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....AND THE METABOLISM OF WHOLE SEEDS IN THE CASTROINTESTINAL

....TRACT OF CANNULATED HOLSTEIN STEERS.....

THESIS ....M.Sc., 1985.....

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THE USE OF WHOLE SUNFLOWER SEEDS IN DAIRY CATTLE RATIONS AND THE METABOLISM OF WHOLE SEEDS IN THE GASTROINTESTINAL TRACT OF CANNULATED HOLSTEIN STEERS

by

Brian Gregory White

A thesis
presented to the University of Manitoba
in partial fulfillment of the
requirements for the degree of
Masters of Science
in
Department of Animal Science

Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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entitled: THE USE OF WHOLE SUNFLOWER SEEDS IN DAIRY CATTLE RATIONS AND THE METABOLISM OF WHOLE SEEDS IN THE GASTROINTESTINAL TRACT OF OF CANNULATED HOLSTEIN STEERS

submitted by Brian White

in partial fulfillment of the requirements for the degree of

Masters Degree (Science)

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THE USE OF WHOLE SUNFLOWER SEEDS IN DAIRY CATTLE RATIONS AND THE METABOLISM OF WHOLE SEEDS IN THE GASTROINTESTINAL TRACT OF CANNULATED HOLSTEIN STEERS

BY

BRIAN GREGORY WHITE

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

ABSTRACT

The following experiments were undertaken to determine (1) the effect of whole sunflower seeds (WSS) on milk production and milk composition of lactating dairy cows, and (2) to determine the effect of WSS on the flow of fat and fatty acids through the gastrointestinal (GI) tract of cannulated steers. Twelve Holstein cows were randomly assigned to four treatments in a Lucas Switchback Design with periods of four weeks in length. Experimental diets were control, 1% $NaHCO_3$, 9% WSS and 1% $NaHCO_3$ plus 9% WSS. WSS had no effect on dry matter (DM) intake or milk yield. Milk fat percentage and milk fat yield measured during week four were elevated ($P<0.05$) when cows consumed WSS compared to the sunflower oil containing control diet. Feeding WSS had no effect on volatile fatty acids or ammonia levels in the rumen. The yield of de novo synthesized fatty acids (butyrate to palmitate), except octanoic acid, at week four were significantly higher ($P<0.05$) for cows consuming WSS than cows consuming the sunflower oil control diet.

Four cannulated Holstein steers were randomly allotted to the diets in a 4 X 4 Latin Square Design. Experimental diets were 4% Sunflower oil (SFO), 10% WSS, 20% WSS and 10% canola meal (LFCM). Addition of fat to the diet, as an oil

or as WSS, significantly reduced ($P<0.05$) the ratio of acetate to propionate compared to the LFCM diet; however no differences ($P<0.05$) occured among the oil diets. Lower inclusion of fat in the diet resulted is significantly more ($P<0.05$) fat being synthesized in the rumen, and there appeared to be a negative effect of high inclusion of fat (SFO and 20%WSS) on fat flow. Increasing the level of dietary fat resulted in significantly ($P<0.05$) more fat being absorbed in the small intestine. There was a decrease output of linoleic acid and a subsequent increase outflow of stearic and palmitic acids in the rumen compared to dietary intake. The three oil diets had a higher ($P<0.05$) flow of octadecanoic acids than the LFCM diet, and only the flow of stearic acid differed ($P<0.05$) among the three oil diets. No differences ($P>0.05$) were observed among the four diets for apparent digestibilities of DM, crude protein, and energy. Fiber digestion was unaffected ($P<0.05$) by the increasing fat levels of the diet, while fat digestiblities increased ($P<0.05$) with increasing dietary inclusion of fat. True digestibility of sunflower oil, determined from the acid solvent extract method was 83.4%.

ACKNOWLEDGEMENTS

Appreciation is extended to Dr. G.D. Phillips, Department Head, for access and use of department facilities. The author is sincerely grateful to his advisor, Dr. J.R. Ingalls, for always being there to provide support and guidance throughout my two years.

Sincere thanks are extended to faculty members on my committe, Dr. A.D. Graham and Dr. R.J. Boila of Animal Science, and Dr. B.R. Stefansson of Plant Science. Thanks are also extended to Dr. H.R. Sharma and for his assistance in editing this manuscript. Warm thanks to Dr. G. Crow and Bob McKay for their assistance with statistical analysis.

I am indept to Dr. N.E. Stanger and Ms. J. Haines for their surgical prepartation of the experimental animals. Also appreciations are extended to the dairy barn staff at the Glenlea Research Station as well as the staff in the Metabolism Unit. It is with gratitude that I acknowledge Mr. J.A. McKirdy, Mr. P.A. Mills, Ms. H. Stelsosky, Ms. H. Bernatsky, Ms. L. Iverson, and other technicians for their unselfish assistance in the labratory.

Finally, I am especially thankful to the graduate students of the Department of Animal Science, many of them good

friends of mine, for allowing an atmosphere where the exchange of ideas and cultural experiences could occur.

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Introduction

In recent years more and more producers are feeding high grain-low roughage rations to dairy cows, in order to more nearly satisfy the energy requirements during early lactation. This practice of feeding more grain and less roughage has resulted in the increased occurance of low milk fat tests, thereby lowering the efficiency of energy utilization (Palmquist and Jenkins, 1980). The inclusion of high caloric density ingredients like oils and fats into the diet would allow for higher forage to concentrate rations thereby preventing low milk fat yields. Also oil seeds, like cottonseed and soybean, which are now readily available due to increased world production, have high energy and protein densities. These high density feedstuff would allow for increased energy efficiency for milk production.

Research has demonstrated that (1) the fatty acid composition of milk fat is influenced by the incorporation of fats and oils, and that (2) the composition of milk fat is not a reflection of the fatty acids in the diet (Storry, 1981). Feeding large amounts of polyenoic oils and fats has resulted in milk fats being composed of saturated and 'trans' monoenoic acids rather than unsaturated fatty acids. Consumed esterfied polyunsaturated fatty acids undergo extensive hydrolysis and hydrogenation in the rumen by the microbial population. Furthermore, there has been research

indicating that feeding fats and oils may negatively affect digestiblities of other nutrients by altering rumen metabolism such to reduce animal performance.

For the above reasons, nutritionists have been interested in feeding fats and oils that are protected from microbial activity in the rumen. Australian workers devised a method of encapsulating the lipid into a formaldehyde-casein complex which allow fats to flow through the rumen unaltered. Due to the acidic conditions of the abomasum, the formaldehyde-casein bonds are weakenend, allowing for subsequent digestion and absorption in the small intestine. This method has provided a means of increasing the proportions of polyunsaturated fatty acids in milk fat; however, the utlilization of this procedure at the producer level is impractical due to the cost of applying the protective coating.

Currently there has been increased interest in feeding whole oil seeds to early lactation, high producing cows because of the high energy, high protein and medium fiber levels of such seeds. However, information concerning the feeding value of whole oil seeds is limited. The increased interest is related to the belief that whole seeds may allow for altered rates of release of the oil into the rumen media because of a natural fibrous coating excapsulating the oil. The negative affects of the oil on microbial activity may thus be diminished.

Sunflower plants, genus name Helinanthus annuus L. are native to North America. In 1983 farmers in Manitoba seeded 43,000 hectares (ha), yielding 1,120 kgs/ha, which is approximately 88% of the Canadian supply of sunflower seeds. Sunflower seeds contain 17 to 20% crude protein and 35 to 40% oil, which has a very high content of linoleic acid (72% of total fatty acids detected). The potential merit of a high fat and protein content has led researchers to examine whole sunflower seeds as an ingredient to be used in dairy rations.

The objective of the present study was: (1) to determine the feeding value of whole sunflower seeds for lactating cows; and (2) to determine the effect of sunflower seeds on the flow of fat and fatty acids through the gastrointestinal tract of cannulated steers.

LITERATURE REVIEW

Lipid Metabolism in the Ruminant

Feeding large amounts of unsaturated fats to monogastric species can alter the fatty acid content of depot fat or milk fat. In contrast, feeding large amounts of unsaturated fats to ruminants results in an increase of stearic acid and substantial amounts of 'trans' isomers (Church, 1969). Since trans isomers and branched and odd number length chains are not characteristic of plant lipids, gastrointestinal synthesis of these fatty acids must have occured. Literature since the midcentury indicates that the synthesis of isomers and branched and odd length chain fatty acids occurs in the rumen by the microflora (Church, 1969).

Gastrointestinal lipid metabolism in the ruminant can be divided into two phases: (1) reticulo-rumen metabolism; and (2) intestinal metabolism. Lipid metabolism in the ruminant has been extensively reviewed by Noble (1978).

Fat intake of dairy cows depends on the type of feed being consumed but the diet is generally less than four percent fat. Ruminants being fed roughages will consume large quantities of fatty acids in the form of glycolipids and phospholipids, whereas when fed concentrates, the fatty acids will be in the form of unesterified fatty acids and

their esterified triglycerides (Church, 1969). A second major difference between roughages and concentrates is that linolenic (C18:3) is the major fatty acid in roughages, whereas linoleic (C18:2) is the major fatty acid in concentrates (Church,1969).

Reticulo-Rumen Metabolism

Reticulo-rumen metabolism of lipids can be subdivided into three steps, of which the first two are intrinsically bound together. The first step involves hydrolysis of dietary lipids; the second involves biohydrogenation of unsaturated fatty acids; and the third involves the synthesis of microbial fatty acids. Although these three processes occur concurrently, for convience they will be discussed seperately. Reticulo-rumen lipid metabolism has been reviewed by Keeney (1970); Viviani (1970); and Harfoot (1978).

Biohydrogenation

Biohydrogenation of unsaturated fatty acids was first demonstrated by Reiser (1951) who observed a transformation of linolenic acid to linoleic acid after incubating linseed oil with rumen contents. Research since then has shown that the biochemical reaction of hydrogenation is not a simple process.

Shorland et al (1957) carried out a number of detailed in vitro experiments incubating oleic, linoleic and linolen-

ic acids with rumen contents. These researchers found that 20% of each acid was completely hydrogenated and that 17, 48, and 67%, respectively were converted to a trans isomer. Ward et al (1964) using labelled unsaturated fatty acids in an in vitro system, and Wilde and Dawson (1966) using an 'artifical rumen' technique, confirmed the general findings of Shorland et al (1957). Shorland et al (1957) suggested that unsaturated fatty acids were hydrogenated at an equal rate. However, data by Ward et al (1964) demonstrated that the hydrogenation of linolenic acid was slower than linoleic and oleic acid.

The first step involved in the multistep biohydrogenation of linolenic and linoleic acid involves an isomerization or migration of the double bond (Kepler and Tove,1967). Linolenic acid undergoes isomerization to form an octadecatrienoic acid with a conjugated diene system which was identified as cis,trans,cis octadeca 9,11,15 trienoic acid (Kemp and Dawson,1968). Similiarly, linoleic acid undergoes isomerization to produce cis,trans octadeca 9,11 dienoic (Kepler et al,1966). Kepler and Tove (1967) demonstrated that the enzyme responsible for the isomerization has a specificity for a free carboxyl acid group of an unesterified fatty acid.

The subsequent pathways after the isomerization are less clear. From studies with washed cell suspensions, it

appears that there are two seperate hydrogenation systems, one reducing the diene (cis,trans octadeca 9,11 dienoic acid) to a monoenoic acid, and a second converting the monoenoic acid to stearic acid (Polan et al,1964). Kelper et al (1966) using an isolated bacterium confirmed the findings of Polan et al (1964), when they observed that the bacterium Butyrivibrio Fibrisolvens could convert the dienoic to monoenoic acid but lacked the capability of reducing monoenoic acid to stearate. Polan et al (1964) suggested that there was a rate limiting step in the hydrogenation of unsaturated fatty acids. After incubating 0.25 mg of linoleic acid with rumen contents, stearic acid represented 32% of the total counts and oleic was only 10% of the total counts. Incubating eight fold the amount resulted in a 23% of the total count being oleic acid and only 10% was stearic acid. Polan et al (1964) concluded that the hydrogenation of linoleic acid to stearate could not occur until the level of the monoenoic acid exceeded that of the dienoic acid. Ward et al (1964) observed a similiar response when linolenic acid was incubated.

The reduction of linolenic acid by rumen bacterium is more confusing. Wilde and Dawson (1966) incubated labelled linolenic acid with rumen contents, and, after the initial isomerase reaction, observed a number of octadecadienoic acids with various degrees of unsaturation and positional isomers. Similiar findings were reported by Kepler and Tove (1967).

In general, the literature indicates that hydrogenation occurs first in the cis 9 bond; then the cis 15 bond; and finally the trans 11 bond to form stearic acid (Kemp and Dawson,1968).

The conversion of oleic acid to stearic acid is much faster than the conversion of the monoenoic acid to stearic and there is essentially no interconversion of oleic to trans monoenoic (Ward et al,1964).

Initial in vitro experiments by Wright (1959,1960) suggested that biohydrogenation activity was present in both bacteria and protozoa. Chalupa and Katches (1968) found that oligotrich protozoa had an ability to hydrogenate; however, holotrich protozoa had no ability to hydrogenate unsaturated fatty acids. Kemp et al (1975) isolated a number of bacteria that were capable of hydrogenating unsaturated fatty acids.

Hydrolysis

Hydrolysis of esterified fatty acids, which is a prerequiste for subsequent hydrogenation of unsaturated fatty acids was first demonstrated by Garton et al (1958). Garton et al (1958) observed that a rumen microbial suspension could hydrolyze the ester linkage between fatty acids and glycerol of linseed oil triglycerides. Hawke and Silock (1969) confirmed the findings of Garton et al (1958); how-

ever, the enzyme responsible for lipolysis is still being investigated. Review of the literature has indicated that lipolysis is an extracellular process. However, whether the enzyme is a cell bound esterase or a lipase that is secreted from the cell, is still uncertain. Henderson (1971) observed a lipase that was secreted while earlier findings by Clarke and Hawke (1970) indicated that the lipases were all cell bound and not released into the surrounding medium. Similiarily, Garton et al (1961) observed that the microbes were in close association with the lipid material, suggesting a cell bound lipase.

Hawke and Silock (1969) demonstrated that hydrogenation occured only after hydrolysis. After incubating labelled synthetic triglycerides with rumen contents, Hawke and Silock (1969) found that free fatty acids cleaved off from the triglycerides were biohydrogenated; however, the fatty acids that remained attached to the triglycerides retained their double bond.

Galactoglycerides, phospholipids, sterols, and esters undergo extensive hydrolysis in the rumen, as discussed by Church (1969), Viviani (1970) and Harfoot (1978).

The glycerol released from esterified lipids is fermented to yield volatile, water soluble, fatty acids (Garton et al, 1961; Viviani, 1970; and Harfoot, 1978).

Synthesis of Lipids by Microbes

Prior to discussion of bacterial and protozoal biosynthesis of lipids, it is pertinent to discuss the lipid composition of the rumen microorganisms.

Rumen bacterial lipids consist of 70% nonphospholipids and 30% phospholipids, whereas rumen protozoa are composed of 30% nonphospholipids and 70% phospholipids (Viviani, 1970). The nonphospholipids consist of large quantities of saturated, trans octadecamonoenoic acids and significant proportions of odd number length, branched chain fatty acids (BCFA) (Viviani,1970). Phospholipids characteristically have large amounts of linoleic and linolenic acids (Katz and Keeney, 1967).

Viviani and Lenaz (1965) demonstrated that rumen bacteria were cabable of synthesizing straight and branched chain C15:0 acids. After feeding a synthetic protein-lipid free diet to sheep, Viviani and Lenaz (1965) observed high levels of branched C15:0 acids, suggesting an active metabolic pathway for de novo synthesis of fatty acids.

Incubating rumen contents with labelled isovalerate and isobutyrate, Allison et al (1962) found extensive labelling in branched long chain fatty acids of both even and odd carbon numbers. Their results were confirmed by Wegner and Foster (1963), who concluded that the incubation of isoval-

erate resulted in branched long chains of odd carbon numbers and isocaproate resulted in even carbon numbers.

Using washed suspensions of rumen protozoa, Emmanual (1974) demonstrated that rumen protozoa are cabable of de novo synthesis of fatty acids from labelled short chain fatty acids. Emmanual (1974) concluded that short chain fatty acids are the precursors for fatty acid synthesis and malonate serves as the extender of carbon chain length.

