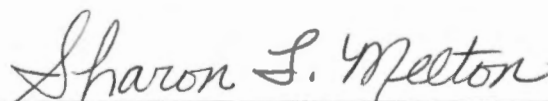


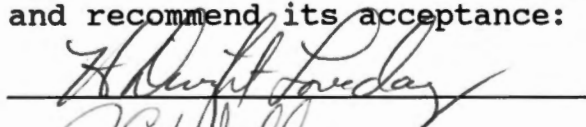
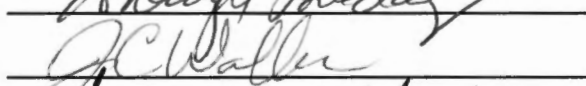
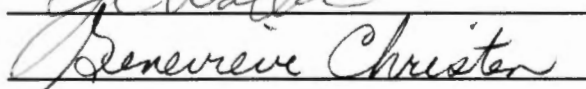
To the Graduate Council:

I am submitting herewith a dissertation written by Dje Yobouet entitled "The Effect of Feeding Protected Dietary Lipid Supplements on the Fatty Acids Composition of the Blood Serum and the Milk Fats of Lactating Ewes". I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Food Science and Technology.



Dr. Sharon L. Melton, Major Professor

We have read this dissertation
and recommend its acceptance:

Accepted for the Council:



Associate Vice Chancellor
and Dean of The Graduate School

**The Effect of Feeding Protected Dietary Lipid
Supplements on the Fatty Acids Composition
of the Blood Serum and the Milk
Fats of Lactating Ewes**

**A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville**

**Dje Yobouet
December, 1994**

AD-VET-MED.

Thesis

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DEDICATION

This Dissertation is dedicated to Bendedou, Dje

and

Abli (Toumodi).

Mi si Koffi Paul oni mami Aya Rosalie n'gna anou mo oni kloua afi

amou man m'man ka man blo.

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ABSTRACT

This work was undertaken to assess the potential of acetaldehyde and diacetyl as alternative protective agents to formaldehyde, a carcinogen, for the commercial production of products from ruminant animals (cattle, sheep, goat) with increased unsaturated fatty acid (18:1, 18:2 and 18:3) contents. In experiment 1, different gels were prepared with emulsions of (1) defatted soy flour-casein-sunflower oil, (2) ground full-fat soybean seeds-casein and (3) ground full-fat sunflower seeds-casein. Each gel protein-oil emulsion was treated with 5% (w/w emulsion) formaldehyde (FA) (37% solution), acetaldehyde (AC), diacetyl (DA) to form a gel or not treated (CO).

The microstructures of type 1 gels were determined by scanning electron microscopy. Protection of the unsaturated fatty acids in all gel types from ruminal biohydrogenation for all treatments (FA, AC and DA) was evaluated *in vitro* before and after incubation with strained rumen fluid at 39°C for 48 hrs. At digestion times, 0, 24 and 48 hrs, the fatty acid contents of digested gels were determined by gas chromatography.

The first experiment showed that the levels of linoleic acid (18:2) were significantly lower in ruminal digests containing the control supplement than in digests containing FA, AC, and DA-treated gel digests, regardless of the type of

oilseed. Meanwhile, the levels of octadecenoic (18:1c+t) and stearic (18:0) acids were higher in the control digest than in the treated ones. Results showed that FA, AC and DA treatment could protect the unsaturated fatty acids than the CO, in all types of gel. Diacetyl, at the level (5%) applied, was less effective in preventing biohydrogenation of the unsaturated fatty acids in the sunflower supplement.

In experiment 2, dietary lipid supplements in gel form were prepared with ground full-fat soybeans, treated with 2.2% FA, AC and DA or nothing (CO). Lactating ewes in a 4x4 Latin square feeding trial design received a daily ration (2.5 to 3.0 Kg) grass hay plus .5 Kg (ground corn, soybean meal, oats and alfalfa pellets) supplemented with 1.0 Kg of one of the lipid supplement gels.

The levels of linoleic acid (18:2) in the ewes blood serum fat increased ($p < 0.05$) by 42, 41. and 25% for FA, AC and DA treatments, respectively, in relation to the control diet. The levels of 18:2 in the ewes milk increased ($p < 0.05$) by 114, 52 and 43% for FA, AC and DA treatments, respectively, in relation to the control diet. Neither storage period nor treatment affected ($p > 0.05$) the fatty acid content of the dietary lipid supplements. Feeding the protected supplements enhanced growth in the lambs but did not affect the weights of the ewes.

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CHAPTER I

INTRODUCTION

Coronary heart disease is the leading cause of death in the United States and many other industrialized countries. Approximately 500,000 lives are lost every year as a consequence of this disease (USDA, 1986; National Research Council, 1989). High blood cholesterol level is one of the risk factors associated with coronary heart disease (Hegsted et al., 1965; Keys et al., 1965; Bonanome and Grundy, 1988). One of the factors contributing to increased blood cholesterol is fatty acids (Rosenberg and Schaefer, 1988; Rassias et al., 1991). People on a diet high in saturated fatty acids tend to have a higher serum cholesterol content compared with those on a diet high in polyunsaturated fatty acids (Keys et al., 1965; Grundy and Vega, 1988; Ginsberg et al., 1990). This relationship between dietary fat and the level of serum cholesterol has encouraged health conscious consumers to limit their consumption of animal products, which have relatively high levels of saturated fatty acids (Mattson et al. 1975; Laine et al., 1982). Thus, vegetable oils (Brock, 1989), which contain high levels of polyunsaturated fatty acids, have enjoyed increased popularity over animal fats. This trend has affected the meat industry, decreasing the consumption of meat

products (USDA, 1986; American Meat Institute, 1989).

The fats of all red meat animals contain 40 to 50% saturated fatty acids (USDA, 1979; Price and Schweigert, 1987). The body fat of monogastric animals can be altered considerably by consuming high levels of dietary polyunsaturated fat, but the fat of ruminant animals cannot be changed through a normal diet. This is attributed to the fact that rumen microorganisms are able to hydrogenate polyunsaturated dietary fats during digestion (Shorland et al., 1955; Garton, 1960; Hungate, 1966; Church, 1983; Van Soest, 1984; Hartfoot and Hazlewood, 1988). In this regard, researchers have undertaken significant steps in order to produce meat and milk from ruminants with higher levels of polyunsaturated fats (Cook and Scott, 1970; Scott et al., 1971; 1972; Wong et al., 1973; Chandler et al., 1973; Edmondson et al., 1974; Scott, 1975; Tracey, 1975). The process is the biomanipulation of ruminant dietary fats. In the early 1970's, Australian researchers produced red meats with high polyunsaturated fatty acid content from ruminants by feeding dietary fat protected from microbial attack by a protein formaldehyde complex (Cook and Scott, 1970; Scott et al., 1971; 1972). Despite their success, there is a drawback on the use of formaldehyde in commercial products. Formaldehyde is not allowed in animal feed in the United States due to its carcinogenicity (Albert et al., 1982; WHO, 1989). In an attempt to find an alternative to formaldehyde,

Newberry (1990) successfully protected unsaturated fats against rumen microbial activity *in vitro* by entrapment of the fat in diacetyl or tannic acid-protein complexes. However, additional research is needed to confirm the results of Newberry's study (1990) and to expand the research to other oils/oilseeds and also to investigate effects of dietary supplements *in vivo*. Therefore, the objectives of this study were: (1) to produce gels containing high levels of linoleic acid oil with sunflower or soybean seeds encapsulated in casein complexes, treated with formaldehyde (FA), acetaldehyde (AC) and diacetyl (DA), (2) to determine the microstructures of the dietary gels by scanning electron microscopy, (3) to evaluate the effectiveness of the gel treatment in protecting dietary fatty acids from biohydrogenation *in vitro*, and (4) to alter the fatty acid composition of sheep serum and milk fats by feeding the gel supplements.

CHAPTER II

LITERATURE REVIEW

Lipids

Lipids are esters of fatty acids and glycerol (Fennema, 1985). They include fats and oils. Normally, fats are lipids that are solid at room temperature while oils are liquid at room temperature. Lipids from animals are mostly fats, oils are predominantly encountered in plants. The number of carbons of the fatty acids and the number of the double bonds between carbons affect the physical and chemical properties of the lipids. Compared with most oils, fats contain higher levels of saturated fatty acids, while oils contain more mono- and polyunsaturated fatty acids than fats (Weiss, 1983; Fennema, 1985).

Lipids are one of the most important constituents in living organisms due to their multiple and essential functions in the body (Holum, 1979; Whitney and Hamilton, 1987). In fact, lipids have different structural roles in the body. First of all, they are constituents of every cell, conferring the cell membrane its permeability to fat soluble substances such as some vitamins and hormones. Lipids are the body's most concentrated reserve of energy. For instance, one gram

of lipids during metabolism furnishes nine calories compared to four calories obtained from the metabolism of one gram of carbohydrate or protein. Lipids offer protection to the organs (heart, kidney, mammary glands, etc.) from temperature extremes and cushion against mechanical shocks (Whitney and Hamilton, 1987).

Lipids, especially polyunsaturated fats, by being precursors to prostaglandins, have a variety of vital regulatory functions in the body. In fact, prostaglandins are hormone-like compounds in many organs. At present time, about 100 of them are known. They have different regulatory activities in the body. For example, one dilates and/or constricts blood vessels therefore affecting the blood pressure. Another alters the transmission of nerve impulses. One is responsible in regulating the body tissues responses to other hormones. The action of some of them on the kidney affects its water excretion. In milk, one of them helps to protect the infants digestion tract against injury (Whitney and Hamilton, 1987; Holum, 1979).

Lipids are important in foods as ingredients, texturizers and energy. The flavor and the aroma of foods are contributed by compounds that are integrated in fat molecules (Fennema, 1985). These molecules are carriers for fat soluble vitamins such as vitamin A, D, E and K. They give palatability (pleasing taste) and add satiety (the feeling of satisfaction and fullness) to foods (Whitney and Hamilton, 1987).

The Rumen Microflora

The digestive tract of ruminants (cattle, sheep, goat) comprises four different compartments: the reticulum, the rumen, the omasum and the abomasum. The particularity of the rumen is its ability to support growth of scores of microorganisms (Hungate, 1988). The pH of the rumen ranges from 5.5 to 7.0 and the temperature from 39 to 40°C. The absence of oxygen makes the rumen an anaerobic growth medium which harbors different types of bacteria (Stewart and Bryant, 1988), protozoa (Williams and Coleman, 1988) and fungi (Orpin and Joblin, 1988) which come from animal feed, predominantly forage. Therefore, the type of microorganisms depends on the type of feed. The majority of the microflora are predominantly obligate anaerobic bacteria; only a few are facultative anaerobes (Hungate, 1966). Most of the bacteria are cocci and short rods with sizes ranging from 0.4 to 1 micron in diameter and 1 to 3 micron in length.

The bacteria of the rumen are differentiated by the substrates they attack. They include mainly cellulose, starch, and hemicellulose digesters, fermenters of sugars; utilizers of acids; and metanogenic, proteolytic and lipolytic bacteria (Hungate, 1966; Chesson and Forsberg, 1988). They metabolize the dietary carbohydrates, proteins, lipids, minerals and other organic compounds to end products such as acetic acid, propionate and butyrate (Hungate, 1966; Church, 1983). Other products such as hydrogen, ethanol, and formic,

lactic and succinic acids are produced by pure ruminal bacteria culture yet they do not constitute final products in the rumen (Hungate, 1966).

The second group of the rumen microflora are the protozoa (Williams and Coleman, 1988). These microorganisms have numerous small hairs, called cilia, which enable them to move. These protozoa have gained the name of ciliates. They are anaerobic, therefore capable of growth in the rumen, and can ferment substances from plant materials for energy. The protozoa of the rumen are mostly ciliates; however, some small flagellates are present. The two different ciliate groups encountered in the rumen are holotrichs which possess cilia over the entire body except the mouth, and endodiniomorphs, which have cilia only on part of the body. Carbon dioxide, hydrogen, formic, acetic, propionic, butyric and lactic acids account for the fermented products by the protozoa (Hungate, 1966). Lipids in the chloroplasts are hydrogenated by the protozoa (Wright, 1959).

The rumen microorganisms take advantage of the dietary substances as a source of energy for their proliferation. The decomposition of these numerous substrates yields useful products including vitamins for the host. Substrates such as cellulose are broken down by bacteria without which this compound would not be metabolized by the ruminant. However, the hydrogenation of the polyunsaturated fats by the microflora to saturated fats (Annison et al., 1959; Garton,

1959; Church, 1983; Hartfoot and Hazlewood, 1988) is not advantageous to humans. In order to correct the course of action of these microorganisms, the dietary fats of the ruminant farm animals have to be protected.

Saturated and Unsaturated Fats and Health Concern

A fatty acid is an organic acid which contains 2 or more carbon atoms. The number of carbon atoms in naturally occurring fatty acids is even. Fatty acids having 2 to 10 carbon atoms are characterized as short chain fatty acids and are common in dairy products. The fatty acids predominantly encountered in fish and meat products contains 14 or more carbon atoms. They are the long chain fatty acids (Fennema, 1985).

Fatty acids are differentiated by the number of hydrogen present in the molecule. Saturated fatty acids carry all the hydrogen atoms possible and have a general molecular formula of $C_nH_{2n}O_2$. No double bond between carbon atoms ($C=C$) is present in the molecule. On the other hand, the presence of one or several double bonds between carbon atoms characterizes the second type as mono or polyunsaturated fatty acids. These different fatty acids are esterified with a molecule of glycerol to give the fat molecule or triacyl glycerol. Fats, which contain higher levels of saturated fatty acids are solid at room temperature, while oils, which are liquid, contain higher levels of unsaturated fatty acids (Fennema, 1985;

Whitney and Hamilton, 1987).

As stated earlier, fatty acids are important components of living systems. Polyunsaturated fatty acids such as linoleic (18:2), linolenic (18:3) and arachidonic (20:4) are recognized as essential fatty acids (EFA). Linoleic and linolenic cannot be synthesized in the animal body. The production of arachidonic acid, requires linoleic acid. Therefore, EFA have to be supplied to the metabolic system only through diet. The deficiency of linoleic acid, for instance, results in the occurrence of symptoms such as reddening and irritation of the skin, possible infections and dehydration, and development of liver abnormalities (Whitney and Hamilton, 1987). In infants, this deficiency results in growth failure.

Despite the numerous benefits of fatty acids in living organisms, their detrimental effects on human health is well established. The controversy between the level of serum cholesterol and the risk of heart disease has made saturated fats, which increase serum cholesterol levels, unpopular. Several epidemiological and research studies in both animals and humans have confirmed this relationship.

The relationship between the type of dietary lipid and the response of the serum cholesterol level was first studied by Kinsell et al. (1952). They reported that human subjects had their serum cholesterol level reduced when their diet included high amounts of vegetable oils. They observed that

people who were subjected to a liquid formula diet containing the oils had their serum cholesterol level reduced by up to 100 mg/dL. In addition, supplementing the diet containing high vegetable oil with 69 g cholesterol/d did not significantly raise the level of serum cholesterol.

Ahrens et al. (1954), fed healthy subjects diets containing butter or vegetable oil margarine and evaluated their influence on the serum cholesterol. They found that the serum cholesterol level of the subjects, who were on the diet including butter, was 20% higher than those whose diet contained the vegetable margarin. These original works led different researchers to investigate the relationship between the dietary lipids and the serum cholesterol response. A study by Trevisan et al. (1990) with 4,917 Italian males and females, 20 to 59 years of age showed indiscriminately that the intake of atherogenic foods (foods high in cholesterol and saturated fats) resulted in the increase of systolic blood pressure and levels of serum glucose and serum cholesterol. The study confirmed other findings that consumption of foods high in saturated fats and cholesterol is associated with an increased risk for atherosclerosis. On the other hand, polyunsaturated fats have been recognized for their reduction in the plasma cholesterol concentrations (Ahrens et al., 1957; Keys et al., 1965). These latter studies showed that the sum of the amounts of the saturated fatty acids, lauric, myristic and palmitic acids raised the cholesterol concentration by

twice the amount that the sum of the equivalent amounts of polyunsaturated fatty acids lowered it (Keys et al., 1965; Grande et al., 1972).

In comparing the effect of dietary fats on the response of the serum cholesterol, Nestel et al. (1973) put five males and a female on two different diets for a period of three to four weeks. The polyunsaturates/saturates ratios in the control and treatment diets were 0.06 to 0.09, and 0.56 to 0.89, respectively. The study showed a 10% reduction in serum cholesterol for the subjects whose diet contained high level of polyunsaturated fats (Nestel et al., 1973). Nestel et al. (1992) fed three diets containing different oil blends. The control contained 21.2, 41.9 and 36.9% polyunsaturated (PUFA), monounsaturated (MUFA) and saturated (SFA) fatty acids respectively. The oil blend 1 was composed of 36.9% PUFA, 42.1% MUFA and 21.0% SFA. Blend 2 contained 36.4% PUFA, 43.2% MUFA and 20.4% SFA. Twenty six subjects of an average age of 42.4 years were randomly divided into three groups. The groups were fed for four weeks with a diet containing a different oil blend, then switched to another diet until all groups had received all diets. Their results showed a significant lowering effect of the serum cholesterol of the diets which contained higher PUFA, than the control. The cholesterol contents of the serum were 214, 175 and 164 mg/dL for subjects on diets containing control, blend 1 and blend 2, respectively. Saturated fatty acids such as lauric (12:0),

myristic (14:0) and palmitic (16:0) acids in the diet have been found to induce an increase in serum cholesterol levels, while monounsaturated and polyunsaturated fats have the opposite effect (Keys et al., 1965).

Ruminal Biohydrogenation of Dietary Lipids

A. Fatty Acid Composition of Ruminant Dietary Lipids

The dietary lipids of domesticated ruminants come from a variety of sources including: forage crops, crushed cereal grains (corn) and crushed oilseeds (soybean, cottonseed, rapeseed). The dry forage contains 6 to 7% lipids of which glycolipids and phospholipids account for about 95%. The minor components of these lipids are phosphatidylinositol and diphosphatidyl glycerol (cardiolipin) (Shorland, 1963). Though the fatty acid composition differs from forage to forage, in general, polyunsaturated fatty acids such as linolenic (18:3) and linoleic (18:2) occur predominantly in the forage lipids as reported in Table 1.

B. Mechanism of the Biohydrogenation of Dietary Lipids

Banks and Hilditch (1931) were the first to report that fats of ruminants' tissues contained more saturated fatty acids than those of non-ruminant herbivores.

Table 1. Fatty acid Composition of Forages and Ruminants Feeds

Sources of	Fatty Acids									
lipids	12:0	14:0	16:1	16:1	17:0	18:0	18:1	18:2	18:3	other
	-----concentration (%)-----									
Forages ^a										
Mix Past.G ^b	2.9	3.3	9.4	3.0	--	1.5	13.2	20-25	30-39	1.4
Mix.Past.G ^c	--	1.1	15.9	2.5	--	3.4	19.2	61.3	0.5	--
Clover Past ^d	--	--	8.9	7.9	--	2.8	9.5	8.1	58.9	3.9
Rations ^f										
Luc. + Con.	0.6	2.1	24.2	4.4	0.9	2.1	9.6	33.3	18.2	4.7
Concentrates	0.1	1.1	15.0	2.9	0.5	0.4	13.2	57.6	6.2	3.0

^aMix Past. G=Mixed Pasture Grass; Clover Past.=Clover Pasture.

^bGarton (1959); ^cGarton (1960); ^dShorland et al. (1955).

^fLuc. + Con.=Lucerne hay + Concentrates.

They suggested that the dietary fats were hydrogenated within the tissues. Latter, others reported that the alteration of the dietary lipids occurred in the rumen before their incorporation in the depot fats (Reiser, 1951; Ward et al., 1964). As the result of this biohydrogenation, it is estimated that approximately 3% of the fatty acids of the ruminant fats is polyunsaturated, the remainder being monounsaturated and saturated acids. As previously stated, domesticated ruminants are mostly raised on cereal grains and pastures whose lipids contain about 13% linoleic acid (18:2) and 60% linolenic acid (18:3).

Other investigations have shown that when the rumen is bypassed by infusion of polyunsaturated fats in the abomasum, the composition of the fatty acids in the animal body fat reflects those from the fat infused in the abomasum (Scott et al., 1971; Chilliard et al., 1991; LaCount et al., 1994). This evidence shows that the dietary fats of ruminants undergo significant modifications during ruminal digestion before they are incorporated in the body fat. As mentioned earlier, the ruminal microbes are solely responsible for these changes.

The mechanism of this process has been partially elucidated by different researchers. When the dietary lipids reach the rumen, they first undergo lipolysis before they are hydrogenated. The enzymes, lipases and phospholipases, produced by the rumen microorganisms are responsible for decomposing the acyl lipids from the diet to glycerol and

individual fatty acids. The glycerol is then fermented while the unsaturated fatty acids are hydrogenated.

Researchers have isolated different bacteria from rumen fluid with lipolytic activity (Hobson and Mann, 1961; Henderson, 1973; 1975; Prins et al., 1975; Hazlewood and Dawson, 1975; 1979). Gram negative anaerobic bacteria from sheep rumen fluid were found to hydrolyze linseed oil triglycerides and tributyrin and to ferment glycerol (Hobson and Mann, 1961). The lipase from lipolytic bacteria was purified and characterized by Henderson (1971) as an extracellular enzyme associated with the cell surface. The optimum activity of this lipase occurred at pH 7.4 and at 20 to 22°C. CaCl_2 or BaCl_2 enhanced its activity while ZnCl_2 and HgCl_2 were inhibitory (Hartfoot and Hazlewood, 1988).

Several studies have led to the elucidation of the mechanism and pathways of the biohydrogenation of the unsaturated fatty acids. It has been suggested that the presence of a free carboxyl group ($-\text{COOH}$) is essential for the process to occur (Kepler et al., 1970; Hazlewood et al., 1976). These studies proposed that upon their hydrolysis, linolenic (cis-9,cis-12,cis-15 octadecatrienoic acid) and linoleic acids (cis-9,cis-12 octadecadienoic acid) are transformed to oleic (18:1) and stearic (18:0) acids. Prior to hydrogenation, the cis-12 double bond in the PUFA is isomerized to the trans-11 form. The outcome of this initial reaction is the production of a conjugated cis-9,trans-11,cis-

15 triene and a cis-9,trans-11 diene from linolenic and linoleic acids, respectively. Figure 1 shows the hydrogenation of linolenic acid (18:3) to trans-11,cis-15 octadecadienoic acid, then to trans-15 and cis-15 octadecenoic acids and finally to stearic acid (Wood et al., 1963; Dawson and Kemp, 1970). Figure 2 illustrates the steps of the biohydrogenation of linoleic acid as suggested by (Harfoot and Hazlewood, 1988).

Two groups of bacteria are involved in this biohydrogenation process (Figures 1 and 2). Group A includes bacteria which are capable of transforming linolenic acid to trans-11,cis-15 octadecadienoic (18:2tc) and trans-11 octadecenoic (18:1t) acids, but cannot hydrogenate them further to stearic acid (18:0). The bacteria, *Butyrivibrio fibrisolvens* belongs to this group. On the other hand, the members of group B have the ability to hydrogenate linoleic (18:2) and trans-11 octadecenoic acids to stearic acid (Hazlewood et al., 1976).

The enzyme, 12-cis, 11-trans isomerase from *Butyrivibrio fibrisolvens* is believed to be responsible for the formation of the cis-9,trans-11 conjugated double bonds (Kepler et al., 1971). These investigators suggested that the presence of both, cis-9,cis-12 bonds and free -COOH group at position 1, in the substrate of this isomerase is necessary for its activity.

Following the isomerization, the hydrogenation of the

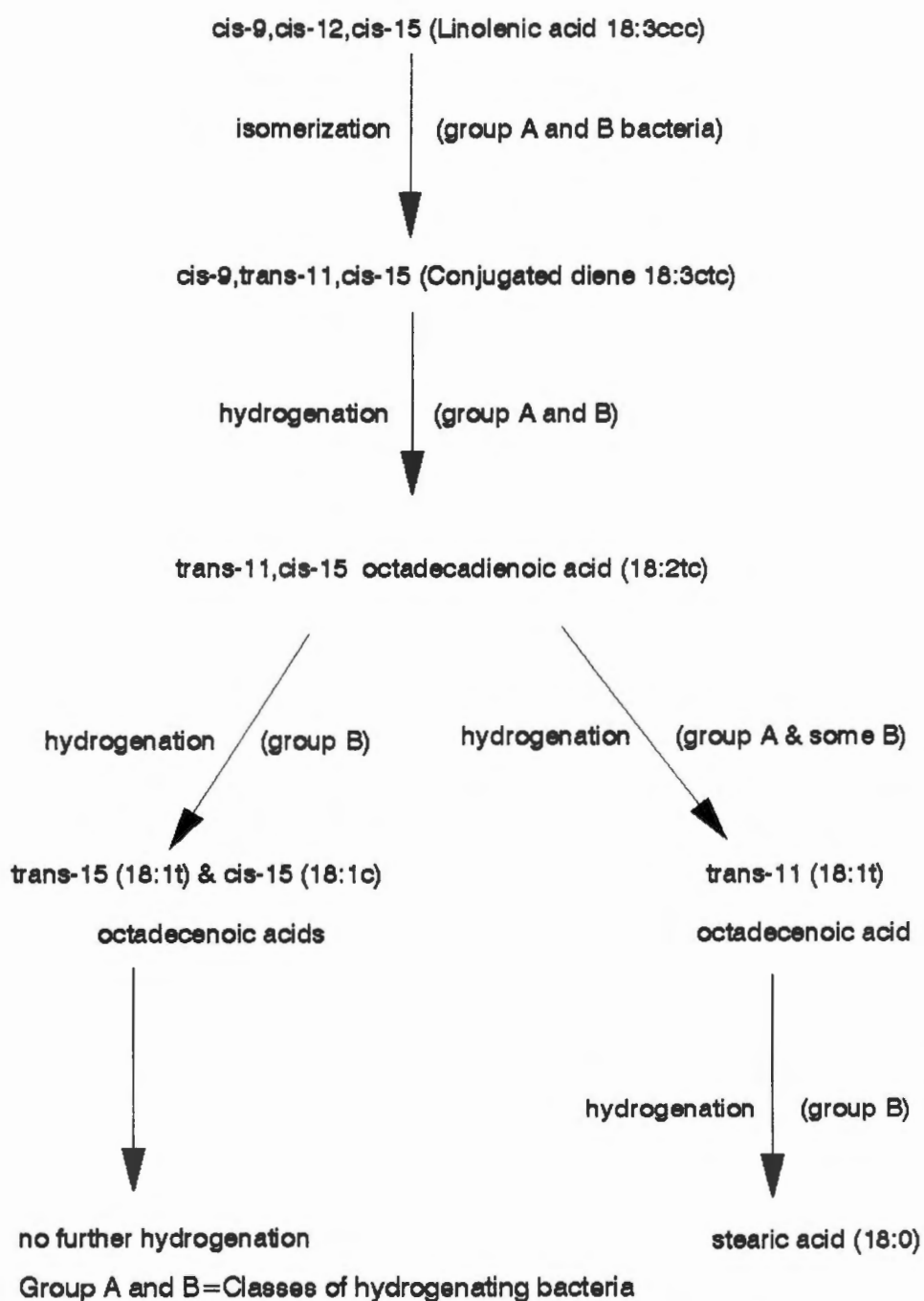
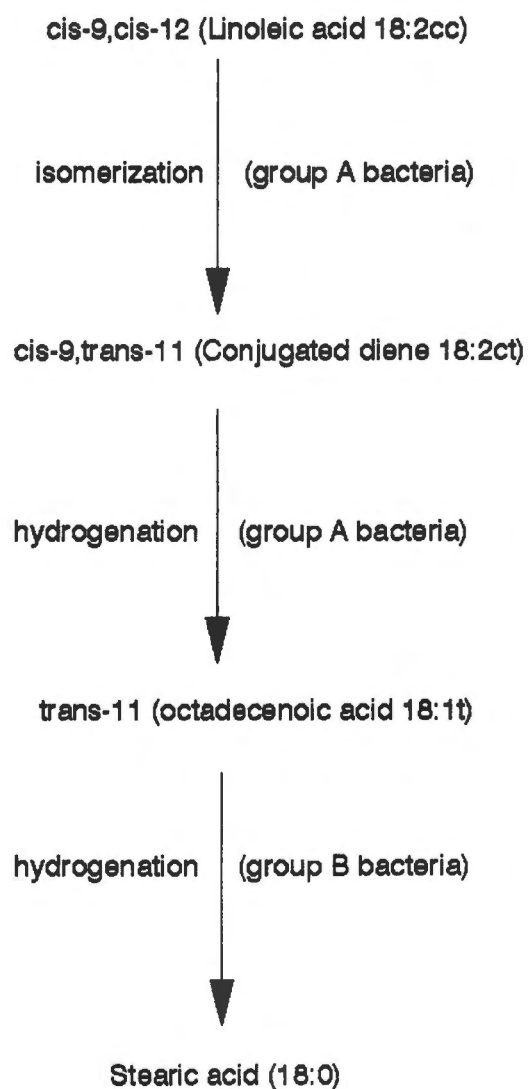


Figure 1. Biohydrogenation of linolenic acid (18:3) (Harfoot and Hazlewood, 1988).

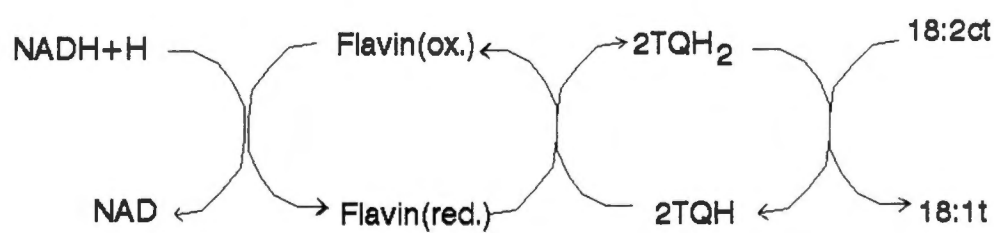


Group A and B=Classes of hydrogenating bacteria

Figure 2. Biohydrogenation of linoleic acid (18:2) (Harfoot and Hazlewood, 1988).

conjugated intermediates is due to the transfer of two hydrogens to each double bond, the cis being saturated first and the trans bond, the last. The mechanism of this process has been studied by researchers for over 20 years (Rosenfeld and Tove, 1971; Hunter et al., 1976). However, it was not until recently that the probable electron donors have been identified as tocopherolquinol (TQH_2). Hughes and Tove (1980a; 1980b) suggested that tocopherolquinone in presence of NADH could be reduced to tocopherolquinol. Thus, two of the reduced molecules (TQH_2) are oxidized, each to the semiquinone, which provides the electron for the reduction of the isomers to more saturated fatty acids. A flavin-like compound is believed to transfer the electrons from the NADH to the tocopherolquinone molecule. The following reactions (Figure 3) involved in the process were proposed by Hughes and Tove (1980a; 1980b).

The cis-9,trans-11 octadecadienoate reductase implicated in this reaction has been isolated and characterized by Hughes et al. (1982). It has a MW of 60,000 and contains 10 mol of fucose and 12 mol of galactose per mol. However, the carbohydrate portion is not required for the activity of this enzyme. On the other hand, it contains 2 mol of Fe/mol, and Fe^{3+} is essential for its activity, suggesting that Fe^{3+} may be involved in the oxidation-reduction reaction (Hughes and Tove, 1980a).



TQH_2 = A-tocopherolquinol

TQH = A-tocopherolquinone

Figure 3. Electron transfer during the Biohydrogenation of Unsaturated Fatty Acids (Hughes and Tove, 1980a).

Protective Additives

A. Formaldehyde

Formaldehyde is the simplest of the aldehyde family. It constitutes one of the most important chemical compounds produced and used in the United States (National Research Council, 1981). Monomeric formaldehyde is a flammable colorless gas that can form a liquid at high vapor pressure and crystallizes at -118°C (National Research Council, 1981; WHO, 1989). It polymerizes at ambient temperature. Its molecular weight is 30.03 g, with a density of 0.7834 g/mL, a boiling point of -21°C , and a melting point of -92°C . It has a pungent odor that is irritating to the membrane of the nose, eye and upper respiratory tract (Newell, 1983). It is highly soluble in water (Loudon, 1984) and the aqueous solution of 30 to 50%, called formalin is the principal form in which formaldehyde is commercialized (National Research Council, 1981; WHO, 1989). Its production was estimated at 5.45 billion pounds in 1983.

Formaldehyde is widely present in the environment. It is a metabolite of biological systems, including human (Newell, 1983) and plants. Levels of formaldehyde in apples, grapes, and pears were 26, 80 and 275 ppb, respectively. It is also produced in cigarette smoke at an estimated 40 ppm (Turoski, 1985). Formaldehyde is produced commercially by

dehydrogenating methanol by two different procedures:



Formaldehyde is converted mostly to urea and phenol-formaldehyde resins, which are used to produce plywood, particle board, foam insulation, and many plastic materials. Other important applications include the production of resins, high-polymeric resins, chelating agents, plastics, disinfectants, textile treatment agents, leather, and dye manufacture (National Research Council, 1981). In addition, formaldehyde has been widely used as a preservative in cosmetic products such as shampoos, bubble baths, nail hardeners, hair conditioners, and bath oils, tablets and salts (Jass, 1985).

Due to its good binding ability to proteins, formaldehyde has been the ideal compound used to protect dietary lipids against ruminal hydrogenation. To date, formaldehyde has constituted the sole aldehyde utilized to produce protein-oil emulsions which have effectively withstood degradation by ruminal microflora (Rawlings and Procter, 1977). This protection leads to a significant increase in the level of dietary polyunsaturated fatty acids reaching the small intestine of ruminants. These acids then are absorbed and incorporated into the adipose tissue (Faichney et al., 1972; Lough et al., 1992).

The quality of ruminant's feed can be improved by

treatment with formalin (37 to 50% aqueous solution of formaldehyde) (Snyman, 1993).

A great number of researchers have significantly raise the level of unsaturated fatty acids in cattle (Dinius, 1975; Cadden et al., 1984; Atwal, 1991), sheep (Lough et al., 1992; Solomon et al., 1992), buffalo (Srivastava and Mudgal, 1984), and in goat products by feeding them in one form or another, lipid-protein-formaldehyde supplements.

Due to its toxicity, the use of formaldehyde on a commercial scale to produce animal feed is not legal in the United States. Epidemiological studies (Blair et al., 1985) have shown that formaldehyde has a wide range of health hazards in humans and animals. The assessment of cancer risks associated with formaldehyde has been done with people who work with it. It is estimated that about 1.6 million workers may be exposed to formaldehyde during its manufacture or its use to produce other commercial items (Blair et al., 1985). Occupational exposure to formaldehyde vapors has resulted in symptoms such as irritation of the eyes and respiratory tract, headache, tiredness and thirst. These symptoms were observed in residents of homes, schools, industrial buildings in which high levels of formaldehyde were found (National Research Council, 1981). Control studies in humans have determined that different doses of formaldehyde induce different symptoms. Table 2 gives the relationship between levels of formaldehyde and symptoms in humans.

Table 2. Health Effects of Formaldehyde at Various Concentrations

Reported Effects	Concentration (ppm)
None reported	0-0.05
Neurophysiological effect	0.05-1.05
Odor threshold	0.05-1.00
Eye irritation	0.05-2.00
Upper airway irritation	0.10-25.00
Lower airway and pulmonary effects	5.0-30.00
Pulmonary edema, inflammation pneumonia	50.0-100
Death	100+

(Newell, 1983).

As a result of ingestion, formaldehyde can cause loss of consciousness, vascular collapse, pneumonia, hemorrhagic nephritis, and abortion in humans (Newell, 1983).

Formaldehyde has been shown to induce cancer in the nasal cavity of rodents (Carr and Kolbye, 1985). One of the most significant carcinogenic studies of formaldehyde was carried out by the Chemical Industry Institute of Technology (CIIT). In the experiment, 240 mice and rats were exposed to 15, 6 or 2 ppm of formaldehyde gas for 6 hr/day, 5 d/week for 2 years. The study showed a appearance of carcinogenic cells in the nasal cavity of the rats after 13 to 14 months exposure at 15 ppm (Marks et al., 1980). Other researchers demonstrated that rats exposed to 14 ppm of formaldehyde gas for 6 hr/d, 5 d/week for life induced squamous carcinomas of the nasal cavity (Albert et al., 1982).

