.
[6] Long chain fatty acids correspond to $C_{18:0}$ to $C_{18:3}$.

Table 5-6. Effects of feeding raw and roasted sunflower seeds (SFS) on milk fatty acids composition of multiparous cows (g kg^{-1} of fatty acids)

	Treatments			SEM[1]	Contrasts[2]	
	Control	Raw SFS	Roasted SFS		1	2
$C_{4:0}$	26.5	25.0	26.5	0.84	0.40	0.16
$C_{6:0}$	26.1	20.3	21.5	1.18	<0.01	0.31
$C_{8:0}$	14.5	10.0	10.6	0.80	<0.01	0.34
$C_{10:0}$	31.7	19.1	20.2	1.77	<0.01	0.37
$C_{12:0}$	41.5	24.6	25.9	2.04	<0.01	0.43
$C_{14:0}$	122.2	90.2	91.5	3.12	<0.01	0.69
$C_{14:1}$	10.2	8.4	7.8	0.43	<0.01	0.21
$C_{15:0}$	12.0	8.9	8.7	0.53	<0.01	0.66
$C_{16:0}$	344.0	240.6	239.0	10.02	<0.01	0.77
$C_{16:1}$	12.0	10.2	9.4	0.62	<0.01	0.25
$C_{18:0}$	117.5	172.8	169.8	6.48	<0.01	0.42
Trans $C_{18:1}$[3]						
Week 1	17.1	41.8	46.9	2.51	<0.01	0.06
Week 2	15.5	42.9	47.4	2.51	<0.01	0.09
Week 3	16.3	41.1	47.0	2.51	<0.01	0.03
Cis $C_{18:1}$	197.5	290.7	281.3	11.38	<0.01	0.26
$C_{18:2}$	21.2	24.9	26.5	1.02	<0.01	0.07
$C_{18:3}$	3.1	2.6	2.5	0.14	<0.01	0.42
CLA[3]						
Week 1	3.7	9.0	11.2	1.26	<0.01	0.05
Week 2	3.3	9.8	12.5	1.26	<0.01	0.01
Week 3	3.3	10.4	12.1	1.26	<0.01	0.12
SCFA[4]	140.3	98.9	105.8	6.16	<0.01	0.24
MCFA[5]	500.0	358.8	356.3	11.99	<0.01	0.72
LCFA[6]	358.9	543.0	538.9	14.50	<0.01	0.72

[1] Standard error of the mean.
[2] Contrasts: 1: control vs. sunflower seed diets; 2: raw sunflower seed diet vs. roasted sunflower seed diet.
[3] Treatment x week interaction is significant ($P < 0.05$).
[4] Short chain fatty acids correspond to $C_{4:0}$ to $C_{12:0}$.
[5] Medium chain fatty acids correspond to $C_{14:0}$ to $C_{16:1}$.
[6] Long chain fatty acids correspond to $C_{18:0}$ to $C_{18:3}$.

Milk fat concentration and yield were higher ($P < 0.05$) only in the first week of treatment for the primiparous cows fed the raw sunflower seed diet than for cows fed the roasted sunflower seed diet. On the other hand, milk fat yield and concentration in milk of multiparous cows were not affected by heat treatment.

5.4.4. Milk Fatty Acid Composition

Milk fatty acid composition of primiparous and multiparous cows is given in Tables 5-5 and 5-6, respectively. For both parities, sunflower seed supplementation reduced ($P < 0.05$) the concentrations of short and medium chain fatty acids and increased ($P < 0.01$) the concentrations of long chain fatty acids. Supplementing dairy cows with sunflower seeds has resulted in similar effects (Casper et al., 1988; Drackley and Schingoethe, 1986; Markus et al., 1996; Schingoethe et al., 1996). Similar results were reported in various studies in which supplemental dietary lipids were provided as oilseeds (Chouinard et al., 1997a; Kennelly, 1996; Mohamed et al., 1988; Mustafa et al., 2003a). Concentrations of linoleic acid and CLA in milk of primiparous and multiparous cows were increased ($P< 0.01$) by sunflower seed supplementation. Increased in milk CLA concentration has been reported following supplementation with various oilseeds such as canola, cottonseed, flaxseed, solin, soybean (Chouinard et al., 1997a; Dhiman et al., 1999b; Dhiman et al., 2000; Ward et al., 2002). Concentration of trans $C_{18:1}$ was increased ($P < 0.01$) for multiparous cows and only tended to be increased ($P = 0.08$) for primiparous cows. Similar results following sunflower seed supplementation have been reported by Casper et al. (1988).