The synthesis of poylunsaturated fatty acids is restricted to aerobic organisms, due to an obligatory requirement of the desaturase enzyme for oxygen. Since the rumen is highly anaerobic, the presence of polyunsaturated fatty acids in the structural lipids of rumen microbes was the consequence of uptake of unsaturated fatty acids from the rumen media (Harfoot, 1978). Emmanual (1974) suggested that rumen protozoa have the capability of synthesizing monounsaturated fatty acids, but that C18:2 and the C18:3 fatty acids of rumen protozoa were of dietary origin.

The ability of bacteria and protozoa to absorb long chain fatty acids has been demonstrated by Harfoot et al (1974) and Broad and Dawson (1975). Using a pure culture of protozoa incubated with labelled long chain fatty acids, Broad and Dawson (1975) showed the relative uptake of fatty acids were linoleate>oleate> palmitate>stearate. Harfoot et al (1974), however found the relative uptake of long chain

fatty acids by bacteria was greatest with saturated fatty acids and decreased with increasing unsaturation.

Besides saturase and limited desaturase activity, Emmanual (1974) showed that protozoa were capable of α oxidation, β oxidation and chain elongation by two carbon units.

Incubating labelled acetate with mixed suspensions of rumen microflora, Patton et al (1970) observed an increase in the de novo synthesis of phospholipids. Patton et al (1970) suggested that protozoa and bacteria were responsible for a large extent of the synthesis of phospholipids. Broad and Dawson (1975) confirmed those findings using a pure culture of protozoa. The protozoa were shown to absorb choline; synthesis phosphatydylcholine; and incorporate the molecule into the cell membrane.

Intestinal Metabolism

Flow of Lipids

After extensive modifications of dietary lipids in the rumen, the digesta passes through the omasum, abomasum and into the small intestine (SI) for absorption (Noble, 1978). In general, lipids passing into the SI consist of free fatty acids that are in close association with particulate matter and structural lipids of microorganisms (Bath and Hill, 1967).

Using re-entrant cannulae, Bath and Hill (1967) were able to demonstrate that the lipid distribution of digesta leaving the rumen was similiar to the digesta passing into the SI, however they did observe an increase in the amount of lipid passing in the duodenum compared with that ingested from the diet. The most likely source of this extra lipid was from the microbial synthesis in the reticulo-rumen and subsequent release in the abomasum (Leat and Harrison, 1969).

Felinski et al (1964) observed that digesta entering the duodenum had a greater content of unsaturated fatty acids and phospholipids than digesta leaving the rumen. When biliary and pancreatic secretions from the duodenum were diverted to outside the body, Leat and Harrison (1969) observed a marked decrease in the phospholipids and unsaturated fatty acids of the duodenal digesta. Leat and Harrison (1969) suggested that these secretions could be the source of the increased unsaturated fatty acids and phospholipids that Felinski et al (1964) observed.

On analyzing the bile and pancreatic secretions, Leat (1965) found no lipids in pancreatic juice, whereas bile had high concentrations of phospholipids, particularly lecithin and lysolecithin. Fatty acid analysis of bile phospholipids revealed high proportions of saturated fatty acids in α positions and high proportions of unsaturated fatty acids in β positions.

Digestion and Absorption

Upon reaching the small intestine, the digesta is mixed with duodenal, biliary and pancreatic secretions, which together are necessary for optimal lipid absorption (Heath and Morris, 1963). Since a majority of the lipids were hydrolzyed in the rumen, digestion in the duodenum can be described as the release of particulate bound fatty acids and the formation of soluble micelles. Leat and Harrison (1969) observed a gradual transfer of fatty acids from particulate matter to micelles as the digesta passed along the SI.

Lennox et al (1968) quantitatively determined, with the use of reentrant cannulas, the site of absorption for unesterified fatty acids. Results clearly indicated that the major site for lipid absorption was between the middle and lower jejunum, with small proportions being absorbed under the acidic conditions of the upper jejunum.

Bickerstaffe et al (1972) suggested that there was no difference in absorption rates between the major fatty acids; however, researchers now believe that the overall rate of absorption is related to fatty acids chain length and degree of saturation. Lennox et al (1968) observed that the monounsaturated fatty acids were absorbed faster than saturated fatty acids: oleic>palmitic> stearic. Similiarly Harrison and Leat (1972) observed changes in absorption due to chain length and degree of saturation.

Role of Bile and Pancreatic Juices

As stated earlier, bile and pancreatic juice are essential for optiminal lipid absorption. Heath and Morris (1963) observed a 10% depression in the absorption of fatty acids when pancreatic juice was diverted, and an even greater depression when bile was diverted. Leat and Harrison (1969) had similiar observations and concluded that bile and pancreatic juice were involved in the solubilization of fatty acids.

Solubilization of fatty acids is primarily due to the action of bile salts, with phospholipids, lecithin and lysolecthin, playing a complimentary role. The role of lecithin and lysolecthin is uncertain; however, it is believed that lysolecithin, a powerful detergent, expands the fatty acid micelle for absorption, similiarly to the action of monoglycerides in absorption of fatty acids in monogastrics (Harrison et al, 1974). Lecithin appears to be a precursor for the synthesis of phospholipids that are responsible for the stabilization of lymph lipid droplets (Leat and Harrison, 1974). Since lecithin is unabsorable, conversion to lysolecithin is a prerequisite for absorption. Resterification to lecithin will occur in the mucosal cell (Leat and Harrison, 1974).

The role of pancreatic juice is less well defined. Review of the literature indicates that the pancreas secretes

two phospholipases, one acid labile and the other acid stable (Leat and Harrison, 1975). The second phospholipase would therefore be predominant and cleave lecithin in the ß position, releasing lysolecithin and unsaturated fatty acids. Pancreatic juice aso contains an acid labile lipase that is capable of hydrolyzing any esterified fatty acids that escape rumen hydrolysis. The optimum pH for this lipase is 7.5, with neglible activity below 5.5; therefore, any hydrolysis would occur after the midjejunum (Noble, 1978).

Transportation of Lipids

Once the fatty acids have been absorbed into the mucosal cells resynthesis into triglyceride occurs via the *a* glycerol phosphate (Bickerstaffe and Annison, 1969; and Leat and Harrison, 1974) and not the monoglyceride pathway as in monogastrics. The monoglyceride pathway is still important when excess triglycerides escape rumen hydrolysis (Scott et al, 1971).

Digestion is a continuous process; therefore, absorption of lipids is continuous and is characterized by the lymph having a permanent milky appearance (Leat and Harrison, 1975). Although lymph transportation is continuous, concentration of lipid in lymph fluctuates due to irregular peristalsis in the jejunum (Leat and Harrison, 1975).

Lymphatic fluid lipids consist of 75% triglycerides and 25% phospholipids with neglible amounts of cholesterol and free fatty acids (Felinski et al, 1964; and Leat and Harrison, 1974). This high percentage of phospholipids results in smaller lipid droplets in the lymph compared to monogastrics which have less than 10% phospholipids (Harrison et al, 1974). Fatty acid analysis of lymph triglycerides and phospholipids revealed the triglycerides were comprised of saturated fatty acids whereas lymph phospholipids had a high percentage of unsaturated fatty acids (Leat and Harrison, 1975). Bickerstaffe et al (1972) suggested that the mucosal cells are capable of converting stearic to oleic; however, Leat and Harrison (1974) stated that there is no desaturase activity present in the mucosal cells.

Under normal conditions, three quarters of the lymph lipid is associated with the very low density lipoproteins (VLDL) and one quarter with chylomicrons. In contrast, cholesterol and phospho lipids are the principal components of the lipid in plasma, with small quantities of triglycerides and free fatty acids; and most of the plasma lipid is transported as high density lipoproteins (HDL) (Puppione, 1978).

Milk Fat Synthesis

The subject of milk synthesis has been extensively reviewed by Dimuck et al (1970), Emery (1973), and Smith and Abraham (1975). In general, short chain fatty acids (4 to

10 carbon numbers) are synthesized de novo from acetate and ß hydroxybutyrate; long chain fatty acids (18 carbons and above) are absorbed from the plasma pool of triglycerides; and the intermediate chain acids (12 to 16 carbons) originate from either source. Approximately 50% of milk fatty acids are synthesised within the mammary gland and the remaining 50% are absorbed from the blood (Palmquist and Mattos, 1978).

Effects of Fats and Oils

The effects of feeding fats and oils on milk production and compostion has been variable (Palmquist and Jenkins, 1980). To discuss the effects of fats and oils on milk production and composition adequately, reference must be made to the effect of fats and oils on rumen metabolism. The effect of saturated and unsaturated fatty acids and the effect of feeding protected fats and whole seeds on milk fat yield and composition will also be discussed.

Feeding fats and oils to lactating dairy cows has resulted in a number of variable responses due to the difference in the composition of the basal diets and the type of fat being incorporated into the diet (Christie, 1978). Steele and Moore (1968a) observed that dietary supplementation of cottonseed oil and tallow increased daily milk yields, whereas Steele and Moore (1968b) and Storry et al (1973) observed no significant change in daily milk yield

with dietary supplementation of cottonseed oil or tallow, respectively. Storry et al (1967) fed dietary coconut oil and red palm oil to lactating dairy cows and observed a non-significant increase in milk yield. Feeding dietary soyabean oil resulted in a nonsignificant increase in daily milk yield (Steele et al, 1971; and Banks et al, 1976).

There has been a similiar inconsistency in the response of daily milk yields when protected fats were fed to lactating dairy cows. Wrenn et al (1977) observed an increase in daily milk yield when protected tallow was fed; however, Dunkley et al (1977) and Smith et al (1978) observed no apparent increase. Goering et al (1977) reported no significant increase in daily milk yield with dietary supplementation of protected soyabean oil and cottonseed oil.

Steele et al (1971) and Hutjens and Schultz (1972) reported increases in milk yield with dietary supplementation of whole soyabeans, however Perry and Macleod (1968) observed no significant change in milk yield. Feeding whole cottonseeds (Anderson et al, 1979) and whole sunflower seeds (McGuffey and Schingoethe, 1982 and Rafalowski and Park, 1982) resulted in an increase in daily milk yields.

Previous research has shown that type of fat in the diet can affect the fatty acid compostion of milk (Storry, 1970). Dietary fat can alter the milk fat composition in a number of ways: (1) a specific fatty acid may be absorbed,

transported to the mammary gland, and incorporated into the milk fat globule unchanged; (2) specific fatty acids can be first altered in the rumen and then incorporated into the globule; (3) desaturation can occur in the mammary gland prior to esterification; (4) long chain fatty acids (LCFA) can alter the VFA metabolism in the rumen, thereby reducing the substrate availability for de novo fatty acid synthesis in the mammary gland; and (5) LCFA can inhibit de novo fatty acid synthesis in the mammary gland.

There has been a lack of consistent responses reported in the literature with respect to the effect of the addition of fats and unesterified fatty acids on milk fat yield (Christie, 1978). Steele and Moore (1968a) and Banks et al (1976) observed significant improvements in daily milk fat yield with lactating dairy cows fed tallow or vegetable oils, whereas Cook et al (1972) and Storry et al (1973) observed no significant change. The yield of milk fat is the reflection of a balance between the yields of the two sources of fatty acids, i.e. de novo synthesized fatty acids (DSFA) and fatty acids absorbed from the plasma (Storry, 1970).

Studying the effects of saturated fatty acids on milk fat secretion, Steele and Moore (1968c) showed that dietary supplementation of lauric acid decreased, stearic and palmitic increased, and myristic acid had no significant effect

on milk fat yield. Similiar observations were reported by Noble et al (1969). Daily milk fat yields were depressed when dietary oleic was fed to lactating cows (Steele and Moore,1968c).

Depressed yields of short and intermediate fatty acids, have been frequently observed with the addition of native oils and unsaturated fatty acids to the diet (Brumby et al, 1972; Noble et al 1969; Steele and Moore, 1968a,b,c; and Storry et al, 1974). Decreased yields of DSFA appears to be a consequence of decreased substrate availability for synthesis in the mammary gland and/or an inhibition of the rate limiting enzyme, acetyl-coA carboxylase, in fatty acid synthesis (Storry et al, 1980).

Steele and Moore (1968d) and Storry et al (1967) observed a decrease in the short chain fatty acids (SCFA) of milk with dietary supplementation of lauric and mystric acids, in a pure or natural esterified form (coconut oil) and subsequent increase in the yield of lauric and mystric acid in the milk fat globule. The decrease in SCFA may have been the result of a reduction of substrate availabitity due to changes in the fermentation pattern in the rumen. Noble et al (1969) observed a similiar decrease in the synthesis of SCFA with dietary supplementation of palmitic and stearic aicd. Noble and his coworkers observed no significant alterations of volatile fatty acid (VFA) production in the

rumen and postulated that palmitic and stearic inhibit DSFA by inhibiting the acetyl-CoA carboxylase enzyme.

Steele and Moore (1968c,d) observed increases in the concentration of palmitoleic and oleic acids of milk fat with dietary supplementations of palmitate and stearate, respectively. Similiar observations were reported by Noble et al (1969). The increase in the monoenoic acids was probably produced by desaturation of saturated fatty acids in the mammary gland (Noble et al, 1969). Desaturation of palmitic to palmitoleic occurs at one fifth the rate of desaturation of stearic to oleic (Bickerstaffe and Annison, 1968).

Dietary supplementation of oleic acid results in increased yields of stearic and oleic acid with a decrease in the DSFA (Steele and Moore, 1968c). Dietary oleic acid appears to effect the availabitity of precursors for de novo synthesis rather than inhibiting the acetyl-CoA carboxylase enzyme (Steele and Moore, 1968c). Steele and Moore (1968c) observed a significant decrease in the relative concentrations of acetate and butyrate, while Storry et al (1969a) observed an increase in milk fat yield and no change in the yield of DSFA, in response to infusion of esterfied oleic directly into the blood. Selner and Schultz (1980) observed no significant difference in milk fat percentage when cows were supplemented with 250 ml or 500 ml of oleic acid.

Dietary supplementation of tallow (Storry et al, 1973) and cottonseed oil (Steele and Moore,1968b) increased the yield and relative percentage of stearic and oleic acid whereas the yields of DSFA decreased. Tallow supplementation had no significant effect on ruminal concentrations of VFA's. Therefore, the reduced synthesis was most likely in response to an inhibition of the rate limiting enzyme, not to a decreased substrate availability.

Dietary supplementation of saturated oils and fatty acids had no effect on the mammary yields of butyrate (Storry et al, 1967; and Steele and Moore, 1968c). The yield would remain unchanged because the primary pathway of synthesis of milk butyrate, is via the crotonyl-CoA reductase route, which is not inhibited by LCFA.

Research on the dietary supplementation of unsaturated vegetable oils has shown that the yields of stearic and oleic acid in milk fat increase while the yields of DSFA decrease and there is little or no changes in the yields of linoleic and linolenic acid (Macleod and Wood,1972 and Banks et al, 1976). The increase in the stearic acid yield is the consequence of rumen hydrogenation whereas the increase in the oleic acid is due to biohydrogenation (of which up to 25% can be of the trans isomer) and due to desaturation of stearic acid (Banks et al, 1976).

The feeding of cod liver oil to dairy cows resulted in depressed milk fat yields (Brumby et al, 1972). The reduction in milk fat yield appeared to be related firstly to, a reduction in the yield of mammary DSFA because of a change in the rumen fermentation pattern and secondly to a reduction in the yields of fatty acids taken up from the plasma. The longer chains (C22:0 to C24:0) inhibit the incorporation of the C18 polyunsaturated fatty acids into triglycerides and inhibit the uptake of the C18 polyunsaturated fatty acids by the mammary gland (Brumby et al, 1972).

Steele et al (1971) observed that the response of milk fat yield and composition depended upon the physical form in which the oil was supplied. Steele et al (1971) reported that milk fat yield was depressed when soybean oil was fed but yield was increased when an equal amount of oil was fed in the form of intact soybean. Similiar observations were reported by Perry and Macleod (1968) and Hutjens and Schultz (1971). There was a reduction in the yields of intermediate fatty acids and increases in the yields of stearic, oleic and linoleic when the two fat rations were fed to lactating cows (Steele et al, 1971).