B. Acetaldehyde

Acetaldehyde (CH_3CHO) has a molecular weight of 44.05 g, a boiling point of 20.8°C , a melting point of -121°C , and a density of 0.7834 g/mL (Loudon, 1984). Acetaldehyde is generated in different living organisms as a result of biochemical reactions. In humans, it is a product of the metabolism of ethanol, using alcohol dehydrogenase in the following reaction:



This is the way alcohol is removed from the blood

(Loudon, 1984). In bacteria and yeasts, acetaldehyde is reduced to ethanol in a variety of fermented products (Loudon, 1984). It is one of the numerous carbonyl compounds produced by lactic acid bacteria: *Lactobacillus*, *Lactococcus*, *Leuconostoc* and *Streptococcus* (Cogan and Jordan, 1990) and yeasts: *Candida* species (Collings et al., 1991), which contribute to the flavor and aroma of cultured dairy products (Abrahamsen and Rysstad, 1991; Gaafar, 1992). It has been recovered at 0.42 ppm in milk (Gaafar and Gaber, 1992). It was produced by *Bifidobacterium* CNRZ 1494 at 8 to 12 ppm and 4 to 5 ppm in cow milk supplemented with yeast and in soya extract, respectively (Murti et al., 1992). Kneifel et al. (1992) found levels of acetaldehyde ranging from 5.5 to 20.7 ppm in 47 types of commercial yoghurts.

Commercially, Acetaldehyde is produced by two major procedures. The most common production method is realized by controlled oxidation of ethylene with an aqueous solution of palladium and cupric chloride in a single step reaction:



The catalyst is regenerated in the second step of the reaction. Other synthesis methods include hydration of acetylene and dehydration of ethanol. However, due to the elevated cost of acetylene and the limited availability of ethanol, these last procedures are less competitive (National Research Council, 1981). Acetaldehyde is used mostly to produce other chemicals including acetic acid and anhydride as

well as other useful compounds.

Acetaldehyde is the least toxic of aldehydes. It has not been shown to have mutagenic or carcinogenic effects. However, when administered orally, it is slightly toxic. Irritation of the eyes, skin and upper respiratory tract can occur as the result of exposure to low concentration of acetaldehyde (National Research Council, 1981). The potential of this non toxic compound as a protective agent for dietary lipid has not yet been studied.

C. Diacetyl

Diacetyl (2,3-butanedione) is an important flavor component of foods. It is produced by bacteria as *Streptococcus*, *Lactococcus lactis* species, *Leuconostoc*, *Lactobacillus*, and *Pediococcus* in cultured dairy products such as butter, sour cream, and buttermilk, yoghurt, cheese in which diacetyl contributes to their characteristic buttery flavor and aroma (Kaneko et al., 1991; Gaafar, 1992; Wangoh et al., 1992). It is also common in wines, brandy, roasted coffee, silage and many other fermented products (Charley, 1986; Abeygunasekera and Jansz, 1989). Diacetyl is considered a GRAS (Generally Recognized as Safe) substance and is used primarily as an imitation flavor (Jay, 1986). Due to its carbonyl groups, diacetyl can react with proteins. The interaction between carbonyl compounds, such as formaldehyde and malonaldehyde, and protein is a well established fact

(Fennema, 1985). Damodaran and Kinsella (1981a) suggested that the interaction between soy proteins and carbonyls is hydrophobic in nature. Their investigation showed that the binding affinity depended on the length of chain as well as the position of the keto group in the chain. The binding affinity tends to increase when the keto group is toward the end of the chain. Between 25 and 45°C, temperature of reaction had little impact on the binding affinity. However, a higher temperature that partially denatured the soy protein enhanced its binding affinity for carbonyls compared to the native protein (Damodaran and Kinsella, 1981b). The carbonyl interaction with proteins gives diacetyl its potential as a protective agent for encapsulated fats in a protein coat against biohydrogenation during ruminal digestion. In a recent study, Newberry (1990) successfully protected soybean oil in a diacetyl treated emulsion from biohydrogenation when incubated with rumen fluid *in vitro*.

The drawback of diacetyl, however may be its antimicrobial properties. In fact, diacetyl is recognized as an effective antimicrobial agent against gram negative bacteria and fungi (Jay, 1982; 1984). Diacetyl antagonizes arginine utilization by reacting with arginine-binding proteins of the gram negative bacteria. Very little research has been done to ascertain the inhibitory effect of diacetyl on food microorganisms. Jay (1982) reported that gram negative bacteria and yeasts were inhibited by 200 ug/mL

diacetyl while 300 ug/mL was the inhibitory level for non-lactic bacteria. It was suggested that diacetyl could be used as an antimicrobial dip for utensils and work surfaces because of its high volatility (Jay, 1982). Should it leach from the treated protected dietary supplement, diacetyl may inhibit the activity of the rumen microflora.

Fatty Acid Composition of Soybean Seeds

Soybean, *Glycine max* belongs to the family of Leguminosae and includes 3 species: *Glycine ussuriensis* (wild), *Glycine max* (cultivated) and *Glycine gracilis* (intermediate). It has been used as human food in Asia for centuries (Salunkhe et al., 1992). With a production of 92.33 million tons in 1988, soybean constitutes the worlds most important oilseed today, accounting for over 30% of the oilseeds produced worldwide (FAO, 1988). The seeds are used as human food and livestock feed and for edible oil. The United States is the major producer with about 70% of soybean production in the world.

The soybean seed is an excellent source of protein and lipid. The protein content ranges from 32 to 50%, while the oil content ranges from 14 to 23% of the seed. Carbohydrates account for one third of the bean, including 5.0% sucrose, 3.8% stachyose and 1.1% raffinose (Salunkhe et al., 1992). Table 3 lists the proximate composition of different parts of soybean seed (Mounts et al., 1987) and Table 4, the major fatty acids component of the oil (USDA, 1984).

Table 3. Proximate composition of soybean seed parts.

Components	Proximate Composition (%)			
	Whole seed	Cotyledons	Hypocotyl	Hull
crude protein	40.0	43.0	41.0	8.0
fat	21.0	23.0	11.0	2.0
carbohydrate	34.0	29.0	43.0	86.0
ash	4.9	5.0	4.4	4.3

(Adapted from Mounts et al., 1987).

Table 4. Fatty acid composition of soybean oil.

Fatty acid	Concentration (%)
Saturated	14.92
14:0	0.11
16:0	10.81
18:0	4.00
Monounsaturated	24.17
16:1	0.21
18:1	23.95
20:1	0.21
Polyunsaturated	60.72
18:2	53.57
18:3	7.14

(USDA, 1984).

Soybean contains 18 to 23% of total lipids which are composed of 88.1% neutral lipids, 9.8% phospholipids and 1.6% glycolipids (Salunkhe et al., 1992). Neutral lipids include triglycerides, free fatty acids, sterols and sterol esters. Palmitic, oleic, linoleic and linolenic acids constitute the main components of neutral lipids (Mahadevappa and Raina, 1978). The proximate composition, the lipid content and the fatty acid content of the bean are influenced by genetic and environmental factors (Salunkhe et al., 1992).

Soybean is an excellent protein source. The seed contains an estimated 35 to 44% of protein, depending on the cultivar. Strains with elevated levels of protein, usually contain less oil. The majority of soybean proteins have molecular weight of 100,000 or higher. Water soluble soy proteins over 90% of the total protein are classified on their sedimentation constants as 2S, 7S, 11S and 15S proteins. The major portion of the globulins comprises the 7S and 11S fractions (Salunkhe et al., 1992).

Soy protein has a high lysine content, making it an ideal supplement to cereal proteins. However, methionine is a limiting amino acid in soybean (Salunkhe et al., 1992). These factors have to be considered when soybean is used for nutritional purposes. The ability of soy proteins to form a gel has been used in food processing. Tofu (soybean curd) is a common soybean protein food in Asia. It is traditionally prepared by coagulating heated soybean milk with magnesium

chloride or calcium sulfate. Today, glucono-delta-lactone (GDL) has become a major soybean protein coagulant in tofu-processing factories due to its ability to easily form homogenous gels (Kohyama and Nishinari, 1993). The gelation property of soybean proteins is attributed to the globulin fractions 7S and 11S (Saio and Watanabe, 1978; Kohyama and Nishinari, 1993). Soybeans were used in this study because of their availability, low cost, their elevated content of oil, containing high amounts of polyunsaturated fatty acids.

Protection of Dietary Lipids Against Biohydrogenation

The biohydrogenation of dietary lipids in the rumen can be circumvented by protecting them in a protein-formaldehyde coat. The technique was introduced by Scott et al. (1971). They produced casein-safflower oil particles by spray-drying and treated the particles with formalin (37% formaldehyde). The supplement was similar to dried milk powder. The microstructure reveals 10 to 60 microns size particles consisting of a casein matrix containing fine inclusions of oil 0.1 to 4.0 micron in diameter. The particles have air space in the center. The principle of protection takes advantage of the emulsifying ability of protein with lipids and the binding ability of formaldehyde to proteins. This bond is stable at a neutral pH which is encountered in the rumen (pH 6 to 7). Therefore, contact between the encapsulated lipids and microbial enzymes is prevented. The

protected lipid particles leave the rumen without being altered. In acidic (pH 2 to 3) condition of the abomasum, the protein-formaldehyde bond is disrupted. The released lipids are carried to the small intestine where they are absorbed by the chylomicrons in the blood stream, then stored in different tissues.

The anaerobic incubation of the formaldehyde-treated and untreated particles *in vitro* with sheep rumen fluid for 20 hr allowed Scott et al. (1970; 1971) to conclude that formaldehyde was efficient in protecting unsaturated fats from microbial hydrogenation. Other researchers (Plowman et al., 1972) latter confirmed the success of the original work. Plowman et al. (1972) also investigated the effect of the casein-formaldehyde ratios as well as the casein-oil ratios on the efficiency of the protection. They found that 4.8% formaldehyde enabled total protection. A 1:1 casein:oil ratio was found to be adequate in producing the casein-oil emulsion.

The resistance of protected dietary lipids to ruminal biohydrogenation was investigated using goat, sheep and cattle. The fate of both protected and unprotected dietary lipids of same composition was determined by feeding these ruminants with a diet containing the lipid supplements. Determination of the fatty acid composition of rumen fluid (Plowman et al., 1972), abomasum (Scott et al., 1971) blood serum, muscles, depot fats, milk and liver showed that biohydrogenation of the dietary polyunsaturated fatty acids

was prevented. As a result, milk, serum, muscle and depot fats of animals fed with formaldehyde protected lipids contained significantly higher levels of polyunsaturated fatty acids than those of their counterparts fed a diet in which the lipids were not protected. This lipid supplement allowed the fat levels in the ruminants diet to be increased to high levels not normally tolerated. Ruminants loose their appetites when dietary fat exceeds 5% (Van Soest, 1984). Protected fats were fed at much higher levels, up to 35% without causing digestive upsets (Van Soest, 1984).

The protected supplement also prevents the alteration by rumen microorganisms of proteins in the particles (Faichney and White, 1977). Dietary protein is normally used by the microflora to make new protein for their own benefit. Microbial protein is not nutritionally efficient for the host animal (Wallace and Cotta, 1988). Scott et al. (1971) suggested that not only can lipids and proteins be protected by this procedure but also fat soluble nutrients, flavor compounds, vitamins and antioxidants.

Scanning Electron Microscopy

Scanning electron microscopy (SME) uses scattered electrons, reflected from the "illuminated" specimen and secondary electrons. This technique is used in biological sciences to identify surface microstructures and shapes of

cells and microorganisms. It has become an indispensable tool in the domain of food research. It is frequently used to determine the microstructure of different food products that the food scientist can correlate with various physical, chemical and microbiological characteristics of food material (Kerr et al., 1981).

Kerr et al. (1983) reported that scanning electron microscopy can be an efficient method for the detection of adulteration in spray-dried dairy products. Their study focussed on the morphological characteristics of 11 different commercially spray-dried dairy products. The microstructure of cassava flour extrudate were determined by Badrie and Mellowes (1991). They found that the microstructure correlated with the textural and physical properties of the flour during extrusion. Tatsumi et al. (1991) used SEM to explain the appearance of carrot after cutting with a culinary knife. Newberry (1990) examined the microstructure of spray dry casein-oil particles after treatment with formaldehyde, acetaldehyde, diacetyl and tannic acid.

Scanning electron microscope was used in our study to determine and compare the microstructures of soybean gels treated with formaldehyde, acetaldehyde and diacetyl and correlate these structures to the ability of the corresponding gels to withstand dissociation in the rumen.

CHAPTER III

MATERIALS AND METHODS

Protein and Lipid Sources

Dried, full-fat soybean (mixed varieties) seeds were donated by Dr. Fred Allen of the Department of Plant and Soil Science, The University of Tennessee, Knoxville; dried full-fat sunflower seeds were purchased from a local food store in Knoxville, TN.; sodium caseinate from bovine milk was purchased from Sigma Chemical Co. (St. Louis, MO); and defatted soy flour (DSF) with a PDI (protein dispersibility index) higher than 75 was obtained from Archer Daniels Midland (ADM) (Decatur, IL). These protein sources were used for the encapsulation of the lipid, and were stored in closed heavy polyethylene bags at room temperature until used.

Both oilseeds and commercial sunflower oil constituted the major lipid sources for the study. Soy lecithin, an emulsifier, which was obtained from a local market in Knoxville, TN, contributed a minor part of the lipid source. The seeds and the emulsifier were stored in a dark environment at room temperature, prior to their use.

Protective Chemical Agents

Reagent grade acetaldehyde (AC), diacetyl (DA), and formaldehyde (FA) were purchased from Sigma Chemical Co. (St. Louis, MO). They were stored in closed containers at 5 to 6°C prior to their use.

Preparation of Protein-Lipid Gels

A. Gels for Experiment 1: *in vitro* Study

A 10% (w/v) aqueous solution of sodium caseinate was prepared at 70°C. The pH of the solution was adjusted to 8.0 with a 50% (w/v) solution of sodium hydroxide. Three different types of batters were prepared, each containing full-fat soybean seeds, sunflower seeds or defatted soybean flour. The dried seeds were ground with a Wiley Mill Model No 2 (Arthur H. Thomas CO, Philadelphia, PA), through a 1 mm screen. The full-fat soybean or sunflower seed batters were each prepared by adding 40 g of ground seeds to 100 mL of the caseinate solution and blending for 2 min in a Waring blender at high speed with 0.1% (0.1% of the amount of oil in the batter) soy lecithin. The defatted soy flour batter was prepared by mixing 40 g of partially hydrogenated commercial sunflower oil with 100 mL solution of caseinate containing 0.1% soy lecithin (0.1% of 40 g of oil) at 70°C. The

caseinate-oil mixture was further emulsified by blending for 2 min. at high speed. Forty grams of DSF was added to the caseinate-oil emulsion which was mixed with a Kitchen Aid Model K5SS (The Hobart M. Co., Troy, OH) dough mixer for 10 min. into a homogeneous batter. Each batter was converted into a gel by mixing 5% (w/w of batter) acetaldehyde (AC), diacetyl (DA), or formaldehyde (37% solution, v/v) (FA) in the mixer. Untreated batter containing each type protein source constituted the controls. Each gel was sealed in polyethylene bags and stored at 5 to 6°C until used. Formaldehyde and acetaldehyde transformed the batter into a gel during treatment or shortly thereafter, while the diacetyl treated batter became a gel after it was stored for 48 hr at room temperature. The untreated batters were stored at -18°C to prevent spoilage. The gels were used to study the ability of formaldehyde, acetaldehyde and diacetyl to prevent or reduce the biohydrogenation of the lipids in the gels by ruminal microorganisms *in vitro*. Only the DSF gels were used in the electron microscopy study, since the protein particle size in the other gels were too coarse for good electron micrographs.

B. Gels for Experiment 2: *in vivo* Study

Dried full-fat soybean seeds (mixed varieties) from the Department of Plant and Soil Sciences, University of Tennessee, Knoxville, were ground through a 1 mm screen with

a Hammer Mill (Viking Manufacturing Co., Jackson, MI). To 27.22 Kg of water, were added .136 Kg of sodium hydroxide and 0.028 Kg of soy lecithin and mixed with a Portable Lightnin Mixer Model S-1 (Mixing Equipment Co. Inc., Rochester, NY). The mixture was heated to 70°C in a steam kettle (Groen M. Co., Chicago, IL). To the caustic solution, were added 3.63 Kg of sodium caseinate and 13.608 Kg of ground full-fat soybeans, and the batter was mixed for 180 min. A given weight (.907 Kg) of a single chemical additive (formaldehyde, acetaldehyde or diacetyl) or nothing (untreated) was mixed with the batter. All treated batters were transformed to gels by the chemical treatment. The untreated and treated batters were divided into 1.0 Kg units, and each unit was packed in polyethylene bags. The untreated batter units were stored at -18°C, while the treated batter (gel) units were stored at 5 to 6°C for 60 d until used.

Microstructure Determination of the Protected Gels

The microstructure of untreated and treated DSF gels were determined using a Scanning Electron Microscope Model-S800 (Hitachi Co., Japan). Gels were cut into strips (approximately 5 x 5 x 20 mm dimensions), then freeze dried, in a Virtis Freeze Dryer, Model FFD-15-WS (The Virtis Co., Gardiner, NY) for 48 hr at freezing and drying temperatures of -60°C and 100°C, respectively or in a vacuum oven at 65°C and -20 kPa. The gel samples were cooled for 10 min and stored in

a desiccator prior to microscopic examination. These dried samples were frozen in liquid nitrogen for 1 to 2 min and fractured. A small fragment was mounted on a carbon foam specimen holder and placed in the SEM for the microstructure analysis. Micrographs were made using accelerating voltages of 2.5 KeV and 1.8 KeV for the oven dried gels and the freeze dried gels, respectively. Both tilt and rotate angles were 0°. Images were obtained using a Secondary Electron Detector (SE) (Joy, 1993).

Biohydrogenation of Oils in Protected and Unprotected Soybean and Sunflower Protein Gels *in vitro*

The effectiveness of the treatments to prevent ruminal biohydrogenation of unsaturated fatty acids in the gels was evaluated by three *in vitro* experiments. Each experiment involved the chemically treated gels and an untreated batter with the gels and batter containing a single protein source (defatted soy flour; ground, full-fat soybean seed or ground, full-fat sunflower seeds). Each experiment consisted of two replications with the three chemically treated (FA, AC and DA) gels and untreated (control) batter sampled at 3 different digestion times (0, 24 and 48 hr) during ruminal fluid incubation constituting a single replication.

For each experiment, enough rumen fluid was collected from a fistulated steer on a grass hay and corn diet at the

Blount County farm of The University of Tennessee, Knoxville, to complete digestion of all replications of a single experiment. The rumen fluid was strained through double layers of cheese cloth into a prewarmed thermos-container and transported to the Department of Food Science and Technology within 20 min, for the laboratory experiment. For the experiments in which the gels and batter contained ground, full-fat soybean or sunflower seed, samples (2.0 g) of the chemically treated (FA, AC and DA) gels and of the untreated batter were weighed in triplicate into individual 250 mL Erlenmeyer flasks. A 20 mL aliquot of strained rumen fluid and 20 mL of Brucella broth (Appendix H; Difco Laboratories, Detroit MI) were added to each Erlenmeyer flask. The flasks were flushed with carbon dioxide and placed into a 38°C in a Shaking Water Bath Model 127 (Fisher Scientific Co.) for incubation. Flasks were shaken continuously through out incubation. At 0, 24 and 48-hr incubation, flasks, each containing a treated gel or untreated control, were removed, and microbial action was stopped by addition of 30 mL methanol and 30 mL of 2N sodium hydroxide to the flask.

For the gels and batter containing defatted soy flour-sunflower oil, 0.5 g samples of each treated gel and the untreated bath were weighed in triplicate into individual 250-mL Erlenmeyer flasks. Rumen liquor (20 mL) and 20 mL of Brucella broth were added to each flask, and after flushing with carbon dioxide, the flasks were treated as previously

described for gels containing the other protein sources.

Lipids and fatty acids of each flask were extracted following the procedure of Scott et al. (1971). After the microbial action was stopped, the mixtures in the 250 mL Erlenmeyer flasks were heated under reflux for 90 min, cooled and acidified with 10 mL of 6N hydrochloric acid (Scott et al., 1971). The lipids including the fatty acids were extracted with 30 mL hexane. After evaporation of the solvent with a rotary evaporator at 60°C under nitrogen, the lipid residue was converted to methyl esters and stored at -18°C prior to gas chromatographic analysis.

Effect of Feeding Protected Lipid Supplements on the Fatty Acids Composition of Sheep Blood and Milk

A. Treatments and Animals

To evaluate the effectiveness of AC, DA and FA treatments in preventing biohydrogenation of dietary lipids, four lactating ewes (mixed breeds, 2 to 3 years old) were used. The weights of the animals were recorded at the beginning and at the end of each experimental period. Each ewe had twin lambs; all sets of twins were born within a four d period. Each ewe and her lambs were housed in a 4.27 x 1.22 m pen at The University of Tennessee Knoxville Experiment Station. The pens were bedded with wood shavings. The ewes were fed twice

daily, in the morning (8 am) and late afternoon (3 pm) with .25 Kg of the basal diet (BD) (ground corn and alfalfa pellets) the composition of which is given in Table 5. The BD was supplemented with 1.0 Kg gel supplement treated with AC, DA, FA or no chemical at all (control). The animals also had free choice of medium quality mixed grass hay (2.5 to 3.0 Kg). The basal diet was provided by the Blount sheep farm. Basal diet (BD) with gel and grass supplements constituted a single treatment.

B. Experimental Design for the Feeding Trial

A 4x4 Latin square design was used in the feeding trial. There were four experimental periods of 10 d each. During any one period, each ewe was fed one of the 3 treatments or the control previously described, with each ewe getting a diet with a different treatment (protected lipid supplement). In subsequent periods, each ewe was fed a treatment different from those it had previously been fed, until each had received all 3 treatments and the control, as shown in Table 6. The feeding trial started with a prefeeding period of 5 d during which all 4 animals were fed the same basal diet (Table 5) and grass hay, without any gel supplement. The first period of the trial followed the prefeeding period. Each experimental period had a 7 d adjustment, during which each ewe was fed one of the treatments, followed by a 3 d collection, during which two samples of gel supplements, milk and blood were collected

Table 5. Composition of basal diet for feeding trial.

Ingredient	Amount (%)
Cracked corn	63.3
Dehy. alfalfa pellets	20.0
Soybean meal (48% CP)	10.0
Rolled oats	5.0
Trace mineral Salt	0.6
Limestone	0.4
Antibiotic	0.05

Dehy.=Dehydrated.

CP=Crude Protein.

Table 6. Assignment of diets with protected dietary fat supplements to animals during the feeding trial.

Feeding Periods	Animals				
	1	2	3	4	
	1	A	D	C	F
	2	F	C	D	A
	3	D	F	A	C
4	C	A	F	D	

A=Acetaldehyde (AC)
C=Control (CO)
D=Diacetyl (DA)
F=Formaldehyde (FA).

as described later for analysis of their fatty acid composition (Lu et al., 1990; Kawas et al., 1991; Nunez-Hernandez et al., 1991). In addition, all animals were weighed at the beginning of each experimental period. During each period, the times for sample collection and for weighing were scheduled as follows:

1. Gel supplements: d 10
2. Milk: d 8 and 10.
3. Blood: d 8 and 10.
4. Weight: d 0 and 10.

Lambs were separated from the ewe the night preceding milk and blood collections. One hundred to 200 mL of milk were obtained by hand in the morning, before the animals were fed. The milk samples were sealed in sterile polyethylene bags, placed in ice and transported to the Department of Food Science and Technology (within 10 min) where they were stored at -18°C until analysis. Blood was collected from the jugular vein by venipuncture into 20 mL vacuum tubes. The tubes were placed in ice and transported to Department of Food Science and Technology where the serum was separated from the blood sample by centrifugation at 2000 g for 20 min, in a Sorvall Superspeed Model RC2-B Automatic Refrigerated Centrifuge (Ivan Sorvall Inc., Newtown, CT). The blood sera were placed into vials, sealed and stored at -18°C until analysis.

The Effect of Storage at 5 to 6°C on the Stability of the Fatty Acids in the Gel Supplements

The soy gel supplements were prepared 60 d prior to the feeding trial and stored at 5 to 6°C. The fat content and the fatty acid composition were determined at 60, 70, 80 and 90 d. This was to assess the oxidative stability of the fatty acids throughout the feeding trial. The fat and fatty acids were extracted and the composition determined for each treatment at every sampling d as described in the following sections.

Chemical Analysis of Gels, Milk and Serum Samples

A. Lipid Extraction from Gels and Milk Samples

Lipids were extracted from gel supplements and milk samples, using the method described by Melton et al. (1979). Fatty acid composition of these samples were prepared and analyzed following the AOCS (1975) procedures. Forty grams of soybean gel or 100 mL of milk were homogenized with 130 mL methyl alcohol for 5 min in a Waring blender. Sixty five mL of a 0.013% butylated hydroxy toluene (BHT) chloroform solution was blended with the methanolic homogenate for 5 min; then, 65 mL of the BHT-chloroform solution was added to the mixture and blended for an additional 20 sec. The emulsion was broken by being blended with 65 mL of aqueous solution

containing 1.5 g zinc acetate for 10 sec. The mixture was filtered through a Whatman #1 filter paper in a Buchner funnel using suction into a filter flask. The solid residue and the filter paper were reblended with 100 mL BHT-chloroform solution for 5 min and the filtrate was added to the first extract. The residual sample was rinsed from the blender jar with 75 mL chloroform and filtered into the extract. The solvent extract was poured into a 500 mL graduated cylinder. The filter flask containing the filtrate was rinsed with 25 mL methanol and the content added to the extract in the cylinder. The graduated cylinder containing the entire extract was flushed with nitrogen gas, tightly covered and stored at 5 to 6°C overnight. The volume of the chloroform layer (lower layer) of the sample extract in the cylinder was recorded and the extract was transferred to a 500 mL separatory funnel. The contents were allowed to separate in the separatory funnel at 5 to 6°C for 4 hr. The chloroform layer was drained into a 500 mL round bottom flask, flushed with nitrogen, covered and stored at 5 to 6°C for further analysis.

To determine the lipid content of each sample, two 50 mL beakers were washed and dried at 90 to 100°C for 10 min, allowed to cool in a desiccator and weighed. Ten mL of the chloroform extract was placed in each beaker and the chloroform was allowed to evaporate under a hood overnight. The lipid residue left in the beaker was further dried in an oven at 90 to 100°C for 30 min in an oven. The weight of the

lipid residue was determined by weighing the beaker after it had cooled in a desiccator for 10 min. The lipid content of the sample was calculated as follows:

$$\text{Total lipid(\%)} = [(\text{lipid wt} \times \text{vol of chloroform}) / (40\text{g} \times 10\text{mL})] \times 100$$

The lipid extract was concentrated on a rotary evaporator at 55°C under vacuum. The concentrate was dissolved in 25 mL chloroform, placed in a 25 mL Erlenmeyer flask, flushed with nitrogen, sealed and stored at -18°C until fatty acid analysis.

To determine the lipid content per mL of chloroform residue, two 50 mL beakers were dried in an oven at 90 to 100°C for 30 min, cooled in a desiccator for 10 min and weighed. One mL of the chloroform extract was pipetted into each beaker and allowed to dry for 3 hr under a hood. The lipid residue was further dried in an oven for 30 min, cooled in a desiccator for 10 min then weighed. The lipid weight/mL chloroform extract allowed to determine the volume of the extract that contained 0.1 g of lipid. The calculation was as follows:

$$\text{mL of Chloroform} = [(0.1\text{g of lipid}) \times (1/(\text{g of lipid/mL}))].$$

The correct volume of chloroform extract containing 0.1 g lipid was pipetted into 125 mL flask and the solvent was evaporated on a rotary evaporator at 55°C under vacuum. The residual lipid was further converted to fatty acid methyl esters (FAME) (AOCS, 1975) before GC analysis.

B. Lipid Extraction from Serum Samples

Serum samples were thawed prior to the extraction of lipid. To 1.5 mL serum, were added 8 mL 0.05% BHT-chloroform solution, and 18 mL methanol. The mixture was homogenized at a slow speed for 2 min with a Virtis Model 23 homogenizer (The Virtis Company Inc., Gardiner, NY), followed by an addition of 9 mL BHT-chloroform solution and homogenization for 30 sec. The resulting emulsion was broken down by addition of 9 mL aqueous solution of zinc acetate and homogenized for 30 min. The extract was poured in a separatory funnel and placed at 5 to 6°C for 1 hr to allow further separation (Bright et al., 1994). The lower layer (chloroform) was decanted into a 125 mL Erlenmeyer flask and the lipid concentrated on a rotary evaporator at 55°C under vacuum. The lipid concentrate was further converted to fatty acid methyl esters (FAME) prior to GC analysis.

C. Preparation of Fatty Acid Methyl Esters

Fatty acid methyl esters were prepared according to AOCS (1975) method. Lipids previously extracted from ruminal digests of treated and untreated soybean seeds gels, sunflower seed gels and defatted soy flour gels; lipids from the blood serum, the milk and the gel supplements were hydrolyzed and converted to methyl esters in the following steps. To the lipid residue in the 125 mL Erlenmeyer flasks were added 4 mL

of 0.5N sodium hydroxide in methanol and a few boiling glass beads. The reaction flasks were attached to condensers, and, their content allowed to boil under reflux for 10 min. Five mL of boron trifluoride (14%) in a methanol solution were added to the contents of the flasks and allowed to react for 2 min. The reactants were then cooled and 7 mL of pentane were added through the condenser. The flask contents were boiled under reflux for 1 min; the flasks were removed from the heating pad and cooled. The flasks were removed from the condensers and the pentane layer containing the FAME was brought to the neck of the flask by adding saturated sodium chloride solution to the content of the reaction flasks. The FAME layer was removed into a vial, a small amount of sodium sulfate was added to the vial in order to absorb the residual water from the FAME. The vial was flushed with nitrogen, sealed and stored at -18°C until analysis by gas chromatography.

D. Chromatographic Analysis of Fatty Acids

The methyl esters were analyzed with a Shimadzu Model GC-9A gas chromatograph equipped with an automatic injection system Model AOC-9 (Shimadzu Corporation, Kyoto, Japan). A 0.25 mm i.d. by 30 m long fused silica SP-2330 column (Supelco, Inc., Bellefonte, PA) was used to separate the methyl esters, which were detected with a flame ionization detector (FID). The injection temperature was 250°C and the

temperature of the column was programmed from 130 to 220°C at 2°C/min. Helium was the carrier gas with a flow set at 50 mL/min, using a split ratio of 1:30. The areas of the peaks corresponding to individual FAME and their relative concentrations were recorded using a Shimadzu data processor, Chromatopac Model CR501 interfaced with an IBM personal computer. All chromatograms were stored using the Chromatopac Data Archive Utility version 3.1 software (Shimadzu Scientific Instruments, Inc., Columbia, MD). Fatty acid methyl esters standards were injected into the column under the same conditions as the sample methyl esters to determine the retention times of individual FAME. Identification of sample methyl esters was achieved by matching the retention times of sample peaks with those of standard peaks. Quantitative analysis of the sample FAME was done as follow. Standards containing known weight percentages of individual FAMES, present in levels similar to those in the samples, were analyzed by GC, and the correction factors (Table 7) relative to palmitic acid (16:0) were calculated according AOCS (1975) method for fatty acid analysis. The area of individual sample FAME was corrected using its correction factor (Table 7). The relative weight percentages of the different fatty acids in each sample were then calculated from GC chromatograms using the corrected areas. The chromatographic conditions did not allow volatile fatty acid such as butyric acid (common in milk) to be detected.

Table 7. Correction factors (CF) for fatty acid methyl ester standards.

Names	Acids	C F
Hexanoic	6:0	1.0000
Octanoic	8:0	1.0000
Nonanoic	9:0	1.0000
Decanoic	10:0	1.0456
Undecanoic	11:0	1.0000
Dodecanoic	12:0	1.0052
Tridecanoic	13:0	1.0000
Myristic	14:0	0.9732
Myristoleic	14:1	1.0042
Pentadecanoic	15:0	1.1247
Palmitic	16:0	1.0000
Palmitoleic	16:1	1.0042
Margaric	17:0	1.0182
Stearic	18:0	1.0182
Oleic	18:1	1.0366
Nonadecanoic	19:0	1.0000
Linoleic	18:2	1.0266
Eicosanoic	20:0	1.1115
Linolenic	18:3	1.0064
Eicosenoic	20:1	1.0144
Eicosadienoic	20:2	0.9700
Eicosatrienoic	20:3	0.9884
Arachidonic	20:4	1.0400
Docosanoic	22:0	1.1298
Eicosapentaenoic	20:5	1.0000
Docosatetraenoic	22:4	1.0000
Docosapentaenoic	22:5	1.0000
Docosaheptaenoic	22:6	1.0000

CF=Correction factor.

Statistical Analysis

All statistical analyses were performed, using GLM procedure (SAS, 1985). The percentage of individual fatty acid for each treatment, weight of animals and total lipid content of gels and milk represented the dependent variables.

A. Effects of *in vitro* Ruminal Digestion on the Lipid of the

Gel Supplements

In the first experiments, for each investigation, the dependent variables were the mean percentages of individual fatty acids (13:0, 14:0, 16:0, 16:1n9, 17:0, 18:0, 18:1n9, 18:1n7, 18:1Iso, 18:2Iso, 18:2n6, 20:0, 18:3n3 and 20:1n11) in different types of gels (soybean seed, sunflower seed and defatted soy flour) for each treatment level (acetaldehyde, diacetyl and formaldehyde or control). Variation was partitioned for gel treatments (control, acetaldehyde, diacetyl and formaldehyde), incubation times (0, 24 and 48 hr), and treatment and time interaction. The analyses of variance are shown in Table 8. A split plot design was used, with the incubation time as the split plot.

The statistical model was as follow:

$$\gamma_{ij} = \mu + T_i + I_j + TxI + \epsilon_{ij}; \text{ where:}$$

γ_{ij} = the response to treatment i at the jth incubation time;

Table 8. Analyses of variance for the ruminal digestion of the dietary gel supplements.

Source	<u>Degrees of freedom</u> incubation
Treatment (T)	3
Time (I)	2
Treatment X Time	6
Replication (R)	1
Error	11
Total	23

μ = the overall percentage mean for every individual fatty acid in each ruminal digest of each type of gels;

T_i = Effect due to gel treatment i ;

I_j = Effect due to incubation time j ;

$T \times I$ = Effect due to treatment and time interaction.

ϵ_{ij} = Random Error associated with the response to treatment i at incubation time j .

Significant differences between the percent means of the experimental treatments were determined by the General Linear Model method (SAS, 1985). When any significant difference was found, the means of the percent fatty acid were separated by the Student-Newman-Keuls test (Ott, 1985).

B. Effects of Feeding the Gel Supplements in vivo on the Serum and Milk Fat

In this experiment, the dependent variables were the least squares mean percents of individual fatty acids including 6:0, 8:0, 9:0, 10:0, 12:0, 14:0, 14:1n7, 15:0I, 15:0, 16:0I, 16:0, 16:1n9, 17:0, 18:0, 18:1n9, 18:2n6, 18:3n3 and 20:1n11, 20:3n6, 20:4n3, 20:5n3, 22:4n6, 22:5n3 and 22:6n3 in milk or serum fat. Variations were partitioned for feeding period, animal and treatments (control, acetaldehyde, diacetyl and formaldehyde). The analyses of variance are shown in Table 9. A 4x4 Latin square design was used, with the

Table 9. Analyses of variance for fat content of the milk and fatty acids in the blood serum and milk of sheep fed dietary gel supplements.

Source	Degrees of freedom
Period (P)	3
Animal (A)	3
Treatment (T)	3
Error	6
Total	15

randomized complete block model:

$$\gamma_{ijk} = \mu + T_i + P_j + A_k + \epsilon_{ijk}; \text{ where:}$$

γ_{ijk} = the response to treatment i in the j^{th} period and
for the k^{th} animal;

μ = the overall LSMeans percent for every individual
fatty acid in the serum or milk;

T_i = Effect due to gel treatment i ;

P_j = Effect due to experimental period j ;

A_k = Effect due to animal k ;

ϵ_{ijk} = Error associated with the response to treatment i
in period j and for animal k .

When any significant difference was found, the least squares means of the percent fatty acid were separated by the Student-Newman-Keuls test (Ott, 1985). This statistical analysis was applied to the milk fat content as well (Table 9).