Roasting sunflower seeds had no effect on milk fatty acid composition of primiparous cows except for linoleic acid which was higher ($P < 0.01$) for cows fed the roasted sunflower seed diet than cows fed the raw sunflower seed diet. Roasting of sunflower seed also tended to increase ($P = 0.07$) concentration of linoleic acid in milk of multiparous cows. In the trial involving multiparous cows, heat treatment of sunflower seeds also increased ($P < 0.05$) the concentration of CLA over that of raw sunflower seed diet in the first and second week of treatment. Heat treatment also increased ($P < 0.05$) the concentration of trans $C_{18:1}$ in the third week of treatment and a tendency ($P = 0.06$

and 0.09) for higher concentration was observed in the first and second week of treatment for multiparous cows. These results suggest that roasting of sunflower seed helped to prevent complete ruminal biohydrogenation of linoleic acid. Emanuelson et al. (1991) found that rate of ruminal lipolysis was lower and less biohydrogenation took place when cows were fed roasted rapeseed than raw rapeseed. Similarly, Reddy et al. (1994) reported that roasting provided a partial protection of fatty acids in soybean from ruminal bacteria, thus reducing the rate of lipolysis and biohydrogenation. Dhiman et al. (2000) reported that milk CLA concentration were higher for cows fed roasted soybeans than for cows fed raw soybeans. On the other hand, milk concentration of oleic and linoleic acids were similar for cows fed roasted or raw cottonseed (Mohamed et al., 1988)

5.5. Conclusions

Dry matter intake of multiparous cows was reduced by sunflower seed supplementation, which was not the case for primiparous cows. Heat treatment of sunflower seeds had no effect on dry matter intake of both multiparous and primiparous cows. Total milk yield and energy-corrected milk yield were not affected by sunflower seed supplementation. However, multiparous cows fed the roasted sunflower seed diet produced more milk than those fed the raw sunflower seed diet. Milk fat yield and milk fat concentration were reduced for multiparous cows fed sunflower seeds. Concentrations of other milk constituents were not affected by sunflower seed supplementation, except for a reduced lactose concentration for primiparous cows. Sunflower seed supplementation decreased SCFA and MCFA concentrations and increased milk LCFA and CLA concentrations. Roasting tended to increase $C_{18:2}$ in multiparous cow's milk and increased $C_{18:2}$ in primiparous cow's milk.

6. General Discussion and Conclusions

The main goal of heating oilseeds is to reduce ruminal protein degradability and therefore increase the concentration of amino acids available for digestion in the small intestine (Van Soest, 1994). In addition, the amount of polyunsaturated fatty acids reaching the small intestine might also increase. Results of the first experiment indicated that roasting sunflower seeds using a particulate medium heat processor, in which salt was heated at 250° C served as medium (4:1 salt to seed ratio, 60 second contact time) is an effective method to reduce ruminal degradability of sunflower seed protein and therefore increase its ruminal undegraded protein value. The heating method did not seem to have detrimental effects on the protein quality of sunflower seeds as indicated by the lack of a statistically significant increase in acid detergent insoluble protein. However, the roasting process reduced the lysine concentration of sunflower seeds. Other researchers also reported no adverse effects of moderate heat treatments on quality of ruminal undegraded protein (McKinnon et al., 1995; Mustafa et al., 1999; Mustafa et al., 2003b).