Unesterified linoleic acid released from whole seeds in the rumen underwent complete biohydrogenation to form stearic acid whereas biohydrogenation of linoleic acid from the soyabean oil produced a number of geometric isomers (Steele

et al, 1971). The former resulted in more linoleic acid in the milk fat whereas the latter resulted in more trans isomers. Similiar observations were reported by Perry and Macleod (1968) and Hutjens and Schultz (1971). The increase in the yield of linoleic acid in the milk fat, when whole soybean were fed to lactating cows, was probably a reflection of delayed release of intracellular oil into the rumen for hydrolysis and hydrogenation by the rumen microflora (Steele et al, 1971).

The decrease in intermediate fatty acids was probably due to the inhibition of the acetyl-CoA carboxylase enzyme, by the increased uptake of LCFA, rather than an alteration of rumen VFA patterns. Perry and Macleod (1968) and Steele et al (1971) observed no significant changes in the proportions of VFA produced in the rumen. Hutjens and Schultz (1971) and Steele et al (1971) observed marked increases in the unsaturation of circulating lipids and Steele et al (1971) observed an increase in the yields of butyrate in milk fat.

There has been a significant increase in the use of whole cottonseeds in the diets for lactating cows, due to the increased availability and the unique feature of being both a high fiber and high energy feedstuff (Smith et al, 1981). Feeding graded levels of whole cottonseed (0,5,15,25%) to dairy cows resulted in increases in the

yield of milk fat and fat-corrected milk and decreases in the yield of protein and solids-non-fat (Smith et al, 1981). Similiar findings were reported by Anderson et al (1979).

Smith et al (1981) reported that the yields of milk fatty acids were altered significantly by feeding of whole seeds. Synthesis of fatty acids within the mammary gland decreased as the level of inclusion of whole cottonseed increased. However, there was a two fold increase in stearic acid and oleic acid on the higher level of supplementations when compared to the control diet. Whole seeds had no effect on the yields of linoleic acid in milk fat (Smith et al, 1981). The depression of intermediate chain fatty acids was attributed to decreased enzyme activity in the mammary gland (Smith et al, 1981), since no effect on the pattern of rumen fermentation was found (Anderson et al, 1979).

Similarily the utilization of whole sunflower seeds in dairy rations has increased. Rafalowski and Park (1982) fed graded levels of whole seeds (0,10,20,30%) to lactating dairy cows and observed a significant increase in the yield of milk and no change in the yield of milk fat. Whole sunflower seeds were found to result in a decrease in the yield of DSFA, no change in the yield of linolenic and an increase in the yield of oleic acid (Rafalowski and Park, 1982; McGuffey and Schingoethe, 1982).

There has been interest within the last 15 years in increasing the level of polyunsaturated fatty acids in the milk by feeding supplements of vegetable oils that are protected against hydrolysis and biohydrogenation in the rumen. The technique of encapsulating fat within a protein coating and then crosslinking with formaldehyde was developed by Australian workers, Scott and coworkers and has since been reviewed by Fogerty and Johnson (1980).

Feeding dietary supplements of protected polyunsaturated oils to lactating cows has increased the daily yield of milk fat as much as 25% over the control diet (Christie,1978). This increase in milk fat yield was a consequence of the additional amounts of linolenic acid being incorporated into the milk fat. As much as 35% of the total fatty acids in milk were linolenic acid (Astrup et al, 1976; Goering et al, 1977 and Wrenn et al, 1977). As linolenic acid concentration of milk fat increased the concentrations of the intermediate fatty acids decreased. A change in rumen fermentation resulted in a diminished availibility of substrate to the mammary gland (Goering et al, 1977).

There has been some interest in protecting saturated fats to increase the intake of dietary energy and efficency of milk production in high yielding dairy cows. Supplementation with protected saturated fats, such as tallow, generally has invoked similiar responses in milk fat yield and

composition as protected polyunsaturated fatty (Storry et al, 1974; Dunkley et al, 1977; Astrup et al, 1976; and Sharma et al, 1978).

The method of protecting whole seeds with formaldehyde has resulted in a cheaper cost due to a decreased requirement for casein (Scott et al, 1971). Chandler et al (1973) observed an increase in daily fat yield and an increase up to 26% in the proportions of linoleic acid in milk fat, with dietary supplementation of formaldehyde treated sunflower seeds (FT-SS). Feeding graded levels of FT-SS, Earle et al (1976) observed similiar results.

Effects on Rumen Metabolism

Fiber digestibility in the rumen has been reported to be depressed by the addition of dietary fats (Devendra and Lewis, 1974); however, the interaction between lipid and fiber digestibility is still unclear. Devendra and Lewis (1974) summarized several theories which have been advanced to explain the interaction: (1) prevention of microbial digestion by physical coating of the fiber with fat; (2) modification of the rumen microbial population due to a toxic effect of fatty acids on microbes; (3) inhibition of the microbial activity due to an effect on cell permeability; and (4) reduced availability of minerals for microbial growth and activity.

Devendra and Lewis (1974) observed physical coating of the fiberous component by maize oil and tallow; most studies support the hypothesis of inhibitory effect of lipids on microbial population and activity. By incubating pure cultures of bacteria with different fatty acids, Henderson (1973) demonstrated that unsaturated fatty acids inhibited rumen microbes, particularly cellulolytic and methanogenic bacteria. Similiar observations were reported in vivo and Czerkawski et al (1975).

Czerkawski (1973) reported no significant change in the total number of bacteria by the addition of linseed oil fatty acids, but did observe a decrease in propionic and lactic production, and a small increase increase in fecal excretion of cellulose. Thus the type of fermentation was adversely affected and similiar observations were reported by Czerkawski et al (1975).

The lipolytic ability of rumen microbes was altered when the fat content of the diet was increased (Czerkawski et al, 1975). Czerkawski et al (1975) observed that the quantity of polyunsaturated fatty acids undergoing hydrogenation was decreased when dietary linseed oil was increased from 60 to 90 g/day. Reduction in the hydrogentation of unsaturated fatty acids is associated with a reduction in the number of protozoa (Clarke and Hawk, 1970), and the number of Butyrivibrio spp bacterium (Polan et al, 1964). Czerkaw-

ski (1973) and Czerkawski et al (1975) observed drastic reductions in the number of protozoa in the rumen.

The adverse effects of fat on fiber digestion were not observed when protected lipids were fed in the form of whole seeds (Smith et al, 1981) and formaldehyde treated lipids (Sharma et al, 1978; Bines et al, 1978; and Mattos and Palmquist, 1974).

Buffers

Researchers over the past three decades have tried to alleviate metabolic disorders such as the low milk fat syndrome, associated with feeding high grain-low roughage diets by the addition of buffers such as sodium bicarbonate ($NaHCO_3$). The effects of $NaHCO_3$ have been variable.

Dry Matter Intake and Milk Yield

Erdman et al (1980) demonstrated a beneficial effect of adding $NaHCO_3$ to a total mixed ration for cows during early lactation. Cows fed rations containing 1.5% $NaHCO_3$ produced greater ($P<0.05$) yields of milk at four weeks; however, this effect was diminished after eight weeks. Similarily cows consumed significantly greater amounts of feed at 5 weeks, but no benefical effects were observed after eight weeks. Similiar findings were reported by Kilmer et al (1980).

Erdman et al (1982a) observed that cows on control diets consumed higher DMI as a percentage of BW during weeks 1 and 2; however cows receiving diets containing $NaHCO_3$ consumed more at the end of the experiment than controls. Erdman et al (1982a) observed no significant effect on milk production. English et al (1982) reported no difference in DMI or milk yield between diets containing $NaHCO_3$ and control diet during the first eight weeks of lactation.

Kilmer et al (1980) studied the effects of adding $NaHCO_3$ to rations for dairy cows pre and postpartum. Results indicated that actual DMI or DMI as a percent of BW was significantly greater for cows fed buffers only postpartum (CB) than control or rations feed pre and postpartum (BB). During the first nine weeks, actual milk yield produced by cows fed CB and BB diets was significantly greater than yields by cows fed the control diet. Kilmer et al (1980) concluded "compared to control, cows fed sodium bicarbonate adapted to rations more rapidly postpartum and there was no benefical effect of adding to the ration prepartum".

Rogers et al (1982) suggested that there was no benefical effect of adding buffers to rations for cows past peak production. Emery et al (1964, 1965) and Miller et al (1965) were in agreement in reporting that the addition of buffers caused a slight decrease in DMI and milk yields for cows in midlactation.

Erdman et al (1982b) compared the sudden addition to gradual addition of $NaHCO_3$. Sudden addition of $NaHCO_3$ to the concentrate resulted in decreased DMI during the first two weeks; however, differences disappeared by week four. Similarily any effect on milk production by the sudden addition of $NaHCO_3$ appeared to be temporary. Erdman et al (1982b) concluded " reductions in concentrate consumption were temporary and there was a slight advantage in milk production to the gradual addition of$NaHCO_3$".

Milk Fat

The effect of buffers on milk fat percentage has been variable. Since milk fat depression is correlated to changes in the molar proportions of volatile fatty acids (VFA) in the rumen (Davis and Brown, 1970), the effect of buffers on VFA production will be discussed. Reference will be made to the effect of buffers on ruminal pH and dilution rate.

The effect of buffers on VFA production and metabolism has been variable; however, a finding that is consistent throughout the literature is that ruminal propionate concentration decreased with the addition of buffers (Davis, 1978). Emery et al (1964,1965) and Miller et al (1965) reported a significant increase in the ruminal molar percentage (molar %) of acetate, a significant reduction in ruminal molar % of propionate and a significant increase in milk fat percentage. Kilmer et al (1980) reported no signinficant

effect of buffers on propionate in the rumen and milk fat percentage.

McCullough (1966) was the first to report a relationship between propionate and milk fat percentage. The data indicated a highly significant negative relationship between the ruminal molar % of propionate and milk fat percentage. On examing the relationship further, McCullough (1966) found the relationship of propionate to fat only held true for a molar % of propionate above 25%. Hence, there would be little benefit for the addition of buffers to diets that result in a molar % of propionate less than 25 as was the case of the experiment of Kilmer's et al (1980). The control ration did not show a milk fat depression and the molar percentate of propionate after eight weeks was 23%.

McCullough (1966) also noted a positive relationship between the molar ratio of acetate to propionate and milk fat percentage. McCullough (1966) showed that there was a linear increase in milk fat percentage as the ration of acetate:propionate increased from a value of 1 up to 2.2. However, there was little change in milk fat percentage above 2.2. Erdman et al (1980) confirmed these findings. Since the relationship between molar % of VFA's in the rumen and milk fat percentage is well established, the next question to be dealt with is the mode of action of buffers.

Research clearly indicates that the quantity of VFA's produced by the rumen microbial population are influenced by ruminal pH (Esdale and Satter, 1972). However, the effect of sodium bicarbonate on rumen pH is variable.

Emery et al (1965); Miller et al (1965) and Rogers et al (1982) found an increase in ruminal pH when the addition of $NaHCO_3$ resulted in an increase molar % of acetate and reduced the molar % of propionate. Kilmer et al (1980) observed no changes in ruminal pH or molar ratio of acetate to propionate with the addition of $NaHCO_3$, and Erdman et al (1980, 1982a) observed a slight increase in ruminal pH and a significant change in in the molar % of acetate and propionate. Erdman et al (1982a) suggested that the changes were related to an increase in the liquid turnover which will be discussed.

Esdale and Satter (1972) showed that ruminal VFA production was not measurably altered between a pH of 6.2 to 6.8. Therefore the effect of exogenous buffers would not be observed with any basal diet which yielded a ruminal pH value between these two values; such was the case in the study of Kilmer et al (1980). Esdale and Satter (1972) futher showed that a pH change between 5.6 and 6.2 resulted in an increase in the total VFA production with a minor increase in molar % of acetate and a decrease in the molar % of propionate. This would explain the results of Rogers et al

(1982) where cows consuming a basal diet had a ruminal pH of 5.98 and through the addition of $NaHCO_3$, the ruminal pH increased to 6.16, with a significant increase in the molar ratio of acetate to propionate.

Since a relationship exists between ruminal pH and VFA production, the question to be dealt with next is whether the altered rumen fermentation in response to an elevated pH is due to a change of microbial population or whether an elevated pH causes existing microbes to shift their metabolism towards an increase in acetate fermentation.

Long term change undoubtly occurs in the rumen microbes in response to decreased pH associated with low roughage feeding (Latham et al, 1973). However, in vitro results from Esdale and Satter (1972) indicate that the immediate changes in the production of VFA is due to altered metabolism of microbes. Results by Thomas et al (1978) confirm Esdale and Satter (1972) findings when they observed that Selonomends, a group of microbes associated with a propionate fermentation remained the major component of microbial population in the rumen of sheep supplemented with mineral salts, even though there was a shift to an acetate type of fermentation.

Since the molar % of propionate is negatively correlated with milk fat percentage and since ruminal pH is a major determinant of propionate production, supplements that increase ruminal pH should have a positive effect on milk fat percentage.

As stated earlier, Erdman et al (1980, 1982a) reported a nonsignificnant increase in ruminal pH with the addition of buffers; however, there was a significant changes in the acetate:propionate ratio. It therefore appears that other factors are important in determining the molar % of acetate and propionate. Hodgson and Thomas (1972) were the first to suggest that propionate production is negatively correlated to rumen dilution rate, defined as the proportion of total rumen volume leaving the rumen per hour. Such a correlation has since been confirmed by Hodgson and Thomas (1975), Harrison et al (1975,1976) and Rogers et al (1979).

Harrison et al (1975) observed that the dilution rate and the pattern of fermentation was altered when artificial saliva was infused into the rumen of sheep, but there was no significant change in the ruminal pH. Rogers and Davis (1982) observed a similiar response upon intraruminal infusion of sodium bicarbonate or salt which caused a significant change in the dilution rate. Bicarbonate infusion caused a significant increase in ruminal pH and molar % of VFA's, when compared to the salt infusion, however molar % of acetate and propionate were similiar for both type of infusions. Salt infusion had no effect on ruminal pH.

The mode of action for the increase in dilution rate or the associated change in the rumen fermentation is still uncertain. Harrison et al (1975) observed that intraruminal

infusion of artifical saliva increased the dilution rate, whereas intraruminal infusion of water resulted in a slight decrease in dilution rate and concluded that tonicity of saliva played an effect on dilution rate. Rogers et al (1979) observed that infusions of water plus mineral salts resulted in an increase in the dilution rate and outflow of water from the rumen.

Rogers et al (1979) observed that the infusion of mineral salts caused a significant increase in rumen osmolarity and a subsequent increase in plasma osmolarity. An increase in plasma osmolarity will cause an increase in water consumption, thereby increasing the dilution rate in the rumen (Rogers et al, 1979). Warner and Stacey (1977) suggested an inverse relationship between plasma osmolarity and salivary flow. Under normal conditions, when the transruminal water flux is minimal, water outflow is equal to water inflow. However, when plasma osmolarity increases, there is an increase in water consumption and a decrease in salivary flow, resulting in an increase in the transruminal waterflux or water outflow is greater than inflow of water (Rogers et al, 1979). Associated with the increased dilution rate was an increase in the molar % of acetate and a decrease in the molar % of propionate (Rogers et al, 1979). These results are consistent with Harrison et al (1975, 1976) and Rogers et al (1982).

Rogers and Davis (1982) observed a positive response in rumen dilution rate in steers fed high grain diets with mineral salt inclusion, but, when steers were fed a high roughage diet, the infusion of mineral salts had no significant effect on dilution rate or pattern of VFA production. Rogers and Davis (1982), however did observe that the dilution rate was two fold higher on a roughage diet compared to a high grain diet.

Rogers and Davis (1982) further noted that mineral salts altered the molar % of acetate; however, the acetate production (moles/day) was not affected and propionate production was significantly decreased by the infusion of mineral salts.

The question arises whether the reduction in propionate production was due to reduced microbial activity or reduced substrate availability to microbes. Rogers and Davis (1982) observed a slight change in microbial activity; however, this could not account for the significant decrease in propionate production. Thus, the decrease must be due to a reduction in substrate availability. Rogers et al (1982) observed that the flow of starch out of the rumen was increased by the infusion of mineral salts. Harrison et al (1976) also observed an increased outflow of hexoses out of the rumen due to an increased dilution rate associated with the infusion of artifical saliva.