C. The Storage Stability of the Gel Supplements for 90 d at 5 to 6°C.

The dependent variables were the least square means of the individual fatty acids (6:0, 8:0, 10:0, 14:0, 16:0, 16:1n7, 16:1n9, 18:0, 18:1n9, 18:2n6, 18:3n3, 20:1n11, 20:3n6, 20:5n3 and 22:4n6) in the dietary gel supplements. The statistical model is:

$$\gamma_{ijk} = \mu + T_i + P_j + R_k + T \times R + \epsilon_{ijk}; \text{ where:}$$

γ_{ijk} = the response to treatment i in the j^{th} period and
for k^{th} replication;

μ = the overall LSMeans percent for every individual

fatty acid in the gel supplements;

T_i = Effect due to gel treatment i ;

P_j = Effect due to experimental period j ;

R_k = Effect due to replication k ;

$P \times T$ = Effect due to treatment and period interaction;

ϵ_{ijk} = Error associated with the response to treatment i
in period j and for replication k .

When any significant difference was found, the least squares means of the percent fatty acid were separated by the Student-Newman-Keuls test (Ott, 1985). The analyses of variance are shown in Table 10.

Table 10. Analyses of variance for the fatty acids in the gel supplements during storage at 5 to 6°C for 60, 70, 80 and 90 days.

Source	<u>Degrees of freedom</u> incubation
Treatment (T)	3
Period (P)	3
Replication (R)	1
PxT	9
Error	12
Total	28

CHAPTER IV

RESULTS AND DISCUSSIONS

Microstructure of Protected Casein-Soybean-Oil Gels by Scanning Electron Microscopy (SEM)

Plate 1 shows Figures 4, 5, 6 and 7 which, represent untreated, formaldehyde (oven drying), acetaldehyde (freeze drying) and formaldehyde (freeze drying) treated gels, respectively. The untreated batter or control, which had a fibrous and open structure (Figure 4), did not gel. The numerous lattices may have been occupied by air and oil which leached out during the heating. During drying, removal of the water and the denaturation of proteins probably destabilized the protein-water-oil emulsion, resulting in the leaching of the oil. This created the porous type of structure in the control gel. The structure of the control batter (Figure 4) contains circular formations that may be layers of protein matrix encapsulating the droplets of oil. On the other hand, the gel treated with formaldehyde and subjected to oven drying showed a more compact structure (Figure 5). Many tiny crevices may have been the spaces occupied by pockets of air, while the larger circular formations may have been created by oil. The binding of formaldehyde to the proteins disrupted

Plate 1. Microstructure of untreated and treated soy gels.

Figure 4. Microstructure of untreated defatted soy flour batter (Oven drying) at 2.5 KeV. (x30).

Figure 5. Microstructure of formaldehyde-treated defatted soy flour gel (Oven drying). at 2.5 KeV. (x40).

Figure 6. Microstructure of acetaldehyde- treated defatted soy flour gel (Freeze drying). at 1.8 KeV (x60).

Figure 7. Microstructure of formaldehyde- treated defatted soy flour gel (Freeze drying). at 1.8 KeV. (x110).

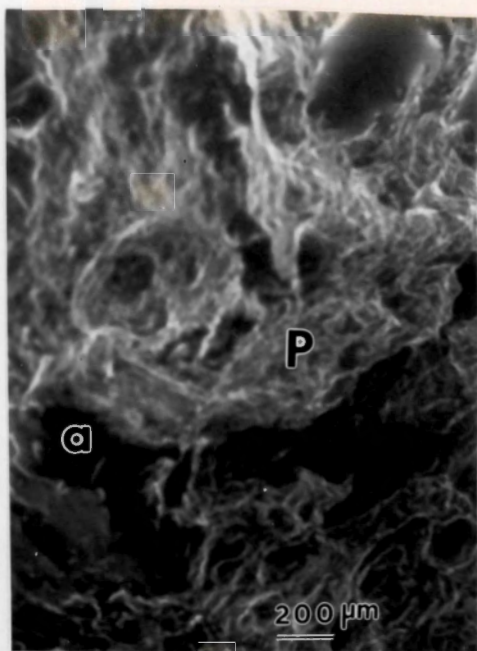


Figure 4

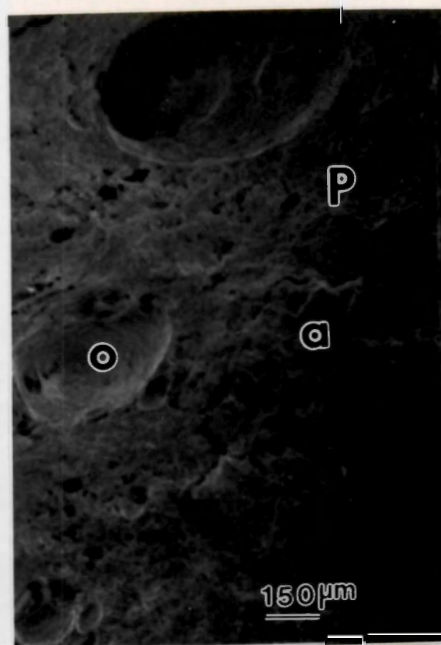


Figure 5

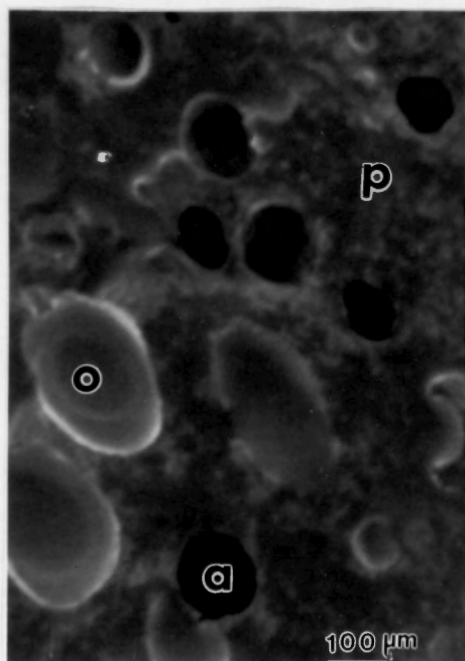


Figure 6

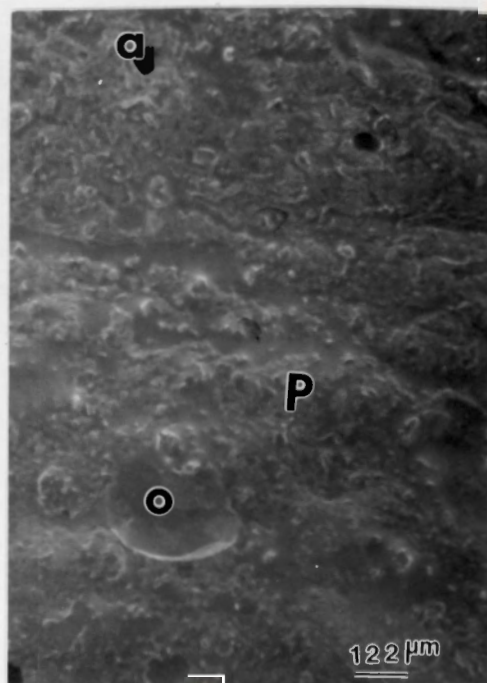


Figure 7

a:air; p:protein; o:oil inclusion

the protein network resulting in the non-fibrous structure. During the formation of the formaldehyde gel, the protein-water-oil emulsion lost some of its water creating an elastic like material. As a result, the gel containing formaldehyde shrunk less during oven drying than did the untreated batter.

Figures 6 and 7 (Plate 1), respectively, display the microstructure of acetaldehyde and formaldehyde treated gels which were freeze dried prior to microscopic analysis. Both treatments resulted in a compact non-fibrous type structures. The gel treated with acetaldehyde (Figure 6) contained many dark holes, circular to oval in shape which may have been inclusions of gas. A second type of structure observed was lighter shaded ovals. These formations may have been deposits of frozen oil. The untreated batter, after freeze drying was so porous that high vacuum could not be reached in the electron microscope, therefore, it was not possible to obtain micrographs.

My observations are in agreement with the description of casein-oil emulsions that had been treated with formaldehyde prior to spray drying in the investigation of Scott et al. (1971). They showed that such spray dried particles contained air spaces and small oil droplets embedded in a casein matrix. Scott et al. (1971) further showed that the formaldehyde-treated spray dried casein-oil emulsion protected the polyunsaturated fatty acids in the oil from biohydrogenation *in vitro* and *in vivo*. Scott et al. (1972) showed that ground

sunflower seeds treated with casein and formaldehyde to form a gel also protected unsaturated fatty acids from biohydrogenation in ruminant animals. Thus, formaldehyde treatment of protein-oil complexes protects against biohydrogenation.

No reports of studies were found which described the microstructure of chemically treated, oil containing gels as prepared in the present investigation. However, because the dried acetaldehyde and formaldehyde-treated protein-oil emulsions had similar structure from SEM observations, it is also likely that acetaldehyde could protect oil polyunsaturated fatty acids from biohydrogenation in ruminants.

In conclusion, the microstructure of protein-oil emulsion gels were successfully determined by scanning electron microscopy. Both oven and freeze drying appeared to be adequate preparation methods for SEM. While oven drying allowed oil to leach out, freeze drying preserved oil inclusions. The drawback of freeze drying is that it was not a feasible preparation method for some type of gels (untreated and diacetyl treated) for SEM analysis due to their porous structure which did not allow the necessary vacuum to be obtained in the electron microscope.

Experiment 1: Effects of Ruminal Fluid on the Fatty Acids Composition of Selected Casein-oil Seeds Emulsion Gels *in vitro*

A. Defatted Soybean Flour-Sunflower Oil Emulsion Gels

The percentage composition of fatty acids and fatty acid types in the reaction media containing defatted soy flour-sunflower oil gels (DSFG) treated with acetaldehyde (AC), diacetyl (DA), formaldehyde (FA), or untreated (CO) are shown, respectively, in Table 11 and Figure 8. Analyses of variance for individual fatty acids and fatty acid types are listed in Appendix A. The gels produced, were possible supplements to the diets of ruminant animals to alter fatty acid composition of ruminant products.

A.1. Saturated Fatty Acids (SFA)

In general, saturated fatty acids responded differently to treatments and reaction time (Figure 8-a). Saturated fatty acids (SFA) found in the digests included tridecanoic (13:0), tetradecanoic (14:0), hexadecanoic (16:0), heptadecanoic (17:0), octadecanoic (18:0), eicosanoic (20:0) (Table 11). The 13:0 acid increased ($P < 0.05$) during the first 24 hr digestion in the digests of all four gels, but from 24 to 48 hr digestion, 13:0 increased only in the digest containing the untreated or control and the acetaldehyde

Table 11. Mean concentrations (%)^a of fatty acids (C13-C18) in rumenal digests of emulsions containing defatted soybean flour and sunflower oil treated with different chemicals.

Fatty acid	Digestion (hrs)	Treatment ^b			
		Control	Formalde- hyde	Acetalde- hyde	Diacetyl
13:0	0	0.00c	0.00c	0.19c	0.00c
	24	1.01b	1.05a	0.48b	1.05a
	48	1.43a	0.79b	0.66a	0.75b
14:0	0	0.19	0.16b	0.20	0.26
	24	0.48	0.53b	0.45	0.56
	48	0.66	2.21a	0.62	0.90
16:0	0	5.08b	7.71c	8.13	7.75b
	24	9.18a	11.06b	9.24	8.77b
	48	10.70a	14.73a	8.96	10.13a
16:1n9	0	0.10	0.11b	0.11	0.10b
	24	0.10	0.14b	0.15	0.07b
	48	0.18	0.38a	0.14	0.20a
17:0	0	0.07	0.19b	0.10b	0.13b
	24	0.32	0.89a	0.56a	0.80a
	48	0.24	0.37b	0.14b	0.20b
18:0	0	1.20c	2.21b	4.24b	6.58c
	24	19.01b	15.21a	10.46a	10.03b
	48	25.24a	16.79a	11.48a	16.23a
18:1c+t	0	21.53b	21.26	18.98	17.19
	24	23.98b	17.95	15.71	16.18
	48	38.94a	18.79	16.91	17.19
18:1I	0	0.00	0.00	0.00	0.00
	24	0.34	0.00	0.00	0.00
	48	0.42	0.15	0.17	0.24

Table 11. (Continued)

Fatty acid	Digestion (hr)	Treatment ^b			
		Control	Formaldehyde	Acetaldehyde	Diacetyl
18:2I	0	0.00	0.00	0.00	0.00
	24	0.67	0.00	0.00	0.00
	48	1.25	0.00	0.11	0.00
18:2n6	0	64.66a	67.15a	67.09a	66.89a
	24	42.72b	50.92b	61.01b	59.63b
	48	16.88c	41.54c	57.34c	49.43c
20:0	0	0.19	0.54b	0.48	0.40b
	24	0.75	1.57a	0.98	1.36ab
	48	0.41	0.17b	0.68	2.53a
18:3n3	0	0.30	0.37b	0.46	0.42b
	24	0.19	0.36b	0.41	0.45b
	48	1.47	3.71a	1.12	1.89a
20:1n11	0	0.15c	0.30	0.30b	0.28b
	24	1.24b	0.35	0.39b	0.81a
	48	2.17a	0.37	1.36a	0.31b

^aFor any one acid means in a column followed by unlike letters are different ($P < 0.05$).

^bControl emulsions had no additives; other emulsions were treated with compound listed.

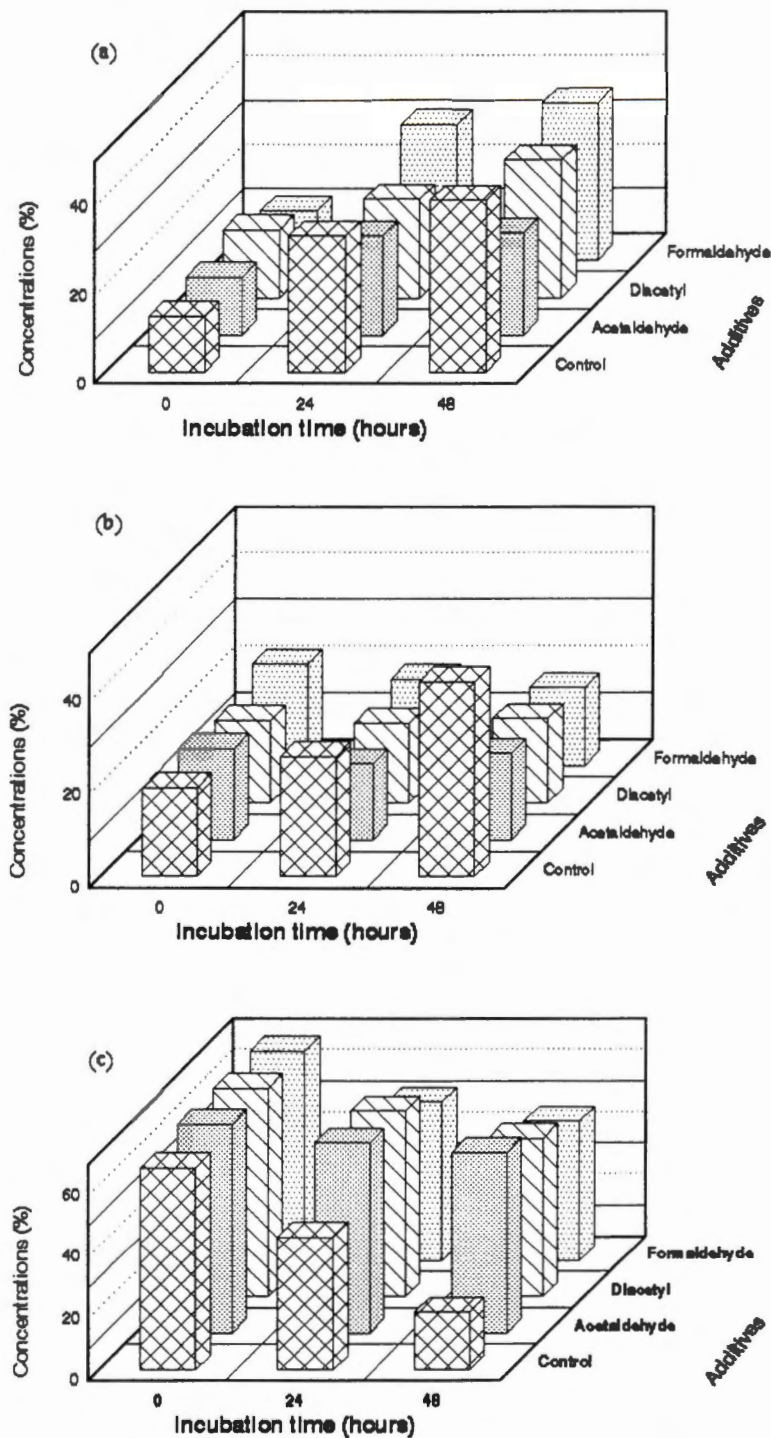


Figure 8. Contents of (a) saturated (SFA), (b) monounsaturated (MUFA) and (c) polyunsaturated (PUFA) fatty acid types in ruminal digests of emulsions containing defatted soybean flour and sunflower oil.

treated gels (Table 11); it decreased in the other gel digests.

Levels of 16:0 increased ($p < 0.05$) as the digestion time increased in the media containing the untreated (between 0 and 24 hr), formaldehyde (across 0 to 48 hr) and diacetyl treated (between 24 and 48 hr) supplements. However, incubation time did not affect ($p > 0.05$) the level of 16:0 in the medium containing acetaldehyde-treated supplement (Table 11). The concentrations of 17:0 increased ($p < 0.05$) in digests containing of treated (FA, AC and DA) gels as the incubation time increased from 0 to 24 hr and then decreased in these digests from 24 to 48 hr. Levels of 17:0 were not affected ($p > 0.05$) by time in the digest containing the untreated supplement.

The levels of 18:0 increased ($p < 0.05$) in all treatments as the digestion time increased from 0 to 24 hr, and further increased in control and diacetyl treated supplements from 24 to 48 hr digestion. These increases were much higher in the digest containing the untreated supplement than in the digest containing chemically treated gels (Table 11).

Significant increases in concentrations of 20:0 were also observed during ruminal digestion of formaldehyde and diacetyl treated gels, while the level of 20:0 was not affected ($p > 0.05$) by time in the digest containing untreated and acetaldehyde treated supplements (Table 11).

The greatest changes in the levels of the SFA during the

48 hr digestion occurred in the amount of 18:0 in the digest containing untreated supplement. Although the percentage 18:0 increased ($p < 0.05$) in the lipids of digests of treated supplements, all three treatments, formaldehyde, acetaldehyde and diacetyl, hindered the formation of 18:0 during ruminal digestion. It is also possible that the gels were not soluble in the ruminal fluid. During incubation, the reaction flasks were continuously shaken, and the control supplement dissociated rapidly, while the chemically treated gels dissociated slowly and incompletely.

The sums of all the SFA for each treatment and incubation time are shown in Figure 8a and the analyses of variance in Appendix A. The overall levels of the SFA increased significantly as the incubation time increased from 0 to 48 hr. The rate of increase was lower in the acetaldehyde-treated digest than the others (Figure 8-a).

A.2. Monounsaturated Fatty Acid (MUFA)

The MUFA recovered in the ruminal digests included, palmitoleic (16:1c), octadecenoic (18:1c, 18:1t and 18:1l), eicosenoic (20:1n11) acids (Table 11). However, because of incomplete separation of 18:1c (oleic acid) and 18:1t (elaidic acid) by the gas chromatographic method, the levels of the two were added together as 18:1c+t acids. Both digestion time and treatment affected ($p < 0.05$) the levels of the individual MUFA (Table 11).

The level of 16:1n9 in the digests containing formaldehyde and diacetyl treated supplements increased ($p < 0.05$) from 24 to 48 hr but not 0 to 24 hr digestion time. On the other hand, the levels of 16:1n9 in digests containing untreated and acetaldehyde treated supplements were not affected ($p > 0.05$) by digestion time (Table 11).

The levels of 18:1c+t increased ($p < 0.05$) from 24 to 48 hr digestion time only in the digest containing untreated supplement, and were not affected ($p > 0.05$) in the digests containing the treated gels. The increase was a result of an active synthesis of 18:1c+t in the digest containing the untreated supplement. The level of oleic isomer (18:1I) was not affected ($p > 0.05$) by digestion time in any digest containing treated or untreated supplements (Table 11).

Both treatment and incubation time significantly ($p < 0.05$) affected the level of eicosenoic acid (20:1n11). The levels of this fatty acid increased ($p < 0.05$) with increasing digestion time in the digests containing untreated (across 0 to 48 hr), acetaldehyde (24 to 48 hr) and diacetyl treated supplements (0 to 24 hr). However, digestion time did not have any effect ($p > 0.05$) on the level of 20:1n11 in the digest containing formaldehyde treated supplement (Table 11).

The sums of the MUFA for each treatment and each digestion time are presented in Figure 8-b. and the analyses of variance in Appendix A. The overall level of MUFA in the digest containing the control supplement increased ($p < 0.05$)

from 0 to 48 hr digestion time. The overall levels of MUFA were not affected by the incubation time in all digests containing chemically treated gels (Figure 8b).

A.3. Polyunsaturated Fatty Acids (PUFA)

The PUFA recovered in the reaction media were linoleic acid (18:2n6), its isomer (18:2I) and linolenic acid (18:3n3). The level of linoleic acid (18:2n6) in the digest containing untreated or treated supplements decreased ($p < 0.05$) as the digestion time increased from 0 to 48 hr (Table 11). However, the reduction in the level of 18:2n6 was much greater in the digest containing the untreated supplement than in the those containing formaldehyde, acetaldehyde, and diacetyl treated gels. On the other hand, 18:2I level in any digest was not affected ($p > 0.05$) by incubation time (Table 11). This result is the consequence of the intense biohydrogenation of 18:2n6 present in the digest containing untreated supplement, while hydrogenation of 18:2n6 in the digest containing treated gels occurred but was inhibited by the chemical treatments.

The level of linolenic acid (18:3n3) in formaldehyde and diacetyl treated gel digests increased ($p < 0.05$) as the digestion time increased from 0 to 48 hr (Table 11). The increase was greater in the digests containing the formaldehyde treated gel than in the diacetyl containing digests.

The levels of PUFA type are presented in Figure 8c and

the analyses of variance in Appendix A. Levels of PUFA were affected ($p < 0.05$) by both incubation time and treatment. The levels of PUFA decreased sharply from 0 to 48 hr digestion time in the digest containing the control supplement. On the other hand, the levels of these acids decreased moderately from 0 to 48 hr incubation time in all the digests containing the chemically treated gels (Figure 8c).

From these results, it can be concluded that when the DSFG was untreated, the unsaturated fatty acids were readily hydrogenated. This is justified by the significant disappearance of 18:2n6 and the appearance of 18:1c+t and 18:0 in the control medium as the digestion time increased from 0 to 48 hr. On the other hand, the hydrogenation was limited, but not completely blocked by treatment with formaldehyde, acetaldehyde and diacetyl (Table 11 and Figure 8).

B. Ground Full-fat Soybean Emulsion Gels

The fatty acid composition of the ruminal digests containing ground full-fat soybean gels, treated (AC, DA, FA) or untreated, are listed in Table 12 and the analyses of variance presented in Appendix B. These gels also were produced as possible dietary lipid supplements for ruminants to prevent biohydrogenation of unsaturated fatty acids in order to alter the fatty acid composition of ruminant animal products. Some of the individual saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids

Table 12. Mean concentrations (%)^a of fatty acids (C13-C18) in ruminal digests of emulsions containing ground full-fat soybeans treated with different chemicals.

Fatty acid	Digestion (hrs)	Treatment ^b			
		Control	Formaldehyde	Acetaldehyde	Diacetyl
13:0	0	0.00b	0.00	0.00	0.00
	24	1.70a	0.00	0.35	0.67
	48	2.12a	0.00	0.76	1.11
14:0	0	0.80	0.78	0.42	0.43b
	24	1.08	0.95	0.43	1.37a
	48	1.44	0.76	0.58	1.24a
16:0	0	18.44b	17.85a	15.87	16.09a
	24	17.45c	16.27b	16.21	14.85b
	48	21.03a	16.66b	16.35	14.59b
16:1n9	0	0.31	0.24	0.21	0.25
	24	0.13	0.20	0.32	0.14
	48	0.21	0.21	0.31	0.23
17:0	0	0.45	0.42	0.26	0.33
	24	0.37	0.33	0.33	0.32
	48	0.24	0.36	0.34	0.32
18:0	0	23.33	15.05	10.67	14.87
	24	16.75	15.49	16.12	14.42
	48	20.19	17.95	16.16	21.12
18:1c+t	0	17.52c	24.98	20.08	18.53c
	24	25.40b	19.48	19.18	21.78b
	48	31.68a	19.56	18.07	27.13a

Table 12. (Continued)

Fatty acid	Digestion (hrs)	Treatment ^b			
		Control	Formaldehyde	Acetaldehyde	Diacetyl
18:2n6	0	58.43a	56.93	60.41	55.61a
	24	46.10a	54.88	56.15	41.27a
	48	18.16b	56.68	52.78	19.24b
20:0	0	0.26b	0.30	0.23	0.37
	24	0.33ab	0.34	0.28	0.38
	48	0.41a	0.22	0.29	0.44
18:3n3	0	0.20	0.38a	0.23	0.17b
	24	0.12	0.14b	0.15	0.12a
	48	0.00	0.20b	0.13	0.00c
20:1n11	0	0.32b	0.21	0.08	0.28c
	24	1.62a	0.23	0.25	0.60b
	48	1.21a	0.34	0.49	5.15a

^aFor any one acid, means in a column followed by unlike letters are different ($P < 0.05$).

^bControl treatment had no additives; other treatments contained listed compound.

were affected significantly by treatment and digestion time but in different ways (Table 12). Also the SFA, MUFA and PUFA types (Figure 9) were affected significantly either by treatment and/or digestion time.

B.1. Saturated Fatty Acids (SFA)

Tridecanoic (13:0), tetradecanoic (14:0), hexadecanoic (16:0), heptadecanoic (17:0), octadecanoic (18:0) and arachidic (20:0) were the SFA recovered at different levels in the reaction media (Table 12). The level of 13:0 increased ($p<0.05$) from 0 to 24 hr digestion only in the digest containing untreated supplement. The levels of 13:0 in digests containing formaldehyde, acetaldehyde and diacetyl treated gels were not affected ($p>0.05$) by digestion time.

Levels of 14:0 in the digests containing untreated, formaldehyde and acetaldehyde treated supplements were not affected ($p>0.05$) by digestion time. On the other hand, the level of 14:0 in the digest containing diacetyl treated gel increased significantly as the incubation time increased from 0 to 24 hr (Table 12).

The level of 16:0 in the digest containing the untreated supplement first decreased from 0 to 24 hr digestion before it increased ($p<0.05$) with increasing digestion time from 24 to 48 hr (Table 12). It decreased ($p<0.05$) slightly in the digests containing formaldehyde and diacetyl treated gels.

The level of 16:0 in the digest containing acetaldehyde

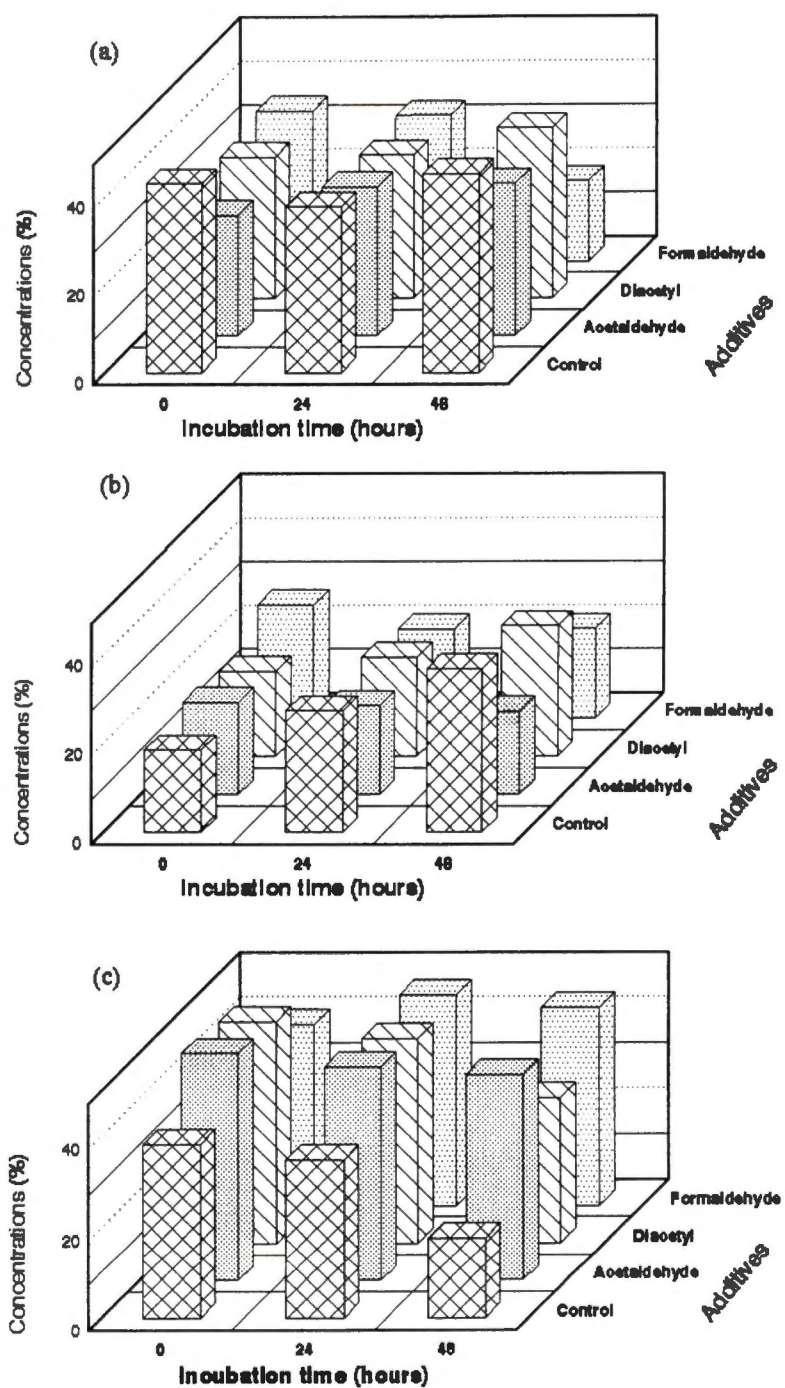


Figure 9. Contents of (a) saturated (SFA), (b) monounsaturated (MUFA) and (c) polyunsaturated (PUFA) fatty acid types in ruminal digests of emulsions containing ground full-fat soybean seeds.

treated gel was not affected ($p>0.05$) by digestion time (Table 12). No significant changes ($p>0.05$) were found in the levels of margaric (17:0), stearic (18:0) and arachidic (20:0) in digests containing untreated and treated supplements across digestion time (Table 12).

The overall levels of SFA are presented in Figure 9a, and the analyses of variance listed in Appendix B. Neither digestion time nor treatment affected ($p>0.05$) this type of fatty acids (Figure 9a).

B.2. Monounsaturated Fatty Acids (MUFA)

Palmitoleic (16:1n9), octadecenoic (18:1c+t), its isomer (18:1I) and eicosenoic (20:1n11) acids accounted for the MUFA encountered in the digests containing untreated and treated supplements. Levels of palmitoleic (16:1n9) acid in digests containing untreated and treated supplements were not affected ($p>0.05$) by incubation time (Table 12).

The levels of oleic plus elaidic (18:1c+t) acids in the digests containing untreated and diacetyl treated supplements increased ($p<0.05$) as the incubation time increased from 0 to 24 hr and from 24 to 48 hr (Table 12). On the other hand, the level of 18:1c+t in the digests containing formaldehyde and acetaldehyde treated supplements was not affected ($p>0.05$) by incubation time (Table 12). The level of 18:1I in the digest containing the untreated supplement increased ($p<0.05$) substantially with increasing digestion time from 24 to 48 hr.

However, its level was not affected ($P>0.05$) by digestion time in digests containing chemically treated gels.

The levels of eicosenoic acid (20:1n11) increased as the incubation time increased from 24 to 48 hr in the control supplement and from 0 to 48 hr in the diacetyl treated supplement. The contents of 20:1n11 in digests containing formaldehyde and acetaldehyde treated gels were not affected by digestion (Table 12).

Figure 9b illustrates the overall levels of MUFA. The analyses of variance for levels of this fatty acid type are listed in Appendix B. Levels of MUFA in the untreated digest significantly increased from 0 to 48 hr incubation (Figure-9b).

B.3. Polyunsaturated Fatty Acids (PUFA)

The PUFA recovered in the reaction media included linoleic acid (18:2n6), its isomer (18:2I) and linolenic acid (18:3n3). The level of linoleic acid isomer (18:2I) in the digests containing untreated supplement increased ($p<0.06$) as the incubation time increased from 24 to 48 hr. This acid was not recovered in digests containing formaldehyde and diacetyl treated supplements. Its level was not affected by incubation time in the digest containing acetaldehyde treated supplement.

The level of linoleic acid (18:2n6) in digests containing untreated and diacetyl treated supplements decreased ($p<0.05$) as the incubation time increased from 24 to 48 hr. The

concentration of 18:2n6 was not affected ($p>0.05$) by digestion time for formaldehyde and acetaldehyde treated gels (Table 12). Linolenic acid (18:3n3) had the same fate as linoleic acid in the control and diacetyl treated supplements (Table 12).

The overall levels of PUFA for treatment and digestion time are illustrated in Figure 9c, and their analyses of variance listed in Appendix B. The levels of this type of fatty acids in the control digest decreased ($p<0.05$) from 0 to 48 hr but in the diacetyl containing digest, the reduction of PUFA occurred between 24 and 48 hr. The amounts of PUFA in the formaldehyde and acetaldehyde treated digests were not affected ($p>0.05$) by digestion (Figure 9c).

These results suggest that linoleic (18:2n6) and linolenic (18:3n3) acids in the untreated full-fat soybean supplement were converted by ruminal microorganisms mostly to octadecenoic acids (18:1c+t) and its isomer (18:1I). About 60% of linoleic acid (18:2n6) and 70% of linolenic acid (18:3n3) in the control digest disappeared during the 48 hr incubation. Meanwhile, the level of octadecenoic acids (18:1c+t and 18:1I) in that digest doubled during the same period. Further hydrogenation of octadecenoic acids (18:1c+t and 18:1I) to stearic acid (18:0) did not occur. The accumulation of 18:1I may be an intermediate compound or a byproduct formed during the hydrogenation of linolenic (18:3n3) and linoleic (18:2n6) acids to 18:1c+t (Table 12).

The same trends found during the digestion of the control gel were also observed in the digest of the diacetyl containing gel. Hydrogenation of linolenic acid (18:3n3) and linoleic acid (18:2n6) occurred but to a lesser extent than in the control gel (Table 12).

For formaldehyde and acetaldehyde treatments, the levels of linolenic (18:3n3), linoleic (18:2n6) and stearic (18:0) acids were not significantly affected during incubation. This lack of significant changes suggests that biohydrogenation was hindered by these latter treatments more than by the diacetyl treatment (Table 12). Thus, formaldehyde and acetaldehyde were more efficient than diacetyl in protecting unsaturated fatty acids in a ground full-fat soybean gel against ruminal biohydrogenation.

C. Ground Full-fat Sunflower Seed Emulsion Gels

The fatty acid composition of the ruminal digests containing full-fat sunflower seeds, treated (AC, DA, FA) or untreated (control) are listed in Table 13 and analyses of variance of the individual fatty acids are listed in Appendix C. The treated gels were produced as possible dietary supplements for ruminant animals for protection against biohydrogenation of unsaturated fatty acids in an effort to alter the fatty acid composition of ruminant animal products. The levels of certain individual SFA, MUFA and PUFA were affected ($p < 0.05$) by both treatment and incubation time. The

Table 13. Mean concentrations (%)^a of fatty acids (C13-C18) in ruminal digests of emulsions containing ground full-fat sunflower seeds treated with different chemicals.