The objective of the second experiment was to determine the effects of feeding diets containing raw and roasted sunflower seeds (7.8% of the diet dry matter) on ruminal fermentation parameters and total tract nutrient utilization. Results showed that sunflower seed supplementation did not alter the ruminal pH, ammonia-nitrogen concentration and total volatile fatty acid concentration suggesting that oil supplementation from sunflower seed up to 5.8% ether extract of the diet did not negatively affect the microbial fermentation in the rumen. The lack of detrimental impact of sunflower supplementation on ruminal fermentation may in part be attributed to the fact that sunflower seeds were fed as whole seeds. This probably slowed down the release of dietary oil in the rumen and therefore reduced the exposure of ruminal bacteria to polyunsaturated fatty acids. Sunflower seed supplementation or heat treatment did not affect total tract nutrient digestibility.

In the third study the effects of feeding diets containing raw and roasted sunflower seeds on milk yield and milk composition were determined using primiparous and multiparous

cows. Results showed that sunflower seed supplementation decreased dry matter intake of multiparous cows. This is likely due to the higher energy content of the sunflower seed diets compared with the control diet. Sunflower seed supplementation had no effect on milk yield despite an increase in the energy density of the ration. Several authors reported similar results where feeding heated oil seeds did not increase milk yield (Chouinard et al., 1997b; Santos et al., 1998). A decrease in microbial protein synthesis in the rumen, poor essential amino acid profile of the ruminal undegraded protein, low intestinal digestibility of the ruminal undegraded protein and sufficient amount of ruminal undegraded protein in the diet before supplementation were suggested to explain the lack of positive response of milk yield to increased rumen undegraded protein (Santos et al., 1998; NRC, 2001).

Sunflower seed supplementation caused milk fat depression in multiparous cows. The observed milk fat depression is likely due to the increase concentrations of trans-10, cis12 conjugated linoleic acid, which is known to be associated with milk fat depression (Baumgard et al., 2000; Bell and Kennelly, 2003). Even if modifications of ruminal fermentation parameters in the first study seemed to be minor, changes in ruminal processes might have been significant enough to alter ruminal biohydrogenation process of linoleic acid and this could have resulted in an increased proportion of trans-10, cis-12 CLA reaching the mammary gland. However, proportions of individual isomers of CLA were not measured during this experiment.

Sunflower seed supplementation altered the fatty acid composition of milk by reducing the concentrations of short and medium chain fatty acids and by increasing the concentrations of long chain fatty acids. Roasting sunflower seeds might have provided a partial protection against ruminal biohydrogenation of fatty acids since concentration of linoleic acid was higher in milk fat of primiparous cows and a similar tendency was observed for linoleic acid and trans oleic acid in multiparous cows. The changes in milk fatty acid profile observed as a result of sunflower seed supplementation are in good agreement with other studies (Casper et al., 1988; Markus et al., 1996; Mohamed et al., 1988).

Sunflower seed supplementation increased the concentration of conjugated linoleic acid (CLA) in the milk of both primiparous and multiparous cows. Supplementation of roasted sunflower seeds increased the concentration of CLA in milk of multiparous cows more than supplementation of raw sunflower seeds. This also suggests that roasting provided some protection against complete ruminal biohydrogenation of fatty acids contained in sunflower seeds.

In conclusion, it is clear that roasting was an effective heat treatment to reduce ruminal degradability of sunflower seeds. However, supplementing dairy cows with roasted sunflower seeds had no advantage over the control diet in term of milk yield.

In future research, it would be interesting to see if modification of the basal diet could prevent the occurrence of milk fat depression as was observed in our study. Possible modifications could include increasing the forage-to-concentrate ratio or increasing the quantity of fiber in the diet. It would also be interesting to determine if increasing the roasting temperature of sunflower seeds would be beneficial in reducing milk fat depression, but also in increasing milk yield, and increasing milk concentrations of long chain fatty acids and conjugated linoleic acid.

7. References

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