The alteration of rumen fluid dilution rate associated with mineral salt infusions affects VFA production in the rumen, without changing ruminal pH, by altering the proportion of nutrients fermented.

Digestiblity

Rogers et al (1982) reported a significant increase in dry matter and organic matter digestibilities, upon the inclusion of mineral salts; however, Rogers and Davis (1982) and Synder et al (1983) reported no significant change in DM or organic matter (OM) digestibilities with the addition of mineral salts. Rogers et al (1982) reported that any change in DM or OM digestibilitiy was related to a change in acid detergent fiber (ADF) and starch digestibility.

Emmanual et al (1969) reported that the optimum pH range for cellulotic activity was between 6.7 and 7.0, and that at lower pH values, the activity decreased. Erdman et al (1982a) reported a linear relationship between rumen pH and ADF digestion, and suggested that any supplement that increased the pH would increase fiber digestion. The effect of buffers on fiber digestion has been extensively reviewed by Merten (1979).

Rogers and Davis (1982); Rogers et al (1982) and Synder et al (1983) reported no significant change in protein or ether extract digestiblities upon $NaHCO_3$ feeding.

Markers

Introduction

To fully understand absorption of nutrients by animals, it is essential to have knowledge of partition of digesta within the gastrointestinal (GI) tract (Faichney, 1975). Two techniques have been developed to estimate net absorption in a particular section of the GI tract. The first method involves the use of reentrant cannulas as discussed by MacRae (1975). The second technique, used to measure flow rate as well as retention times and volumes, involves the use of markers.

Type of Markers

The two purposes of dietary markers are (1) to measure the rate of passage of digesta along the GI tract; and (2) to act as an inert reference to measure digestiblitiy, volumes or flow rates (MacRae,1974).

Warner (1981) catagorized markers into four catagories: (1) dietary markers which are normal constituents of the feed, which go undigested and unabsorbed through the GI tract; eg, lignin; (2) liquid phase markers which stay in solution throughout the GI tract; eg, polyethylene glycol (PEG) and/ or the complex of chromium (Cr) or Cobalt (Co) with ethylenediamine tetra acetic acid (CrEDTA or CoEDTA,respectively); (3) particulate markers which are insolu-

ble throughout the gut; eg, chromic oxide (Cr_2O_3); and (4) particle markers which are originally in solution and become physically bound or chemically associated with the diet; eg, rare earth elements like dysprosium (Dy). The use of markers has been extensively reviewed by Kotb and Luckey (1972), MacRae (1974), and Faichney (1975).

Faichney (1975) summarized the criteria for an ideal GI marker as: (1) must be nonabsorbable, therefore complete recovery should occur; (2) it should have no effect on the GI tract as well as the microbial population; (3) it must be physically similiar to or intimately associated with the material it is marking; and (4) there should be a specific and accurate method for analysis.

To date none of the available markers satisfy all of the criteria (Uden et al, 1980) because all markers are difficult to determine accurately and some markers are not fully recovered (eg CrEDTA). However, markers can be used effectively as long as the limitations of a marker are known (Warner, 1981) and as Theurer (reported in Young et al, 1975) states "the recovery of a marker may not be complete, as long as it is consistent".

Liquid Phase Markers

Two water soluble markers for measuring rumen liquid volume and dilution rate are PEG and CrEDTA. Both markers

are absorbed to a small degree and in the case of CrEDTA, half of the absorbed marker is excreted in the urine (Downes and MacDonald, 1964). Downes and MacDonald (1964) reported that only 95% of the original dose of CrEDTA was recovered in the feces and 2.5% appeared in the urine. PEG recovery in the feces was slightly less, and no significant amount appeared in the urine. Similiar digestibility coefficients were calculated with either marker (Downes and MacDonald, 1964); however, due to the lack of a specific and sensitive method of analysis for PEG, the popularity of this marker as a reference marker is limited. ^{51}CrEDTA, on the other hand, is used quite extensively due to the simple accurate analysis (Downes and MacDonald, 1964). With the ability to account for small urinary excretions, simple corrections can be made during the digestion trial (Kotb and Luckey, 1972).

Uden et al (1980) compared a new liquid marker (CoEDTA) to CrEDTA and found that the new marker was comparable. Both markers were excreted in the urine at low levels (2-3%); however, the lossses for CoEDTA were some what higher.

PEG, CrEDTA and CoEDTA were examined by Teeter and Owens (1983) to understand the behavior and similiarity of water soluble markers (WSM) more fully. In general, there was no significant difference between the WSM in estimating rumen volume or dilution rates, suggesting that all three

markers are biologically similiar. This is in contrast with Goodal and Kay's (1973) data which suggests that PEG consistently yielded lower rumen volume estimates than CrEDTA.

In general all the markers tested by Teeter and Owens (1983) underestimated rumen volume when compared to total evacuation by an average of 4%. Teeter and Owens (1983) relate this to a number of factors including that WSM are being absorbed and that WSM are being bound to particulate matter. Warner (1981) suggested that CrEDTA binds to ruminal particulate matter under conditions yet undefined, and this binding could cause an overestimation of dilution rate. Teeter and Owens (1983) also observed an increase in dilution rate four hours after feeding, however whether the increase is due to an increase in rumen volume or to an increase in liquid flow is unknown. Earlier results by Warner and Stacey (1968) agree that there is an increase in dilution rate immediately after feeding.

Solid Markers

Chromium Sesquoxide

Chromium sesquoxide (Cr_2O_3) first utilized by Edin in 1918 (reported in Kotb and Luckey, 1972) is probably still the most commonly used inert marker used in digestibility trials (Chamberlain and Thomas, 1983). The only requirement for the use of Cr_2O_3 in digestibility trials is that it must

be fully recovered and, unlike other particulate markers, Cr_2O_3 does not require close association to digesta (MacRae, 1974). Kotb and Luckey (1972) indicated a number of studies showing that Cr_2O_3 passes along the GI tract unabsorbed and can be quantitatively recovered in the feces. Earlier methods for Cr_2O_3 determination were titration and colorimetry; however, these methods were tedious and researchers questioned the accuracy of the analytical procedures (Utley et al, 1970). The use of atomic absorption spectroscopy, introduced in 1955 by Walsh (reported in Williams et el, 1962) provided a rapid and accurate method for determining Cr_2O_3. Cr_2O_3 could also be determined in feed and feces samples at lower levels with the new method (Williams et al, 1962).

Utley et al (1970) used radioactive $^{51}Cr_2O_3$ to determine if Cr_2O_3 was absorbed and secondly to determine if the coeffecients of apparent digestiblities of DM, crude protein, and ADF derived from markers were similiar to those obtained with the total fecal collection technique. Results indicated the 98% of the ingested $^{51}Cr_2O_3$ was recovered and that coefficient of digestibilities were not significantly different. Data by Crampton and Llyod (1951) indicated that Cr_2O_3 gave lower estimates of digestibilites if the material was unground.

Even though recovery of Cr_2O_3 is a major concern, Cr_2O_3 is used in digestiblity trials because of its low cost, be-

ing safe to use, and lack of any special animal accomodations (Chamberlain and Thomas, 1983).

Dysprosium

The use of rare earth elments for estimating particulate phase digesta flow has been discussed by Ellis (1968); Hartnell and Satter (1979b) and Crooker et al (1982).

One of the most distinquishing properties in addition to being essentially undigestible and unabsorbable by livestock, is that rare earth markers posses an affinity for particulate matter, and therefore might be expected to flow through the GI tract closely associated with feed particles (Ellis, 1968). This affinity would reduce variation in concentrations of fecal markers attributed to the differential flow rates of feed particles and markers from the reticulum-rumen (Corbett et al reported in Kotb and Luckey, 1972).

Although rare earth markers have a high affinity for particulate matter, movement of marker among different particulate fractions has been observed. Research by Faichney and Griffth (1978) shows an exchange occurred between large and small particle fractions. Retention times are thus underestimated. Hartnell and Satter (1979a) observed that when rare earth markers were applied to grain and hay particles, an average of 92.6 and 99.0% remained associated with the original grain or hay particles, respectively. In sub-

sequent experiments, Hartnell and Satter (1979b) found that the concentration of markers on stained hay particles was 15 times greater than that found on stained grain samples. Differences between the affinities of hay and grains for markers can be explained by the fact that grains are more readily digested than hay. Upon digestion the released markers may be reabsorbed unto hay particles, or form insoluble hydroxides and get entrapped by hay particles or the markers by be engulfed by bacteria that get entrapped in hay particles (Hartnell and Satter, 1979b).

Crooker et al (1982) observed significant marker movement. Incubating four feeds with one of two markers, in polyester rumen bags for 12 hours, Crooker et al (1982) observed increases in marker contamination on feed residues, when compared to inital levels.

Crooker et al (1982) suggested that the difference between the two studies could be related to the specific binding capacity of feeds for markers. Feed particles have a specific binding capacity for markers and when excess markers are added, weak binding sites would occur. During rumen fermentation, dissociation would occur at the weak sites accounting for most of the observed marker movement (Crooker et al, 1982). Dissociation of strong binding sites can occur under acidic conditions, as found in the abomasum (Crooker et al, 1982).

The use of radioisotopes in digestion trials has gained considerable attention (Kennelly et al, 1980); however, the use of radioisotopes necessitates the complete collection and approved disposal of feeds and feces, as well as safe disposal of experimental animal carcasses at the end of the experiment (Young et al, 1976). These problems can be eliminated by the inclusion of nonradioactive stable isotopes, which can be activated after the samples have been collected (Young et al, 1976). The basic principle of activation is that the stable isotope undergoes a nuclear transformation when immersed in an intense flux of neutrons to produce a radioactive nucleotide which emits radiation of characteristic energies (Young et al, 1975).

Instrumental neutron activation analysis (INAA) to determine concentrations of stable isotope markers in feed and digesta provides a degree of sensitivity not available to other methods such as mass spectrometry and atomic absorption (Kennelly et al, 1980).

The use of dysprosium (Dy) as a representative of rare earth markers in digestion trials has been demonstrated by Ellis (1968); Young et al, (1975, 1976) and Kennelly et al (1980). Dy was recommended as an inert indicator in digestion trials due to consistent recoveries with minimal variations, reliable analysis procedure and short half life (T1/2 = 1.26 min) compared to other markers where the half life is

much longer (Chromium, T1/2 = 27.8 days). With longer half lifes, more backgroud activity occurs and therefore analysis time would take longer and adequate storage facilities are necessary for active material (Kennelly et al, 1980).

Marker Technique

Marker technique will be considered in relation to the method of administration and sampling (Faichney, 1975). Markers can be given continuously or as a pulse dose. Samples are taken from sections of the tract at successive times or the animal can be slaughtered and then the digesta collected (Faichney, 1975). Different possiblities can occur including continuous infusion with time sequence sampling and pulse dose with total collection.

Continuous infusion with time sequence sampling requires that the animal be cannulated at different points along the GI tract. The technique is used primarily to measure flow rate; however, volume and mean retention times for a particular section of the GI tract can be determined when samples are taken after infusion of markers has stopped (Faichney, 1975).

Faichney (1975) and Warner (1981) have extensively reviewed the different marker techniques for digestion experiments.

FEEDING WHOLE SUNFLOWER SEEDS AND SODIUM BICARBONATE TO DAIRY COWS IN MID LACTATION

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ABSTRACT

Twelve holstein cows, 13 to 15 weeks postcalving, were randomly assigned to four treatments in a Lucas Switchback design with periods of four weeks in length. Experimental diets were control, 1% $NaHCO_3$, 9% whole sunflower seeds (WSS) and 1% $NaHCO_3$ plus 9% WSS. Diets without WSS were balanced with sunflower meal, sunflower oil and wheat straw. The isonitrogenous, isocaloric total mixed 65:28:7 (concentrate:cornsilage:hay) diets were fed ad libitum once a day.

Total dry matter intake (DMI) and milk yield were not different ($P>0.05$) among treatments. Milk fat percentage and yield were significantly ($P<0.05$) increased by dietary inclusion of WSS. Diet had no effect ($P>0.05$) on milk protein or lactose. Total Volatile Fatty Acids (VFA) and the molar percentage of individual VFA and rumen ammonia were unaffected ($P>0.05$) by dietary treatments. Molar percentage of short chain fatty acids (SCFA) in milk did not change ($P>0.05$) with dietary inclusion of $NaHCO_3$ and WSS alone but there was a significant ($P<0.05$) effect on molar % of SCFA when both ingredients were fed. Cows which consumed WSS had significantly ($P<0.05$) lower polyunsaturated fatty acids in milk fat than control animals. Yields of de novo synthesized fatty acids (butyrate to palmitate) at week four in general were significantly ($P<0.05$) increased when cows consumed WSS

but no treatment ($P>0.05$) was found to affect the yield of octanoic acid. Although not significant ($P>0.05$) $NaHCO_3$ tended to result in higher levels of de novo synthesized fatty acids.

INTRODUCTION

The common practice of feeding dairy cows high ratios of fermentable carbohydrates to fibrous constituents has resulted in a significant reduction in milk fat yield (Davis and Brown, 1970). Early research indicated a benefical effect on milk fat depression by the addition of buffers to the diet (Emery et al, 1961,1964,1965; and Miller et al,1965). More recent research on feeding $NaHCO_3$ at parturition to aid cows in the switch from high forage ration prepartum to a high grain diet postpartum have resulted in conflicting findings on milk fat percentage (Erdman et al, 1980; Kilmer er al, 1980,1981; and Erdman et al, 1982a).

High producing cows in early lactation are often in negative energy balance because energy intake is less than the high energy output required for milk production. Researchers have attempted to increase energy intake by the inclusion of 3 to 5% oil or fat in the diet. However dietary supplementation of certain oils and unsaturated fatty acids may cause a reduction in milk fat secretion (Davis and Brown, 1970).

There has also been considerable attention given to the metabolic effects of feeding protected fats and oils to dairy cows (Macleod and Wood, 1972a,b; Mattos and Palmquist,1974; Palmquist and Conrad, 1980; Sharma et al, 1978; and Wrenn et al, 1977); however, information concerning the feeding of whole oil seeds, which may have a natural protection against rumen microflora, is limited. Several sources of whole oil seeds have been studied including soybean seeds (vanDijk et al, 1983 and Anderson et al, 1984); cottonseeds (Anderson et al,1979 and Smith et al,1981); and sunflower seeds (McGuffey and Schingoethe,1982; and Rafalowski and Park, 1982).

This study was undertaken to evaluate the addition of $NaHCO_3$ and/or whole sunflower seeds to a diet expected to cause a milk fat depression.

MATERIALS AND METHODS

Twelve holstein cows, 13 to 15 weeks post calving, were randomly allotted to four treatments in a Lucas Switchback Design with periods of four weeks in length. The four test rations were control; 1% $NaHCO_3$; 9% Whole Sunflower Seeds (WSS) and 1% $NaHCO_3$ plus 9% WSS (Table 1). The 65:28:7 (concentrate:corn silage:long hay) diet were fed ad libitum once a day as a total mixed ration. All diets were formulated to be isonitrogenous and isocaloric. Diets without WSS were balanced by the addition of sunflower meal, sunflower oil and chopped straw.

Table 1. Ingredient and Chemical Composition of Total Mixed Rations for Cows Receiving Sodium Bicarbonate and/or Whole Sunflower Seeds (WSS)

Ingredients (kgs)	Diets			
	Control	$NaHCO_3$	WSS	$NaHCO_3$+ WSS
Rolled Barley	20.0	19.5	20.0	20.0
Triticale	25.1	24.7	25.1	24.8
Soybean Meal	8.1	8.1	8.5	8.5
Whole Sunflower Seeds	-	-	9.0	9.0
Sunflower Oil	3.1	3.1	-	-
Sunflower Meal	4.1	4.1	-	-
Wheat Straw	1.8	1.8	-	-
Dicalcium Phosphate	0.8	0.8	0.7	0.7
Limestone	1.0	1.0	1.0	1.0
Salt	0.4	0.4	0.4	0.4
Vitamin Premix†	0.4	0.4	0.4	0.4
$NaHCO_3$	-	1.0	-	1.0
Hay	7.0	7.0	7.0	7.0
Corn Silage	28.0	28.0	28.0	28.0
Total	100.0	100.0	100.0	100.0

Nutrient Composition of Concentrates, Hay and Corn Silage by Analysis (% DM)

	Control	$NaHCO_3$	WSS	$NaHCO_3$+ WSS	Hay	Silage
Protein	19.0	18.9	18.3	17.6	21.9	10.1
Ether Extract	4.1	3.9	5.7	5.6	2.5	3.4
ADF	8.6	8.6	8.1	7.7	28.4	33.1
Calcium	1.1	1.1	0.9	0.7	-	-
Phosphorus	0.8	0.8	0.8	0.8	-	-
Energy (Kcal/g)	4.3	4.2	4.4	4.4	-	-

†Provided per kilogram of diet: 3500 IU vitamin A, 1750 IU vitamin D, 10 IU vitamin E, 44 mg ZnO, 45 mg MnO_2*H_2O, 0.3 mg $CoCl_2$, 1 mg KI, 116 g MgO.