Fatty acid	Digestion (hrs)	Treatment ^b			
		Control	Formalde- hyde	Acetalde- hyde	Diacetyl
13:0	0	0.00b	0.00	0.00c	0.00b
	24	0.24a	0.00	0.20b	0.33a
	48	0.22a	0.00	0.55a	0.48a
14:0	0	0.32b	0.22	0.28b	0.22b
	24	0.94a	0.33	0.58a	0.60a
	48	0.85a	0.26	0.72a	0.57a
16:0	0	7.48	7.27	7.30	7.78
	24	8.19	7.80	7.43	8.12
	48	8.45	7.66	7.16	8.67
16:1n9	0	0.24	0.13	0.12	0.16
	24	0.31	0.14	0.14	0.14
	48	0.20	0.15	0.14	0.12
17:0	0	0.07	0.11	0.06	0.12
	24	0.15	0.13	0.12	0.15
	48	0.15	0.12	0.12	0.18
18:0	0	6.34	6.48	6.12	3.05a
	24	7.42	2.84	2.69	18.99b
	48	8.15	6.96	5.06	24.85c
18:1c+t	0	26.33b	27.97b	25.18	32.24b
	24	34.58b	33.17a	32.02	29.14b
	48	62.20a	27.34b	32.56	40.47a

Table 13. (Continued)

Fatty acid	Digestion (hrs)	Treatment ^b			
		Control	Formaldehyde	Acetaldehyde	Diacetyl
18:2n6	0	58.43a	56.93	60.41	55.61a
	24	46.10a	54.88	56.15	41.27a
	48	18.16b	56.68	52.78	19.24b
20:0	0	0.26b	0.30	0.23	0.37
	24	0.33ab	0.34	0.28	0.38
	48	0.41a	0.22	0.29	0.44
18:3n3	0	0.20	0.38a	0.23	0.17b
	24	0.12	0.14b	0.15	0.12a
	48	0.00	0.20b	0.13	0.00c
20:1n11	0	0.32b	0.21	0.08	0.28c
	24	1.62a	0.23	0.25	0.60b
	48	1.21a	0.34	0.49	5.15a

^aFor any one acid, means in a column followed by unlike letters are different ($P < 0.05$).

^bControl treatment had no additives; other treatments contained listed compound.

effect of digestion time on amounts of individual saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids were dependent upon treatment (Table 13). The acid types, SFA, MUFA and PUFA, also were affected significantly in different ways by digestion time for each treatment (Figure 10).

C.1. Saturated Fatty Acids (SFA)

Tridecanoic (13:0), tetradecanoic (14:0), hexadecanoic (16:0), heptadecanoic (17:0), octadecanoic (18:0) and arachidic (20:0) account for the SFA recovered at different levels in the reaction media (Table 13). Levels of tridecanoic (13:0) and tetradecanoic (14:0) acids in the digests containing untreated, acetaldehyde and diacetyl treated supplements increased ($p < 0.05$) as the incubation time increased from 0 to 24 hr (Table 13). In addition, the content of 13:0 in the digests containing acetaldehyde increased from 24 to 48 hr digestion time. The concentrations of 13:0 and 14:0 in the digests containing the formaldehyde treated supplement was not affected ($p > 0.05$) by incubation time (Table 13).

The contents of palmitic (16:0) and margaric (17:0) acids in all digests containing untreated and treated supplements were not affected ($p > 0.05$) by incubation time (Table 13). However, the content of stearic acid (18:0) increased ($p < 0.05$) in the digests containing diacetyl treated supplements, but

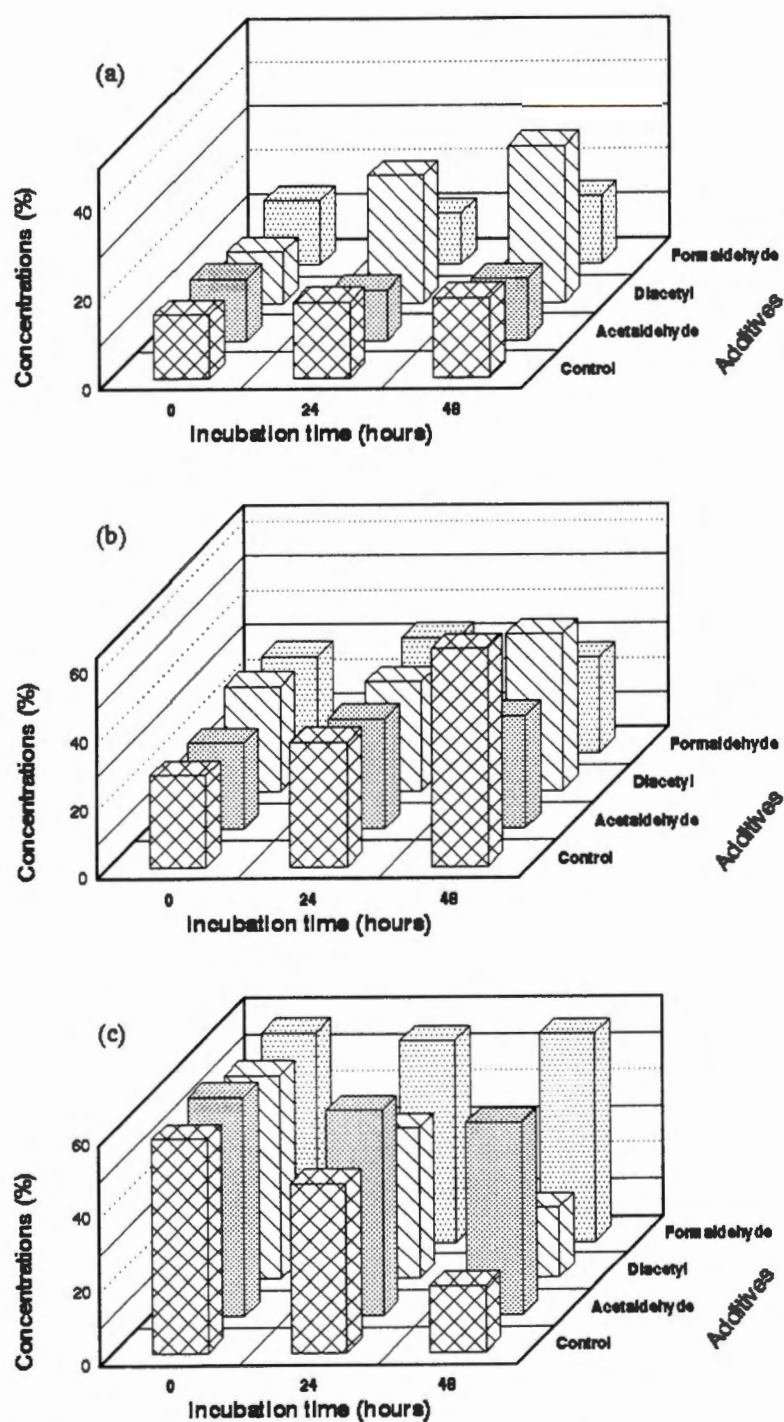


Figure 10. Contents of (a) saturated (SFA), (b) monounsaturated (MUFA) and (c) polyunsaturated (PUFA) fatty acid types in ruminal digests of emulsions containing ground full-fat sunflower seeds.

was not significantly affected by digestion time in digests containing untreated, formaldehyde and acetaldehyde treated supplements (Table 13).

The level of arachidic acid (20:0) was not significantly affected by the incubation time in any digest containing chemically treated supplements. On the other hand, the content of 20:0 in the digests containing the control supplement increased ($p < 0.05$) with increasing incubation time between 0 and 48 hr (Table 13).

Figure 10a illustrates the overall levels of SFA and Appendix C lists the analyses of variance for that type of fatty acids. The level of SFA increased ($p < 0.05$) from 0 to 48 hr incubation in the diacetyl treated digest. The level of SFA in other treated gels and the control were not affected ($p > 0.05$) during the digestion.

C.2. Monounsaturated Fatty Acids (MUFA)

The MUFA recovered in digests containing full-fat sunflower seed supplements included palmitoleic (16:1n9), octadecenoic (18:1c+t), and eicosenoic acids (20:1n11) (Table 13). The levels of palmitoleic acid (16:1n9) were not affected ($p > 0.05$) by the incubation time in any digests (Table 13).

The levels of octadecenoic acids (18:1c+t) in the digests containing untreated and diacetyl treated supplements increased ($p < 0.05$) from 24 to 48 hr incubation time, but were

not affected ($p>0.05$) by digestion time in digests containing formaldehyde and acetaldehyde treated gels. The increase in the level of 18:1c+t was greater in the digests containing the untreated supplement than in the digests containing the diacetyl treated gel (Table 13).

The level of eicosenoic acid (20:1n11) increased ($p<0.05$) with incubation time only in the digests containing the untreated and diacetyl treated supplements. Its level, on the other hand, was not affected ($p>0.05$) in the digests containing the formaldehyde and acetaldehyde treated gels (Table 13).

The overall levels of MUFA are illustrated in Figure 10b and its analysis of variance is presented in Appendix C. The levels of this type of fatty acid increased ($p<0.05$) in digests containing the control and diacetyl treated supplements, between 0 and 48 hr incubation. Its levels in the formaldehyde and acetaldehyde treated digests, however, were not affected ($p>0.05$) by the digestion time (Figure 10b).

C.3. Polyunsaturated Fatty Acids (PUFA)

Linoleic (18:2n6) and linolenic (18:3n3) acids, the only individual PUFA recovered in the digests, are listed in Table 13. The levels of 18:2n6 in the digests containing untreated and diacetyl treated supplements were reduced ($p<0.05$) from 24 to 48 hr digestion time. On the other hand, 18:2n6 levels in the formaldehyde and acetaldehyde treated supplements did not

change ($p>0.05$) during incubation (Table 13). The level of linolenic acid (18:3n3) was less than 1% in all media at all incubation times and was not affected ($p>0.05$) by digestion time (Table 13).

The overall levels of PUFA are illustrated in Figure 10c and its analysis of variance presented in Appendix C. The levels of this type of fatty acid decreased ($p<0.05$) in the digests containing the control and diacetyl treated gels between 0 and 48 hr incubation time. On the other hand, the levels of PUFA in the digests containing formaldehyde and acetaldehyde were not affected ($p>0.05$) by the digestion time.

In the digests containing untreated sunflower seed supplements, hydrogenation of linoleic acid (18:2n6) occurred, with the product being mainly octadecenoic acid (18:1c+t). Hydrogenation of 18:2n6 also occurred in diacetyl treated sunflower seeds supplements, but the products included both 18:1c+t and 18:0. Formaldehyde and acetaldehyde were excellent protective agents against biohydrogenation of linoleic acid (18:2n6) in the ground full-fat sunflower gel.

D. Discussion for *in vitro* Experiments

Although a great number of *in vitro* studies have been done with protective dietary lipid supplements for ruminants (Cook and Scott, 1970; Scott et al., 1971; Plowman et al., 1971), none of the studies have investigated dietary fat

supplements in the gel form as it was studied in this research. All of the studies reported, except for the one by Ashes et al. (1979), have focussed only on spray dried supplements (casein-oil emulsions) treated with nothing or with formaldehyde only.

The results using *in vitro* rumen fluid digestion of different types (soy flour sunflower oil, ground full-fat soybean seeds or sunflower seeds) gels treated with nothing or with formaldehyde, acetaldehyde or diacetyl showed that with increasing digestion time changes occurred in the SFA (Figures 8a, 9a and 10a), and in particular, the 18:0 contents (Tables 11, 12 and 13). The type of changes, however, were dependent upon the type of gel. In the gels with soybean flour, the levels of 18:0 increased significantly during the incubation period (Table 11). On the other hand, the gels containing ground full-fat soybean or sunflower seeds had no changes in their contents of 18:0 throughout the incubation period (Tables 12 and 13, respectively). The increase in 18:0 content in the soy flour containing gel combined with decreases in the levels of 18:2n6 and increases in amounts of 18:1c+t in the same gel (Table 11) show that hydrogenation did occur. In the other gels (soybean and sunflower seeds) compared with the soy flour containing gel, the lack of increases in the 18:0 content and lower decreases in the amount of 18:2n6 in the control gel indicate that hydrogenation was more extensive in the soy flour containing

gel. This may have been due to differences in the particle size and the state of the oil in the gels. Since the oil was added to the soy flour gel, this oil may not have been an integral part of the gel or bound to any other components in the flour. Therefore, it could easily leach into the rumen fluid during incubation and was readily available to the hydrogenation by the microorganisms. The oil in the ground full-fat seeds was in a different state and environment. Here, the oil was still inside the cells and may have been in a bound state. In such an environment, the oil was not easily available to attack by microorganisms. The oilseed particle had to be digested before the oil was exposed and hydrogenated.

The concentration of 18:1c+t (oleic plus elaidic acids) increased in all control gels (Tables 11, 12 and 13) during 48 hr incubation with rumen fluid, but the rate and extent of the increase was dependent upon the type of gel. In this respect, my results agreed with those reported by Plowman et al. (1971) who found a large increase in the concentration of oleic acid in spray dried safflower oilcasein emulsions during a 48 hr incubation with rumen fluid.

The levels of MUFA in the control gels (Figures 8b, 9b and 10b) during 48 hr incubation with rumen fluid showed similar trends as the levels of 18:1c+t, the major MUFA. The greatest increase in the concentration of MUFA in the soy flour sunflower oil gels and in ground full-fat sunflower seed

gels occurred from 24 to 48 hr digestion; whereas, in the ground full-fat soybean seed gel, the MUFA level increased steadily from 0 to 24 and 24 to 48 hr digestion. The increase in MUFA levels, and particularly the 18:1c+t levels, is tangible proof that more unsaturated acids were hydrogenated during rumen fluid digestion or that they were produced by other means.

The reduction in levels of 18:2n6 and 18:3n3 (Tables 11, Table 12 and 13) and of PUFA (Figures 8c, 9c and 10c) during the 48 hr incubation period combined with concurrent increases in the more saturated fatty acids (18:0 and 18:1c+t) is additional proof that hydrogenation occurred during rumen fluid digestion. The decreases in the PUFA concentrations, individual and as a fatty acid type, during *in vitro* rumen fluid digestions are in agreement with the researchers (Cook and Scott, 1970; Scott et al., 1971; Plowman et al., 1971) who used a similar system to investigate biohydrogenation in spray dried soybean oil-casein emulsions. In particular, the continued reduction in levels of 18:2cc and 18:3n3 in any of the control gels are in agreement with results reported by Plowman et al. (1971). These latter researchers found reduction in levels of soybean oil 18:2cc and 18:3n3 increased with increasing incubation time with rumen fluid up to 48 hr.

When the gels were treated with formaldehyde, acetaldehyde or diactyl, the rate of biohydrogenation of the PUFA was decreased from that of the control or untreated gel

(with the exception of the diacetyl treatment in the gel containing sunflower seeds). The amount or decrease for each treatment, however, was dependent upon the type of gel. Generally, it can be assumed for any one type of gel, that when averaged across digestion time for a single treatment, levels of 18:2n6, 18:1c+t and 18:0 are indicative of the amount of biohydrogenation that has occurred for that treatment. For a treatment of any one type gel, lower levels of 18:2n6 and higher levels of 18:1c+t and 18:0 indicate that a greater amount of biohydrogenation occurred during digestion.

Averaged across digestion time, acetaldehyde treated gels containing ground full-fat soybean seeds had the highest level ($p<0.05$) of 18:2n6 among all gels (Table 13); acetaldehyde treated gels containing soy flour and sunflower oil had the lowest levels of 18:0 among all gels (Table 14). Acetaldehyde treated gels containing the soy flour and sunflower oil also had the same concentration of 18:2n6 as the formaldehyde or diacetyl treated gels of the same type. In gels containing ground full-fat sunflower seeds, acetaldehyde treated gels had ($p<0.05$) the same level of 18:2n6 as formaldehyde treated gels and more 18:2n6 than the diacetyl treated gels (Table 14). In all gels, the control or untreated gels had lower levels of 18:2n6 and higher concentrations of 18:1c+t than those treated with acetaldehyde or formaldehyde. These observations lead to the conclusion that across type of gel, the acetaldehyde

Table 14. Mean^a concentrations^b (%) of stearic (18:0), octadecenoic (18:c+t), and linoleic (18:2n6) acids in different types of gels incubated from 0 to 48 hr with rumen fluid.

<u>Type gel</u> Fatty acid	Treatment			
	Control	Form- aldehyde	Acet- aldehyde	Diacetyl
<u>Soy flour</u>				
<u>sunlf. oil</u>				
18:0	15.15a	11.40b	8.72c	10.95b
18:1c+t	28.15a	19.33b	17.20c	16.85c
18:2n6	31.72b	53.20a	61.81a	58.65a
<u>Full-fat</u>				
<u>soybean</u>				
18:0	20.09	16.16	14.32	16.79
18:1c+t	24.86a	21.34ab	19.11b	22.44ab
18:2n6	25.96c	37.25b	42.17a	37.03b
<u>Full-fat</u>				
<u>sunflower</u>				
18:0	7.30b	5.43b	4.62b	15.63a
18:1c+t	16.63b	13.70b	13.06b	25.10a
18:2n6	40.90b	56.16a	56.45a	38.71b

^aAveraged across 0, 24 and 48 hr digestion times (n=6).

^bMeans in any row followed by unlike letters are different at the p<0.05 level.

treatment provided slightly more protection against biohydrogenation than did formaldehyde and diacetyl treatments and much more than the control. The fact that the formaldehyde treatment inhibited biohydrogenation of the unsaturated fatty acids in the sunflower oil in the gels containing soy flour and ground full-fat sunflower seeds are in agreement with Scott et al. (1971), who showed that spray dried formaldehyde sunflower oil particles resisted biohydrogenation after 48 hr incubation with ruminal fluid from sheep.

The amount of protection against biohydrogenation provided by the diacetyl treatment was dependent also upon the type of gel. In the gel containing soy flour and sunflower oil, the diacetyl treated gel contained ($p < 0.05$) the same levels of 18:2n6 and 18:1c+t but a lower amount of 18:0 than the acetaldehyde treatment (Table 14). The acetaldehyde gel also contained ($p < 0.05$) the same concentrations of 18:0 and 18:2n6 but less 18:1c+t than the formaldehyde treatment (Table 14). In the soy flour containing gel, the untreated gel contained lower levels of 18:2n6 and higher amounts of 18:1c+t and 18:0 than the diacetyl treatment. These results suggest that the diacetyl treatment is not as effective as the acetaldehyde treatment for preventing *in vitro* biohydrogenation but may be slightly more effective than the formaldehyde treatment in doing so for the gel containing soy flour and sunflower oil.

In the gel containing ground full-fat soybeans, the level of 18:0 was the same in all treatments (Table 14). The diacetyl treatment contained the same amounts of 18:1c+t and 18:2n6 as the formaldehyde treatment, but more 18:2n6 than the control. These results seem to indicate that diacetyl and formaldehyde treatments were about the same in preventing biohydrogenation in the ground full-fat soybean containing gel. However, the significant interaction between treatment and digestion time for these acids (Appendix B, Table 12) show that for some reason the formaldehyde treatment at 0 hr had a lower level of 18:2n6 than the corresponding diacetyl treatment. The 18:2n6 content of the formaldehyde containing digest was not reduced further by biohydrogenation, but that of the diacetyl containing digest was (Table 12). This information demonstrates that formaldehyde treatment was better at inhibiting biohydrogenation in ground, full-fat soybean containing gels than the diacetyl treatment.

In the gels containing ground full-fat sunflowers, the diacetyl treatment did not prevent biohydrogenation at all. The diacetyl treatment contained ($p < 0.05$) higher levels of 18:0 and 18:1c+t than any of the other treatments including the control and also contained the same level of 18:2n6 as the control gel (Table 14). The diacetyl and control treatments had lower amounts of the 18:2n6 than the formaldehyde or acetaldehyde treatments (Table 14) indicating that biohydrogenation occurred at a faster rate in the control and

diacetyl treatments. The reason for the ineffectiveness of the diacetyl treatment in preventing biohydrogenation in the sunflower seed containing gel may have been that even when the diacetyl was at a concentration of 5%, it could not form a gel as firm as did acetaldehyde and formaldehyde. The reason for the lowered activity of the diacetyl in the sunflower seed type gel is unknown at this time.

All three chemical treatments of protein-oil gels showed inhibition of biohydrogenation of polyunsaturated fatty acids. With the exception of formaldehyde, the extent to which the treatments would inhibit biohydrogenation *in vivo* is unknown. With the present health concerns of the American consumers about saturated fat, it is important that ruminant products containing higher levels of polyunsaturated fatty acids be produced (American Heart Association, 1988). Since formaldehyde is a carcinogen and cannot be used to produce human food, it is imperative to test the ability of acetaldehyde and diacetyl, both GRAS chemical compounds, against *in vivo* biohydrogenation in ruminant animals.

Experiment 2: The Effects of Protected Soy Gels on the Fatty Acid Composition of Sheep Blood Serum and Milk Fats

The fatty acid contents of the blood serum and the milk fat of the four lactating ewes were determined prior and during feeding protected dietary soy gels containing soybean

oil. The compositions of individual fatty acid and types of fatty acids in the serum and milk for each sheep and feed treatment are presented in the following sections. Analyses of variance for all the data are listed in Appendices D and E.

A. Effect of Feeding Protected Dietary Fat Supplements

on the Fatty Acid Composition of Sheep Blood Serum Fat

Table 15 presents the least squares mean concentrations of individual fatty acids of sheep blood serum after the sheep have been fed untreated (control) and formaldehyde, acetaldehyde or diacetyl treated dietary fat supplements. The overall levels of saturated (SFA), monounsaturated (MUFA), n3-polyunsaturated (PUFA-n3) and n6-polyunsaturated (PUFA-n6) fatty acid types, recovered from the blood serum of sheep prior (Appendix J) and during (Table 15) the experimental feeding trial, are illustrated in Figure 11. Analyses of variances for individual fatty acids and fatty acid types are given in Appendix D.

A.1. Saturated Fatty Acids (SFA)

SFA recovered in the blood serum fat after feeding protected dietary fat supplements were: octanoic (8:0), tetradecanoic (14:0), pentadecanoic (15:0), hexadecanoic (16:0), and octadecanoic (18:0). The contents of these acids in the blood serum fat were not affected ($p>0.05$) by

Table 15. Fatty acid composition (%)^a of blood serum fat from lactating ewes fed untreated or treated soybean gel supplements.

Fatty Acids	^b Treatments			
	Control	Formaldehyde	Acetaldehyde	Diacetyl
8:0	6.37 (2.2)	7.42 (2.2)	7.07 (2.9)	5.04 (2.2)
12:0	0.19	ND	2.23 (1.8)	ND
14:0	0.79 (.09)	0.72 (.12)	0.79 (.12)	0.94 (.09)
15:0	0.52	0.90	0.50	0.72
16:0	16.95 (1.4)	15.50 (1.4)	15.51 (1.4)	16.10 (1.4)
16:1n9	0.63 (.33)	0.51	0.76	0.61 (.12)
17:0	1.38 (.94)	0.81 (.33)	0.56	0.92 (.14)
18:0	19.41 (1.2)	18.13 (.93)	19.54 (1.2)	20.81 (.93)
18:1n9	24.38 (3.3)	16.58 (3.3)	16.23 (3.3)	18.42 (3.3)
18:2n6	23.85b(2.5)	33.98a(2.5)	33.64a(2.3)	29.90ab (3)
18:3n3	2.46 (.20)	1.98 (.20)	1.88 (.20)	1.99 (.20)
20:4n3	2.47 (.33)	3.29 (.43)	3.36 (.33)	2.60 (.33)
22:5n3	0.96 (.09)	1.35 (.09)	1.23 (.12)	1.02 (.09)
22:6n3	0.76 (.12)	1.11 (.21)	1.05 (.16)	0.75 (.12)

^aFor any fatty acid means in a row followed by different letters are significantly different ($p < .05$).

^bControl had no additives; other treatments contained listed compounds.

(SE)=Standard Error.

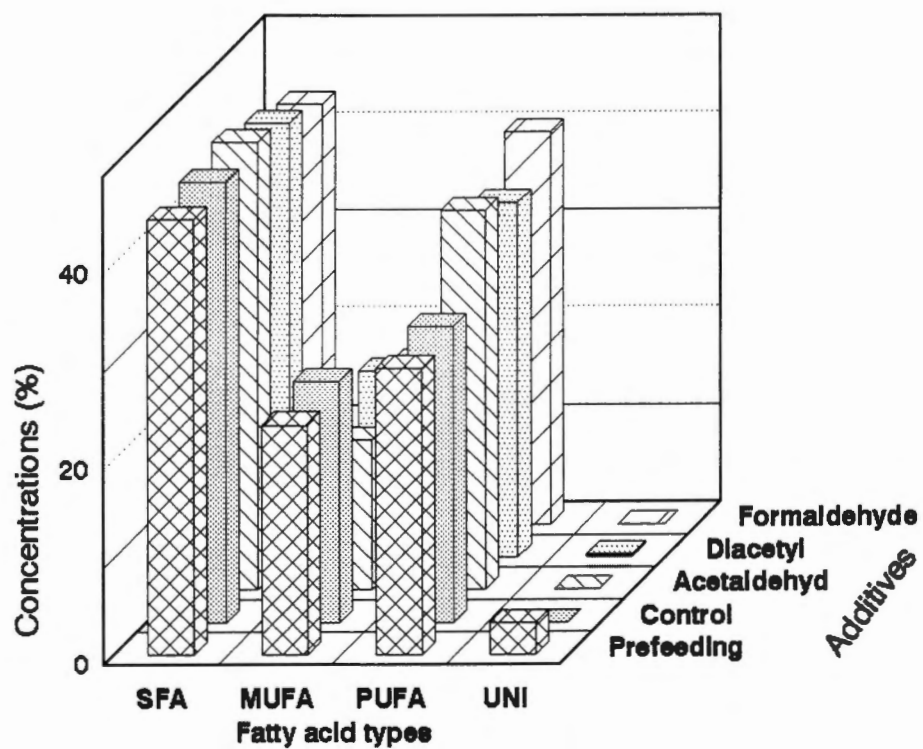


Figure 11. Fatty acid types in the blood serum fat of sheep prior and after feeding protected dietary fat supplements.

treatments (Table 15). Sums of the percentages of SFA for each treatment are illustrated in Figure 11. The overall levels of the SFA were not different ($p>0.05$) among treatments (Appendix D).

A.2. Monounsaturated Fatty Acids (MUFA)

The only MUFA recovered were 16:1n9 (palmitoleic acid) and 18:1n9 (oleic acid) (Table 15), the levels of which did not differ ($p>0.05$) among treatments (Table 15 and Figure 11).

A.3. Polyunsaturated Fatty Acids (PUFA)

The PUFA include the PUFA-n3: linolenic (18:3n3), docosapentaenoic (22:5n3), docosahexaenoic (22:6n3) acids and the PUFA-n6: linoleic (18:2n6) and eicosatetraenoic (20:4n6) acids. The overall content of PUFA was not affected ($p>0.05$) by treatment (Appendix D). Among the individual PUFA, only the concentration of 18:2n6 was affected ($p<0.05$) by treatment (Table 15). Compared to the control, feeding chemically treated dietary fat supplements increased ($p<0.05$) the levels of 18:2n6 in the blood serum fat for formaldehyde and acetaldehyde treatments. However, feeding the diacetyl treatment resulted in a level of 18:2n6 in the blood serum that was not different ($p>0.05$) from that of feeding the control or the formaldehyde or acetaldehyde treated supplements (Table 15).

B. Effect of Feeding Protected Dietary Fat Supplements on the Fat Level and Fatty Acid Composition of Sheep Milk Fat

B.1. Fat content

The fat contents of milk from ewes fed control and protected dietary fat supplements is listed in Table 16 and the analysis of variance in Appendix E. The level of fat in the milk decreased as the number of feeding periods increased. This change in the milk fat is probably due to the lactation pattern as the lactation period increased. Treatment did not significantly affect the level of fat in the milk (Table 16).

B.2. Fatty Acids

Tables 17 lists the levels of individual fatty acids and Figure 12 illustrates the concentrations of type of fatty acids (SFA, MUFA, PUFA and UNI) in the milk fat from sheep fed diets supplemented with treated or untreated protected soybean gels. Analyses of variance for effect of feeding period, animal and treatment for each fatty acid and fatty acid type are given in Appendix F. Short chain fatty acids (4:0) which elution times are before 6:0 may have not been separated under the gas chromatographic conditions. This is why 4:0 was not present in both serum and milk fat.

Table 16. Least squares means^a of fat concentration^b in milk of lactating sheep fed fat supplements untreated (CO) or treated with FA, AC and DA.

<u>Independent variable</u>	Fat content in milk (mg/100 mL)	Standard error
<u>Feeding Period</u>		
1	1140.62a	(100.29)
2	1064.10a	(100.29)
3	847.56b	(100.29)
4	655.16c	(100.29)
<u>Animal</u>		
1	1078.73	(100.29)
2	853.90	(100.29)
3	974.06	(100.29)
4	800.75	(100.29)
<u>Treatment</u>		
Control	1017.72	(100.29)
Acetaldehyde	1030.57	(100.29)
Diacetyl	855.53	(100.29)
Formaldehyde	803.61	(100.29)

^an = 4.

^bFor any least square mean of fat content followed by unlike letters are significantly different (p<0.05).

Table 17. Fatty acid composition (%)^a of milk fat from lactating ewes fed untreated or treated soybean gel supplements.

Fatty Acid	^b Treatments			
	Control	Formaldehyde	Acetaldehyde	Diacetyl
*6:0	2.01 (.44)	1.74 (.34)	1.65 (.34)	1.66 (.34)
8:0	2.05 (.20)	2.00 (.20)	1.87 (.20)	2.15 (.20)
9:0	0.06 (.01)	0.05 (.01)	0.05 (.01)	0.06 (.01)
10:0	7.01 (.44)	7.09 (.44)	7.03 (.44)	7.96 (.44)
11:0	0.11 (.01)	0.11 (.01)	0.12 (.01)	0.14 (.01)
12:0	3.86 (.20)	4.07 (.20)	3.94 (.20)	4.31 (.20)
14:0	8.44 (.43)	8.47 (.43)	8.61 (.43)	9.53 (.43)
14:1n5	0.18 (.02)	0.18 (.02)	0.21 (.02)	0.24 (.02)
15:0I	0.47 (.02)	0.53 (.02)	0.51 (.02)	0.53 (.02)
15:0	1.06 (.07)	1.14 (.07)	1.07 (.07)	1.15 (.07)
16:0I	0.19b(.01)	0.26a(.01)	0.21b(.01)	0.19b(.01)
16:0	23.24 (.62)	22.57 (.62)	23.40 (.62)	23.07 (.80)
16:1n9	0.47 (.12)	0.46 (.12)	0.31 (.12)	0.26 (.16)
16:1n7	0.93 (.10)	0.58 (.10)	0.76 (.10)	0.95 (.10)
17:0	0.44 (.05)	0.42 (.05)	0.37 (.05)	0.42 (.05)
Unk1	0.82 (.07)	0.68 (.07)	0.78 (.07)	0.87 (.07)
Unk2	0.32 (.01)	0.30 (.01)	0.26 (.01)	0.33 (.01)
18:0	11.89ab (1)	9.73b(1.0)	11.65ab (1)	12.79a(1.0)
18:1n9	26.14 (1.5)	24.16 (1.5)	25.15 (1.5)	28.29 (1.5)
19:0	0.91a(.05)	0.41b(.05)	0.46b(.05)	0.51b(.05)
18:2n6	4.93c(.44)	10.53a(.44)	7.52b(.44)	7.06b(.44)
Unk3	0.14 (.03)	0.12 (.02)	0.14 (.02)	0.11 (.02)
18:3	1.48c(.11)	2.38a(.11)	1.84b(.11)	1.89b(.11)
20:1n9	0.98 (.14)	0.80 (.14)	0.85 (.14)	1.25 (.14)
20:2n6	0.14 (.01)	0.11 (.01)	0.09 (.01)	0.12 (.01)
20:3n6	0.06 (.01)	0.10 (.01)	0.08 (.01)	0.11 (.01)
22:0	0.25 (.04)	0.28 (.03)	0.27 (.03)	0.38 (.06)
20:4n6	0.42 (.05)	0.30 (.05)	0.29 (.05)	0.28 (.05)
20:5n3	0.19 (.01)	0.17 (.01)	0.17 (.01)	0.19 (.01)
22:4n6	0.54 (.23)	0.09 (.18)	0.00 (.23)	0.00 (.23)
22:5n6	0.67 (.25)	0.39 (.33)	0.19 (.25)	0.21 (.25)
22:6n3	0.29 (.12)	0.06 (.12)	0.08 (.09)	0.09 (.09)

^aFor any one fatty acid means in a row followed by different letters are significantly different ($p < .05$).

^bControl had no additives; other treatments contained listed compounds.

ND=Not Determined

*Fatty acids before 6:0 were not separated under the chromatographic conditions

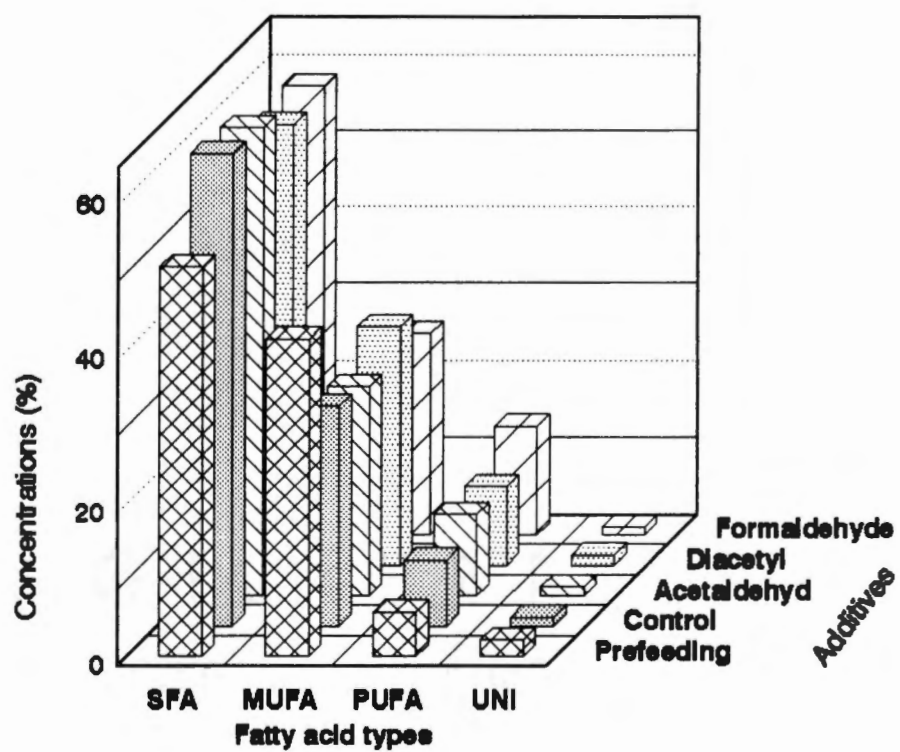


Figure 12. Fatty acid types in the blood serum fat of sheep prior and after feeding protected dietary fat supplements.

B.3. Saturated Fatty Acids (SFA)

Fatty acids in SFA include hexanoic (6:0), octanoic (8:0), nonanoic (9:0), decanoic (10:0), undecanoic (11:0), lauric (12:0), tetradecanoic (14:0), pentadecanoic (15:0), methyl-tetradecanoic (15:0I), hexadecanoic (16:0), methyl-pentadecanoic (16:0I), heptadecanoic (17:0), octadecanoic (18:0), nonadecanoic (19:0) and docosanoic (22:0) acids (Table 17). The percentage of SFA was not affected ($p>0.05$) by treatment (Figure 12). With the exception of 16:0I, 18:0 and 19:0, the contents of other individual SFA did not change ($p>0.05$) with treatment. The level of 18:0 was lower ($p<0.05$) in the milk from ewes fed formaldehyde treated supplement than in the milk from ewes fed the diacetyl treated supplements (Table 17). Ewes fed the control diet had a higher 19:0 level in the milk fat than did ewes fed other supplements, while ewes fed the formaldehyde treated supplement had the highest level of 16:0I (Table 17).

B.4. Monounsaturated Fatty Acids (MUFA)

The MUFA include: myristoleic (14:1), trans-palmitoleic (16:1n7), palmitoleic (16:1n9), oleic (18:1n9) and eicosenoic (20:1n11), with oleic acid (18:1n9) being the major fatty acid (Table 17). Treatment did not affect ($p>0.05$) the levels of any individual MUFA (Table 17) or the overall level of MUFA in the milk fat (Figure 12).

B.5. Polyunsaturated Fatty Acids (PUFA)

PUFA include 2 types of fatty acids, the PUFA-n3 and the PUFA-n6. The overall contents of these PUFA were 8.60, 14.20, 10.30 and 10.58%, for the control, formaldehyde, acetaldehyde, and diacetyl treatments, respectively (Figure 12). The PUFA-n3 include linolenic (18:3n3), eicosapentaenoic (20:5n3), docosapentaenoic (22:5n3) and docosahexaenoic (22:6n3) acids (Table 17). The overall content of the PUFA-n3 was not affected ($p>0.05$) by treatment (Figure 12). Among the individual PUFA-n3, only the level of linolenic (18:3n3) was affected ($p<0.05$) by treatment. The levels of 18:3n3 were significantly higher in the milk from ewes fed treated supplements than in the milk from ewes fed the untreated supplement. Also, the content of 18:3n3 in the milk from ewes fed formaldehyde treated supplement was the highest of all treatments (Table 17).

The PUFA-n6 recovered in the milk fat were linoleic (18:2n6), eicosadienoic (20:2n6), eicosatrienoic (20:3n6) eicosatetraenoic (20:4n6) and docosatetraenoic (22:4n6) acids (Table 17). The total concentration of PUFA-n6 fatty acid was affected by treatment ($p<0.05$). Levels of PUFA-n6 were 5.75% (control), 11.18% (formaldehyde treatment), 8.10% (acetaldehyde treatment) and 7.32% (diacetyl treatment). The total levels of the PUFA-n6 fatty acids in the milk fat were higher for all the treatments than the control, and the formaldehyde treatment had the highest level of PUFA-n6

(Figure 12). The major acid of PUFA-n6 present in the milk fat was linoleic acid (18:2n6). The levels of 18:2n6 in the milk from ewes fed the chemically treated supplements were higher ($p < 0.05$) than the levels in milk from ewes fed the control supplement, with the percentage of 18:2n6 in the milk produced on formaldehyde treatment being the highest of all (Table 17). The level of 20:2n6 was also affected ($p < 0.05$) by treatment with ewes fed the control supplement having the highest 20:2n6 concentration in the milk and ewes fed the acetaldehyde supplement, the lowest (Table 17). The acid, 20:2n6 is present however in very small amounts ($< 0.20\%$). The levels of all the other individual PUFA were not significantly changed by treatment (Table 17).

B.6. Unidentified Fatty Acids (UNI)

Unidentified fatty acids (UNI) were composed of Unk1, Unk2 and Unk3 fatty acids (Table 17). The levels of these fatty acids were very low ($< 1.0\%$) and were not affected ($p > 0.05$) by treatment.

C. Summary for Fatty Acids in Serum and Milk

From the *in vivo* experiments, it can be concluded that the contents of linoleic acid (18:2n6) in blood serum fat and milk fat were affected by feeding protected dietary lipid supplements to sheep. Compared with the control, or untreated

supplement, the formaldehyde, acetaldehyde, and diacetyl treated supplement,, increased the 18:2n6 contents in sheep blood serum fat by 42, 41 and 25% and in sheep milk fat by 114, 52 and 43%, respectively.

D. Storage Stability of Dietary Fat Supplements

The fatty acid composition for the untreated (control) and treated gels used for dietary supplements are listed in Table 18 and the analyses of variance in Appendix G. Neither storage time nor treatment significantly affected the fatty acid composition of the gel supplements.

D.1. Saturated Fatty Acids (SFA)

The major SFA were palmitic (16:0) and stearic (18:0) in the gels. Other individual SFA recovered at less than 1% concentration include hexanoic (6:0), octanoic (8:0), decanoic (10:0) and tetradecanoic (14:0) (Table 18). The levels of the SFA were not significantly affected by storage time or by treatments. These fatty acids contain no carbon-carbon double bonds; therefore, they are resistant to autoxidation (Table 18).

D.2. Monounsaturated Fatty Acids (MUFA)

MUFA included cis and trans hexadecenoic acids (16:1n9 and 16:1n7), oleic (18:1n9) and eicosenoic (20:1n11). Oleic

Table 18. Fatty Acid Composition (%) of Soybean Gels Stored at 5-6°C for 60, 70, 80 and 90 days.

		Treatments ^a			
Fatty Storage acids (days)		Control	Formaldehyde	Acetaldehyde	Diacetyl
6:0	60	0.11	0.34	1.41	0.51(.3)
	70	ND	ND	ND	ND
	80	0.11	ND	ND	ND
	90	ND	ND	ND	ND
8:0	60	0.24(.04)	0.24(.07)	ND	0.30
	70	0.44(.01)	0.85(.08)	0.50	0.71(.24)
	80	0.40(.15)	0.26(.02)	0.63(.05)	0.18(.10)
	90	0.57(.01)	0.64(.12)	0.69(.07)	1.07
10:0	60	0.06(.01)	0.07(.01)	0.06	0.13(.03)
	70	0.05	0.05(.01)	0.05	0.33(.09)
	80	0.05	0.08(.01)	0.08(.01)	0.28(.06)
	90	ND	0.6	ND	0.15
14:0	60	0.19(.01)	0.15(.01)	0.20	0.18(.01)
	70	0.16(.01)	0.15(.01)	0.21	0.18(.01)
	80	0.16(.01)	0.16(.01)	0.16(.01)	0.16(.01)
	90	0.15(.01)	0.18(.01)	0.16(.01)	0.13
16:0	60	11.81(.1)	12.31(.01)	12.37	11.47(.01)
	70	10.74(1)	12.26(.04)	12.18	11.34(.07)
	80	11.40(.1)	11.98(.03)	12.45(.2)	11.29(.04)
	90	11.41(.1)	12.02(.04)	11.92(.1)	11.19
16:1n7	60	ND	ND	0.05	ND
	70	ND	ND	ND	ND
	80	ND	ND	0.06(.01)	ND
	90	ND	ND	0.05	ND
16:1n9	60	0.07(.01)	0.08(.001)	0.07(.001)	0.07(.001)
	70	ND	ND	ND	ND
	80	0.07(.01)	0.07(.01)	0.07(.002)	0.07(.0)
	90	0.08(.01)	0.08(.01)	0.08(.01)	0.07(.01)
18:0	60	3.51(.38)	3.53(.23)	3.25	3.54(.01)
	70	7.67(4.2)	3.53(04)	3.32	3.54(.02)
	80	3.81(.01)	3.46(.30)	3.43(.52)	3.58(.03)
	90	3.79(.01)	3.75(.01)	3.75(.05)	3.50

Table 18. (Continued)

Fatty acids	Storage (days)	Treatments ^a			
		Control	Formaldehyde	Acetaldehyde	Diacetyl
18:1n9	60	22.87(.19)	21.70(.05)	20.80	20.34(.01)
	70	22.54(.22)	21.70(.06)	21.82	20.36(.06)
	80	22.14(.03)	21.70(.11)	22.31(.14)	20.58(.16)
	90	21.96(.02)	21.46(.08)	21.40(.23)	20.20
18:2n6	60	53.51(.18)	53.66(.07)	52.60	54.89(.11)
	70	50.68(3.1)	52.97(.09)	52.82	54.93(.06)
	80	53.76(.07)	53.94(.18)	52.70(.67)	54.91(.01)
	90	53.86(.004)	53.74(.03)	53.61(.22)	54.74
18:3n3	60	6.84(.01)	6.97(.006)	6.90	7.96(.02)
	70	6.95(.38)	6.93(.02)	7.05	8.18(.07)
	80	7.10(.01)	7.15(.03)	6.72(.28)	7.98(.08)
	90	7.16(.01)	7.04(.09)	7.25(.08)	8.17
20:1n9	60	0.29(.001)	0.28(.001)	0.27	0.20(.06)
	70	ND	ND	ND	ND
	80	0.31(.004)	0.29(.01)	0.28(.01)	0.28(.01)
	90	0.31(.001)	0.23(.04)	0.24(.05)	0.17
20:3n6	60	0.48(.01)	0.44(.003)	0.46	0.40(.01)
	70	0.43(.001)	0.42(.01)	0.43	0.38(.01)
	80	0.47(.003)	0.46(.002)	0.49(.01)	0.42(.01)
	90	0.45(.001)	0.44(.006)	0.44(.01)	0.40
20:5n3	60	0.07(.004)	0.20(.03)	0.35	ND
	70	0.09(.01)	0.24(.40)	0.12	0.08(.01)
	80	0.07(.02)	0.25(.00)	0.43(.07)	0.8(.002)
	90	0.06(.001)	0.22(.005)	0.25(.03)	0.06
22:4n6	60	ND	0.20(.001)	0.19	0.16(.03)
	70	0.27(.06)	0.92(.90)	1.52	0.16(.03)
	80	0.21(.001)	0.20(.00)	0.20(.003)	0.17(.01)
	90	0.20(.001)	0.15(.003)	0.18(.004)	0.18(.003)

^aControl had no additives; other treatments contained listed compounds. ND=Not Determined; SE=Standard Error.

acid (18:1n9) was the major MUFA; other MUFA were present at levels less than 0.5% (Table 18). No significant changes occurred in the contents of these acids throughout the storage period. These results indicate the absence of oxidation reactions that may have reduced the levels of MUFA as storage lasts longer (Table 18).

D.3. Polyunsaturated Fatty Acids (PUFA)

The major fatty acids of PUFA were linoleic (18:2n6) and linolenic (18:3n3) acids. Other PUFA type acids, including eicosatrienoic (20:3n6), eicosapentaenoic (20:5n3) and docosatetraenoic (22:4n6), were present at levels less than 0.50% (Table 18). The levels of individual PUFA were not affected significantly by storage period or treatment. The lack of significant storage effects indicate that the PUFA were not oxidized during storage. These results indicate that none of the gels had significant changes in their fatty acid composition during the experiment feeding period which was 40 d long and that all animals received the same amounts of PUFA at any feeding period, regardless of the treatment.

D.4. Summary of Fatty Acids in Treated Gel Supplements.

Among the research reports on protected dietary lipid supplements for ruminants, only one deals with the gel form (Scott et al., 1972). These researchers successfully made

gels with ground safflower kernels treated with formaldehyde and fed them to lactating goats. Feeding the formaldehyde treated safflower gel increased the level of linoleic acid (18:2) from 4 to about 20% in milk fat. Since then, the potential of their method has not been well studied. Thus, little scientific data exist concerning this matter. This study is the only one of its kind to investigate the effect of storage on the fatty acid composition of soybean gels produced with the carcinogen, formaldehyde, and non carcinogenic additives, acetaldehyde and diacetyl.

The major fatty acids in the gels and their percentages are in agreement with the fatty acid composition in soybean oil as reported by Weiss (1983). The levels of myristic (14:0) ranged from 0.10 to 0.20%, palmitic (16:0), from 10 to 12%, stearic (18:0), from 3 to 4%, oleic (18:1), from 20 to 23%, linoleic (18:2), from 50 to 55% linolenic (18:3), from 6 to 8% and eicosenoic (20:1) 0.10 to 0.30%. My soy gel supplements contained high levels of unsaturated fatty acids and low levels of saturated fatty acids. Treatment of the soy gels with acetaldehyde, diacetyl and formaldehyde prevented spoilage during storage at a low temperature (5 to 6°C). The untreated gel, however, had to be stored at -18°C to prevent its degradation. At 5 to 6°C the control or untreated gel rapidly degraded.

E. Effect of Feeding Protected Dietary Fat Supplements

on the Weight of the Animals During the Feeding Trial

The weights of lactating ewes and for their lambs are listed for each treatment and each feeding period in Table 19. The lambs significantly gained weight as the feeding period increased from 1 to 4. Ewes weighed more during periods 2, 3 and 4 than during period 1. The results of increasing weight in lambs with increasing time are normal since these young lambs are in their rapid growth phase.

The weight of the ewes was not affected ($p>0.05$) by treatment, but lamb weight differed among treatments. Lambs on ewes fed the formaldehyde and the acetaldehyde treated whole soybean gels weighed more than the lambs on ewes fed the control or diacetyl treated soybean gels (Table 19). However, the maximum difference in lamb weights was less than .67 Kg.

F. Discussion of *in vivo* Feeding Trial Results

F.1. Blood Serum Fatty Acids

When sheep were fed the basal diet (Table 5) (.25 Kg/d) and dried grass hay (2.5 to 3.0 Kg/d), the levels of SFA, MUFA, PUFA-n3, PUFA-n6 and unidentified fatty acids (UNI) in the blood serum fat ranged, respectively, from 40 to 52%, 22 to 25%, 35%, 18 to 27% and 2 to 5% (Table 15). The average

level (56%) of unsaturated fatty acids for the four animals was higher than the level (44%) of saturated fatty acids. Across treatments, the contents of linoleic (18:2n6) and linolenic (18:3n3) averaged, 21 and 2%, respectively. Since these fatty acids cannot be synthesized *de novo* by higher animals, they had to be provided by dietary lipids. The levels of 18:2n6 found in the blood serum fat of sheep fed the full-fat soybean gel supplements are higher than those (2%) reported in other ruminant animals such as goats on a diet containing lucerne chaff and oats (1:1, w/w) (Scott et al., 1971). My data, however, are more in agreement with other researchers (Rule and Beitz, 1986) who found a 38% level of 18:2 in the plasma of steers fed a diet containing 57.7% cracked corn, 27% corn silage and 10.8% soybean meal. The most abundant fatty acid in corn oil which is present at approximately 6% of the corn kernel, is 18:2n6 (Weiss, 1983). It is not known how much of the corn 18:2n6 escaped the rumen of the sheep and transferred into the plasma of the sheep fed the control diet. It would be ideal to eliminate the corn supplement in the control diet in order to determine the effect of my dietary gel supplements on the fatty acid profile of the blood serum fat.

When the animals were fed grass hay, the basal diet and 1.0 Kg untreated or chemically treated full-fat soybean gels, no significant change in the levels of the major individual SFA (16:0 and 18:0) among treatments were found (Table 15).

Table 19. Least squares means^a of the weights of lactating ewes and lambs fed treated or untreated dietary lipid supplements.

<u>Independent variable</u>	<u>Ewes</u>		<u>Lambs</u>	
	Weight (kg)	Standard error	Weight (kg)	Standard error
<u>Treatment</u>				
Control	61.76	(0.33)	14.47b	(0.17)
Formaldehyde	62.03	(0.35)	15.14a	(0.18)
Acetaldehyde	61.07	(0.43)	15.07a	(0.23)
Diacetyl	61.53	(0.38)	14.76b	(0.20)
<u>Feeding period</u>				
1	60.33b	(0.35)	10.94d	(0.18)
2	62.08a	(0.38)	13.07c	(0.20)
3	62.37a	(0.35)	15.70b	(0.18)
4	61.62a	(0.35)	19.73a	(0.18)

^aFor any independent variable, means in a column followed by unlike letters are different at the $p < 0.05$ level.

These results are not in agreement with the findings of Scott et al. (1974) who reported a reduction from 17 to 9.2% in the level of 16:0 and from 32.2 to 18.4% in the level of 18:0 in the plasma of goats fed 2.0 Kg/d lucerne and oat (1:1) plus .5 Kg of spray dried linseed oil-caseinate particles treated with formaldehyde. The lack of change in 18:1n9 in the blood serum from sheep fed the chemically treated soybean gel supplements also is not in agreement with Scott et al. (1974). They found a reduction from 38.7 to 25.4% in the level of 18:1n9 in the plasma of goats fed a formaldehyde treated casein linseed oil supplement.

Feeding sheep formaldehyde , acetaldehyde and diacetyl treated soybean gels increased ($p < 0.05$) the 18:2n6 content in the blood serum, from 23.9% to 34.0, 33.6 and 29.9%, respectively (Table 15). Scott et al. (1974) reported an increase of from 2.0 to 11.7% in the level of 18:2 in the plasma of goats fed the formaldehyde-caseinate-linseed oil supplement. However, no changes were in the serum content of 18:3n3 in sheep fed the chemically treated soybean gel supplements in my study (Table 15). Scott et al. (1974) reported an increase from 3.5 to 30.8% in the plasma of goats fed linseed oil-casein treated with formaldehyde. These major disagreements between results of the present study and those of Scott et al. (1974) are somewhat more understandable considering that linseed oil belongs to the family of linolenic acid oils and has 50% 18:3n3, while soybean oil is

a linoleic acid oil containing 54% 18:2n6 (Salunhke et al., 1992). Also, Scott et al. (1974) fed goats with spray dried formaldehyde-casein-linseed oil emulsions, while in the present study, sheep were fed with full-fat soybean gels treated with formaldehyde, acetaldehyde or diacetyl. The digestibility of these different forms of fat supplements may be quite different.

F.2. Effect on Milk Fat

The fatty acids recovered from the milk samples were listed in Table 17. The average levels of major fatty acids in the milk fat of sheep fed basal diet (.25 Kg/d) in addition to 2.5 to 3.0 Kg/d grass hay are presented in Appendix K. The average contents of SFA, MUFA, PUFA-n3, PUFA-n6 and UNI were respectively 50.65, 41.30, 1.30, 4.50 and 2.18% (Appendix K). In contrast to the fatty acid profile of the blood serum, milk had a very low level of polyunsaturated fatty acids (5.86%) and high levels of saturated (50.65%) and monounsaturated fatty acids (41.30%) (Appendix K). USDA (1985) reported different average levels of SFA (70%), MUFA (25%) and PUFA (5%) in the milk fat of sheep. Perhaps the corn supplement used in this study boosted the content of monounsaturated fatty acids, and in particular, 18:1n9. Feeding chemically treated dietary soybean gel supplements to sheep resulted in significant alteration of the milk content of 18:0, 18:2 and 18:3, but not 16:0 and 18:1n9 (Table 17). Milk from sheep fed

the diacetyl treated supplement had higher levels of 18:0 than did milk from sheep fed the formaldehyde treated supplement (12.79 versus 9.73%). The levels of 18:2 in the milk significantly increased from 4.93% in sheep fed the untreated supplement to 10.53, 7.52 and 7.06%, respectively, for those fed the formaldehyde, acetaldehyde, and diacetyl treated supplements (Table 17). The level of 18:3n3 also increased when sheep fed the chemically treated supplements were compared with those fed the control supplement. Sheep fed the control supplement contained 1.48% 18:3n3 compared with 2.38, 1.84 and 1.89% 18:3n3 in the milk of sheep on the formaldehyde, acetaldehyde and diacetyl treated supplements, respectively. In goats milk, Scott et al. (1974) reported a reduction in 16:0 level from 11.5 to 7.2%, a negligible increase in 18:0, a reduction in 18:1n9 from 42.8 to 24.1%, and increases in 18:2n6 content from 2.6 to 10.0% and in the 18:3n3 level from 1.5 to 22.8% when goats were fed formaldehyde treated casein-linseed oil particles. In another study, Scott et al. (1972) fed a diet containing .8 Kg lucerne chaff, .8 Kg oats and .8 Kg safflower seed-casein-formaldehyde gel to lactating goats for 3 days after feeding them a control diet for 6 d. The control diet contained 2.0 Kg lucerne chaff and oats (1:1, w/w). They observed a remarkable alteration of major fatty acids in the milk as a result. The relative percentages of 14:0 and 16:0, respectively, reduced 5 and 12%. The levels of stearic and linolenic acids did not change,

while the relative percentages of oleic (18:1) and linoleic acids increased, respectively, 3 and 25%.

Several reasons may have contributed to the moderate alteration in the PUFA contents of the blood serum and milk fat of sheep fed chemically treated full-fat soybean gels. First, the way in which the gels were prepared in bulk for the *in vivo* study may have resulted in a heterogenous mixture of the chemical additive in their structures. The soybean mixture was not homogenized in a colloid mill as suggested by Scott et al. (1972). In this ground full-fat soybean (1 mm particle size) were mixed with water and the caseinate solution by using portable mixer. The solid level in the batter was high (32 to 34%), and the batter gelled before all the targeted amount (5%) of an additive could be incorporated. In fact, the gel fed to the ewes contained about 2% additive. As a result, many portions of the gel may have had little or no additive. Therefore, fatty acids in the several pockets of the gel may have not been fully protected by the protein-oil gel, and thus, were biohydrogenated in the rumen.

A second theory concerning why the 18:2n6 level in the sheep blood serum and milk fat was less than expected is that the fatty acids in the gel supplements were digested before they could be absorbed into the blood stream with subsequent incorporation into the milk fat. However Scott et al. (1972) found that feeding spray dried or gelled casein-safflower oil formaldehyde supplements to goats increased the relative

percentage of 18:2n6 in the milk by increments of 16 and 24%, respectively. Therefore, other factors must be responsible for the results of this study.

Perhaps the nature of the oilseed (soybean) used in my protected gels may have affected the digestibility of the gel supplements and the absorption of their fatty acids. Bitman et al. (1973) prepared protected dietary fat supplements for ruminants: spray dried safflower oil-casein-formaldehyde particles and ground soybean (<3mm) or full-fat soy flour, treated, with 10 and 15% by weight formaldehyde, respectively. The treated soybean particles were dried at 65°C in a forced air oven for 20 hr. Feeding the dietary ground soy supplements to lactating cows resulted in doubling of the level of 18:2n6 in their milk fat to 6 to 7%, compared with 20 to 30% 18:2n6 reached by feeding the safflower oil particles. The results of Bitman et al. (1973) using the soybean supplements are in agreement with results of this experiment feeding soybean supplements which resulted in a doubling of the levels of 18:2n6 to 7 to 10% in sheep milk. Bitman et al. (1973) postulated, "The physical nature of the oil and protein in the soybean structure may be such that formaldehyde treatment affords little protection to the oil or the oil is too protected in the formaldehyde soybean preparations and passes out into the feces without absorption in the body".

CHAPTER V

CONCLUSION

The objectives of this study were: (1) to prepare dietary lipid supplements in gel form containing high levels of linoleic acid (18:2) oil or oilseeds (sunflower or soybean) encapsulated in casein complexes, which were treated with formaldehyde (FA), acetaldehyde (AC) and diacetyl (DA); (2) to determine the microstructure of the dietary gels by scanning electron microscopy; (3) to evaluate the effectiveness of the gel treatment in protecting dietary fat acids from ruminal biohydrogenation *in vitro*; and (4) to alter the fatty acid composition of the blood serum and milk fats from lactating ewes fed the gel supplements. These objectives were fulfilled by the experiments described herein.

My study showed that emulsion prepared with casein-soy flour, ground full-fat soybean or ground, full-fat sunflower seeds formed firm gels easily when they were treated with 5% (w/w of emulsion) formaldehyde or acetaldehyde. On the other hand, 5% diacetyl formed the gel with the soy materials but failed to make the gel with the sunflower. The pH at which the gels formed was greater than 8.0.

The microstructures of the gels containing sunflower oil-defatted soy flour were determined after they were dried (oven

or freeze drying). The structure of the gels were dependent on treatment and drying method and showed similar features as spray dried dietary lipid supplements. Scanning electron microscopy of gel containing ground full-fat oilseeds was not possible because of the coarse texture of ground seeds.

The evaluation of the protective ability of the treated gels against biohydrogenation was achieved by incubating the gels at 39°C with strained rumen fluid for up to 48 hr. In the digests containing each type of gel, both treatment and incubation time affected the levels of fatty acids.

When the gels were not treated, hydrogenation of PUFA occurred, regardless of the type of gel. The level of hydrogenation increased with increasing digestion time from 0 to 48 hr. For the untreated gels, the rate of hydrogenation appeared to be greatest in the gels containing defatted soy flour-sunflower oil. This may have been due to the state of the oil in these gels. In the gel containing defatted soy flour, some of the oil may not have been bound to the protein as in the gels containing ground oilseeds. In these latter gels, the oil was encapsulated in a natural bound state; therefore, it required more time and metabolic activity to digest the protected materials. However, the 48 hr digestion time was insufficient to achieve complete hydrogenation of the PUFA even in the untreated batter control.

In the digests containing formaldehyde, acetaldehyde and diacetyl treated gels, hydrogenation of PUFA occurred in all

types of gels, but the level of protection was much higher against hydrogenation in the treated than in the untreated gels. Acetaldehyde treatment protected against biohydrogenation slightly more than formaldehyde or diacetyl treatment in soy flour gels, and the diacetyl treatment was more effective than the formaldehyde treatment. In gels containing ground full-fat soybeans, acetaldehyde treatment protected 18:2 more than did formaldehyde or diacetyl treatment. In gels containing ground full-fat sunflower seeds, diacetyl failed to protect against *in vitro* biohydrogenation of 18:2; however, acetaldehyde and formaldehyde treatment prevented biohydrogenation of 18:2 to a great extent. The reason for the failure of the diacetyl treatment to offer any protection against biohydrogenation was probably due to the fact that diacetyl, at the 5% level did not form as firm a gel as those formed with formaldehyde and acetaldehyde.

Based on the *in vitro* results and the importance of soybeans as a cash crop in Tennessee, it was decided to utilize the ground, full-fat soybeans for evaluation of the effectiveness of formaldehyde, acetaldehyde and diacetyl in protecting dietary lipids *in vivo*. The possibility to increase the PUFA levels in the blood serum and the milk fat was assessed by feeding lactating ewes a diet supplemented with ground, full-fat soybean gels, treated with the previously listed chemicals or untreated, in a 4x4 Latin

square feeding trial.

The fatty acids recovered in the blood serum fat included: SFA (caprylic, 8:0; lauric, 12:0; myristic, 14:0; pentadecanoic, 15:0; palmitic, 16:0; margaric, 17:0; and stearic, 18:0), MUFA (palmitoleic, 16:1n9; and oleic, 18:1) and PUFA (linoleic, 18:2; linolenic, 18:3; arachidonic, 20:4; 7,10,13,16,19-docosapentaenoic, 22:5; and 4,7,10,13,16,19-docosaheptaenoic, 22:6). The level of only one fatty acid (18:2) in the blood serum was significantly affected by treatment. The levels of 18:2 were 23.85, 33.98, 33.65 and 29.90%, respectively, for the control, formaldehyde, acetaldehyde and diacetyl treatments. With reference to the control, feeding treated dietary fat supplements resulted in increases ($p < 0.05$) in the levels of 18:2 in the blood serum fat by 42, 41 and 25% for formaldehyde, acetaldehyde and diacetyl treatments, respectively.

The fatty acids encountered in the milk fat were: SFA (caproic, 6:0; caprylic, 8:0; capric, 10:0; undecanoic, 11:0; lauric, 12:0; myristic, 14:0; isopentadecanoic, 15:0I; pentadecanoic, 15:0; Isopalmitic, 16:0I; palmitic, 16:0; margaric, 17:0; stearic, 18:0; nonadecanoic, 19:0; and docosanoic, 22:0), MUFA (myristoleic, 14:1; palmitelaidic, 16:1t; palmitoleic, 16:1n9; oleic, 18:1; and 11-eicosenoic acid, 20:1), PUFA (linoleic, 18:2; linolenic, 18:3; cis,cis-11,14-eicosadienoic, 20:2; cis,cis,cis-8,11,14-eicosatrienoic, 20:3; arachidonic, 20:4; 5,8,11,14,17-eicosapentaenoic, 20:5;

7,10,13,16-docosatetraenoic acid, 22:4; 7,10,13,16,19-docosapentaenoic acid, 22:5; and 4,7,10,13,16,19-docosahexaenoic acid, 22:6) and unknown fatty acids. The levels of 18:0, 18:2 and 18:3 were affected ($p < 0.05$) by treatment. Compared with the control, diacetyl, acetaldehyde, and formaldehyde treatments, respectively, increased the 18:2n6 content in the milk fat by 43, 52 and 114%. The level of the fat in the sheep milk was unaffected ($p > 0.05$) by treatment, but it was less (0.9%) than that reported in sheep milk (2.3%) (USDA, 1984).

The fatty acids in the protected supplements were resistant to oxidation at 5 to 6°C storage for 90 days. None of the PUFA were affected by either storage period or treatment.

The weights of ewes and lambs were affected by the feeding period, but treatment did not affect ($p < 0.05$) the weight of the ewes. On the other hand, lambs gained more weight when their mothers were fed formaldehyde or acetaldehyde treated fat supplements. The difference between the lowest and highest lamb weights averaged across treatments was only 0.67 kg.

This study was undertaken to find compounds which were not carcinogenic and which could protect unsaturated fatty acids in a dietary lipid supplement against biohydrogenation in ruminants. Acetaldehyde, *in vitro*, proved to be more effective than formaldehyde and diacetyl. Depending upon the

type of oilseed material, diacetyl also showed some protective potentials. Acetaldehyde and diacetyl are both considered "Generally Recognized As Safe" by the United States Food and Drug Administration for use in human foods. This study is the first step in an attempt to maximize the potential ability of these two compounds in producing ruminant products (meat and dairy) with altered fatty acid compositions. More research is needed (1) to investigate the levels of acetaldehyde and diacetyl which can provide the maximum protection of unsaturated fatty acids in other oils/oilseeds (canola, safflower, corn and linseed) against biohydrogenation and (2) the study of *in vivo* digestibility of different forms of acetaldehyde and diacetyl treated fat supplements (gel and spray dried). Since the treated dietary fat supplements were in the form of protein gels, it is also possible that acetaldehyde and diacetyl treated protein supplements could cause dietary protein to bypass the rumen and be digested in the abomasum. This latter possibility also needs to be investigated. Hence, the ability of this method to protect other important compounds (essential amino acids, vitamins, antioxidants and flavors) that can improve the nutritive quality of ruminants' diet or their products for human consumption may be investigated as well. Thus consumers could have the choice among possible arrays of meats products which fat content respond to their health concern.

By improving the method of preparation of the protected

dietary lipid supplements in order to obtain a more uniform chemical treatment and higher amounts of lipid and protective additive, the fatty acid composition from ruminants' products (meat and dairy) can be profoundly altered to meet consumers' demand.

LIST OF REFERENCES

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- Abeygunasekera, D.D., Jansz, E.R. 1989. Effect of the maturation process on fermented cocoa bean I: free amino acids and volatile carbonyls. J. Nat. Sci. Coun. of Sri-Lanka. 17 (1):23-33.
- Abrahamsen, R.K. and Rysstad, G. 1991. Fermentation of goats' milk with yoghurt starter bacteria: a review. Cultur. Dairy Prod. J. 26 (3):20-26.
- Ahrens, E.H.Jr., Hirsh, J., Insull, W.Jr. 1957. The influence of dietary fats on serum-lipid levels in man. Lancet I. 943-953.
- Albert, R.E., Sellakumar, A.R., Laskin, S., Kuschner, M., Nelson, N., Snyder, C.A. 1982. Gaseous formaldehyde and hydrogen chloride induction of nasal cancer in rat. J. Nat. Cancer Inst. 68:597-602.
- American Heart Association. 1988. Dietary guidelines for healthy American adults: A statement for physicians and health professionals. Circulation 77:721A.
- American Meat Institute. 1989. Meat Facts. Am. Meat Inst., Washington D.C.
- Annison, E.F. and Lewis, D. 1959. *Metabolism in the Rumen*, A.R.C. Institute of Animal Physiology, 23. John Wiley and Sons Inc., New York.
- AOCS. 1975. *Official and Tentative Methods of Analysis*, 4th ed. American Oil Chemists' Society, Champaign, IL.
- Ashes, J.R., Gulati, S.K., Cook, L.J., Scott, T.W. and Donnelly, J.B. 1979. Assessing the biological effectiveness of protective lipid supplement for ruminants. J. Amer. Oil Chem. Soc. 56:522-527.
- Atwal, A. S., Hidioglou, M., and Kramer, J. K. G. 1991. Effects of feeding Protec and α -tocopherol on fatty acid composition and oxidative stability of cow's milk. J. Dairy Sci. 74:140-145.
- Badrie, N. and Mellowes, W. A. 1991. Texture and microstructure of cassava (*Manihot esculenta* Crantz) flour extrudate. J. Food Sci. 56 (5):1319-1322.

- Banks, A. and Hilditch, T.P. 1931. The glyceride structure of beef tallows. *Biochem. J.* 25:1168-1182.
- Bitman, L.P., Dryden, H.K., Goering, T.R., Wrenn, T.R., Yoncoskie, R.A. and Edmondson, L.F. 1972. Efficiency of transfer of polyunsaturated fats into milk. *J. Amer. Oil Chem. Soc.* 50 (3):93-98.
- Blair, A., Walrath, J. and Malker, H.. 1985. Review of Epidemiologic Evidence Regarding Cancer and Exposure to Formaldehyde. Ch. 18, *In Formaldehyde Analytical Chemistry and Toxicology*. Turoski, V. (Ed.), 261-273. American Chemical Society, Washington, D.C.
- Bonanome, A., and Grundy, S.M. 1988. Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. *New Eng. J. Med.* 318:1244-1248.
- Bright, J.M., Sullivan, P.S., Melton, S.L., Schneider, J.F. and McDonald, T.P. 1994. The effects of n-3 fatty acid supplementation on bleeding time, plasma fatty acid composition, and in vitro platelet aggregation in cats. *J. Vet. Int. Med.* 8 (4):247-252.
- Brock, R.A. 1989. Oil consumption continues upward trend. *Snack World.* 9:14.
- Cadden, A. M., Urquhart, A., and Jelen, P. 1984. Storage stability of canola based protected lipid feed supplement and its effect on characteristics of milk and butter. *J. Dairy Sci.* 67:1414-1420.
- Chandler, N.J., Robinson, I.B., Ripper, I.C. and Fowler, P. 1973. The effect of feeding formaldehyde treated sunflower seed supplement on the yield and composition of milk fat. *Austr. J. Dairy Technol.* 12:179.
- Carr, C.J. and Kolbye, J.A. Jr. 1985. Evaluation of Potential Carcinogenic Hazard. Ch. 22, *In Formaldehyde Analytical Chemistry and Toxicology*. Turoski, V. (Ed.), 335-340. American Chemical Society, Washington, D.C.
- Cartter, J.L. and Hopper, T.H. 1942. Influence of Variety, Environment and Fertility Level on the Chemical Composition of Soybean Seed. *Tech. Bull. No 7.* U.S. Dept. of Agriculture, Washington, DC.
- Charles, S. S. 1979. Lipid Nutrition. *In Animal Feed and Pet Foods, Recent Developments*. Charles, S.S. (Ed.), 17-26. Noyes Data Corporation, Park Ridge, N.J.

- Charley H. 1986. *Food Science*. 2nd ed. Mcmillan Publishing Company, New York.
- Chesson, A. and Forsberg, C.W. 1988. Polysaccharide Degradation by Rumen Microorganisms. Ch. 8, *In The Rumen Microbial System*. Hobson, P.N. (Ed.), 251-285. Elsevier Publishing Co. Inc., New York.
- Chilliard, Y., Bauchart, D., Gagliostro, G., Ollier, A., and Vermorel, M. 1991. Duodenal rapeseed oil infusion in early and midlactation cows. 1. Intestinal apparent digestibility of fatty acids and lipids. *J. Dairy Sci.* 74:490-498.
- Church, D. C. 1983. *Digestive Physiology and Nutrition of Ruminants*, 4th ed. O & B Books, Inc., Corvallis, OR.
- Clary, J.J., Gibson, J.E. and Waritz, R.S. 1983. *Formaldehyde, Toxicology, Epidemiology Mechanisms*. Marcel Dekker, Inc., New York, N.Y.
- Cogan, T.M. and Jordan, K.N. 1990. Flavour production by cultures in cheese and butter. 2nd Cheese Symposium. Cogan, T.M. (Ed.), 11-18.
- Collings, A., Holmes, A.R., and Shepherd, M.G. 1991. Secretion of end-products of metabolism by *Candida* species. *Biomed. Letters*. 46 (184):285-296.
- Cook, L.J. and Scott, T.W. 1970. Protection of dietary polyunsaturated fatty acids from ruminal microbial hydrogenation. *Proc. Austr. Biochem. Soc.* 3:393.
- Cook, L.J., Scott, T.W., Ferguson, K.A. and McDonald, I.W. 1970. Production of polyunsaturated ruminant body fats. *Nature* 228:178-179.
- Cook, L.J., Scott, T.W. and Faichney, G.J. 1975. Fatty acid interrelationships in plasma, liver, muscle and adipose tissues of cattle fed safflower oil protected from ruminal hydrogenation. *Lipids* 7 (2):83-89.
- Daeschel, M.A. 1989. Antimicrobial substances from lactic acid bacteria for use as food preservatives. *J. Food Technol.* 43 (1):164-167.
- Damodaran, S. and Kinsella, J.E. 1981a. Interaction of carbonyls with soy protein: Thermodynamic effects. *J. Agr. Food Chem.* 29 (6):1249-1253.