Cows were allowed to adjust to the experimental rations for the first two weeks of each period. Dry matter intake (DMI) and milk yield were recorded daily during the last two weeks, and milk samples were taken every second day during the last two weeks for analysis of fat, protein and lactose. Samples of concentrates, hay, corn silage and weighbacks were taken once weekly and composited monthly for chemical analysis.

During the third and/or fourth week, just prior to feeding and three hours after feeding, rumen liquor was taken via a stomach tube, and heparinized venous tail blood samples were taken using vacutainer tubes. Following seperation of plasma from the blood by centrifugation, the rumen and plasma samples were frozen for further analysis.

Chemical and Statistical Analysis

All feed and weighback samples were oven dried at 60°C for three days to determine dry matter percentage. Nitrogen and ether extract were determined according to Association of Official Analytical Chemist (1980). Gross energy was determined by Parr Adiabatic Oxygen Bomb Calorimeter and acid detergent fiber (ADF) according to Goering and Van Soest (1970).

Volatile fatty acids (VFA) in rumen fluid were determined by gas chromotography according to Erwin et al (1961)

and ruminal ammonia was analyzed by an ammonia electrode (Model 95-10, Orion Research,Cambridge, MA). Urea nitrogen of blood serum was analyzed by an autoanalyzer (Technicon Model No AAII-1). The method employed is a modification of the procedure of Marsh et al (1965). Milk fat, protein and lactose were measured by infra red spectroscopy (Milkoscan Model 203, Fosselectric, Cornwall, Ont).

Fat was extracted from weekly milk samples according to Lambert (1964) and the molar ratios of fatty acids of milk were determined by injections of a methylated sample (Metcalfe et al, 1966) onto a GP 3% SP2310/2% SP2300 on 100/120 Chromosorb WAW 8 ft by 1/4 in packed column. Peak areas for each fatty acid were analyzed using a Varian 402 Data System and expressed as a percentage of total fatty acids detected. Identification of each fatty acids was determined on the basis of retention time of a milk standard. Milk fatty acid yield was calculated by multiplying weekly milk yield by weekly yield of fat by molar % of individual fatty acids.

The data collected for all measurements except for milk fatty acid yields were analyzed statistically as a combined total of Week 3 and Week 4; whereas milk fatty acid yields were analyzed weekly. Data were analyzed by the General Linear Models of the Statistical Analysis Systems (Barr et al, 1976). Duncans Multiple Range Test was used to detect the significant ($P<0.05$) differences among treatment least square means (Steel and Torrie,1976).

RESULTS

Diets without seeds tended to cause greater weight gains but the effect was not significant ($P>0.05$) (Table 2). Total DMI, total crude protein intake and intake of ether extractable materials did not differ ($P>0.05$) among treatments (Table 2).

Dietary treatments (Table 2) had no effect on mean daily milk yield or fat corrected milk; however, supplementing the diets with WSS increased ($P<0.05$) milk fat percentage. The addition of $NaHCO_3$ to the control or WSS containing diet tended to increase fat percentage and fat yield; however, these differences were not significant ($P>0.05$). There was no dietary effect ($P>0.05$) on the percentage of milk protein or lactose.

Rumen ammonia nitrogen and blood urea nitrogen levels were not influenced ($P>0.05$) by dietary treatments (Table 3). Total VFA concentrations and molar percentage of acetate, propionate, butyrate and valerate did not differ ($P>0.05$) among treatments (Table 3).

The molar proportions of milk fat short chain fatty acids (C4:0-C10:0) were significantly higher ($P<0.05$) with the combination diet than the control diet (Table 4). There was no dietary effect ($P>0.05$) on medium chain (C12:0 to C16:0) fatty acids.

Table 2. Treatment Effects on Least Square Means for Daily Dry Matter Intake, Milk Production and Milk Composition.

Parameters	Diets				SE
	Control	$NaHCO_3$	WSS	$NaHCO_3$ + WSS	
Dry Matter Intake (kg)	22.3	24.1	21.5	21.0	1.30
Protein Intake (kg)	3.7	3.9	3.5	3.5	0.2
Fat Intake (kg)	1.01	0.88	1.04	0.94	0.07
Milk Yield (kg)					
Actual	24.5	25.8	24.8	25.2	0.9
4% FCM†	17.7	19.1	20.0	20.7	0.9
Milk Composition					
Fat %	2.16b	2.25b	2.70a	2.78a	0.14
Fat Yield (g)	526.5b	585.5b	672.3ab	705.8a	44.6
Protein %	3.3	3.2	3.2	3.2	0.05
Lactose %	4.8	4.8	4.8	4.8	0.06
Weight Gain (kg/day)	0.26	0.28	0.13	0.16	0.07

†Fat Corrected Milk

a,b means on the same row with different letters differ ($P<0.05$)

Table 3. Treatment Effects on Least Square Means for Rumen Metabolites and Blood Urea Nitrogen (BUN).

Metabolites (Hours postfeeding)		Diets				SE
		Control	$NaHCO_3$	WSS	$NaHCO_3$+ WSS	
Rumen Fluid						
Ammonia·Nitrogen	0h	6.3	7.9	7.7	8.3	1.1
(mg/100mL)	3h	7.2	6.5	7.4	7.4	1.1
Total VFA†	0h	4.1	3.4	4.1	3.7	0.5
(mmoles/100mL)	3h	4.9	4.8	5.0	4.2	0.6
VFA (molar %)						
Acetate (C_2)	0h	62.0	58.0	59.9	61.5	2.0
	3h	56.6	57.6	57.4	62.2	1.4
Propionate (C_3)	0h	21.5	25.5	22.9	24.0	1.9
	3h	22.2	24.3	23.9	22.5	1.7
Butyrate (C_4)	0h	12.0	12.8	11.9	11.2	1.2
	3h	15.8	13.5	13.5	11.8	1.2
Valerate (C_5)	0h	1.7	1.5	1.6	1.0	0.4
	3h	2.0	1.8	2.0	1.1	0.3
IsoVFA (iC_4+iC_5)	0h	2.3	2.2	3.1	1.9	0.5
	3h	3.2	2.5	3.2	1.9	0.5
C_2/C_3 Ratio	0h	2.9	2.4	2.8	2.7	0.3
	3h	2.6	2.5	2.5	2.9	0.2
BUN (mg/100mL)	0h	14.7	13.2	12.4	13.8	0.9
	3h	14.5	14.7	12.0	15.5	0.9

†Volatile Fatty Acids

Table 4. Treatment Effects on Least Square Means of Molar Percentage of Fatty Acids in Milk Fat.

Fatty Acids (molar %)	Diets				SE
	Control	$NaHCO_3$	WSS	$NaHCO_3$+ WSS	
C 4:0†	1.02	1.17	1.25	1.28	0.09
C 6:0	1.18	1.40	1.50	1.50	0.08
C 8:0	0.97	1.04	1.05	1.17	0.12
C10:0	2.03	2.29	2.47	2.62	0.13
C12:0	2.65	2.92	2.90	3.10	0.13
C14:0	10.5	10.2	11.3	11.1	0.8
C14:1	2.10	1.91	1.73	1.92	0.11
C16:0	23.9	23.7	23.6	25.3	0.6
C16:1	2.86	2.63	2.39	2.47	0.20
C18:0	9.4	8.5	9.9	9.9	0.8
C18:1	38.2	38.7	36.9	34.8	0.9
C18:2	3.76a	3.61ab	3.19c	3.35bc	0.12
Short (C4-C10)	5.19b	5.90ab	6.26ab	6.63a	0.35
Medium (C12-C16)	41.9	41.4	41.9	43.9	1.0
Long (C18 and up)	52.9	52.7	51.8	49.4	1.1
Saturated	53.1	53.2	55.8	57.5	1.1
Unsaturated	46.9	46.8	44.2	42.5	1.1

†Expressed as the number of carbons:number of bonds.

a,b,c means on the same row with different letters differ ($P<0.05$)

Overall there was a trend for the whole seeds diet to a higher molar percentage of de novo synthesis fatty acids (C4:0 to C16:0) than the pure oil diets. Feeding WSS tended to reduce the proportions of oleic (C18:1) and significantly decreased ($P<0.05$) the concentration of linoleic (C18:2) acid. There was no significant ($P>0.05$) difference among treatments with respect to the ratio of saturated to unsaturated fatty acids.

There was no significant difference ($P>0.05$) among treatments during week 3 for milk fat yield and yield of individual fatty acids (Table 5), however during week 4, there was a significant increase ($P<0.05$) in the yield of milk fat and DSFA's (butyrate to palmitate) with dietary supplementation of WSS.

DISCUSSION

Milk Fat Depression

Research by Broster et al (1978) and Macleod et al (1983) indicated a linear and quadratic effect of increasing ration energy density on milk yield and fat composition. Macleod et al (1983) reported that a 65:35 concentrate:forage ration similiar to that used in the present experiment resulted in a significant decrease in milk fat percentage due to the low ADF content. A minimum of 21% ADF is recommended (NAS-NRC,1978) to maintain milk fat percentage.

Table 5. Treatment Effects on Least Square Means of Fatty Acid Yields per Week

Fatty Acids (g/week)	Weeks	Diets				SE
		Control	$NaHCO_3$	WSS	$NaHCO_3$+ WSS	
Fat Yield	3	479.3	588.4	672.4	663.5	59.8
	4	525.1c	579.5bc	681.7ab	744.6a	42.6
C 4:0†	3	5.7	7.2	9.2	8.5	1.4
	4	5.4c	7.7bc	8.4ab	11.1a	1.1
C 6:0	3	6.6	8.9	10.9	10.3	1.7
	4	6.0c	8.8bc	10.2ab	13.6a	1.3
C 8:0	3	4.7	6.5	7.8	7.7	1.3
	4	5.1b	6.5b	7.3ab	10.0a	1.2
C10:0	3	11.6	14.8	17.1	18.2	2.5
	4	9.8c	13.8bc	17.4ab	21.5a	2.0
C12:0	3	14.6	18.0	19.6	21.1	2.4
	4	12.5c	17.4bc	20.9ab	24.6a	2.0
C14:0	3	55.3	61.1	79.9	75.2	8.3
	4	49.5c	61.1bc	72.4ab	83.6a	6.0
C14:1	3	9.8	11.7	11.4	12.2	1.0
	4	9.2b	10.2b	12.5ab	15.2a	1.2
C16:0	3	114.7	141.9	156.4	171.3	17.0
	4	121.7c	138.3bc	168.0ab	187.4a	11.1
C16:1	3	12.0	14.7	14.7	14.5	1.5
	4	13.7b	14.6ab	16.1ab	18.9a	1.5
C18:0	3	40.8	54.7	64.8	70.1	10.8
	4	47.9	60.9	69.6	73.8	10.6
C18:1	3	179.6	217.1	247.0	221.0	17.4
	4	201.9	219.8	244.4	253.0	16.4
C18:2	3	16.7	20.0	21.5	23.6	2.5
	4	20.8	21.8	22.9	23.0	1.7

†Expressed as the number of carbons:number of bonds.

a,b,c means on the same row with different letters differ ($P<0.05$)

The diets fed in the present experiment contained an average of 16.1% (15.9 to 16.2%) ADF.

Physical form of roughages influences milk fat percentage and yield (Sutton, 1981). Corn silage in the present study was finely chopped (4.72 mm) and data by Sudweeks et al (1979) indicated that finely chopped corn silage significantly reduced the ratio of acetate to propionate, which could decrease milk fat percentage.

Palmquist and Jenkins (1980) indicated that feeding polyunsaturated oils may decrease milk fat production by altering rumen fermentation with the result of a lower acetate:propionate ratio and/or by inhibiting the acetyl-CoA carboxylase enzyme responsible for de novo synthesized fatty acids (DSFA) within the mammary gland. Feeding sunflower oil in the control diet may have altered the rumen fermentation but this was not indicated by the molar ratios of VFA or total VFA as the levels are similiar for all diets (Table 4). Total rumen levels of VFA in the present study were lower than the quantities generally reported in other studies (Chalupa et al, 1984). There may have been dilution due to contamination with saliva when using a stomach tube. Also availability of acetate for synthesis of DSFA in the mammary gland could be low, resulting in low yields of individual fatty acids and depressed milk fat. Feeding sunflower oil may have decreased the yields of DSFA's by inhibiting the

acetly CoA carboxylase enzyme due to the significant increase in linoleic acid in milk. The inhibition of the enzyme by polyunsaturated fatty acids and oils has been observed by Mattos and Palmquist (1974), Storry et al (1973), and Macleod et al (1972a).

The combination of a high concentrate, low ADF diets with 3.1% of the diet as highly unsaturated sunflower oil and finely chopped corn silage played a role in the reducing fat test for cows consuming the control diet.

Effects of Buffers

Although differences between the control diet and $NaHCO_3$ were nonsignificant (Table 2), the diet containing $NaHCO_3$ exhibited the same trends of increasing DMI and milk production that were reported by Erdman et al (1980,1982) and Kilmer et al (1980,1981). Similar nonsignificant differences in body weight gains and percentage of milk protein were reported by Emery et al (1965) and Donker and Marx (1980).

Although several workers (Miller et al, 1965; Emery et al, 1965; Erdman et al, 1982; and Rogers et al, 1982) have reported an increase in milk fat percentage and yield with $NaHCO_3$, the lack of effect on fat test in the present experiment (Table 2) agrees with the results of Donker and Marx, 1980; Erdman et al, 1980; and Kilmer et al, 1980.

In contrast to other studies, where researchers observed a significant (Rogers et al, 1982 and Erdman et al, 1982) or nonsignificant (Erdman et al, 1980, and Kilmer et al, 1980,1981) increase in the ratio of acetate to propionate with the inclusion of $NaHCO_3$ to fat depressing diets, no difference occured in the present study (Table 3). Changes in the proportions of acetate to propionate have been related to increased liquid turnover rates in the rumen (Harrison et al,1975 and Rogers et al,1982).

Although $NaHCO_3$ had no apparent effect on changing rumen fermentation, the effect of $NaHCO_3$ (Table 4) on increased synthesis of SCFA (butyrate to decanoeic acid) suggests increased acetate availability for milk fat synthesis within the mammary gland.

Effects of WSS

DMI and milk production were not affected ($P>0.05$) by the inclusion of 9% WSS in the diet (Table 2), results which are in agreement with results reported by McGuffey and Schingoethe (1982) and Rafalowski and Park (1982). Rafalowski and Park (1982) observed a significant improvement in milk yields with 10% inclusion (4% of total ration), but a nonsignificant increase as the level of inclusion increased to 8 or 12% of the diet.

Although several workers (Mattos and Palmiquist, 1974; Sharma et al, 1978; and Dunkley et al, 1977) have reported a decrease in the percentage of milk protein when protected fats were fed, no effect was found in the present study (Table 2). A decrease in the percent of milk protein could be due to a dilution effect due to increased milk yield; however, Emery (1978) suggested that feeding excess protected lipids could decrease carbohydrate availability to the rumen microbes, thereby depressing production of microbial protein.

There was an improvement ($P<0.05$) in milk fat test with dietary inclusion of WSS (Table 2). Feeding approximately the same level of WSS (8% of the diet), Rafalowski and Park (1982) observed a nonsignificant improvement in milk fat test. Similiarly Smith et al (1981) observed a significant increase in fat yields from cows fed graded levels of cottonseed. Although Rafalowski and Park (1982) did not feed cottonseed, they suggest that the difference in the response to cottonseed compared to the sunflower seed, was possibly due to the unequal characteristics (ie the seed coat of the sunflower seed having a lower digestibility) of the two oil seeds. In a comparative study, Anderson et al (1984) confirmed these findings when they observed cows consuming cottonseed produced more milk with a higher percentage of fat than cows consuming WSS.