- Damodaran, S. and Kinsella, J.E. 1981b. Interaction of carbonyls with soy protein: conformational effects. *J. Agr. Food Chem.* 29 (6):1253-1257.
- Dawson, R.M.C. and Kemp, P. 1969. The effect of defaunation on the phospholipids and on the hydrogenation of unsaturated fatty acids in the rumen. *Biochem. J.* 115:351-352.
- Dawson, R.M.C. and Kemp, P. 1970. Biohydrogenation of Dietary Fats in Ruminants. In *Physiology of Digestion and Metabolism in the Ruminant*. Phillipson, A.T. (Ed.), 504-518. Oriel Press, Newcastle-upon-Tyne.
- Dinius, D.A., Oltjen, R.R.; Lyon, C.K.; Kohler, G.O., and Walker, H.G. 1974. Utilization of a formaldehyde treated casein-safflower oil complex by growing and finishing steers. *J. Anim. Sci.* 39:124-133.
- Dollear, F.G., Krauczunas, P. and Markley, K.S. 1940. The chemical composition of some high iodine number soybean oils. *Oil soap* 17:120-126.
- Edmondson, L.F., Yoncoskie, R.A., Rainey, N.H., Douglas, F.W.Jr. and Bitman, J. 1974. Feeding encapsulated oils to increase the polyunsaturation in milk and meat fats. *J. Amer. Oil Chem. Soc.* 51:72-76.
- Ekeren, P.A., Smith, D.R., David, K.L. and Smith, S.B. 1992. Ruminal biohydrogenation of fatty acids from high-oleic sunflower seeds. *J. Anim. Sci.* 70:2574-2580.
- Faichney, G.J., Davies, A.L., Scott, T.W. and Cook, L.J. 1972. The incorporation of linoleic acid into the tissues of growing steers offered a dietary supplement of formaldehyde-treated casein-safflower oil. *Austr. J. Biol. Sci.* 25:205-212.
- Faichney, G.J. and White, G.A. 1977. Formaldehyde treatment of concentrated diets for sheep. I: Partition of the digestion of organic matter and nitrogen between stomach and intestines. *Austr. J. Agric. Res.* 28:1055-1067.
- FAO. 1988. *Production Yearbook*. Food and Agriculture Organization, Rome, Italy.
- Fennema, O.R. 1985. *Food Chemistry*, 2nd ed. Marcel Dekker, Inc., New York.
- Gaafar, A.M. 1992. Volatile flavour compounds of yoghurt. *Intern. J. Food Sci. Technol.* 27 (1):87-91.

- Gaafar, A.M. and Gaber, F.I. 1992. Volatile flavour compounds of sunlight-exposed milk. Egypt. J. Dairy Sci. 20 (1):111-115.
- Garton, G.A.; Lough, A.K. and Vioque, E. 1959. The effect of sheep rumen contents on triglycerides *in vitro*. Biochem. J. 73:46.
- Garton, G.A. 1960. Fatty acid composition of the lipids of pasture grasses. Nature. 187:511-512.
- Gibson, J.E. 1983. *Formaldehyde, Toxicity*. Gibson, J.E. (Ed.). Hemisphere Publishing Corporation, Washington, DC.
- Ginsberg, H.N., Barr, S.L. and Gilbert, A. 1990. Reduction of plasma cholesterol levels in normal men on an American Heart Association step 1 diet or a step 1 diet with added monounsaturated fat. N. Engl. J. Med. 322:574-579.
- Grande, F., Anderson, J.T. and Keys, A. 1972. Diets of different fatty acid composition, producing identical serum cholesterol levels in man. Amer. J. Clin. Nutr. 25:53-60.
- Grundy, S.M. and Vega, G.L. 1988. Plasma cholesterol responsiveness to saturated fatty acids. Am. J. Clin. Nutr. 47:822-824.
- Harrap, B.S. 1973. Recent developments in the production of dairy products with increased levels of polyunsaturation. Austr. J. Dairy Technol. 9:101-104.
- Hartfoot, C.G. and Hazlewood, G.P. 1988. Lipid Metabolism in Rumen. Ch. 9, *In The Rumen Microbial Ecosystem*. Hobson, P.N. (Ed.), 285-322. Elsevier Publishing Co. Inc., New York.
- Hazlewood, G.P. and Dawson, R.M. 1975. Isolation and properties of a phospholipid-hydrolysing bacterium from ovine rumen fluid. J. Gen. Microbiol. 89:163-174.
- Hazlewood, G.P., Kemp, P., Lander, D. and Dawson, R.M. 1976. C18 unsaturated fatty acid hydrogenation patterns of some rumen bacteria and their ability to hydrolyse exogenous phospholipid. Br. J. Nutr. 35:293-297.
- Hazlewood, G.P. and Dawson, R.M. 1979. Characteristics of a lipolytic and fatty acid-requiring *Butyrivibrio* sp. isolated from the ovine rumen. J. Gen. Microbiol. 112: 15-27.

- Hegsted, D.M., McGandy, R.B., Myers, M.L. and Stare, F.J. 1965. Quantitative effects of dietary fat on serum cholesterol in man. *Amer. J. Clin. Nutr.* 17:281-295.
- Henderson, C. 1971. A study of the lipase produced by *Anaerovibrio lipolytica*, a rumen bacterium. *J. Gen. Microbiol.* 65:81-89.
- Henderson, C. 1973. The effect of fatty acids on pure cultures of rumen bacteria. *J. Agric. Sci.* 81:107-111.
- Henderson, C. 1975. The isolation and characterization of strains of lipolytic bacteria from the ovine rumen. *J. Appl. Bacteriol.* 39:101-109.
- Hobson, P.N. and Mann, S.O. 1961. Isolation of glycerol-fermenting and lipolytic bacteria from the rumen of the sheep. *J. Gen. Microbiol.* 25:227-240.
- Hogan, J.P., Connell, P.J. and Mills, S.C. 1972. The digestion of safflower oil-casein particles protected against ruminal hydrogenation in sheep. *Austr. J. Agric. Res.* 23:87-95.
- Holum, J.R. 1979. *Elements of General and Biological Chemistry*. 5th ed. John Wiley and Sons Inc., New York.
- Hughes, P.E and Tove, S.B. 1980a. Identification of an endogenous electron donor for biohydrogenation as α -tocopherolquinol. *J. Biol. Chem.* 255:4447-4452.
- Hughes, P.E and Tove, S.B. 1980b. Identification of deoxy- α -tocopherolquinol as another endogenous electron donor for biohydrogenation. *J. Biol. Chem.* 255:11802-11806.
- Hughes, P.E, Hunter, W.J. and Tove, S.B. 1982. Biohydrogenation of unsaturated fatty acids: purification and properties of cis-9,trans-11 octadecadienoate reductase. *J. Biol. Chem.* 257:3643-3649.
- Hungate, R.E. 1966. *The Rumen and its Microbes*. Academic Press, New York and London.
- Hungate, R.E. 1988. Introduction: The Ruminant and the Rumen. Ch. 1, *In The Rumen Microbial Ecosystem*. Hobson, P.N. (Ed.), 1-19. Elsevier Publishing Co. Inc., New York.

- Hunter, W.J., Baker, F.C., Rosenfeld, I.S., Keyser, J.B. and Tove, S.B. 1976. Biohydrogenation of unsaturated fatty acids. VII. Hydrogenation by a cell-free preparation of *Butyrivibrio fibrisolvens*. J. Biol. Chem. 251:2241-2247.
- Jass, H. E. 1985. History and Status of Formaldehyde in the Cosmetics Industry. Ch. 15, *In Formaldehyde Analytical Chemistry and Toxicology*. Turoski, V. (Ed.) 229-236. American Chemical Society, Washington, D.C.
- Jay, J.M. 1982. Antimicrobial properties of diacetyl. Appl. Env. Microb. 44 (3):525-532.
- Jay, J.M. 1986. *Modern Food Microbiology*. 3rd ed. Van Nostrand Reinhold Company, New York.
- Johnson, A.R. 1974. Biomanipulation of ruminant fats. Food Manufac. 49:19-24.
- Joy, D. 1993. Personal communication. University of Tennessee, Knoxville.
- Kaneko, T., Watanabe, Y. and Suzuki, H. 1991. Differences between *Lactobacillus casei* subsp. *casei* 2206 and citrate-positive *Lactococcus lactis* subsp. *lactis* 3022 in the characteristics of diacetyl production. Appl. Env. Microbiol. 57 (10):3040-3042.
- Kawas, J.R., Lopes, J., Danelon, D.L. and Lu, C.D. 1991. Influence of forage to concentrate ratios on intake, digestibility, chewing and milk production of dairy goats. Small Rumin. Res. 4:11-18.
- Kinsell, L.W., Partridge, J., Boling, L., Margen, S. and Michaels, G. 1952. Dietary modification of serum cholesterol and phospholipid levels. J. Clin. Endocrinol. 12:909-913.
- Kneifel, W. Ulberth, F., Erhard, F. and Jaros, D. 1992. Aroma profiles and sensory properties of yoghurt and yoghurt related products. I. Screening of commercially available starter cultures. Milchwissenschaft. 47 (6):362-365.
- Kemp, P. and Lander, D.J. 1983. The hydrogenation of -linolenic acid by pure cultures of two rumen bacteria. Biochem. J. 216:519-522.
- Kemp, P. and Lander, D.J. 1984. Hydrogenation *in vitro* of α -linolenic acid to stearic acid by mixed cultures of pure strains of rumen bacteria. J. Gen. Microbiol. 130: 527-533.

- Kemp, P. , White, R.W. and Lander, D.J. 1975. The hydrogenation of unsaturated fatty acids by five bacteria isolates from the sheep rumen , including a new species. J. Gen. Microbiol. 90:100-114.
- Kemp, P., Lander, D.J. and Gunstone, F.D. 1984a. Hydrogenation of some cis and trans octadecenoic acids to stearic acid by a rumen *Fusocillus* sp. Br. J. Nutr. 52:165-170.
- Kepler, C.R. and Tove, S.B. 1967. Biohydrogenation of unsaturated fatty acids. III. Purification and properties of a linoleate 12-cis, 11-trans isomerase from *Butyrivibrio fibrisolvens*. J. Biol. Chem. 242: 5686-5692.
- Kepler, C.R., Tucker, W.P. and Tove, S.B. 1970. Biohydrogenation of unsaturated fatty acids. IV. Substrate specificity and inhibition of linoleate 12-cis, 11-trans isomerase from *Butyrivibrio fibrisolvens*. J. Biol. Chem. 245:3612-3620.
- Kepler, C.R., Tucker, W.P. and Tove, S.B. 1970. Biohydrogenation of unsaturated fatty acids. V. Stereospecificity of proton addition and mechanism of action of linoleic acid 12-cis,11-trans isomerase from *Butyrivibrio fibrisolvens*. J. Biol. Chem. 246:2765-2771.
- Kerr, T.J., Clinton, J.W., Evans, A.L. and Rigsby, W.E. 1983. Structural Characterization of Spray-dried Dairy Products by Scanning Electron Microscopy. In *Developments in Industrial Microbiology*. 475-484. Society for Industrial Microbiology, Arlington, VA.
- Keys, A., Anderson, J.T., and Grande, F. 1965. Serum cholesterol response to changes in the diet. IV. Particular saturated fatty acids in the diet. Metab. Clin. Exp. 14:776-787.
- Kohyama, K. and Nishinari, K. 1993. Rheological studies on the gelation process of soybean 7S and 11S Proteins in the presence of glucono-delta-lactone. J. Agric. Food. Chem. 41:8-14.
- LaCount, D.W., Drackley, J.K.; Laesch, S.O. and Clark, J.H. 1994. Secretion of oleic acid in milk fat in response to abomasal infusion of canola or high oleic sunflower fatty acids. J. Dairy Sci. 77:1372-1385.

- Laine, D.C., Snodgrass, C.M., Dawson, E.A., Ener, A.M., Kuba K. and Frantz, I.D. 1982. Lightly hydrogenated soy oil versus other vegetable oils as a lipid-lowering dietary constituent. *Amer. J. Clin. Nutr.* 35:683-690.
- Loudon, G. Mark. 1984. Aldehydes and Ketones. Ch.21., *In Organic Chemistry*. Eastman, C.L. (Ed.), 869-962. Addison Wesley Publishing Company, Inc., Reading, MA.
- Lough, D.S., Solomon, M.B., Rumsey, T.S., Elsasser, T.H., Slyter, L.L., Kahl, S. and Lynch, G.P. 1992. Effects of dietary canola seed and soy lecithin in high-forage diets on cholesterol content and fatty acid composition of carcass tissues of growing ram lambs. *J. Anim. Sci.* 70:1153-1158.
- Lu, C.D., Potchoiba, M. J., Sahlu, T. and Kawas, J. R. 1990. Performance of dairy goats fed soybean meal or meat and bone meal with or without urea during early lactation. *J. Dairy Sci.* 73 (3):726-734.
- Lubis, D., Van Horn, H. H., Harris, B. Jr., Bachman, K. C., and Emanuele, S. M. 1990. Responses of lactating dairy cows to protected fats or whole cottonseed in low or high forage diets. *J. Dairy Sci.* 73:3512-3525.
- Mahadevappa, V.P. and Raina, P.L. 1978. Nature of some Indian legume lipids. *J. Agric. Food Chem.* 26:1241-1244.
- Marks, T. A., Worthy, W. C., Staples, R. E. 1980. Influence of formaldehyde and sonacide (potentiated acid glutaraldehyde) on embryo and fetal development in mice. *Teratol.* 22 (1):51.
- Mattson, F.H., Hollenback, E.J., and Kligman, A.M. 1975. Effect of hydrogenated fat on the plasma cholesterol and triglyceride levels in man. *Amer. J. Clin. Nutr.* 28:726-731.
- Melton, S.L., Moyers, R.E. and Playford, C.G. 1979. Lipids extracted from soy products by different procedures. *J. Amer. Oil Chem. Soc.* 56:489-497.
- Morgan, M., Heller, R.F. and Swerdlow, A. 1989. Changes in diet and coronary heart disease mortality among social classes in Great Britain. *J. Epidem. Com. Health* 43:162-167.

- Mounts, T.L., Warner, K.A., List, G.R., Friedrich, J.P. and Koritala, S. 1987. *Soybeans Improvement, Production and Uses*, 2nd ed. Wilcox, J. R. (Ed.), 850. American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc. Madison, WI.
- Murphy, J.J., and Morgan, D. J. 1983. Effect of inclusion of protected and unprotected tallow in the supplement on the performance of lactating dairy cows. *Anim. Prod.* 37:203-209.
- Murti, T.W., Roger, S., Bouillanne, C., Landon, M. and Desmazeaud, M.J. 1992. Growth of *Bifidobacterium* sp CNRZ 1494 in soya extract and cow milk. Effects on aromatic compounds. *Sciences des Aliments*. 12 (3) 429-439.
- National Research Council. 1981. Commercial Production, Properties, and Uses of the Aldehydes. In *Formaldehyde and Other Aldehydes*. National Academy Press, Washington, DC.
- National Research Council. 1988. *Designing Foods, Animal Product Options in the Market Place*. National Academy Press, Washington, DC.
- National Research Council. 1989. *Diet and Health-implications for Reducing Chronic Disease Risk*. National Academy Press, Washington, DC.
- Nestel, P.J., Havenstein, N., White, H.M., Scott, T.J. and Cook, L.J. 1973. Lowering of plasma cholesterol and enhanced sterol excretion with the composition of polyunsaturated ruminant fats. *New Eng. J. Med.* 288 (8):279-382.
- Nestel, P. J., Noakes, M., Belling, G. B., McArthur, R., Clifton, P. M. and Abbey M. 1992. Plasma cholesterol lowering potential of edible-oil blends. *Am. J. Clin. Nutr.* 55:46-50.
- Newberry, S.D.B. 1990. Biohydrogenation of Soybean Oil in Spray Dried Casein-Oil Emulsions from Different Treatments. University of Tennessee, Knoxville, Master's Thesis.
- Nunez-Hernandez, G., Wallace, J.D., Holechek, J.L., Galyean, M.L. and Cardenas, M. 1991. Condensed Tannins and nutrient utilization by lambs and goats fed low-quality diets. *J. Anim. Sci.* 69:1167-1177.

- Oprin, C.G. and Joblin, K.N. 1988. The Rumen Anaerobic Fungi. Ch. 4, In *The Rumen Microbial Ecosystem*. Hobson, P.N. (Ed.), 129-149. Elsevier Publishing Co. Inc., New York.
- Ott, L. 1984. *An Introduction to Statistical Methods and Data Analysis*. 2nd ed. PWS Publishers, Boston, MA.
- Plowman, R.D.; Bitman, J.; Gordon, C.H.; Dryden, L.P. Goering, H.K. and Wrenn, T.R. 1971. Milk fat with increased polyunsaturated fatty acids. *J. Dairy Sci.* 55 (2): 204-207.
- Price, J.F. and Schweigert, B.S. 1987. *The Science of Meat and Meat Products*, 3rd ed. Food and Nutrition Press Inc., Westport, CT.
- Prins, R.A., Lankhorst, A., Van der Meer, P. and Van Nevel, C.J. 1975. Some characteristics of *Anaerovibrio lipolytica*, a rumen lipolytic organism. *Ant. van Leeuwenhoek J. Microbiol. Serol.* 41:1-11.
- Rassias, G., Kestin, M. and Nestel, P.J. 1991. Linoleic acid lowers LDL cholesterol without a proportionate displacement of saturated fatty acid. *Eur. J. Clin. Nutr.* 45:315-320.
- Rawlings, R.M. and Procter, D. 1979. Ruminant Feeds and Feed Supplements. In *Animal Feeds and Pet Foods. Recent Developments*. Sodano, C.S. (Ed.), 17-25. Noyes Data Corporation, Park Ridge, NJ.
- Reiser, R. 1951. Hydrogenation of polyunsaturated fatty acids by the ruminant. *Fed. Proc.* 10:236.
- Rizek, R.L. and Jackson, E.M. 1980. Current food consumption practices and nutrient sources in the American diet. U.S.D.A. Hyatville, MD.
- Rosenberg, I.H. and Schaefer, E.J. 1988. Dietary saturated fatty acids and blood cholesterol. *New Engl. J. Med.* 318:1270-1271.
- Rosenfeld, I.S. and Tove, S.B. 1971. Biohydrogenation of unsaturated fatty acids. IV. Source of hydrogen and stereospecificity of reduction. *J. Biol. Chem.* 246: 5025-5030.
- Rule, D.C. and Beitz, D.C. 1986. Fatty acids of adipose tissue, plasma, muscle and duodenal ingesta of steers fed extruded soybeans. 1986. *J. Amer. Oil. Chem. Soc.* 63(11):1429-1436.

- Saio, K. and Watanabe, T. 1978. Differences in functional properties 7S and 11S soybean proteins. *J. Texture Stud.* 9:135-157.
- Salunkhe, D.K., Chavan, J.K., Adsule, R.N. and Kadam, S.S. 1992. Soybean. Ch. 2, *In World Oilseeds Chemistry, Technology and Utilization*, 9-23. Van Nostrand Reinhold, New York.
- SAS Institute Inc. 1985. *SAS User's Guide: Basics*, Version 5 Edition, SAS Institute Inc., Cary, NC.
- Scott, T.W., Cook, L.J. and Mills, S.C. 1971. Protection of dietary polyunsaturated fatty acids against microbial hydrogenation in ruminants. *J. Amer. Oil Chem. Soc.* 48:358-364.
- Scott, T.W., Bready, Royal, A.J. and Cook, L.J. 1972. Oil seed supplements for the protection of polyunsaturated ruminant milk fat. *Search* 3 (5):170-171.
- Scott, T.W. 1975. Polyunsaturated meat and dairy products. *Cereal Food World* 20 (2):72-76.
- Shorland, F.B., Weenink, R.O., Johns, A.T., and McDonald, I.R.C. 1957. The effect of sheep-rumen contents on unsaturated fatty acids. *Biochem. J.* 67:328-333.
- Shorland, F.B., Weenink, R.O. and Johns, A.T. 1955. Effect of the rumen on dietary fat. *Nature*. 175:1129-1130.
- Shorland, F.B. 1963. The Distribution of Fatty Acids in Plant Lipids, *In Chemical Plant Taxonomy*. Swain, T.W. (Ed.), Academic Press, New York.
- Snyman, L.D. 1993. Effect of exposure on the chemical composition in vitro dry matter digestibility of untreated and formalin treated green chop. *Anim. Feed Sci. and Technol.* 44:275-280.
- Solomon, M.B., Lynch, G.P. and Lough, D.S. 1992. Influence of dietary palm oil supplementation on serum lipid metabolites, carcass characteristics, and lipid composition of carcass tissues of growing ram and ewe lambs. *J. Anim. Sci.* 70:2746-2751.
- Srivastava, A. and Mudgal, V.D. 1984. Effect of feeding protected fat to buffaloes milk production. *Indian J. Anim. Sci.* 1122-1125.

- Stewart, C.S. and Bryant, M.P. 1988 The Rumen Bacteria. Ch. 2, *In The Rumen Microbial Ecosystem*. Hobson, P.N. (Ed.), 21-75. Elsevier Publishing Co. Inc., New York.
- Sutton, J.D., Knight, R., McAllan, A.B. and Smith, R.H. 1983. Digestion and synthesis in the rumen of sheep given diets supplemented with free and protected oils. *Br. J. Nutr.* 49:419-432.
- Tatsumi, Y., Watada, A. E. and Wergin, W. P. 1991. Scanning Electron Microscopy of carrot stick surface to determine cause of white translucent appearance. *J. Food Sci.* 56 (5):1357-1359.
- Tracey, M.V. 1975. Altering fatty acid composition of ruminant products. *Cereal Food World.* 20 (2):77-81.
- Trevisan, M., Krogh V, Freudenheim, J.L., Blake, A., Muti, P., Panico, S., Farinaro, E., Mancini, M., Menotti, A. and Ricci, G. 1990. Diet and coronary heart disease risk factors in a population with varied intake. *Prev. Med.* 19 (3):231-241.
- Turoski, V. 1985. *Formaldehyde Analytical Chemistry and Toxicology*. American Chemical Society, Washington, D.C.
- USDA. 1979. Composition of Foods, Fats, and Oils, Raw, Processed, Prepared. Handbook No. 8-4. U.S. Dept. of Agriculture, Washington, D.C.
- USDA. 1984. Composition of Foods, Vegetable and Vegetable Products. Handbook No. 8-11. U.S. Dept. of Agriculture, Washington, D.C.
- USDA. 1986. National Food Consumption Survey, Continuing Survey of Food Intakes by Individuals. Human Nutrition Information Service. Rpt. No. 85. U.S. Dept. of Agriculture, Washington, D.C.
- Van Soest, P.J. 1984. *Nutritional Ecology of the Ruminant*. O & B Books, Inc., Corvallis, OR.
- Vincent, G. R., Shelford, J. A., Peterson, R. G., and Krishnamurti, C. R. 1984. Effects of feeding canola-meal-protected-tallow or soybean-meal-protected in the low roughage diet of dairy cows in early lactation. *Can. J. Anim. Sci.* 64:81-91.
- Viviani, R. 1970. Metabolism of long chain fatty acids in the rumen. *Adv. Lipid Res.* 8:267-346.

- Wallace, R.J. and Cotta, M.A. 1988. Metabolism of Nitrogen-Containing Compounds. Ch. 7, *In The Rumen Microbial Ecosystem*. Hobson, P.N. (Ed.), 217-250. Elsevier Publishing Co. Inc., New York.
- Wangoh, J., Schulthess, W. and Struebi, P. 1992. Flavouring of the cultured milk product Mala with fruits available in Kenya. *Milchwissenschaft*. 47 (1):27-31.
- Ward, P.F.V., Scott, T.W. and Dawson, R.M.C. 1964. The biohydrogenation of unsaturated fatty acids in the ovine digestive tract. *Biochem. J.* 92:60-68.
- Weiss, T.J. 1983. *Food Oils and their Uses*. 2nd ed. AVI Publishing Co., Inc. Westport, CT.
- Whitney, E. N. and Hamilton, E.M.N. 1987. *Understanding Nutrition*. 4th ed. Wet Publishing Co. St. Paul N.Y.
- WHO. 1989. *Formaldehyde*. World Health Organization, Geneva.
- Williams, A.G. and Coleman, G.S. 1988. The Rumen Protozoa. Ch. 3, *In The Rumen Microbial Ecosystem*. Hobson, P.N. (Ed.), 77-127. Elsevier Publishing Co. Inc., New York.
- Wong, N.P., Walter, H.E., Vestal, J.H., Lacroix, D.E. and Alford, J.A. 1973. Cheddar cheese with increased polyunsaturated fatty acids. *J. Dairy Sci.* 56 (10): 171-175.
- Wood, R.D., Bell, M.C., Grainger, R.B. and Teekel, R.A. 1963. Metabolism of labelled linoleic-1-¹⁴C acid in the sheep rumen. *J. Nutr.* 79:62-68.
- Wright, D.E. 1959. Hydrogenation of lipids by rumen protozoa. *Nature*. 184:875-876.

APPENDICES

APPENDIX A

APPENDIX A. Analyses of variances for individual and fatty acids type in ruminal digests of untreated or treated emulsions containing defatted soy flour and sunflower oil

A-1. Analyses of variances for tridecanoic (13:0) and myristic (14:0) acids in ruminal digests of treated emulsions containing defatted soybean flour and sunflower oil.

FATTY ACID = TRICANDECANOIC (13:0)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	0.2543743	0.0847914	1.93	0.1826
TIME	2	4.9519812	2.4759906	56.48	0.0001
TREAT*TIME	6	0.5523600	0.0920600	2.10	0.1356
TREAT=1 ^a					
TIME	2	2.1585423	1.0792711	24.62	0.0001
TREAT=2 ^a					
TIME	2	1.1976074	0.5988037	13.66	0.0010
TREAT=3 ^a					
TIME	2	0.9787599	0.4893799	11.16	0.0010
TREAT=4 ^a					
TIME	2	1.1694316	0.5847158	13.34	0.0010
REP	1	0.0336127	0.0336127	0.77	0.3999
Error	11	0.4821851	0.0438350		
Corrected Total	23	6.2745133			

FATTY ACID = TETRADECANOIC OR MYRISTIC (14:0)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	1.0516867	0.3505622	6.29	0.0096
TIME	2	3.1178046	1.5589023	27.97	0.0001
TREAT*TIME	6	2.3600565	0.3933428	7.06	0.0028
TREAT=1 ^a					
TIME	2	0.1150462	0.0575231	1.03	*
TREAT=2 ^a					
TIME	2	4.7742164	2.3871082	42.83	0.0001
TREAT=3 ^a					
TIME	2	0.1789548	0.0894774	1.61	0.1288
TREAT=4 ^a					
TIME	2	0.4096437	0.2048219	3.67	0.0638
REP	1	0.0593927	0.0593927	1.07	0.3241
Error	11	0.6130839	0.0557349		
Corrected Total	23	7.2020244			

*=p>0.25.

^aTreat=1 is the control treatment; Treat=2 is formaldehyde treatment; Treat=3 is acetaldehyde treatment and Treat=4 is diacetyl treatment.

A-2. Analyses of variance for palmitic (16:0) and palmitoleic (16:1c) acids in ruminal digests of treated emulsions containing defatted soybean flour and sunflower oil.

FATTY ACID=HEXADECANOIC OR PALMITIC (16:0)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	21.890740	7.296913	15.76	0.0003
TIME	2	34.678798	17.339399	37.44	0.0001
TREAT*TIME	6	24.692893	4.115482	8.89	0.0011
TREAT=1 ^a					
TIME	2	3.1549283	1.5774641	3.41	0.0754
TREAT=2 ^a					
TIME	2	49.189838	24.594919	53.01	0.0001
TREAT=3 ^a					
TIME	2	1.3217269	0.6608635	1.43	*
TREAT=4 ^a					
TIME	2	5.7051981	2.8525991	6.16	0.0218
TREAT=4 ^a					
REP	1	0.594219	0.594219	1.28	0.2814
Error	11	5.094036	0.463094		
Corrected Total	23	86.950685			

FATTY ACID = 9-HEXADECENOIC OR PALMITOLEIC (16:1n9)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	0.0275493	0.0091831	3.68	0.0468
TIME	2	0.0611167	0.0305584	12.25	0.0016
TREAT*TIME	6	0.0633588	0.0105598	4.23	0.0188
TREAT=1 ^a					
TIME	2	0.0127625	0.0063813	2.56	0.1351
TREAT=2 ^a					
TIME	2	0.0892436	0.0446218	17.89	0.0010
TREAT=3 ^a					
TIME	2	0.0024050	0.0012025	0.48	*
TREAT=4 ^a					
TIME	2	0.0200643	0.0100322	4.02	0.0492
REP	1	0.0000181	0.0000181	0.01	0.9336
Error	11	0.0274348	0.0024941		
Corrected Total	23	0.1794778			

*=p>0.25.

^aTreat=1 is the control treatment; Treat=2 is formaldehyde treatment; Treat=3 is acetaldehyde treatment and Treat=4 is diacetyl treatment.

A-3. Analyses of variance for margaric (17:0) and stearic (18:0) acids in ruminal digests of treated emulsions containing defatted soybean flour and sunflower oil.

FATTY ACID=HEPTADECANOIC OR MARGARIC (17:0)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	0.2358017	0.0786006	3.95	0.0389
TIME	2	1.1413360	0.5706680	28.69	0.0001
TREAT*TIME	6	0.2214016	0.0369003	1.86	0.1772
TREAT=1 ^a					
TIME	2	0.0374227	0.0187113	0.94	*
TREAT=2 ^a					
TIME	2	0.5330836	0.2665418	13.40	0.0010
TREAT=3 ^a					
TIME	2	0.2565787	0.1282894	6.45	0.0158
TREAT=4 ^a					
TIME	2	0.5356526	0.2678263	13.47	0.0010
REP	1	0.0094750	0.0094750	0.48	0.5044
Error	11	0.2187768	0.0198888		
Corrected Total	23	1.8267910			

FATTY ACID=OCTADECANOIC OR STEARIC (18:0)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	152.92603	50.97534	24.80	0.0001
TIME	2	772.71265	386.35632	187.94	0.0001
TREAT*TIME	6	175.03045	29.17174	14.19	0.0001
TREAT=1 ^a					
TIME	2	534.54539	267.27269	130.01	0.0001
TREAT=2 ^a					
TIME	2	256.14008	128.07004	62.30	0.0001
TREAT=3 ^a					
TIME	2	61.423526	30.711763	14.94	0.0010
TREAT=4 ^a					
TIME	2	95.634103	47.817052	23.26	0.0001
REP	1	5.88745	5.88745	2.86	0.1187
Error	11	22.6134	2.0558		
Corrected Total	23	1129.1699			

*=p>0.25.

^aTreat=1 is the control treatment; Treat=2 is formaldehyde treatment; Treat=3 is acetaldehyde treatment and Treat=4 is diacetyl treatment.

A-4. Analyses of variance for cis plus trans-9-octadecenoic (18:1c+t) and oleic acid isomer (18:1I) acids in ruminal digests of treated emulsions containing defatted soybean flour and sunflower oil.

FATTY ACID= cis plus trans-9-OCTADECENOIC (18:1c+t)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr >F
TREAT	3	504.32775	168.10925	69.86	0.0001
TIME	2	86.12091	43.06045	17.89	0.0003
TREAT*TIME	6	293.47873	48.91312	20.33	0.0001
TREAT=1 ^a					
TIME	2	355.40723	177.70362	73.84	0.0001
TREAT=2 ^a					
TIME	2	11.880300	5.940150	2.47	0.1457
TREAT=3 ^a					
TIME	2	10.945200	5.472600	2.27	0.1691
TREAT=4 ^a					
TIME	2	1.3669000	0.6834500	0.28	*
REP	1	0.47040	0.47040	0.20	0.6670
Error	11	26.47180	2.40653		
Corrected Total	23	910.86958			

FATTY ACID=OLEIC ISOMER (18:1I)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	0.1649543	0.0549848	1.68	0.2278
TIME	2	0.2477710	0.1238855	3.79	0.0559
TREAT*TIME	6	0.0958307	0.0159718	0.49	0.8038
TREAT=1 ^a					
TIME	2	0.1971660	0.0985830	3.02	0.0928
TREAT=2 ^a					
TIME	2	0.0316729	0.0158364	0.48	*
TREAT=3 ^a					
TIME	2	0.0402001	0.0201001	0.62	*
TREAT=4 ^a					
TIME	2	0.0745628	0.0372814	1.14	*
REP	1	0.0326101	0.0326101	1.00	0.3392
Error	11	0.3593057	0.0326642		
Corrected Total	23	0.9004718			

*=P>0.25.

^aTreat=1 is the control treatment; Treat=2 is formaldehyde treatment; Treat=3 is acetaldehyde treatment and Treat=4 is diacetyl treatment.

A-5. Analyses of variance for linoleic isomer (18:2I) and linoleic (18:2n6) acids in ruminal digests of treated emulsions containing defatted soybean flour and sunflower oil.

FATTY ACID=LINOLEIC ISOMER (18:2I)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr >F
TREAT	3	1.6185742	0.5395247	1.71	0.2232
TIME	2	0.4881456	0.2440728	0.77	0.4857
TREAT*TIME	6	1.2157230	0.2026205	0.64	0.6969
TREAT=1 ^a					
TIME	2	1.5684335	0.7842168	2.48	0.1457
TREAT=2 ^a					
TIME	2	0	0	.	.
TREAT=3 ^a					
TIME	2	0.0149016	0.0074508	0.02	*
TREAT=4 ^a					
TIME	2	0.1205334	0.0602667	0.19	*
REP	1	0.7436311	0.7436311	2.35	0.1534
Error	11	3.4787647	0.3162513		
Corrected Total	23	7.5448385			

FATTY ACID=OCTADECADIENOIC OR LINOLEIC (18:2n6)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	1448.1650	482.7217	93.95	0.0001
TIME	2	2530.6514	1265.3257	246.27	0.0001
TREAT*TIME	6	834.2738	139.0456	27.06	0.0001
TREAT=1 ^a					
TIME	2	2288.6474	1144.3237	222.71	0.0001
TREAT=2 ^a					
TIME	2	671.83901	335.91951	65.38	0.0001
TREAT=3 ^a					
TIME	2	96.852996	48.426498	29.93	0.0001
TREAT=4 ^a					
TIME	2	307.58581	153.79291	29.90	0.0001
REP	1	14.2673	14.2673	2.78	0.1238
Error	11	56.5179	5.1380		
Corrected Total	23	4883.8754			

*=p>0.25.

^aTreat=1 is the control treatment; Treat=2 is formaldehyde treatment; Treat=3 is acetaldehyde treatment and Treat=4 is diacetyl treatment.

A-6. Analyses of variance for eicosanoic (20:0) and linolenic (18:3n3) acids in ruminal digests of treated emulsions containing defatted soybean flour and sunflower oil.

FATTY ACID=EISOCANOIC OR ARACHIDIC (20:0)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	2.9414909	0.9804970	10.82	0.0013
TIME	2	2.3154687	1.1577343	12.77	0.0014
TREAT*TIME	6	4.8531854	0.8088642	8.93	0.0011
TREAT=1 ^a					
TIME	2	0.1825241	0.0912620	1.01	*
TREAT=2 ^a					
TIME	2	2.0905772	1.0452886	11.54	0.0010
TREAT=3 ^a					
TIME	2	0.3288565	0.1644282	1.81	0.2230
TREAT=4 ^a					
TIME	2	4.5666964	2.2833482	25.20	0.0001
REP	1	0.0606276	0.0606276	0.67	0.4308
Error	11	0.996889	0.090626		
Corrected Total	23	11.167661			

FATTY ACID=octadecatrienoic or linolenic (18:3n3)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	2.487656	0.829219	2.32	0.1317
TIME	2	14.577316	7.288658	20.39	0.0002
TREAT*TIME	6	5.604423	0.934070	2.61	0.0796
TREAT=2 ^a					
TIME	2	1.7688734	0.884437	2.47	0.1457
TREAT=2 ^a					
TIME	2	14.952185	7.476092	20.92	0.0001
TREAT=3 ^a					
TIME	2	0.6276295	0.3138148	0.88	*
TREAT=4 ^a					
TIME	2	2.8330511	1.4165255	3.96	0.0500
REP	1	0.019119	0.019119	0.05	0.8213
Error	11	3.931916	0.357447		
Corrected Total	23	26.620430			

*=p>0.25.