Yields of DSFA (except C8:0) at week 4 were increased ($P<0.05$) when whole seed were fed (Table 5). This was in contrast to the results of Palmquist and Conrad (1980); Sharma et al (1978); Smith et al (1981); McGuffey and Schingoethe (1982); and Rafalowski and Park (1982) who observed a decrease in SCFA when whole seed or protected oils were fed to lactating cows. However, in their experiments, oil was not included in the control diet. The increased yields of DSFA could be related to the increased availability of acetate but rumen fluid results (Table 3) did not support this concept. Similiar nonsignificant changes where reported by Rafalowski and Park (1982). The results may suggest however that there could be a decreased inhibition of the acetyl-CoA carboxylase when whole seed are consumed.

There was a significant ($P<0.05$) decrease in the percentage of linoleic acid and a nonsignificant decrease in oleic acid in milk fat when whole seeds were fed (Table 4). The decrease could be correlated to the decreased inhibition within the mammary gland of the acetyl-CoA carboxylase enzyme for DSFA. The decrease in the unsaturated C18 acids was probably related to the fibrous coating of the whole seeds. A study by Park et al (1982) revealed that sunflower hulls had low DM and ADF digestibilities suggesting a slower release of polyunsaturated fatty acids which would allow a more complete biohydrogentation of unsaturated C18 fatty acids to stearate. The data (Table 4) seem to favour this ex-

planation, since there was a nonsignificant increase in stearic acid. Similiar observations were reported by Rafalowski and Park (1982).

In summary, the addition of $NaHCO_3$ appeared to have little effect on milk fat level from cows receiving an oil containing fat depressing diet. The addition of whole sunflower seeds in place of straw and sunflower oil appeared to improve fat test.

ACKNOWLEDGEMENTS

In acknowledgements we would like to thank Agricultural Canada for financial aid, the Manitoba Department of Agriculture for analysis of milk samples, and the Glenlea Research Station Staff for their care of the experimental animals.

THE EFFECT OF WHOLE SUNFLOWER SEEDS ON THE FLOW OF FAT AND FATTY ACIDS THROUGH THE GASTROINTESTINAL TRACT OF CANNULATED STEERS

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ABSTRACT

Four Holstein steers, each cannulated in the rumen, abomasum and ileum, were randomly alloted to the experimental diets in a 4 X 4 Latin Square Design. Diets contained (1) 4% Sunflower Oil (SFO); (2) 10% whole sunflower seeds (WSS); (3) 20% WSS and (4) 10% canola meal (LFCM). Dysprosium was used to calculate the flow of the particulate digesta through the gastrointestinal (GI) tract.

The ratio of rumen acetate to propionate was increased ($P<0.05$) when the LFCM diet was fed compared to the three oil containing diets with no differences ($P>0.05$) among the oil containing diets. Nitrogen (N) retention as a percent of N intake was higher ($P>0.05$) for the three oil diet compared to the LFCM diet. Lower inclusion of fat in the diet resulted in significantly ($P<0.05$) more fat being synthesized in the rumen, while higher fat inclusion (SFO and 20%WSS) had a negative effect on fat flow through the rumen. Increasing the level of dietary fat resulted in significantly ($P<0.05$) more fat being absorbed in the small intestine. There was a decreased ($P<0.05$) output of linoleic acid and a subsequent increased ($P<0.05$) output of stearic and palmitic acids in the rumen compared to the dietary intake. The three oil diets had a higher ($P<0.05$) flow of octadecanoic acids through the GI tract than the LFCM diet, and only the flow of stearic acid through the rumen differed ($P<0.05$)

among the three oil diets. Apparent digestibilities of dry matter (DM), crude protein (CP), and energy were not different ($P>0.05$) among the four diets. Fiber digestibility was unaffected by the increasing fat levels. Fat digestion coefficients increased with increasing dietary intake of fat, and calculated true digestibility of sunflower oil using the acid solvent extract method was 83.4%.

INTRODUCTION

Ruminants have a high capacity to synthesis and deposit triglycerides in adipose tissue and to synthesis and secrete them in milk. This ability plus the increased use of cereal grains in the diets, has resulted in the production of meat and milk containing a very high content of neutral fats (Scott and Cook, 1975).

The feeding of large amounts of unsaturated fatty acids to ruminants results in an increase of saturated and monoenoic acids rather than an increase of polyenoic acids in the adipose tissue and milk lipids (Christie, 1978). Including high levels of polyenoic acids in the diet has generally caused changes in the fermentation pattern within the rumen, resulting in decreased feed intake and production, and decreased digestibility of various nutrients (Palmquist and Jenkins,1980).

Recently attention has been directed towards the feeding of protected lipids to ruminants (Mattos and Palmquist, 1974; Macleod et al, 1977; Sharma et al, 1978; and Wrenn et al, 1977). Unprotected dietary fat is subjected to hydrolysis and hydrogenation by rumen microorganisms; however, by encapsulating the fat with formaldehyde treated casein, the fat is protected against microbial action. There is a subsequent release of the fat in the acidic conditions of the abomasum, allowing for increased absorption of polyenoic acids in the small intestine (Scott and Cook, 1975).

World supplies of whole oil seeds has increased in the past decade. This is of interest to researchers since these seeds have the potential merit of being high oil and protein content with a medium fiber level which may provide some degree of protection in the rumen. feeding value of these oil seeds is limited. Several whole oil seeds that have been studied include, soybean seeds (Van Dijk et al, 1983; and Anderson et al, 1984); cottonseeds (Anderson et al, 1979; and Smith et al, 1981); canola seeds (Kennelly et al, 1982) and sunflower seeds (McGuffey and Schingoethe, 1982; and Rafalowski and Park, 1982).

The purpose of this experiment was to determine the digestibility of whole sunflower seeds; and to examine the flow of fat through the gastrointestinal tract as influenced by whole oil seeds.

MATERIALS AND METHODS

In vitro Study

The rate of disappearance of dry matter (DM), crude protein (CP) and acid detergent fiber (ADF) of whole and ground sunflower seeds (WSS and GSS, respectively) were determined using nylon bags according to Rae (1983). Five gram samples of WSS and GSS were placed in nylon bags and two replicate bags were suspended in the rumen of fistulated steers for 0, 3, 6, 12, 18, 24, 36, and 48 hours. After removal from the rumen the nylon bags were throughly rinsed and oven dried at 60°C in a forced air oven to a constant weight. The residue of each bag was removed, replicates were composited for a time period and the residue was analyzed for CP and ADF. Disappearance was expressed as a percentage of total feed DM, CP and ADF lost from the sample over time.

In vivo Study

Four Holstein steers averaging 200 kgs, were fistulated in the rumen, abomasum and terminal ileum (approximately 15cm from the ileal-cecal junction). The latter two fistulas were fitted with a flexible silicone "T"-shaped cannula. Plastisol plugs were prepared to prevent leakage and accumulation of material in the barrel of the cannulae. The rumen fistulas were fitted with a Bar Diamond flexible cannula (Parma, Idaho).

The steers were fed four diets in a 4 X 4 Latin Square Design. Diets were calculated to be isonitrogenous, containing (1) 4% sunflower oil (SFO); (2) 10% WSS; (3) 20% WSS and a fourth diet to contain 10% canola seeds (Table 6); however, due to a mixing error during the preparation of the feed, canola meal was used rather than whole canola seeds. Each experimental period consisted of a 11 day preliminary period for adjustment, a 6 day balance trial, and a 4 day digesta collection period. For the first 8 days, steers were individually fed the experimental diets once a day in pens. From Days 9 to 21, steers were placed in raised metabolism crates and fed continuously using a belt conveyor. Animals had free access to clean water. Feed samples were taken daily for the last four days of each period and composited for chemical analysis.

Dysprosium (Dy), a particulate marker, was mixed in a premix with soyabean meal and then added to the diet to result in a concentration of 25 ppm.

Digestiblity and N Balance trial

Feces were collected daily in a galvinized pan beneath the crates, weighed and a 5% sample by weight was taken and frozen. Urine was collected in a plastic water bottle containing 25ml of 10% HCL. The volume of urine was measured daily, and a 10% sample by volume was frozen. Daily feces and urine samples were composited and subsamples were taken at the end of each period.

Table 6. Ingredient and Chemical Composition of Mixed Rations Fed to Holstein Steers

Ingredients (kgs)	Diets			
	SFO	10%WSS	20%WSS	LFCM
Hay	20.0	20.0	20.0	20.0
Rolled Barley	70.6	65.0	55.0	65.0
Sunflower Oil	4.0	-	-	-
Sunflower Seeds	-	10.0	20.0	-
Canola Seeds	-	-	-	10.0
Urea	0.5	0.1	0.1	0.1
Limestone	0.5	0.5	0.5	0.5
Rock Phosphate	0.4	0.4	0.4	0.4
TM Salt	0.5	0.5	0.5	0.5
Vitamin Premix†	0.5	0.5	0.5	0.5
Molasses	3.0	3.0	3.0	3.0
Total	100.0	100.0	100.0	100.0
Nutrient Composition by Chemical Analysis (%DM)				
Dry Matter	89.3	87.7	89.4	87.8
Crude Protein	14.5	14.1	14.1	15.9
Acid Detergent Fiber	14.1	14.1	16.3	14.0
Ether Extract	6.0	4.0	9.5	2.0
Calcium	1.1	0.8	0.4	0.7
Phosphorus	0.7	0.7	0.6	0.6
Energy (Kcal/kg)	4.4	4.4	4.8	4.3

†Provided per kg of diet: 3500 IU vitamin A, 1750 IU vitamin D, 10 IU vitamin E, 44 mg ZnO, 45 mg MnO_2*H_2O, 0.3 mg $CoCl_2$, 1 mg KI, 116 g MgO.

Fecal samples were freeze dried, ground and stored for chemical analysis.

Collection of Digesta

Ruminal, abomasal and ileal digesta samples were collected via cannulae for two days following the balance trial. Samples were collected at 8:00 am and 3:30 pm each day, starting with the feces, followed by ileal, abomasal and ruminal digesta. Samples taken at each location along the gastrointestinal tract were composited for each period.

400 ml ruminal digesta was collected in plastic containers and the pH was measured and recorded. 300 ml abomasal digesta was collected. No fixed amount of ileal digesta was collected because of the irregular flow of material. All samples were immediately frozen.

During the last two days of each period, samples of rumen digesta were collected at times 0, 6, 12, 24, 36 and 48 hrs after the continuous infusion of dysprosium had stopped. At the end of the experiment, the rumen samples were composited for each diet across steers and periods. Marker mean retention time (MRT) was calculated from the decline of dysprosium concentration over time using the following equation: $C=C^{\circ}$ times e exponent $(-kt)$; where C is marker concentration at time t; C° is equilibrium concentration and k is dilution rate and is the reciprocal of marker MRT (Faichney, 1975).

Chemical Analyses

A 100 mL subsample of all composites was freeze dried to determine DM of total digesta. The remaining fluid was centrifuged using a Sorvall Superspeed RC2-B Automatic Refrigerated Centrifuged at 48,200g. Three hundred ml of the liquid phase and two X 100mL of the particulate fraction was freeze dried. All samples were then ground and stored for chemical analysis.

Total nitrogen, ether extract (EE) and acid solvent extract (ASE) of fat were determined according to the Association of Official Analytical Chemists (1980). Fecal soaps were determined by subtracting the EE of feces from acid solvent extracted fecal fat. Gross energy was determined by Parr Adiabatic Oxygen Bomb calorimetry and ADF was determined according to Goering and Van Soest (1970).

Volatile fatty acids (VFA) in rumen fluid were determined by gas chromotography according to Erwin et al (1961) and rumen ammonia was analyzed by an ammonia electrode (Model 95-10, Orion Research, Cambridge, MA).

Fifty milligram samples of feed, liquid and solid phases of rumen, abomasum and ileum digesta, and feces were sent to the Slowpoke facility at the University of Alberta, Edmonton, Alberta where concentration of Dy was estimated by neutron activation (Kennelly et al, 1980).

The four diets and the solid phases of ruminal, abomasal and ileal digesta were acid hydrolyzed and amino acid content was determined by a Beckman Model No 116/119 Amino Acid Analyzer with sample injector. Fatty acids of the fat extracts from feed, solid abomasal and ileal fractions, and feces were methylated according to Metcalfe et al (1966) and analyzed on a Varian gas chromotography system using a GP 3% SP2310/2% SP2300 on 100/120 Chromosorb WAW 8' by 1/4" packed column. Identification of each fatty acid was made on the basis of the retention time of a standard. A Varian 402 Data System expressed identified palmitic, stearic, oleic and linoleic acids as a percentage of the total of these four fatty acids.

Calculation of Passage of Digesta.

Dy was used to determine the flow of solid digesta through the gastrointestinal tract using the following equation:

$$\text{Flow of DM} = \frac{(\text{Concentration of Dy/g of diet DM}) \times \text{Dry Matter Intake}}{(\text{Concentration of Dy/g of digesta DM})}$$

The level of Dy used in the calculation was the theoretical level added to the diet.

The flow of other nutrients at a cannulated site was calculated by multiplying the flow of digesta DM by the concentration of nutrient in the digesta at that sampling site

of the tract. Changes in the flow of DM, nitrogen, fat and fatty acids through the rumen, small intestine, and large intestine were calculated from the difference between pre- and post- compartments, i.e. the flow through the rumen was determined by subtracting abomasal fatty acids from dietary fatty acids.

Statistical Analysis

The data collected was analyzed using the Latin Square Design procedure of the Statistical Analysis System (Barr et al, 1976). Duncan's Multiple Range Test ($P<0.05$) was used to detect the significant diferences between treatment means (Steele and Torrie, 1976).

RESULTS AND DISCUSSION

In vitro Study

The rate of disappearance of DM, CP, and ADF of WSS and GSS from nylon bags are shown in Figure 1. Grinding the whole sunflower seeds appeared to increase the availability of the CP and DM for microbial degragation, with a relatively small effect on ADF disappearance. Research by Park et al (1982) indicates that sunflower hulls fed to heifers have a low DM and ADF digestibilities; therefore the effectiveness of the sunflower's fibrous hulls in encapasulating and protecting the oil and meal from rapid degradation by microbial action is apparent.

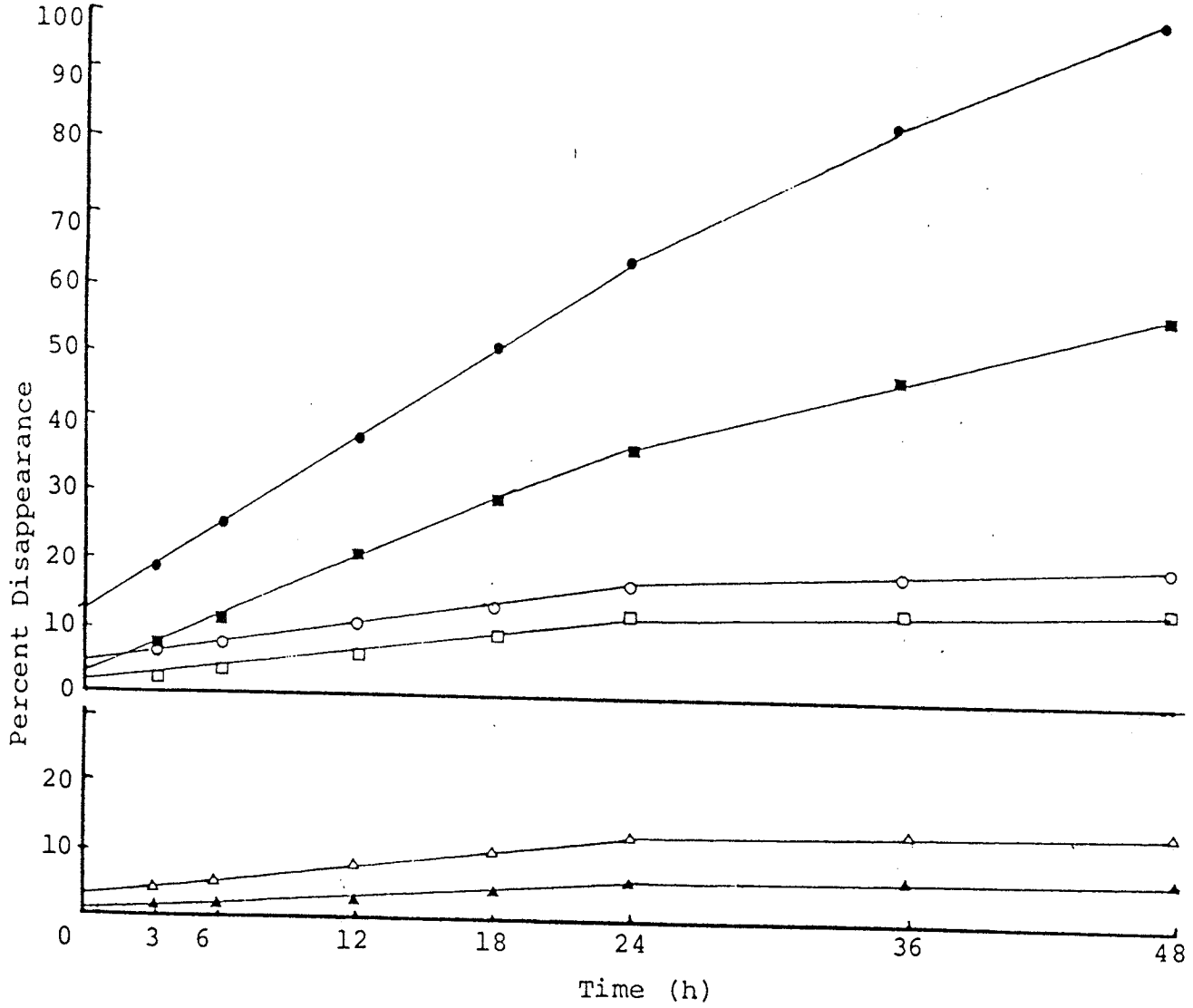

Figure 1. Percent Disappearance of Dry Matter (square), Crude Protein (circle) and Acid Detergent Fiber (triangle) of Whole Sunflower Seeds (open) and Ground Sunflower Seeds (solid) from Rumen Situated Using Nylon Bags for a Steer Fed Ration 2 (10% WSS)

In vivo Study

The mean percentage recovery of dysprosium in the feces of Holstein steers was 94.6±10.7 for the four treatments. The recovery of Dy did not differ significantly ($P>0.05$) among the treatments.

Rumen Parameters

Including pure oil reduced ($P<0.05$) rumen pH as compared to the 10% WSS and LFCM diet; however, no difference ($P>0.05$) was noted between the SFO and the 20% WSS diet (Table 7). Total VFA and rumen ammonia levels were not different ($P>0.05$) among treatments; however, the three oil diets tended to decrease total VFA concentrations. Bines et al (1978) observed similiar decreases of total VFA concentrations when protected lipids were fed to dairy cows. The LFCM diet tended to have a higher but not signifigant molar percentage of acetate, a lower ($P<0.05$) molar percentage of propionate and a higher ($P<0.05$) ratio of acetate to propionate than the three oil diets (Table 7). Similiar decreases in the proportions of acetate and increases in the proportions of propionate were observed by Shaw and Ensor (1959); Steele and Moore (1968b,c) and Noble et al (1969b) when codliver oil, tallow and cottonseed oil, and long chain fatty acids, respectively, were added to the diet. Studies by Czerkawski (1973), Czerkawski et al (1975) and Chalupa et al (1984) indicate that the long chain fatty acids depress

Table 7. Treatment Effects on Rumen Metabolite Means for Steers Fed Experimental Diets.

Parameter	Diets				
	SFO	10%WSS	20%WSS	LFCM	SE
Rumen Fluid					
Ammonia·Nitrogen (mg/100mL)	20.4	17.1	20.2	27.5	3.2
Total VFA† (mmoles/100mL)	5.98	5.93	5.69	7.20	0.6
Rumen pH	5.92b	6.05a	5.99ab	6.15a	0.04
VFA (molar %)					
Acetate (C_2)	58.7	59.6	59.6	63.0	3.1
Propionate (C_3)	24.5a	25.1a	22.9a	18.4b	0.9
Butyrate (C_4)	13.2	10.6	12.4	13.3	2.3
Valerate (C_5)	1.7	2.2	2.0	2.2	0.3
IsoVFA (iC_4+iC_5)	1.9	2.6	3.1	3.0	0.4
C_2/C_3 Ratio	2.5b	2.4b	2.6b	3.5a	0.2

†Volatile Fatty Acids
a,b means on the same row with different letters differ ($P<0.05$)

acetate production by altering the type of fermentation by the bacterium.

Nitrogen Retention

Although equal amounts of diet were fed, slight differences in DM and CP content of the diets resulted in nitrogen intake differing ($P<0.05$) among the four treatments (Table 8). Total nitrogen intake and excretion was highest ($P<0.05$) for the steers fed LFCM diet. This might have been expected with the higher level of nitrogen in LFCM diets as a result of the use of canola meal rather than canola seeds. Nitrogen retention as a percentage of nitrogen intake (Table 4) tended to be higher with the oil diets compared to the LFCM diet with little difference in the amounts retained. Increased nitrogen utilization at low protein intakes (14% vs 16%), because of the relative high efficiency of nitrogen recycling, were reported by Satter and Roffer (1975).

Flow of Digesta

There were no dietary effects observed on the flow of DM or nitrogen through the rumen, small intestine and large intestine of the cannulated steers (Table 9). Lower inclusion of fat, 10% WSS and LFCM, resulted in an increase ($P<0.05$) flow of fat through the rumen. There appeared to be some negative effects on rumen flow of fat with the higher fat

Table 8. Nitrogen (N) Retention in Holstein Steers Fed Experimental Diets During the 6 Day Balance Trial.

Parameter	Diets				
	SFO	10%WSS	20%WSS	LFCM	SE
DM Intake	4265.5a	4165.7d	4246.5b	4170.5c	1.4
N Intake	102.7b	96.5d	98.6c	108.6a	0.2
N Excreted in Feces	25.8	29.3	26.5	34.6	3.0
N Excreted in Urine	21.6a	14.5b	20.3ab	23.2a	1.7
Total N Excreted	47.4b	43.9b	46.8b	57.8a	2.9
N Retained	55.3	52.7	51.8	50.8	2.9
N as a % of N Intake					
Feces	25.1	30.1	26.7	31.6	2.9
Urine	20.9ab	14.8b	20.4ab	21.4a	1.8
Retention	53.9	55.1	52.9	47.0	3.0

a,b means on the same row with different letters differ ($P<0.05$)

Table 9. Intake, Fecal Excretion and Flow of Dry Matter, Nitrogen, and Fat through the Rumen, Small Intestine (SI) and Large Intestine (LI) of Cannulated Steers.

Nutrients / Diets	Intake (g)	Net Appearance or Disappearance			Excreted (g)
		Rumen	SI	LI	
Dry Matter					
SFO	4265.5a	-2827.0	-216.2	-149.3	1073.0
10%WSS	4165.8d	-2576.2	-297.2	-101.0	1191.3
20%WSS	4246.5b	-2720.9	-274.9	-189.2	1061.4
LFCM	4170.5c	-2751.9	-179.4	-230.0	1009.2
SE	1.4	157.4	98.4	129.2	75.6
Nitrogen					
SFO	102.8b	-53.3	-21.1	+1.9	29.8
10%WSS	96.5d	-38.3	-32.7	+7.1	32.7
20%WSS	98.6c	-47.6	-24.3	+0.9	27.7
LFCM	108.3a	-52.9	-27.7	+5.3	33.2
SE	0.2	4.5	3.2	3.3	2.9
Fat					
SFO	258.9b	-13.6b	-202.4b	+14.4	57.4
10%WSS	168.3c	+56.1a	-187.5b	+21.1	57.9
20%WSS	403.5a	-77.0b	-283.4c	+15.0	58.1
LFCM	85.1d	+39.7a	-103.2a	+16.8	38.4
SE	1.4	19.5	20.8	2.4	6.1

a,b,c,d means within the same column within Dry Matter, nitrogen and fat with different letters differ ($P<0.05$).

diets. Increases or decreases in the flow of fat through the rumen may be due to fluctuations in the quantity of fat supplied by the rumen microbes. Clarke and Hawke (1970) observed significant reduction in the number of protozoa and Polan et al (1964) observed significant reduction in the number of bacteria when large amounts of pure oil were supplied in the diet. In general, increasing the level of dietary fat resulted in significantly more fat being absorbed in the small intestine. There was a slight increase ($P>0.05$) in the flow of fat through the large intestine (Table 9) which could be related to microbial activity in the hind gut (Ward et al, 1964).

Dietary intake of palmitic, stearic, oleic and linoleic acids differed ($P<0.05$) among the four diets (Table 10) except stearic acid for the SFO and 10% WSS diets. There was a slight increase in the flow of palmitate through the rumen compared to dietary intake, however differences among diets were nonsignificant ($P>0.05$). There was a substantial increase in the flow of stearic acid; decrease in the flow of linoleic acid, and little change in the flow of oleic acid through the rumen of the cannulated steers relative to dietary intake. Reduction of polyenoic acids and the subsequent increase in saturated fatty acids in the rumen has been observed by Polan et al (1964); Kepler and Tove (1967) and Wilde and Dawson (1966). After hydrolysis of esterfied fatty acids, biohydrogenation of unsaturated fatty acids occurs by the microbes in the rumen (Hawke and Silcock, 1969).

Table 10. Intake, Fecal Excretion and Flow of Palmitic, Stearic, Oleic and Linoleic Acids through the Rumen, Small Intestine (SI) and Large Intestine (LI) of Cannulated Steers.

Fatty Acids / Diets	Intake (g)	Net Appearance or Disappearance			Excreted (g)
		Rumen	SI	LI	
Palmitic					
SFO	28.0b	+15.6	-39.0	+5.5	10.1
10%WSS	25.1c	+9.2	-29.7	+5.1	9.7
20%WSS	33.3a	+11.8	-41.5	+2.9	6.5
LFCM	18.6d	+9.5	-24.9	+7.7	10.9
SE	0.2	5.1	5.3	1.4	1.6
Stearic					
SFO	9.2b	+70.7a	-49.2b	+1.8	32.4
10%WSS	9.4b	+63.6a	-46.9b	+6.3	32.2
20%WSS	19.7a	+68.3a	-53.4b	+4.6	39.2
LFCM	2.6c	+22.4b	-9.8a	+2.7	12.5
SE	0.2	9.5	10.0	2.6	2.9
Oleic					
SFO	50.5b	+5.2	-50.2b	+2.7	8.2
10%WSS	32.6c	+5.1	-33.4a	+3.7	8.0
20%WSS	64.5a	-6.9	-54.0b	+3.4	7.0
LFCM	19.4d	+6.8	-23.9a	+5.7	7.9
SE	0.6	4.9	4.4	1.3	1.5
Linoleic					
SFO	170.9b	-104.8b	-64.0a	+4.5	6.7
10%WSS	101.3c	-21.8a	-77.5a	+6.0	8.0
20%WSS	285.9a	-150.1c	-134.5b	+4.1	5.4
LFCM	44.4d	+1.2a	-44.6a	+6.0	7.0
SE	3.0	9.3	10.4	1.8	2.0

a,b,c,d means within the same column within palmitic, stearic oleic and linoleic with different letters differ ($P<0.05$).

Since octadecamonoenoic acids (oleic acid and the trans isomers) are being created because of the incomplete hydrogenation of linoleic to stearic, and because oleic acid undergoes hydrogenation itself, this could account for the nonsignificant difference in the quantity of oleic flowing through the rumen. Increases in palmitic and stearic acid could also be related to microbial synthesis of these fatty acids (Viviani and Lenaz, 1965).

The three oil containing diets had a higher ($P<0.05$) quantity of stearic acid flowing through the rumen than the LFCM diet (Table 10). Differences between the LFCM and the oil containing diets in respect to the flow rates of stearic acid could be explained by the differences in intake (Table 10) and also by the quantity of linoleic acid being hydrogenated by microbes.

There was no difference ($P>0.05$) in the flow of palmitic acid through the small intestine among the four diets (Table 10). There was significantly ($P<0.05$) more stearic acid absorbed in the small intestine with the high oil diets than the LFCM diet, probably due to the higher quantity of digesta passing into the small intestine. Higher ($P<0.05$) quantities of oleic acid were absorbed with the SFO and 20% WSS diets.

There were no difference ($P>0.05$) among the four diets with respect to the flow of palmitic, stearic, oleic and li-

noleic acids through the the large intestine (Table 10). The nonsignificant (P>0.05) increase in the flow of fatty acids through the large intestine could be due microbial activity in the hindgut or to endogoenous losses, i.e. increases in the quantity of linoleic acid in the feces could be related to metabolic losses when lecithin is hydrolyzed to lysolecithin, releasing unsaturated fatty acids from the β position (Leat and Harrison, 1974). The higher excretion of stearic acid than the other three fatty acids in the feces (Table 10) could be due to the differences in absorption rates of fatty acids, which changes with chain length and degree of saturation (Harrison and Leat, 1972). It should also be noted from Table 10, that summation of fatty acid intake and the changes through the gastrointestinal tract, within diets, closely correlate to the fatty acid values excreted in the feces.

Digestibilities

No dietary difference (P>0.05) was observed in the digestiblities of DM and CP at the abomasum and ileum (Table 11). Fiber digestibility in the rumen has been reported to be depressed by the addition of dietary fat (Devandra and Lewis, 1974); however, no differences (P>0.05) in fiber digestion occured among the four diets in the present study (Table 11). Apparent digestibilities of DM, CP, ADF, and energy were not different (P>0.05) among the four diets.

Table 11. Digestibilities, Using Marker Technique, of DM, CP, ADF, and EE in the Abomasum, Ileum, and Feces of Cannulated Steers Plus Digestibiilities Determined by Total Fecal Collection.

Nutrient Digestibilities (% of Intake)	Diets				SE
	SFO	10%WSS	20%WSS	LFCM	
Abomasum					
DM	66.4	62.1	64.1	66.1	3.7
CP	52.4	39.8	48.3	48.7	4.5
ADF	59.7	46.3	52.8	49.7	8.5
EE	ND	ND	ND	ND	-
Ileum					
DM	71.3	69.1	70.6	70.4	3.3
CP	72.7	73.5	72.8	74.4	2.5
ADF	47.5	36.9	45.5	44.7	5.7
EE	83.4ab	78.3b	89.3a	74.7b	2.5
Feces					
DM	74.9	71.5	75.0	75.7	1.8
CP	70.9	66.2	71.9	69.3	2.8
ADF	50.8	38.4	51.2	49.3	4.4
EE	77.7a	65.5b	85.5a	54.7c	2.8
ASE	52.7ab	32.8bc	67.8a	23.7c	6.6
Energy	72.7	68.8	73.8	70.7	2.2
Total Collection					
DM	78.2	74.6	76.2	74.9	2.4
CP	74.9	69.9	73.3	68.4	2.9
ADF	57.8	44.9	53.7	47.5	4.9
EE	80.4a	69.8ab	86.4a	53.4b	4.9
ASE	57.3ab	39.0bc	68.9a	21.9c	8.3
Energy	76.2	72.1	75.1	73.6	2.6
Fecal Soaps†	6.5	4.6	6.6	2.6	1.1

DM - Dry Matter; CP - Crude Protein; ADF - Acid Detergent Fiber; EE - Ether Extract; ASE - Acid Solvent Extract;

†Determined by subtracting fat extracted with ether from fat extracted with the acid solvent

a,b,c means on the same row with different letters differ ($P<0.05$)

Digestion coefficients for EE and ASE of fat, increased ($P<0.05$) as the level of dietary fat increased. Similiar observations on increased fat digestibility with increasing level of fat were observed by Sharma et al (1978) and Smith et al (1981). True digestibilites of EE and ASE can be calculated by plotting digested fat versus concentration of fat in the ration (Figure 2) (Lucas in Van Soest, 1982). True digestibilities of EE and ASE of fat were 95.6% and 83.4%, respectively. Although differences between the slopes of EE and ASE were not significantly ($P>0.05$) different, increasing the amount of oil tended to increase the amount of soaps being excreted in the feces. Correlations between apparent digestibilities of nutrients determined by total fecal collection and marker technique, except for EE and ASE, were low, but this might be expected due to the narrow range of digestibilities.