^aTreat=1 is the control treatment; Treat=2 is formaldehyde treatment; Treat=3 is acetaldehyde treatment and Treat=4 is diacetyl treatment.

A-7. Analyses of variance for eicosenoic (20:1) acids and saturated fatty acid type in ruminal digests of treated emulsions containing defatted soybean flour and sunflower oil.

FATTY ACID=11-EICOSENOIC OR GADOLEIC (20:1n11)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	2.7733394	0.9244465	38.86	0.0001
TIME	2	2.3322614	1.1661307	49.02	0.0001
TREAT*TIME	6	3.0210332	0.5035055	21.17	0.0001
TREAT=1 ^a					
TIME	2	3.6080538	1.8040269	75.80	0.0001
TREAT=2 ^a					
TIME	2	0.0050764	0.0025382	0.10	*
TREAT=3 ^a					
TIME	2	1.3821940	0.6910970	29.04	0.0001
TREAT=4 ^a					
TIME	2	0.3579703	0.1789851	7.52	0.0100
REP	1	0.0074619	0.0074619	0.31	0.5867
Error	11	0.2616777	0.0237889		
Corrected Total	23	8.3957735			

FATTY ACID TYPE = SATURATED

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	214.6026	71.5342	24.73	0.0001
TIME	2	1515.5209	757.7604	262.01	0.0001
TREAT*TIME	6	213.3750	35.5625	12.30	0.0003
TREAT=1 ^a					
TIME	2	703.50893	351.75447	121.63	0.0001
TREAT=2 ^a					
TIME	2	660.47963	330.23982	114.19	0.0001
TREAT=3 ^a					
TIME	2	121.05270	60.52635	20.93	0.0010
TREAT=4 ^a					
TIME	2	243.85463	121.92732	96.58	0.0019
REP	1	12.0842	12.0842	4.18	0.0656
Error	11	31.8134	2.8921		
Corrected Total	23	1987.3962			

*=p>0.25.

^aTreat=1 is the control treatment; Treat=2 is formaldehyde treatment; Treat=3 is acetaldehyde treatment and Treat=4 is diacetyl treatment.

A-8. Analyses of variance for monounsaturated and polyunsaturated fatty acid type in ruminal digests of treated emulsions containing defatted soybean flour and sunflower oil.

FATTY ACID TYPE = MONOUNSATURATED

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	591.94605	197.31535	73.88	0.0001
TIME	2	121.86730	60.93365	22.81	0.0001
TREAT*TIME	6	339.75317	56.62553	21.20	0.0001
TREAT=1 ^a					
TIME	2	439.58170	219.79085	82.29	0.0001
TREAT=2 ^a					
TIME	2	10.670400	5.335200	2.00	0.2008
TREAT=3 ^a					
TIME	2	10.587433	5.293717	1.98	0.2031
TREAT=4 ^a					
TIME	2	0.7809333	0.3904667	0.15	*
REP	1	0.17510	0.17510	0.07	0.8026
Error	11	29.3798	2.6709		
Corrected Total	23	1083.1215			

FATTY ACID TYPE = POLYUNSATURATED

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	1365.3881	455.1294	92.96	0.0001
TIME	2	2160.8477	1080.4238	220.67	0.0001
TREAT*TIME	6	791.5183	131.9197	26.94	0.0001
TREAT=1 ^a					
TIME	2	2080.3204	1040.1602	212.46	0.0001
TREAT=2 ^a					
TIME	2	531.02583	265.5129	54.23	0.0001
TREAT=3 ^a					
TIME	2	84.16123	42.0806	8.59	0.0017
TREAT=4 ^a					
TIME	2	256.8585	128.4292	26.23	0.0001
REP	1	9.3500	9.3500	1.91	0.1944
Error	11	53.8561	4.8960		
Corrected Total	23	4380.9602			

*=p>0.25.

^aTreat=1 is the control treatment; Treat=2 is formaldehyde treatment; Treat=3 is acetaldehyde treatment and Treat=4 is diacetyl treatment.

APPENDIX B

APPENDIX B. Analyses of variances for individual and fatty acids type in ruminal digests of untreated or treated emulsions containing ground full-fat soybean seeds.

B-1. Analyses of variance for tridecanoic (13:0) and myristic (14:0) acids in ruminal digests of untreated or treated emulsions containing ground full-fat soybean

FATTY ACID=TRIDECANOIC (13:0)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F-Value	Pr > F
TREAT	3	5.0696734	1.6898911	8.03	0.0041
TIME	2	3.4581940	1.7290970	8.21	0.0066
TREAT*TIME	6	3.1496099	0.5249350	2.49	0.0898
TREAT=1 ^a					
TIME	2	5.0526449	2.5263224	12.00	0.0010
TREAT=2 ^a					
TIME	2	2	0	0	.
TREAT=3 ^a					
TIME	2	0.5793695	0.2896848	1.37	*
TREAT=4 ^a					
TIME	2	1.2512137	0.6256068	2.97	0.0950
REP	1	0.3730317	0.3730317	1.77	0.2100
Error	11	2.315475	0.210498		
Corrected Total	23	14.739998			

FATTY ACID=TETRADECANOIC OR MYRISTIC (14:0)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	1.4156590	0.4718863	3.85	0.0417
TIME	2	0.7644247	0.3822124	3.12	0.0846
TREAT*TIME	6	0.7841439	0.1306907	1.07	0.4372
TREAT=1 ^a					
TIME	2	0.4200237	0.2100118	1.71	0.2347
TREAT=2 ^a					
TIME	2	0.0447174	0.0223587	0.18	*
TREAT=3 ^a					
TIME	2	0.0316366	0.0158183	0.13	*
TREAT=4 ^a					
TIME	2	1.0521910	0.5260955	4.29	0.0439
REP	1	0.0083811	0.0083811	0.07	0.7986
Error	11	1.3485947	0.1225995		
Corrected Total	23	4.3212034			

*=p>0.25.

^aTreat=1 is the control treatment; Treat=2 is formaldehyde treatment; Treat=3 is acetaldehyde treatment and Treat=4 is diacetyl treatment.

B-2. Analyses of variance for palmitic (16:0) and palmitoleic (16:1n9) acids in ruminal digests of untreated or treated emulsions containing ground full-fat soybean

FATTY ACID=HEXADECANOIC OR PALMITIC (16:0)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	46.968896	15.656299	60.26	0.0001
TIME	2	4.514336	2.257168	8.69	0.0055
TREAT*TIME	6	14.701684	2.450281	9.43	0.0008
TREAT=1 ^a					
TIME	2	13.680326	6.840163	26.33	0.0001
TREAT=2 ^a					
TIME	2	2.7267300	1.3633650	5.48	0.0233
TREAT=3 ^a					
TIME	2	0.2368294	0.1184147	0.46	*
TREAT=4 ^a					
TIME	2	2.5721351	1.2860676	3.85	0.0558
REP	1	2.862985	2.862985	11.02	0.0068
Error	11	2.857813	0.259801		
Corrected Total	23	71.905714			

FATTY ACID=9-HEXADECENOIC OR PALMITOLEIC (16:1n9)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	0.0297794	0.0099265	0.58	0.6393
TIME	2	0.0292667	0.0146334	0.86	0.4508
TREAT*TIME	6	0.0548494	0.0091416	0.54	0.7711
REP	1	0.0028561	0.0028561	0.17	0.6903
TREAT=1 ^a					
TIME	2	0.0309470	0.0154735	0.91	*
TREAT=2 ^a					
TIME	2	0.0234277	0.0117138	0.67	*
TREAT=3 ^a					
TIME	2	0.0152156	0.0076078	0.45	*
TREAT=4 ^a					
TIME	2	0.0145259	0.0072629	0.45	*
REP	1	0.0028561	0.0028561	0.17	0.6903
Error	11	0.1877456	0.0170678		
Corrected Total	23	0.3044974			

*=p>0.25.

^aTreat=1 is the control treatment; Treat=2 is formaldehyde treatment; Treat=3 is acetaldehyde treatment and Treat=4 is diacetyl treatment.

B-3. Analyses of variance for margaric (17:0) and stearic (18:0) acids in ruminal digests of untreated or treated emulsions containing ground full-fat soybean

FATTY ACID=HEPTADECANOIC OR MARGARIC (17:0)

General Linear Models Procedure
Dependent Variable: PERCENT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	0.0127403	0.0042468	0.42	0.7440
TIME	2	0.0100936	0.0050468	0.50	0.6219
TREAT*TIME	6	0.0533498	0.0088916	0.87	0.5435
TREAT=1 ^a					
TIME	2	0.0462612	0.0231306	2.27	0.1691
TREAT=2 ^a					
TIME	2	0.0094453	0.0047227	0.46	*
TREAT=3 ^a					
TIME	2	0.0076951	0.0038475	0.00	*
TREAT=4 ^a					
TIME	2	0.0000418	0.0000209	0.06	*
Rep	1	0.0033116	0.0033116	0.33	0.5798
Error	11	0.1119000	0.0101727		
Corrected Total	23	0.1913954			

FATTY ACID=OCTADECANOIC OR STEARIC (18:0)

General Linear Models Procedure
Dependent Variable: PERCENT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	104.41975	34.80658	2.07	0.1621
TIME	2	48.55645	24.27822	1.45	0.2771
TREAT*TIME	6	100.84511	16.80752	1.00	0.4711
TREAT=1 ^a					
TIME	2	43.271536	21.635768	1.28	*
TREAT=2 ^a					
TIME	2	9.790325	4.895162	0.29	*
TREAT=3 ^a					
TIME	2	39.913304	19.956652	1.19	*
TREAT=4 ^a					
TIME	2	56.426396	28.213198	1.68	0.2383
REP	1	2.42599	2.42599	0.14	0.7111
Error	11	184.73226	16.79384		
Corrected Total	23	440.97955			

*=p>0.25.

^aTreat=1 is the control treatment; Treat=2 is formaldehyde treatment; Treat=3 is acetaldehyde treatment and Treat=4 is diacetyl treatment.

B-4. Analyses of variance for cis plus trans 9-octadecenoic (18:1c+t) and oleic acid isomer (18:1I) acids in ruminal digests of untreated or treated emulsions containing ground full-fat soybean.

FATTY ACID = CIS PLUS TRANS-9-OCTADECENOIC (18:1c+t)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	103.17043	34.39014	4.01	0.0374
TIME	2	74.58831	37.29415	4.35	0.0406
TREAT*TIME	6	245.76539	40.96090	4.77	0.0124
TREAT=1 ^a					
TIME	2	201.21203	100.60602	11.72	0.0010
TREAT=2 ^a					
TIME	2	39.755200	19.877600	2.32	0.1632
TREAT=3 ^a					
TIME	2	4.0354333	2.0177167	0.26	*
TREAT=4 ^a					
TIME	2	75.351033	37.675517	4.39	0.0420
REP	1	21.85042	21.85042	2.55	0.1389
Error	11	94.39178	8.58107		
Corrected Total	23	539.76633			

FATTY ACID=OLEIC ISOMER (18:1I)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	12.203712	4.067904	4.85	0.0218
TIME	2	5.454265	2.727132	3.25	0.0777
TREAT*TIME	6	11.887270	1.981212	2.36	0.1029
TREAT=1 ^a					
TIME	2	16.968160	8.484080	10.11	0.0010
TREAT=2 ^a					
TIME	2	0.0427343	0.021367	0.03	*
TREAT=3 ^a					
TIME	2	0.0574951	0.028747	0.03	*
TREAT=4 ^a					
TIME	2	0.2731452	0.136572	0.16	*
REP	1	0.187567	0.187567	0.22	0.6456
Error	11	9.227298	0.838845		
Corrected Total	23	38.960110			

*=p>0.25.

^aTreat=1 is the control treatment; Treat=2 is formaldehyde treatment; Treat=3 is acetaldehyde treatment and Treat=4 is diacetyl treatment.

B-5. Analyses of variance for t,t-octadecadienoic (18:2tt) acids and linoleic (18:2n6) acids in ruminal digests of untreated or treated emulsions containing ground full-fat soybean

FATTY ACID=OCTADECADIENOIC ISOMER (18:2tt)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	2.0466981	0.6822327	1.18	0.3612
TIME	2	1.0991472	0.5495736	0.95	0.4156
TREAT*TIME	6	3.2046844	0.5341141	0.93	0.5133
TREAT=1 ^a					
TIME	2	4.3016615	2.1508307	3.73	0.0612
TREAT=2 ^a					
TIME	2	0	0	.	.
TREAT=3 ^a					
TIME	2	0.0021701	0.0010850	0.00	
TREAT=4 ^a					
TIME	2	0	0		
REP	1	0.7552812	0.7552812	1.31	0.2770
Error	11	6.3505296	0.5773209		
Corrected Total	23	13.4563405			

FATTY ACID=9,12-OCTADECADIENOIC OR LINOLEIC (18:2n6)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	845.18912	281.72971	49.94	0.0001
TIME	2	348.95334	174.47667	30.93	0.0001
TREAT*TIME	6	317.57350	52.92892	9.38	0.0009
TREAT=1 ^a					
TIME	2	400.60537	200.30269	35.50	0.0001
TREAT=2 ^a					
TIME	2	30.926318	15.463159	2.74	0.1140
TREAT=3 ^a					
TIME	2	31.518714	15.759357	2.79	0.1082
TREAT=4 ^a					
TIME	2	203.47643	101.73821	18.03	0.0001
REP	1	1.92650	1.92650	0.34	0.5708
Error	11	62.0579	5.6416		
Corrected Total	23	1575.7003			

*=p>0.25.

*Treat=1 is the control treatment; Treat=2 is formaldehyde treatment; Treat=3 is acetaldehyde treatment and Treat=4 is diacetyl treatment.

B-6. Analyses of variance for arachidic (20:0) and linolenic (18:3n3) acids in ruminal digests of untreated or treated emulsions containing ground full-fat soybean

FATTY ACID=EICOSANOIC OR ARACHIDIC (20:0)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	0.2414517	0.0804839	2.65	0.1010
TIME	2	0.1090959	0.0545479	1.79	0.2116
TREAT*TIME	6	0.0978780	0.0163130	0.54	0.7703
TREAT=1 ^a					
TIME	2	0.1135548	0.0567774	1.87	0.2160
TREAT=2 ^a					
TIME	2	0.0109714	0.0054857	0.18	*
TREAT=3 ^a					
TIME	2	0.0790159	0.0395080	1.30	*
TREAT=4 ^a					
TIME	2	0.0034317	0.0017159	0.06	*
REP	1	0.0211955	0.0211955	0.70	0.4215
Error	11	0.3343871	0.0303988		
Corrected Total	23	0.8040082			

FATTY ACID=OCTADECATRIENOIC OR LINOLENIC (18:3n3)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	19.216705	6.405568	30.53	0.0001
TIME	2	17.108470	8.554235	40.77	0.0001
TREAT*TIME	6	12.864525	2.144088	10.22	0.0006
TREAT=1 ^a					
TIME	2	17.264774	8.632387	41.14	0.0001
TREAT=2 ^a					
TIME	2	1.2495953	0.6247977	2.98	0.0950
TREAT=3 ^a					
TIME	2	1.3999682	0.6999841	3.34	0.0785
TREAT=4 ^a					
TIME	2	10.058658	5.029329	23.97	0.0001
REP	1	0.102576	0.102576	0.49	0.4990
Error	11	2.308073	0.209825		
Corrected Total	23	51.600350			

*=p>0.25.

^aTreat=1 is the control treatment; Treat=2 is formaldehyde treatment; Treat=3 is acetaldehyde treatment and Treat=4 is diacetyl treatment.

B-7. Analyses of variance for eicosenoic (20:1n11) acid and saturated fatty acid type in ruminal digests of untreated or treated emulsions containing ground full-fat soybean

FATTY ACID=EICOSENOIC OR GADOLEIC (20:1n11)

General Linear Models Procedure
Dependent Variable: PERCENT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	1.0740481	0.3580160	11.71	0.0010
TIME	2	1.0060242	0.5030121	16.45	0.0005
TREAT*TIME	6	1.5409506	0.2568251	8.40	0.0014
TREAT=1 ^a					
TIME	2	0.3386692	0.1693346	5.54	0.0228
TREAT=2 ^a					
TIME	2	0.0635540	0.0317770	1.04	*
TREAT=3 ^a					
TIME	2	0.0097398	0.0048699	0.16	*
TREAT=4 ^a					
TIME	2	2.1350118	1.0675059	34.91	0.0001
REP	1	0.0040500	0.0040500	0.13	0.7228
Error	11	0.3364006	0.0305819		
Corrected Total	23	3.9614735			

FATTY ACID TYPE = SATURATED

General Linear Models Procedure
Dependent Variable: PERCENT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	337.85263	112.61754	8.18	0.0038
TIME	2	97.14981	48.57490	3.53	0.0656
TREAT*TIME	6	82.63859	13.77310	1.00	0.4716
TREAT=1 ^a					
TIME	2	60.639100	30.319550	2.20	0.1773
TREAT=2 ^a					
TIME	2	7.384433	3.692217	0.27	*
TREAT=3 ^a					
TIME	2	62.751233	31.375617	2.31	0.1644
TREAT=4 ^a					
TIME	2	49.013633	24.506817	1.77	0.2277
REP	1	7.04167	7.04167	0.51	0.4895
Error	11	151.52303	13.77482		
Corrected Total	23	676.20573			

*=p>0.25.

^aTreat=1 is the control treatment; Treat=2 is formaldehyde treatment; Treat=3 is acetaldehyde treatment and Treat=4 is diacetyl treatment.

B-8. Analyses of variance for monounsaturated fatty acid and polyunsaturated fatty acid type in ruminal digests of untreated or treated emulsions containing ground full-fat soybean

FATTY ACID TYPE = MONOUNSATURATED

General Linear Models Procedure
Dependent Variable: PERCENT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	285.59755	95.19918	6.61	0.0082
TIME	2	203.60691	101.80345	7.06	0.0106
TREAT*TIME	6	451.91929	75.31988	5.23	0.0089
TREAT=1 ^a					
TIME	2	487.18343	243.59172	16.90	0.0010
TREAT=2 ^a					
TIME	2	43.334033	21.667017	1.50	*
TREAT=3 ^a					
TIME	2	2.6656333	1.332816	0.09	*
TREAT=4 ^a					
TIME	2	122.34310	61.17155	4.24	0.0449
REP	1	31.99350	31.99350	2.22	0.1643
Error	11	158.51665	14.41060		
Corrected Total	23	1131.63390			

FATTY ACID TYPE = POLYUNSATURATED

General Linear Models Procedure
Dependent Variable: PERCENT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	1012.8902	337.6301	33.59	0.0001
TIME	2	472.7789	236.3895	23.52	0.0001
TREAT*TIME	6	403.6433	67.2739	6.69	0.0035
TREAT=1 ^a					
TIME	2	482.6448	241.322	24.01	0.0001
TREAT=2 ^a					
TIME	2	44.1156	22.0578	2.19	0.1785
TREAT=3 ^a					
TIME	2	45.5192	22.7596	2.26	0.1738
TREAT=4 ^a					
TIME	2	304.1425	152.0712	15.13	0.0010
REP	1	6.6571	6.6571	0.66	0.4330
Error	11	110.5529	10.0503		
Corrected Total	23	2006.5223			

*=p>0.25.

^aTreat=1 is the control treatment; Treat=2 is formaldehyde treatment; Treat=3 is acetaldehyde treatment and Treat=4 is diacetyl treatment.

APPENDIX C

APPENDIX C. Analyses of variances for individual and type of fatty acids type in ruminal digests of untreated or treated emulsions containing ground full-fat sunflower seeds

C-1. Analyses of variance for tridecanoic (13:0) and myristic (14:0) acids in ruminal digests of untreated or treated emulsions containing ground full-fat sunflower seeds

FATTY ACID=TRIDECANOIC (13:0)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	0.2739166	0.0913055	60.18	0.0001
TIME	2	0.3493114	0.1746557	115.12	0.0001
TREAT*TIME	6	0.2736022	0.0456004	30.06	0.0001
TREAT=1 ^a					
TIME	2	0.0711240	0.0355620	23.44	0.0001
TREAT=2 ^a					
TIME	2	0	0	.	.
TREAT=3 ^a					
TIME	2	0.3112944	0.1556472	102.59	0.0001
TREAT=4 ^a					
TIME	2	0.2404951	0.1202476	79.26	0.0001
REP	1	0.0010251	0.0010251	0.68	0.4285
Error	11	0.0166882	0.0015171		
Corrected Total	23	0.9145435			

FATTY ACID=TETRADECANOIC OR MYRISTIC (14:0)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	0.5748449	0.1916150	22.39	0.0001
TIME	2	0.6374600	0.3187300	37.25	0.0001
TREAT*TIME	6	0.1938875	0.0323146	3.78	0.0273
TREAT=1 ^a					
TIME	2	0.4349813	0.2174907	25.42	0.0001
TREAT=2 ^a					
TIME	2	0.0124581	0.0062290	0.73	*
TREAT=3 ^a					
TIME	2	0.2034861	0.1017431	11.89	0.0010
TREAT=4 ^a					
TIME	2	0.1804220	0.0902110	10.54	0.0010
REP	1	0.0264502	0.0264502	3.09	0.1065
Error	11	0.0941328	0.0085575		
Corrected Total	23	1.5267754			

*=P>0.25.

^aTreat=1 is the control treatment; Treat=2 is formaldehyde treatment; Treat=3 is acetaldehyde treatment and Treat=4 is diacetyl treatment.

C-2. Analyses of variance for palmitic (16:0) and palmitoleic (16:1n9) acids in ruminal digests of untreated or treated emulsions containing ground full-fat sunflower seeds

FATTY ACID=HEXADECANOIC OR PALMITIC (16:0)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	3.0759488	1.0253163	5.64	0.0137
TIME	2	1.2669422	0.6334711	3.48	0.0673
TREAT*TIME	6	0.9208804	0.1534801	0.84	0.5619
TREAT=1 ^a					
TIME	2	1.0167782	0.5083891	2.79	0.1082
TREAT=2 ^a					
TIME	2	0.3022423	0.1511212	0.83	*
TREAT=3 ^a					
TIME	2	0.0694149	0.0347075	0.19	*
TREAT=4 ^a					
TIME	2	0.7993871	0.3996935	2.20	0.1773
REP	1	0.0110493	0.0110493	0.06	0.8098
Error	11	2.0001932	0.1818357		
Corrected Total	23	7.2750139			

FATTY ACID=PALMITOLEIC (16:1n9)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	0.0559941	0.0186647	3.18	0.0671
TIME	2	0.0038948	0.0019474	0.33	0.7245
TREAT*TIME	6	0.0100546	0.0016758	0.29	0.9318
TREAT=1 ^a					
TIME	2	0.0116276	0.0058138	0.99	*
TREAT=2 ^a					
TIME	2	0.0003829	0.0001914	0.03	*
TREAT=3 ^a					
TIME	2	0.0003054	0.0001527	0.026	*
TREAT=4 ^a					
TIME	2	0.0016336	0.0008168	0.14	*
REP	1	0.0002939	0.0002939	0.05	0.8270
Error	11	0.0645451	0.0058677		
Corrected Total	23	0.1347825			

*=P>0.25.

^aTreat=1 is the control treatment; Treat=2 is formaldehyde treatment; Treat=3 is acetaldehyde treatment and Treat=4 is diacetyl treatment.

C-3. Analyses of variance for margaric (17:0) stearic (18:0) acids in ruminal digests of untreated or treated emulsions containing ground full-fat sunflower seeds

FATTY ACID=HEPTADECANOIC OR MARGARIC (17:0)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	0.0078117	0.0026039	1.34	0.3126
TIME	2	0.0142459	0.0071230	3.66	0.0606
TREAT*TIME	6	0.0036185	0.0006031	0.31	0.9190
TREAT=1 ^a					
TIME	2	0.0090225	0.0045112	2.31	0.1644
TREAT=2 ^a					
TIME	2	0.0004073	0.0002036	0.10	*
TREAT=3 ^a					
TIME	2	0.0052134	0.0026067	1.34	*
TREAT=4 ^a					
TIME	2	0.0032213	0.0016107	0.83	*
REP	1	0.0001952	0.0001952	0.10	0.7576
Error	11	0.0214355	0.0019487		
Corrected Total	23	0.0473068			

FATTY ACID=OCTADECANOIC OR STEARIC (18:0)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	459.39026	153.13009	10.50	0.0015
TIME	2	133.26639	66.63320	4.57	0.0360
TREAT*TIME	6	411.70036	68.61673	4.70	0.0131
TREAT=1 ^a					
TIME	2	3.3237923	1.6618961	0.11	*
TREAT=2 ^a					
TIME	2	20.257759	10.128879	0.69	*
TREAT=3 ^a					
TIME	2	12.376471	6.188236	0.42	*
TREAT=4 ^a					
TIME	2	509.00873	254.50437	17.44	0.0010
REP	1	30.66598	30.66598	2.10	0.1750
Error	11	160.4860	14.5896		
Corrected Total	23	1195.5090			

*=P>0.25.

^aTreat=1 is the control treatment; Treat=2 is formaldehyde treatment; Treat=3 is acetaldehyde treatment and Treat=4 is diacetyl treatment.

C-4. Analyses of variance for cis plus trans 9-octadecenoic acid (18:1c+t) and oleic acid isomer (18:1I) in ruminal digests of untreated treated emulsions containing ground full-fat sunflower seeds

FATTY ACID = CIS PLUS TRANS-9-OCTADECENOIC (18:1c+t)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	514.85301	171.61767	11.05	0.0012
TIME	2	668.66811	334.33405	21.52	0.0002
TREAT*TIME	6	988.41722	164.73620	10.60	0.0005
TREAT=1 ^a					
TIME	2	1411.4284	705.7142	45.43	0.0001
TREAT=2 ^a					
TIME	2	40.993633	20.496817	1.32	*
TREAT=3 ^a					
TIME	2	67.652433	33.826217	2.18	0.1797
TREAT=4 ^a					
TIME	2	137.01083	68.50542	4.41	0.0416
REP	1	2.80850	2.80850	0.18	0.6789
Error	11	170.8855	15.5350		
Corrected Total	23	2345.6324			

*=P>0.25.

^aTreat=1 is the control treatment; Treat=2 is formaldehyde treatment; Treat=3 is acetaldehyde treatment and Treat=4 is diacetyl treatment.

C-5. Analyses of variance for linoleic (18:2n6) and arachidic (20:0) acids in ruminal digests of untreated or treated emulsions containing ground full-fat sunflower seeds

FATTY ACID=OCTADECADIENOIC OR LINOLEIC (18:2n6)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	1648.9180	549.6393	46.00	0.0001
TIME	2	1815.0270	907.5135	75.95	0.0001
TREAT*TIME	6	1294.3541	215.7257	18.05	0.0001
TREAT=1 ^a					
TIME	2	1703.1989	851.5995	71.27	0.0286
TREAT=2 ^a					
TIME	2	4.9785207	2.4892603	0.21	*
TREAT=3 ^a					
TIME	2	58.475728	29.237864	2.45	0.1480
TREAT=4 ^a					
TIME	2	1342.7279	671.3640	56.19	0.0001
REP	1	13.3147	13.3147	1.11	0.3138
Error	11	131.4344	11.9486		
Corrected Total	23	4903.0482			

FATTY ACID=EICOSANOIC OR ARACHIDIC (20:0)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	0.0524007	0.0174669	10.15	0.0017
TIME	2	0.0185528	0.0092764	5.39	0.0233
TREAT*TIME	6	0.0147742	0.0024624	1.43	0.2867
TREAT=1 ^a					
TIME	2	0.0220897	0.0110448	6.42	0.0160
TREAT=2 ^a					
TIME	2	0.0022114	0.0011057	0.64	*
TREAT=3 ^a					
TIME	2	0.0034745	0.0017373	0.64	*
TREAT=4 ^a					
TIME	2	0.0055514	0.0027757	1.61	0.2464
REP	1	0.0001225	0.0001225	0.07	0.7945
Error	11	0.0189292	0.0017208		
Corrected Total	23	0.1047793			

*=P>0.25.

^aTreat=1 is the control treatment; Treat=2 is formaldehyde treatment; Treat=3 is acetaldehyde treatment and Treat=4 is diacetyl treatment.

C-6. Analyses of variance for linolenic (18:3n3) and eicosenoic (20:1n11) acids in ruminal digests of untreated or treated emulsions containing ground full-fat sunflower seeds

FATTY ACID=OCTADECATRIENOIC OR LINOLENIC (18:3n3)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	0.0803390	0.0267797	3.38	0.0581
TIME	2	0.1134739	0.0567370	7.16	0.0102
TREAT*TIME	6	0.0314010	0.0052335	0.66	0.6833
TREAT=1 ^a					
TIME	2	0.0415578	0.0207789	2.62	0.1281
TREAT=2 ^a					
TIME	2	0.0612511	0.030625	3.86	0.0553
TREAT=3 ^a					
TIME	2	0.0116501	0.0058251	0.73	*
TREAT=4 ^a					
TIME	2	0.0304159	0.0152080	1.92	0.2101
REP	1	0.0081056	0.0081056	1.02	0.3336
Error	11	0.0871987	0.0079272		
Corrected Total	23	0.3205182			

FATTY ACID=EICOSENOIC (20:1n11)

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	12.343882	4.114627	46.62	0.0001
TIME	2	10.501586	5.250793	59.49	0.0001
TREAT*TIME	6	21.113397	3.518900	39.87	0.0001
TREAT=1 ^a					
TIME	2	1.7697837	0.8848919	10.01	0.0010
TREAT=2 ^a					
TIME	2	0.0162337	0.0081169	0.09	*
TREAT=3 ^a					
TIME	2	0.1763832	0.0881916	1.00	*
TREAT=4 ^a					
TIME	2	29.652583	14.826292	167.99	0.0001
REP	1	0.024695	0.024695	0.28	0.6073
Error	11	0.970838	0.088258		
Corrected Total	23	44.954398			

*=P>0.25.

^aTreat=1 is the control treatment; Treat=2 is formaldehyde treatment; Treat=3 is acetaldehyde treatment and Treat=4 is diacetyl treatment.

C-7. Analyses of variance for saturated and monounsaturated fatty acid type in ruminal digests of untreated or treated emulsions containing ground full- fat sunflower seeds

FATTY ACID TYPE = SATURATED

General Linear Models Procedure
Dependent Variable: PERCENT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	552.19575	184.06525	14.19	0.0004
TIME	2	197.50636	98.75318	7.61	0.0084
TREAT*TIME	6	435.56557	72.59426	5.59	0.0070
TREAT=1 ^a					
TIME	2	15.334633	7.667317	0.59	*
TREAT=2 ^a					
TIME	2	16.369033	8.184517	0.63	*
TREAT=3 ^a					
TIME	2	9.4422333	4.7211167	0.36	*
TREAT=4 ^a					
TIME	2	591.92603	295.96302	22.80	0.0001
REP	1	27.47760	27.47760	2.12	0.1735
Error	11	142.7295	12.9754		
Corrected Total	23	1355.4748			

FATTY ACID TYPE = MONOUNSATURATED

General Linear Models Procedure
Dependent Variable: PERCENT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	614.8224	204.9408	13.16	0.0006
TIME	2	844.6791	422.3395	27.11	0.0001
TREAT*TIME	6	1006.1016	167.6836	10.76	0.0005
TREAT=1 ^a					
TIME	2	1450.2085	725.1043	46.55	0.0001
TREAT=2 ^a					
TIME	2	40.144533	20.072267	1.29	*
TREAT=3 ^a					
TIME	2	74.115900	37.057950	2.38	0.1562
TREAT=4 ^a					
TIME	2	286.31170	143.15585	9.19	0.0047
REP	1	2.2448	2.2448	0.14	0.7115
Error	11	171.3577	15.5780		
Corrected Total	23	2639.2056			

*=p>0.25

^aTreat=1 is the control treatment; Treat=2 is formaldehyde treatment;
Treat=3 is acetaldehyde treatment and Treat=4 is diacetyl treatment.

C-8. Analyses of variance for polyunsaturated fatty acid type in ruminal digests of untreated or treated emulsions containing ground full-fat sunflower seeds

FATTY ACID TYPE = POLYUNSATURATED

General Linear Models Procedure					
Dependent Variable: PERCENT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
TREAT	3	1669.8650	556.6217	46.99	0.0001
TIME	2	1841.3897	920.6949	77.73	0.0001
TREAT*TIME	6	1299.2393	216.5399	18.28	0.0001
TREAT=1 ^a					
TIME	2	1719.2532	859.6266	72.57	0.0001
TREAT=2 ^a					
TIME	2	5.8705333	2.9352667	0.25	*
TREAT=3 ^a					
TIME	2	59.973300	29.986650	2.53	0.1500
TREAT=4 ^a					
TIME	2	1355.5320	677.7660	57.22	0.0001
REP	1	13.9690	13.9690	1.18	0.3001
Error	11	130.2924	11.8448		
Corrected Total	23	4954.7556			

*=P>0.25

^aTreat=1 is the control treatment; Treat=2 is formaldehyde treatment; Treat=3 is acetaldehyde treatment and Treat=4 is diacetyl treatment.