No differences ($P>0.05$) occured in the flow of essential amino acids through the rumen, abomasum and ileum (Appendix Table 1).

Mean retention time of the particulate phase of digesta in the rumen for SFO, 10% WSS, 20% WSS and LFCM were 20.4, 25.6, 23.3, and 29.4 hours, respectively. There was a trend for the oil diets to have a lower rumen retention time than the LFCM diet. An explanation for this is not apparent.

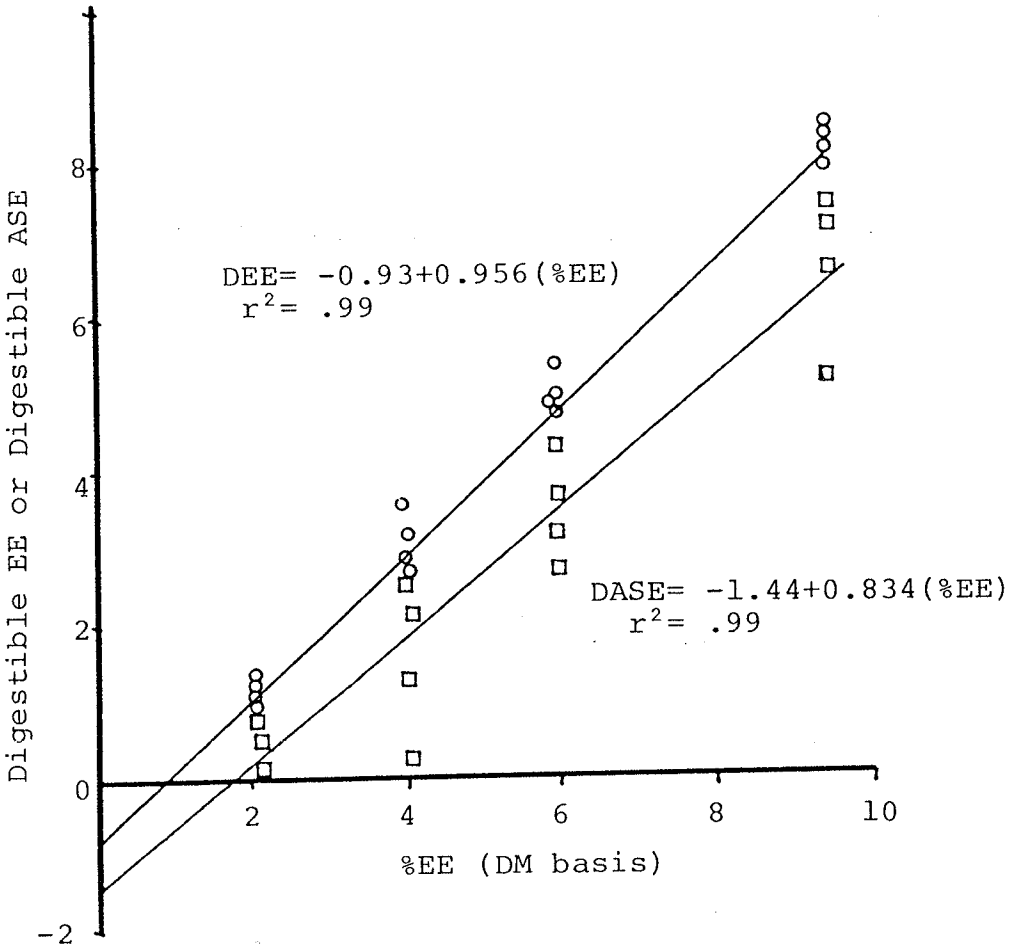

Figure 2. The Interrelationship (on a Dry Matter Basis) between Digestible Ether Extract (EE) or Digestible Acid Solvent Extract (ASE) and Percent Ether Extract (%EE) in the diet.

Data by Rafalowski and Park (1982) and Experiment 1 (Table 2) suggests that there was improved milk fat percent and yield when whole sunflower seeds are fed to lactating dairy cows. In a comparison study between whole sunflower seeds and sunflower oil, we observed improvement in the yield of de novo synthesized fatty acids with the WSS diets. The pure oil appeared to have some inhibiting effect on these acids, thereby reducing milk fat yield. Rafalowski and Park's (1982) research indicates that the fat depressing effects of sunflower oil may have been conteracted by the relatively high fiberous coating of the seeds. Data from the present study agrees with their findings.

Rafalowski and Park (1982) and Experiment 1 (Table 4) both report a significant reduction in the molar percentage of linoleic acid in milk fat when cows consumed WSS compared to the control diets. The present study tends to suggest that this reduction is due to a longer retention of the whole seeds in the rumen, thereby allowing microbes longer access to hydrogenate polyunsaturated fatty acids. The present results also contradicts Rafalowski and Park (1982) and Experiment 1 findings, in so much that more linoleic acid was absorbed (Table 10) in the small intestine with the 20% WSS than the other diets. However Rafalowski and Park (1982) do suggest that the 10% WSS diets are more energy efficient for milk production than the 20% WSS and higher levels of inclusions.

ACKNOWLEDGEMENTS

In acknowledgement I would like to thank Agriculture Canada and the National Research Council for financial aid to carry out this project.

GENERAL DISCUSSION

Dysprosium as a Marker

The use of rare earth neutron activated markers such as dysprosium, to measure digestibilities and the flow of nutrients through the gastrointestinal tract of ruminants is promising. A knowledge of the limitations of such markers is necessary for the technique to be used accurately.

Digestiblities calculated from the marker technique and total fecal collection method were similiar. Correlations ($r^2<.30$) were low. A major reason for the poor correlation was the difficulty in estimating the concentration of dysprosium in the feed. As reported in the Material and Methods, dysprosium was mixed into the ration at a low concentration of 10.8 mg/g. The accuracy in obtaining a subsample depends upon adequate mixing of the premix into the total diet, with adequate sampling of the feed. In the present study, feed did not appear to have been subsampled adequately and the size of sample sent to University of Alberta was less than one-sixth the normal size necessary for proper analysis. Therefore, the subsample that was analyzed did not provide the best estimate of the concentration of dysprosium in the diet.

A second reason for the discrepancy in the apparent digestiblities, marker vs total collection (Table 11), as well

as a concern when determining the flow of digesta, would be the flow of labelled particles through the rumen, as well as migration of labelled particles with a redistribution of label among particles. Dysprosium was bound to soybean meal, and upon digestion of the meal in the rumen, the dysprosium may be released, be bound to other material or microbes, or form insoluble hydroxides (Crooker et al, 1982). If the dissociated marker becomes particle bond to larger particles of feed (ie hay), the estimation of flow of digesta out of the rumen will be low because of a longer retention time in the rumen. In order to estimate the flow of digesta it is necessary to label various components in the feed in a manner that would produce the least error.

There are no certainties that the digesta samples collected to determine the flow of digesta are true representatives of total digesta. Caution is required when sampling the rumen due to the layering of solid and liquid phases. In the rumen the larger feed particles are on top of the liquid fraction, therefore proper dispersion of solid and liquid phases of the rumen is necessary prior to any sampling. A knowledge of the relationship of soluble to insoluble materials in the liquid fraction is also a must prior to removal of any samples. Obtaining adequate abomasal and ileal samples is uncontrolable due to the small quantities involved and the irregularity of digesta flow.

Once the limitations in the use of rare earth markers are known, markers can be successfully used to estimate the rate of absorption and excretion of components along the digestive tract of ruminants.

Effect of Whole Sunflower Seeds

The yield of milk fat is dependent upon a balance between the yield of de novo synthesized fatty acids (DSFA) within the mammary gland and the yield of long chain fatty acids (LCFA) that are absorbed from the blood (Palmquist and Jenkins, 1980). This concept has led researchers to attempt to maintain or increase milk fat yield by (1) altering rumen fermentation to provide more acetate for synthesis of fatty acids within the mammary gland and (2) to increase the polyunsaturated fatty acid content of the diet thereby increasing the quantity of LCFA, particularly unsaturated fatty acids, absorbed from the plasma. The second procedure has had limited success because increased consumption of unsaturated fatty acids appears to reduce the yield of DSFA ($P<0.05$) at the expense of the slight increased uptake of LCFA. The present data indicate that cows consuming extracted sunflower oil in the diet had significantly lower yields of DSFA resulting in a decrease ($P<0.05$) in milk fat test compared to the WSS diets (Table 2). The decreased yield of DSFA could be related to an inhibiting effect of unsaturated fatty acids on acetate production in the rumen

by altering the metabolism of the microbes or related to an inhibiting effect of LCFA on the acetyl-CoA carboxylase enzyme, which is responsible for chain elongation within the mammary gland (Steele and Moore, 1968a,b; Banks et al, 1976 and Palmiquist and Conrad, 1978.

Data from the present study would tend to support the second conclusion since (1) no differences were observed on the production of acetate in the rumen between the extracted oil diet and the whole seed diet; and (2) cows consuming the free oil diet had significantly higher molar percentage of linoleic acid in the milk fat. The increased content of linoleic acid, which normally undergoes biohydrogenation in the rumen, is probably due to the oil decreasing the number of microbes responsible for hydrogentation of unsaturated fatty acids (Czerkawski, 1973 and Czerkawski et al, 1975).

Decreased fiber digestibilities have been observed when free oil was added to the diet, probably due to physical coating of the fiber by the oil (Devandra and Lewis, 1974). Also, alterations in the pattern of rumen fermentation have been related to the fiber levels in the diet (Davis and Brown, 1970). Although the present data does not show decreased fiber digestibilities (Table 11) with the free oil versus the whole seed, the decreased rumen production of total VFA and the decreased molar percentage of acetate with the oil containing diets may suggest altered fiber metabolism.

The negative effects of unsaturated fatty acids on milk fat yield and rumen fermentation can be reduced by feeding natural unextracted seeds (Rafalowski and Park, 1982 and Smith et al, 1981). The present data agrees, since cows consuming whole seeds had significantly higher milk fat yields than cows consuming the free oil, while producing equal quantities of milk (Table 2). The fibrous hull encapsulating the sunflower oil, which has a low DM and ADF digestibility (Park et al, 1982), tends to allow more oil, and specifically more LCFA to be supplied in the diet without the adverse affects on rumen parameters. The increased consumption of fatty acids plus the fact that whole seeds have a longer retention time, allowing microbes to hydrogenate unesterfied unsaturated fatty acids has resulted in the increased outflow of long chain saturated fatty acids from the rumen to be absorbed in the small intestine.

Although more oil can be successfully fed and more saturated fatty acids absorbed with the 20% WSS diets, the economics of feeding this quantity in the diet should be questioned. The present data indicate that complete hydrogenation of linoleic acid does not occur in the rumen even with the whole seeds; therefore, problems with decreased synthesis of fatty acids within the mammary gland could occur. Rafalowski and Park (1982) have observed decreased ($P>0.05$) molar percentage of short chain fatty acids with the 20% inclusion compared to the 10% inclusion. Since

sunflower seeds have 72% of total fatty acids as linoleic acid, incomplete hydrogenation may be related to the excess quantity being consumed at the 20% inclusion level (Polan et al, 1964).

Rafalowski and Park (1982) conclude that 10% inclusion of sunflower seeds into the diet resulted in the greatest output of milk, while maintaining adequate milk fat test, and the greatest feed efficiency. The present data tends to support their findings, and also suggests that a higher efficiency of energy utilization can occur when the oil is added in the form of 10% whole seeds rather than an equal quantity of extracted oil because of the higher levels of fat in the milk produced.

CONCLUSIONS

(1) Cows fed diets containing whole sunflower seeds compared to to cows consuming diets containing extracted sunflower oil, consumed equal quantities of DM and produced similiar quantities of milk; however milk fat percentage was significantly ($P<0.05$) higher for cows consuming WSS, suggesting that energy was more efficiently used for milk production.

(2) The decreased milk fat yield from cows consuming diets containing free sunflower oil was related to decreased ($P<0.05$) yields of de novo synthesis fatty acids within the mammary gland.

(3) Ruminants can consume significantly ($P<0.05$) more oil with the whole seeds rather than as free oil, without adversely affecting rumen microbial fermentation.

(4) Whole sunflower seeds had no effect on the apparent digestibilities of DM, CP, ADF and energy compared to SFO.

(5) Calculated true digestibility of sunflower oil was 95.6% based on ether extract and 83.4% based on the acid solvent extract analysis.

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Table 1A. The Flow of Essential Amino Acids through the Rumen, Abomasum and Ileum of Cannulated Steers (g/day)

Location / Amino Acids	Diets				SE
	SFO	10%WSS	20%WSS	LFCM	
Intake					
Lys	20.8b	17.0c	21.2b	23.5a	0.08
His	10.6d	11.1c	12.3b	13.1a	0.03
Arg	28.7c	26.5d	37.0a	35.0b	0.13
Thr	19.5c	18.8d	19.8b	22.0a	0.05
Val	29.6d	33.2c	37.7b	38.9a	0.11
Ile	21.3c	21.2c	24.2b	24.7a	0.05
Leu	37.9d	39.3c	41.6b	45.3a	0.09
Try	14.5d	16.2c	17.1b	20.3a	0.07
Phe	30.0a	27.8c	29.0b	30.0a	0.04
Cys	8.6c	9.6b	8.0d	11.6a	0.04
Met	17.4c	19.3b	16.4d	21.8a	0.07
Total AA	526.0c	494.1d	532.3b	573.2a	1.1
%EAA of TAA†	45.5d	48.5c	49.6b	49.9a	0.05
Rumen					
Lys	27.7	28.8	27.7	32.5	2.6
His	8.8	10.0	10.8	9.9	0.9
Arg	22.5	26.2	25.9	25.5	2.4
Thr	19.0	20.0	19.0	22.0	1.7
Val	27.6	24.7	28.6	27.8	2.7
Ile	22.9	20.9	22.7	33.8	1.9
Leu	34.9	34.3	36.9	39.7	2.8
Try	12.1b	12.2b	11.9b	15.8a	1.0
Phe	25.6	27.5	27.5	33.8	3.1
Cys	8.0	9.2	7.3	10.6	1.3
Met	16.0	18.3	17.1	20.1	1.9
Total AA	449.4	464.3	466.5	499.3	37.5
%EAA of TAA	50.2b	50.0b	50.5b	52.6a	0.6

Table 1A Con't. The Flow of Essential Amino Acids through the Rumen, Abomasum and Ileum of Steers (g/day).

Location / Amino Acids	Diets				
	SFO	10%WSS	20%WSS	LFCM	SE
Abomasum					
Lys	15.2	17.5	13.8	18.5	2.0
His	5.1	5.3	4.1	5.3	0.6
Arg	11.2	12.5	9.9	12.0	1.5
Thr	9.7	11.3	9.1	11.6	1.4
Val	13.6	14.9	13.2	15.2	1.8
Ile	13.1	14.4	10.1	15.4	2.0
Leu	18.5	20.1	14.5	21.3	2.6
Tyr	7.7	8.6	6.4	9.5	1.2
Phe	13.3	14.6	10.8	14.9	1.6
Total AA	212.2	238.6	181.2	248.2	30.0
%EAA of TAA†	50.7	50.0	50.4	49.8	0.3
Ileum					
Lys	8.9	6.7	7.7	8.1	0.8
His	2.0	2.6	2.5	2.7	0.3
Arg	5.1	4.7	3.1	5.1	0.7
Thr	5.6	4.0	5.4	5.1	0.6
Val	7.5	5.3	7.0	6.7	0.8
Ile	5.9	4.5	5.5	6.0	0.5
Leu	9.2	7.8	8.3	9.3	1.0
Tyr	4.9	3.6	3.8	4.5	0.5
Phe	7.3	6.4	6.3	7.1	0.7
Total AA	155.7	98.2	108.8	114.7	27.3
%EAA of TAA	41.5	46.3	45.8	47.6	3.6

†Percent Essential Amino Acids of Total Amino Acids.

a,b,c,d means on the same row within Intake, Rumen, Abomasum and Ileum with different letters differ ($P<0.05$).