APPENDIX D

APPENDIX D. Analyses of variance for fatty acids in the
blood serum fat of sheep fed protected dietary fat
supplements

D-1. Analyses of variance for fatty acids in the blood serum fat of sheep fed protected dietary fat supplements

Fatty Acid=Octanoic Capric (8:0)

General Linear Models Procedure					
Dependent Variable: PERCENT		Percent of Total Frequency			
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	131.23113	14.58124	0.71	0.6943
PER	3	22.346731	7.44891	0.36	0.7848
AN	3	88.228002	29.409334	1.42	0.3398
TRT	3	13.194584	4.398195	0.21	0.8835
Error	5	103.29221	20.65844		
Corrected Total	14	234.52334			

Fatty Acid=Dodecanoic or Lauric (12:0)

General Linear Models Procedure					
Dependent Variable: PERCENT		Percent of Total Frequency			
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	2.8228345	1.4114172	.	.
PER	0	0	.	.	.
AN	0	0	.	.	.
TRT	0	0	.	.	.
Error	0	.	.	.	.
Corrected Total	2	2.8228345			

Fatty Acid=Tetradecanoic or Myristic (14:0)

General Linear Models Procedure					
Dependent Variable: PERCENT		Percent of Total Frequency			
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	0.6299691	0.0699966	1.97	0.2686
PER	3	0.1601808	0.0533936	1.50	0.3428
AN	3	0.2430401	0.0810134	2.28	0.2217
TRT	3	0.0775628	0.0258543	0.73	0.5872
Error	4	0.1423488	0.0355872		
Corrected Total	13	0.7723178			

Fatty Acid=Pentadecanoic (15:0)

General Linear Models Procedure					
Dependent Variable: PERCENT		Percent of Total Frequency			
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	0.1820491	0.0227561	.	.
PER	3	0.0862020	0.0287340	.	.
AN	2	0.0197489	0.0098744	.	.
TRT	3	0.1191623	0.0397208	.	.
Error	0	.	.	.	.
Corrected Total	8	0.1820491			

D-2. Analyses of variance for fatty acids in the blood serum fat of sheep fed protected dietary fat supplements

Fatty Acid=Hexadecanoic or Palmitic (16:0)

General Linear Models Procedure					
Dependent Variable: PERCENT Percent of Total Frequency					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	48.511739	5.390193	0.63	0.7418
PER	3	21.921520	7.307173	0.86	0.5114
AN	3	20.058686	6.686229	0.79	0.5440
TRT	3	6.531533	2.177178	0.26	0.8547
Error	6	51.047465	8.507911		
Corrected Total	15	99.559203			

Fatty Acid=Hexadecenoic or Palmitoleic (16:1n9)

General Linear Models Procedure					
Dependent Variable: PERCENT Percent of Total Frequency					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	0.1918541	0.0274077	.	.
PER	1	0.0223379	0.0223379	.	.
AN	1	0.0130267	0.0130267	.	.
TRT	1	0.0023203	0.0023203	.	.
Error	0	.	.	.	.
Corrected Total	7	0.1918541			

Fatty Acid=Heptadecanoic or Margaric (17:0)

General Linear Models Procedure					
Dependent Variable: PERCENT Percent of Total Frequency					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	1.5653363	0.2608894	.	.
PER	1	0.5845495	0.5845495	.	.
AN	2	0.8248837	0.4124418	.	.
TRT	2	0.3885764	0.1942882	.	.
Error	0	.	.	.	.
Corrected Total	6	1.5653363			

Fatty Acid=Octadecanoic or Stearic (18:0)

General Linear Models Procedure					
Dependent Variable: PERCENT Percent of Total Frequency					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	34.555380	3.839487	0.64	0.7395
Error	6	36.164129	6.027355		
PER	3	13.525315	4.508438	0.75	0.5619
AN	3	6.651792	2.217264	0.37	0.7793
TRT	3	14.378273	4.792758	0.80	0.5398
Corrected Total	15	70.719509			

D-3. Analyses of variance for fatty acids in the blood serum fat of sheep fed protected dietary fat supplements

Fatty Acid=Octadecenoic or Oleic (18:1n9)

General Linear Models Procedure						
Dependent Variable: PERCENT Percent of Total Frequency						
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	
Model	9	437.34490	48.59388	1.05	0.4933	
PER	3	144.53943	48.17981	1.04	0.4385	
AN	3	121.77527	40.59176	0.88	0.5025	
TRT	3	171.03021	57.01007	1.24	0.3761	
Error	6	276.78272	46.13045			
Corrected Total	15	714.12762				

Fatty Acid=Octadecadienoic or Linoleic (18:2n6)

General Linear Models Procedure						
Dependent Variable: PERCENT Percent of Total Frequency						
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	
Model	9	500.76103	55.64011	2.15	0.1813	
PER	3	115.93516	38.64505	1.50	0.3081	
AN	3	119.17958	39.72653	1.54	0.2986	
TRT	3	265.64629	88.54876	3.43	0.0930	
Error	6	154.98931	25.83155			
Corrected Total	15	655.75034				

Fatty Acid=Octadecatrienoic or Linolenic (18:3n3)

General Linear Models Procedure						
Dependent Variable: PERCENT Percent of Total Frequency						
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	
Model	9	2.4589374	0.2732153	1.56	0.3017	
PER	3	1.6748917	0.5582972	3.20	0.1050	
AN	3	0.5637724	0.1879241	1.08	0.4273	
TRT	3	0.2202734	0.0734245	0.42	0.7452	
Error	6	1.0479096	0.1746516			
Corrected Total	15	3.5068471				

Fatty Acid=Eicosatetraenoic or Arachidonic (20:4n6)

General Linear Models Procedure						
Dependent Variable: PERCENT Percent of Total Frequency						
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	
Model	9	8.7328315	0.9703146	2.12	0.2105	
PER	3	2.2297953	0.7432651	1.63	0.2955	
AN	3	3.4831660	1.1610553	2.54	0.1700	
TRT	3	2.3179965	0.7726655	1.69	0.2831	
Error	5	2.2840703	0.4568141			
Corrected Total	14	11.0169018				

D-4. Analyses of variance for fatty acids in the blood serum fat of sheep fed protected dietary fat supplements

Fatty Acid=Docosapentaenoic (22:5n3)

General Linear Models Procedure					
Dependent Variable: PERCENT Percent of Total Frequency					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	1.6505477	0.1833942	5.29	0.0407
PER	3	0.2727502	0.0909167	2.62	0.1628
AN	3	0.8294695	0.2764898	7.97	0.0237
TRT	3	0.3850189	0.1283396	3.70	0.0965
Error	5	0.1734143	0.0346829		
Corrected Total	14	1.8239620			

Fatty Acid=Docosahexaenoic (22:6n3)

General Linear Models Procedure					
Dependent Variable: PERCENT Percent of Total Frequency					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	0.7996944	0.0888549	1.43	0.4246
PER	3	0.0244073	0.0081358	0.13	0.9356
AN	3	0.6046737	0.2015579	3.24	0.1800
TRT	3	0.2596310	0.0865437	1.39	0.3962
Error	3	0.1865382	0.0621794		
Corrected Total	12	0.9862327			

APPENDIX E

APPENDIX E. Analysis of variance for the fat content of
the milk of sheep fed untreated or treated dietary fat
supplements

E-1. Analysis of variance for the fat content of the milk of lactating sheep fed untreated or treated fat supplements

General Linear Models Procedure					
Dependent Variable: CON					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	921799.03	102422.11	2.55	0.1339
PER	3	578551.67	192850.56	4.79	0.0492
AN	3	186086.98	62028.99	1.54	0.2978
TRT	3	157160.38	52386.79	1.30	0.3571
Error	6	241414.78	40235.80		
Corrected Total	15	1163213.81			

APPENDIX F

APPENDIX F. Analyses of variance for concentrations of fatty acids in the milk fat of sheep fed untreated or treated dietary fat supplements

F-1. Analyses of variance for concentrations of fatty acids (C6-C10) in milk fat of sheep fed untreated or treated dietary fat supplements

Fatty Acid=Hexanoic or Caproic (6:0)

General Linear Models Procedure					
Dependent Variable: PERCENT Percent of Total Frequency					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	5.6657783	0.6295309	1.34	0.3929
PER	3	3.3327224	1.1109075	2.36	0.1884
AN	3	1.3275332	0.4425111	0.94	0.4877
TRT	3	0.2349642	0.0783214	0.17	0.9148
Error	5	2.3568939	0.4713788		
Corrected Total	14	8.0226721			

Fatty Acid=Octanoic or Caprylic (8:0)

General Linear Models Procedure					
Dependent Variable: PERCENT Percent of Total Frequency					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	0.4980747	0.0553416	0.34	0.9290
PER	3	0.1962779	0.0654260	0.40	0.7572
AN	3	0.1391735	0.0463912	0.28	0.8350
TRT	3	0.1626233	0.0542078	0.33	0.8026
Error	6	0.9773013	0.1628835		
Corrected Total	15	1.4753760			

Fatty Acid=Nonanoic (9:0)

General Linear Models Procedure					
Dependent Variable: PERCENT Percent of Total Frequency					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	0.0008403	0.0000934	0.56	0.7801
PER	3	0.0003929	0.0001310	0.79	0.5761
AN	3	0.0001968	0.0000656	0.39	0.7679
TRT	3	0.0000977	0.0000326	0.20	0.8935
Error	3	0.0004999	0.0001666		
Corrected Total	12	0.0013402			

Fatty Acid=Decanoic or Capric (10:0)

General Linear Models Procedure					
Dependent Variable: PERCENT Percent of Total Frequency					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	5.5883254	0.6209250	0.81	0.6261
PER	3	1.4071763	0.4690588	0.61	0.6307
AN	3	1.6600238	0.5533413	0.72	0.5736
TRT	3	2.5211252	0.8403751	1.10	0.4193
Error	6	4.5860209	0.7643368		
Corrected Total	15	10.1743463			

F-2. Analyses of variance for concentrations of fatty acids (C11-C14) in milk fat of sheep fed untreated or untreated dietary fat supplements

Fatty Acid=Undecanoic (11:0)

General Linear Models Procedure					
Dependent Variable: PERCENT Percent of Total Frequency					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	0.0097995	0.0010888	1.32	0.3794
PER	3	0.0078694	0.0026231	3.18	0.1058
AN	3	0.0000402	0.0000134	0.02	0.9969
TRT	3	0.0018899	0.0006300	0.76	0.5541
Error	6	0.0049446	0.0008241		
Corrected Total	15	0.0147441			

Fatty Acid=Dodecanoic or Lauric (12:0)

General Linear Models Procedure					
Dependent Variable: PERCENT Percent of Total Frequency					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	1.9699880	0.2188876	1.34	0.3729
PER	3	0.5115447	0.1705149	1.04	0.4389
AN	3	0.9837347	0.3279116	2.01	0.2147
TRT	3	0.4747086	0.1582362	0.97	0.4669
Error	6	0.9807012	0.1634502		
Corrected Total	15	2.9506892			

Fatty Acid=Tetradecanoic of Myristic (14:0)

General Linear Models Procedure					
Dependent Variable: PERCENT Percent of Total Frequency					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	21.871666	2.430185	3.27	0.0814
PER	3	9.7179897	3.2393299	4.36	0.0594
AN	3	8.9314132	2.9771377	4.01	0.0698
TRT	3	3.2222634	1.0740878	1.45	0.3199
Error	6	4.457049	0.742841		
Corrected Total	15	26.328715			

Fatty Acid=Tetradecenoic or Myristoleic (14:1)

General Linear Models Procedure					
Dependent Variable: PERCENT Percent of Total Frequency					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	0.0141932	0.0015770	0.92	0.5636
PER	3	0.0028448	0.0009483	0.55	0.6649
AN	3	0.0024982	0.0008327	0.49	0.7048
TRT	3	0.0088502	0.0029501	1.72	0.2618
Error	6	0.0102945	0.0017157		
Corrected Total	15	0.0244877			

F-3. Analyses of variance for concentrations of fatty acids (C14-C15) in milk fat of sheep fed untreated and treated dietary fat supplements

Fatty Acid=Methyl-tetradecanoic Isomer (15:0I)

General Linear Models Procedure					
Dependent Variable: PERCENT		Percent of Total Frequency			
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	0.0441669	0.0049074	2.24	0.1688
PER	3	0.0250330	0.0083443	3.81	0.0766
AN	3	0.0103670	0.0034557	1.58	0.2896
TRT	3	0.0087670	0.0029223	1.34	0.3478
Error	6	0.0131238	0.0021873		
Corrected Total	15	0.0572907			

Fatty Acid=Pentadecanoic (15:0)

General Linear Models Procedure					
Dependent Variable: PERCENT		Percent of Total Frequency			
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	0.3740504	0.0415612	1.97	0.2112
PER	3	0.2273402	0.0757801	3.59	0.0856
AN	3	0.1203356	0.0401119	1.90	0.2307
TRT	3	0.0263746	0.0087915	0.42	0.7477
Error	6	0.1266547	0.0211091		
Corrected Total	15	0.5007051			

Fatty Acid=Palmitic Isomer (16:0I)

General Linear Models Procedure					
Dependent Variable: PERCENT		Percent of Total Frequency			
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	0.0366864	0.0040763	9.73	0.0110
PER	3	0.0082883	0.0027628	6.59	0.0345
AN	3	0.0168249	0.0056083	13.38	0.0080
TRT	3	0.0113282	0.0037761	9.01	0.0185
Error	5	0.0020952	0.0004190		
Corrected Total	14	0.0387816			

Fatty Acid=Hexadecanoic or Palmitic (16:0)

General Linear Models Procedure					
Dependent Variable: PERCENT		Percent of Total Frequency			
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	84.918936	9.435437	6.11	0.0302
PER	3	55.662412	18.554137	12.01	0.0101
AN	3	32.054570	10.684857	6.91	0.0314
TRT	3	1.562816	0.520939	0.34	0.8001
Error	5	7.726666	1.545333		
Corrected Total	14	92.645602			

F-4. Analyses of variance for concentrations of fatty acids (C16-C17) in milk fat of sheep fed treated and untreated dietary fat supplements

Fatty Acid=Hexadecenoic (16:1n7)

General Linear Models Procedure						
Dependent Variable: PERCENT Percent of Total Frequency						
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	
Model	9	0.5182561	0.0575840	0.94	0.5590	
PER	3	0.3535517	0.1178506	1.93	0.2430	
AN	3	0.1241405	0.0413802	0.68	0.6022	
TRT	3	0.1145482	0.0381827	0.63	0.6290	
Error	5	0.3053374	0.0610675			
Corrected Total	14	0.8235935				

Fatty Acid=Hexadecenoic or Palmitoleic (16:1n9)

General Linear Models Procedure						
Dependent Variable: PERCENT Percent of Total Frequency						
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	
Model	9	0.4464140	0.0496016	1.33	0.3747	
PER	3	0.0592421	0.0197474	0.53	0.6774	
AN	3	0.0342701	0.0114234	0.31	0.8197	
TRT	3	0.3529018	0.1176339	3.16	0.1069	
Error	6	0.2230650	0.0371775			
Corrected Total	15	0.6694790				

Fatty Acid=Heptadecanoic or Margaric (17:0)

General Linear Models Procedure						
Dependent Variable: PERCENT Percent of Total Frequency						
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	
Model	9	0.1218815	0.0135424	1.14	0.4511	
PER	3	0.1107563	0.0369188	3.12	0.1096	
AN	3	0.0001911	0.0000637	0.01	0.9994	
TRT	3	0.0109342	0.0036447	0.31	0.8194	
Error	6	0.0710424	0.0118404			
Corrected Total	15	0.1929239				

Fatty Acid=Unknown1

General Linear Models Procedure						
Dependent Variable: PERCENT Percent of Total Frequency						
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	
Model	9	0.2869681	0.0318853	1.58	0.2978	
PER	3	0.1400873	0.0466958	2.31	0.1759	
AN	3	0.0660175	0.0220058	1.09	0.4227	
TRT	3	0.0808634	0.0269545	1.33	0.3482	
Error	6	0.1211800	0.0201967			
Corrected Total	15	0.4081481				

F-5. Analyses of variance for concentrations of fatty acids (C17-C19) in milk fat of sheep fed untreated or treated dietary fat supplements

Fatty Acid=Unknown2

General Linear Models Procedure					
Dependent Variable: PERCENT		Percent of Total Frequency			
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	0.0699492	0.0077721	13.38	0.0053
PER	3	0.0123748	0.0041249	7.10	0.0298
AN	3	0.0448534	0.0149511	25.74	0.0018
TRT	3	0.0098316	0.0032772	5.64	0.0463
Error	5	0.0029044	0.0005809		
Corrected Total	14	0.0728536			

Fatty Acid=Octadecanoic or Stearic (18:0)

General Linear Models Procedure					
Dependent Variable: PERCENT		Percent of Total Frequency			
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	71.892966	7.988107	1.89	0.2254
PER	3	29.286871	9.762290	2.31	0.1758
AN	3	22.704103	7.568034	1.79	0.2485
TRT	3	19.901992	6.633997	1.57	0.2913
Error	6	25.327518	4.221253		
Corrected Total	15	97.220484			

Fatty Acid=Octadecenoic or Oleic (18:1n9)

General Linear Models Procedure					
Dependent Variable: PERCENT		Percent of Total Frequency			
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	110.74889	12.30543	1.33	0.3751
PER	3	32.381058	10.793686	1.17	0.3964
AN	3	40.931794	13.643931	1.48	0.3123
TRT	3	37.436035	12.478678	1.35	0.3436
Error	6	55.38573	9.23096		
Corrected Total	15	166.13462			

Fatty Acid=Nonadecanoic (19:0)

General Linear Models Procedure					
Dependent Variable: PERCENT		Percent of Total Frequency			
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	1.6870027	0.1874447	16.66	0.0014
PER	3	0.7193258	0.2397753	21.32	0.0013
AN	3	0.3200780	0.1066927	9.49	0.0108
TRT	3	0.6475989	0.2158663	19.19	0.0018
Error	6	0.0674906	0.0112484		
Corrected Total	15	1.7544933			

F-6. Analyses of variance for concentrations of fatty acids (18-C20) in milk fat of sheep fed untreated or treated dietary fat supplements

Fatty Acid=Octadecadienoic or Linoleic (18:2n6)

General Linear Models Procedure						
Dependent Variable: PERCENT Percent of Total Frequency						
		Sum of	Mean			
Source	DF	Squares	Square	F Value	Pr > F	
Model	9	85.429627	9.492181	12.21	0.0032	
PER	3	17.999966	5.999989	7.72	0.0175	
AN	3	3.379581	1.126527	1.45	0.3190	
TRT	3	64.050080	21.350027	27.47	0.0007	
Error	6	4.662861	0.777144			
Corrected Total	15	90.092488				

Fatty Acid=Unknown3

General Linear Models Procedure						
Dependent Variable: PERCENT Percent of Total Frequency						
		Sum of	Mean			
Source	DF	Squares	Square	F Value	Pr > F	
Model	9	0.0148189	0.0016465	0.95	0.5563	
PER	3	0.0043731	0.0014577	0.84	0.5278	
AN	3	0.0076694	0.0025565	1.47	0.3284	
TRT	3	0.0097164	0.0032388	1.87	0.2530	
Error	5	0.0086826	0.0017365			
Corrected Total	14	0.0235016				

Fatty Acid=Octadecatrienoic or Linolenic (18:3n3)

General Linear Models Procedure						
Dependent Variable: PERCENT Percent of Total Frequency						
		Sum of	Mean			
Source	DF	Squares	Square	F Value	Pr > F	
Model	9	3.2609918	0.3623324	7.06	0.0137	
PER	3	1.4196356	0.4732119	9.22	0.0115	
AN	3	0.1989468	0.0663156	1.29	0.3599	
TRT	3	1.6424094	0.5474698	10.67	0.0081	
Error	6	0.3079684	0.0513281			
Corrected Total	15	3.5689602				

Fatty Acid=Eicosenoic or Gadoleic (20:1n11)

General Linear Models Procedure						
Dependent Variable: PERCENT Percent of Total Frequency						
		Sum of	Mean			
Source	DF	Squares	Square	F Value	Pr > F	
Model	9	0.7556280	0.0839587	1.13	0.4553	
PER	3	0.1349289	0.0449763	0.61	0.6340	
AN	3	0.1229605	0.0409868	0.55	0.6643	
TRT	3	0.4977387	0.1659129	2.24	0.1839	
Error	6	0.4440575	0.0740096			
Corrected Total	15	1.1996855				

F-7. Analyses of variance for concentrations of fatty acids (C20) in milk fat of sheep fed untreated or treated dietary fat supplements

Fatty Acid=Eicosadienoic (20:2n6)

General Linear Models Procedure						
Dependent Variable: PERCENT Percent of Total Frequency						
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	
Model	9	0.0231797	0.0025755	13.60	0.0024	
PER	3	0.0127520	0.0042507	22.44	0.0012	
AN	3	0.0065017	0.0021672	11.44	0.0068	
TRT	3	0.0039260	0.0013087	6.91	0.0226	
Error	6	0.0011365	0.0001894			
Corrected Total	15	0.0243162				

Fatty Acid=Eicosatrienoic (20:3n6)

General Linear Models Procedure						
Dependent Variable: PERCENT Percent of Total Frequency						
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	
Model	9	0.0177046	0.0019672	7.17	0.0662	
PER	3	0.0145106	0.0048369	17.63	0.0208	
AN	3	0.0014460	0.0004820	1.76	0.3275	
TRT	3	0.0023715	0.0007905	2.88	0.2040	
Error	3	0.0008232	0.0002744			
Corrected Total	12	0.0185277				

Fatty Acid=Docosanoic or Behenic (22:0)

General Linear Models Procedure						
Dependent Variable: PERCENT Percent of Total Frequency						
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	
Model	9	0.5884769	0.0653863	22.83	0.0426	
PER	3	0.1979152	0.0659717	23.04	0.0419	
AN	3	0.0038888	0.0012963	0.45	0.7428	
TRT	3	0.0087455	0.0029152	1.02	0.5303	
Error	2	0.0057271	0.0028635			
Corrected Total	11	0.5942039				

Fatty Acid=Eicosatetraenoic or Arachidonic (20:4n3)

General Linear Models Procedure						
Dependent Variable: PERCENT Percent of Total Frequency						
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	
Model	9	0.4671838	0.0519093	4.82	0.0345	
PER	3	0.3161153	0.1053718	9.78	0.0100	
AN	3	0.1003282	0.0334427	3.11	0.1104	
TRT	3	0.0507402	0.0169134	1.57	0.2915	
Error	6	0.0646125	0.0107687			
Corrected Total	15	0.5317963				

F-8. Analyses of variance for concentrations of fatty acids (C22) in milk fat of sheep fed untreated or treated dietary fat supplements

Fatty Acid=Eicosapentaenoic (20:5n3)

General Linear Models Procedure						
Dependent Variable: PERCENT Percent of Total Frequency						
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	
Model	9	0.0327244	0.0036360	4.25	0.0461	
PER	3	0.0054656	0.0018219	2.13	0.1977	
AN	3	0.0249579	0.0083193	9.73	0.0101	
TRT	3	0.0023009	0.0007670	0.90	0.4955	
Error	6	0.0051322	0.0008554			
Corrected Total	15	0.0378566				

Fatty Acid=Docosatetraenoic (22:4n6)

General Linear Models Procedure						
Dependent Variable: PERCENT Percent of Total Frequency						
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	
Model	9	1.4118863	0.1568763	1.25	0.4767	
PER	3	0.3149425	0.1049808	0.83	0.5575	
AN	3	0.5178375	0.1726125	1.37	0.4007	
TRT	3	0.5791062	0.1930354	1.53	0.3669	
Error	3	0.3776001	0.1258667			
Corrected Total	12	1.7894864				

Fatty Acid=Docosapentaenoic (22:5n3)

General Linear Models Procedure						
Dependent Variable: PERCENT Percent of Total Frequency						
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	
Model	9	2.0420295	0.2268922	0.89	0.5884	
PER	3	0.7057960	0.2352653	0.92	0.4950	
AN	3	0.6232647	0.2077549	0.81	0.5394	
TRT	3	0.5986817	0.1995606	0.78	0.5536	
Error	5	1.2787597	0.2557519			
Corrected Total	14	3.3207893				

Fatty Acid=Docosahexaenoic (22:6n3)

General Linear Models Procedure						
Dependent Variable: PERCENT Percent of Total Frequency						
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F	
Model	9	0.2413796	0.0268200	0.79	0.6470	
PER	3	0.0781126	0.0260375	0.77	0.5681	
AN	3	0.0656438	0.0218813	0.65	0.6248	
TRT	3	0.0929624	0.0309875	0.92	0.5091	
Error	4	0.1353362	0.0338341			
Corrected Total	13	0.3767158				

APPENDIX G

APPENDIX G. Analyses of variance for fatty acid content of soybean gels stored at 5-6°C (treated) or at -18°C (untreated) for 60, 70, 80 and 90 days.

G-1. Analyses of variance for fatty acid content of soybean gels stored at 5-6°C (treated) or at -18°C (untreated) for 60, 70, 80 and 90 days.

Fatty Acid=Caproic or Hexanoic (6:0)

General Linear Models Procedure					
Dependent Variable: PERCENT Percent of Total Frequency					
		Sum of	Mean		
Source	DF	Squares	Square	F Value	Pr > F
Model	5	1.3761331	0.2752266	.	.
PER	1	0.1803466	0.1803466	.	.
TRT	3	0.9769953	0.3256651	.	.
REP	1	0.2187912	0.2187912	.	.
PER*TRT	0	0.0000000	.	.	.
Error	0	.	.	.	.
Corrected Total	5	1.3761331	.	.	.

Fatty Acid=Caprylic or Octanoic (8:0)

General Linear Models Procedure					
Dependent Variable: PERCENT Percent of Total Frequency					
		Sum of	Mean		
Source	DF	Squares	Square	F Value	Pr > F
Model	15	1.4939201	0.0995947	4.63	0.0071
PER	3	0.7409014	0.2469671	11.48	0.0010
TRT	3	0.0838321	0.0279440	1.30	0.3235
REP	1	0.0326005	0.0326005	1.52	0.2439
PER*TRT	8	0.5213146	0.0651643	3.03	0.0460
Error	11	0.2365872	0.0215079		
Corrected Total	26	1.7305073			

Fatty Acid=Decanoic or Capric (10:0)

General Linear Models Procedure					
Dependent Variable: PERCENT Percent of Total Frequency					
		Sum of	Mean		
Source	DF	Squares	Square	F Value	Pr > F
Model	14	0.2035138	0.0145367	3.59	0.0478
PER	3	0.0097040	0.0032347	0.80	0.5325
TRT	3	0.1313315	0.0437772	10.82	0.0051
REP	1	0.0000653	0.0000653	0.02	0.9025
PER*TRT	7	0.0395761	0.0056537	1.40	0.3351
Error	7	0.0283302	0.0040472		
Corrected Total	21	0.2318440			

Fatty Acid=Tetradecanoic or Myristic (14:0)

General Linear Models Procedure					
Dependent Variable: PERCENT Percent of Total Frequency					
		Sum of	Mean		
Source	DF	Squares	Square	F Value	Pr > F
Model	16	0.0097984	0.0006124	3.80	0.0120
PER	3	0.0031553	0.0010518	6.52	0.0073
TRT	3	0.0019193	0.0006398	3.97	0.0354
REP	1	0.0001121	0.0001121	0.70	0.4207
PER*TRT	9	0.0061655	0.0006851	4.25	0.0114
Error	12	0.0019354	0.0001613		
Corrected Total	28	0.0117337			

G-2. Analyses of variance for fatty acid content of soybean gels stored at 5-6°C (treated) or at -18°C (untreated) for 60, 70, 80 and 90 days.

Fatty Acid=Hexadecanoic or Palmitic (16:0)

General Linear Models Procedure					
Dependent Variable: PERCENT Percent of Total Frequency					
SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F
Model	16	6.7803741	0.4237734	4.40	0.0065
PER	3	0.5494772	0.1831591	1.90	0.1831
TRT	3	4.9378591	1.6459530	17.09	0.0001
REP	1	0.0160664	0.0160664	0.17	0.6901
PER*TRT	9	1.0538305	0.1170923	1.22	0.3679
Error	12	1.1556027	0.0963002		
Corrected Total	28	7.9359769			

Fatty Acid=Hexadecenoic (16:1n7)

General Linear Models Procedure					
Dependent Variable: PERCENT Percent of Total Frequency					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.0000569	0.0000190	.	.
PER	2	0.0000549	0.0000275	.	.
TRT	0	0.0000000	.	.	.
REP	1	0.0000256	0.0000256	.	.
PER*TRT	0	0.0000000	.	.	.
Error	0	.	.	.	.
Corrected Total	3	0.0000569			

Fatty Acid=Hexadecenoic or Palmitoleic (16:1n9)

General Linear Models Procedure					
Dependent Variable: PERCENT Percent of Total Frequency					
Source	DF	Squares	Square	F Value	Pr > F
Model	12	0.0001624	0.0000135	1.40	0.3108
PER	2	0.0000359	0.0000179	1.86	0.2109
TRT	3	0.0000617	0.0000206	2.13	0.1666
REP	1	0.0000079	0.0000079	0.82	0.3883
PER*TRT	6	0.0000489	0.0000082	0.85	0.5661
Error	9	0.0000869	0.0000097		
Corrected Total	21	0.0002493			

Fatty Acid=Octadecenoic or Stearic (18:0)

General Linear Models Procedure					
Dependent Variable: PERCENT Percent of Total Frequency					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	16	34.439606	2.152475	0.75	0.7091
PER	3	4.308887	1.436296	0.50	0.6888
TRT	3	7.131534	2.377178	0.83	0.5032
REP	1	2.251636	2.251636	0.78	0.3930
PER*TRT	9	18.163645	2.018183	0.70	0.6965
Error	12	34.420417	2.868368		
Corrected Total	28	68.860023			

G-3. Analyses of variance for fatty acid content of soybean gels stored at 5-6°C (treated) or at -18°C (untreated) for 60, 70, 80 and 90 days.

Fatty Acid=Octadecenoic or Oleic (18:1n9)

General Linear Models Procedure					
Dependent Variable: PERCENT Percent of Total Frequency					
		Sum of	Mean		
Source	DF	Squares	Square	F Value	Pr > F
Model	16	17.523425	1.095214	46.19	0.0001
PER	3	0.781530	0.260510	10.99	0.0009
TRT	3	14.559587	4.853196	204.68	0.0001
REP	1	0.145458	0.145458	6.13	0.0291
PER*TRT	9	0.995209	0.110579	4.66	0.0078
Error	12	0.284531	0.023711		
Corrected Total	28	17.807956			

Fatty Acid=Octadecadienoic or Linoleic (18:2n6)

General Linear Models Procedure					
Dependent Variable: PERCENT Percent of Total Frequency					
		Sum of	Mean		
Source	DF	Squares	Square	F Value	Pr > F
Model	16	35.211329	2.200708	1.35	0.3017
PER	3	5.305780	1.768593	1.09	0.3916
TRT	3	15.826092	5.275364	3.24	0.0602
REP	1	2.074939	2.074939	1.28	0.2807
PER*TRT	9	9.457401	1.050822	0.65	0.7402
Error	12	19.513816	1.626151		
Corrected Total	28	54.725145			

Fatty Acid=Octadecatrienoic or Linolenic (18:3n3)

General Linear Models Procedure					
Dependent Variable: PERCENT Percent of Total Frequency					
		Sum of	Mean		
Source	DF	Squares	Square	F Value	Pr > F
Model	16	6.4690600	0.4043162	10.52	0.0001
PER	3	0.1858314	0.0619438	1.61	0.2386
TRT	3	5.5178369	1.8392790	47.84	0.0001
REP	1	0.0517975	0.0517975	1.35	0.2683
PER*TRT	9	0.3228985	0.0358776	0.93	0.5310
Error	12	0.4613281	0.0384440		
Corrected Total	28	6.9303881			

Fatty Acid=Eicosenoic or Gadoleic (20:1n11)

General Linear Models Procedure					
Dependent Variable: PERCENT Percent of Total Frequency					
		Sum of	Mean		
Source	DF	Squares	Square	F Value	Pr > F
Model	12	0.0332224	0.0027685	1.42	0.3046
PER	2	0.0088184	0.0044092	2.26	0.1604
TRT	3	0.0188179	0.0062726	3.21	0.0759
REP	1	0.0006648	0.0006648	0.34	0.5739
PER*TRT	6	0.0068808	0.0011468	0.59	0.7338
Error	9	0.0175699	0.0019522		
Corrected Total	21	0.0507923			

G-4. Analyses of variance for fatty acid content of soybean gels stored at 5-6°C (treated) or at -18°C (untreated) for 60, 70, 80 and 90 days.

Fatty Acid=Eicosatrienoic (20:3n6)

General Linear Models Procedure						
Dependent Variable: PERCENT						
		Sum of	Mean			
Source	DF	Squares	Square	F Value	Pr > F	
Model	16	0.0260030	0.0016252	18.11	0.0001	
PER	3	0.0074968	0.0024989	27.85	0.0001	
TRT	3	0.0133120	0.0044373	49.45	0.0001	
REP	1	0.0009069	0.0009069	10.11	0.0079	
PER*TRT	9	0.0014780	0.0001642	1.83	0.1628	
Error	12	0.0010768	0.0000897			
Corrected Total	28	0.0270798				

Fatty Acid=Eicosapentenoic (20:5n3)

General Linear Models Procedure						
Dependent Variable: PERCENT						
		Sum of	Mean			
Source	DF	Squares	Square	F Value	Pr > F	
Model	15	0.3403657	0.0226910	14.48	0.0001	
PER	3	0.0235833	0.0078611	5.02	0.0197	
TRT	3	0.2164019	0.0721340	46.04	0.0001	
REP	1	0.0017654	0.0017654	1.13	0.3112	
PER*TRT	8	0.0632155	0.0079019	5.04	0.0079	
Error	11	0.0172335	0.0015667			
Corrected Total	26	0.3575992				

Fatty Acid=Docosatetraenoic (22:4n6)

General Linear Models Procedure						
Dependent Variable: PERCENT						
		Sum of	Mean			
Source	DF	Squares	Square	F Value	Pr > F	
Model	15	2.5589161	0.1705944	31.89	0.0001	
PER	3	1.4039758	0.4679919	87.48	0.0001	
TRT	3	0.5405008	0.1801669	33.68	0.0001	
REP	1	0.0035852	0.0035852	0.67	0.4304	
PER*TRT	8	1.2369461	0.1546183	28.90	0.0001	
Error	11	0.0588488	0.0053499			
Corrected Total	26	2.6177649				

APPENDIX H

APPENDIX H. Composition of the Brucella broth

H-Composition of Brucella broth

Ingredient per liter	Weight (g)
Bacto tryptone	10
Bacto Peptamin	10
Bacto dextrose	1
Bacto yeast extract	2
Sodium chloride	5
Sodium bisulfite	0.1

APPENDIX I

APPENDIX I. Analyses of variance for weights (Kg) of
the ewes and their lambs fed untreated or treated
dietary fat supplemnets

I-Analyses of variance for weights (Kg) of the ewes and their lambs fed untreated or treated dietary fat supplement

Animal=Lactating ewes

General Linear Models Procedure					
Dependent Variable: WT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr >F
MODEL	9	1281.6314	142.4035	282.96	0.0001
PER	3	9.6494	3.2165	6.39	0.0268
AN	3	1185.4156	395.1385	785.15	0.0001
TRT	3	1.5744	0.5248	1.04	0.4391
Error	6	3.0196	0.5033		
Corrected Total	15	1284.6510			

Animal=Lambs

General Linear Models Procedure					
Dependent Variable: WT					
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	226.82032	25.20226	177.22	0.0001
PER	3	170.05320	56.68440	398.60	0.0001
AN	3	43.23190	14.41063	101.33	0.0001
TRT	3	1.12107	0.37369	2.63	0.1440
Error	6	0.85325	0.14221		
Corrected Total	15	227.67357			

APPENDIX J

APPENDIX J. Composition of individual and fatty acids type in the blood serum fat from lactating ewes before and after the feeding trial

J-1. ^aComposition of individual fatty acids in the blood serum fat from lactating ewes before the feeding trial

Fatty Acids	Animals			
	1	2	3	4
-----Concentration (%) Lsmean (SE)-----				
6:0	5.99	18.6	ND	ND
8:0	5.60 (.03)	ND	5.06 (.02)	5.88 (.02)
14:0	ND	0.56 (.01)	0.48 (.01)	0.56 (.01)
15:0	ND	ND	ND	ND
16:0	14.24 (.11)	12.39 (.11)	15.63 (.11)	15.26 (.11)
16:1n9	0.60 (.01)	0.62 (.01)	0.62 (.01)	0.82 (.01)
17:0	4.52 (.03)	1.23 (.03)	1.37 (.03)	2.31 (.03)
18:0	17.40 (.08)	19.49 (.08)	18.20 (.08)	18.32 (.08)
18:1n9	21.80 (.11)	20.88 (.11)	23.41 (.11)	23.90 (.11)
18:2n6	22.26 (.15)	17.09 (.15)	22.42 (.15)	21.32 (.15)
18:3n3	1.92 (.01)	2.16 (.01)	1.87 (.01)	1.97 (.01)
20:1n11	ND	0.54 (.01)	0.49 (.01)	0.58 (.01)
20:4n3	3.82 (.01)	1.65 (.01)	4.87 (.01)	3.50 (.01)
22:5n3	1.25 (.02)	1.09 (.02)	1.74 (.02)	1.36 (.02)
22:6n3	0.79 (.04)	1.07 (.04)	1.32 (.04)	0.74 (.04)

^aFor any fatty acid means in a row followed by different letters are significantly different ($p < .05$).

SE=Standard Error.

ND=Not Determined.

J-2. ^aComposition of type of fatty acids in serum fat from lactating ewes before the feeding trial

Fatty Acids		Animals			
Type	1	2	3	4	
-----Concentration (%) Lsmean (SE)-----					
SFA	41.95a(.17)	52.80a(.17)	41.11b(.17)	42.33b(.17)	
MUFA	22.46c(.10)	22.05c(.10)	24.52b(.10)	25.29a(.10)	
PUFA	30.97b(.25)	23.96d(.25)	32.91a(.25)	29.62c(.13)	
PUFAn3	3.97b(.06)	4.32d(.06)	4.93a(.06)	4.07b(.06)	
PUFAn6	26.08b(.15)	18.74d(.15)	27.29a(.15)	24.82c(.15)	
UNI	5.54a(.19)	2.05c(.19)	2.10c(.19)	3.44b(.19)	

^aFor any fatty acid means in a row followed by different letters are significantly different ($p < .05$).

SE=Standard Error.

SFA=Saturated Fatty Acids.

MUFA=Monounsaturated Fatty Acids.

PUFA=PolyUnsaturated Fatty Acids.

PUFAn3=n3-PolyUnsaturated Fatty Acids.

PUFAn6=n6-PolyUnsaturated Fatty Acids.

UNI=Unidentified.

J-3. ^aComposition of type of fatty acids in the blood serum fat from lactating ewes fed untreated or treated soybean gel supplements

Fatty Acids	^b Treatments			
	Control	Formaldehyde	Acetaldehyde	Diacetyl
Type	-----Concentrations (%) Lsmean (SE)-----			
SFA	45.03 (1.36)	43.07 (1.36)	45.78 (1.36)	44.73 (1.36)
MUFA	24.69 (3.49)	16.71 (3.49)	15.42 (3.49)	19.03 (3.49)
PUFA	30.28 (3.15)	40.22 (3.15)	38.80 (3.15)	36.25 (3.15)
PUFAn3	3.96 (0.40)	3.81 (0.40)	3.46 (0.40)	3.74 (0.40)
PUFAn6	26.32b(2.9)	36.81a(2.9)	35.34ab(2.9)	32.50b(2.9)

^aFor any fatty acid means in a row followed by different letters are significantly different ($p < .05$).

^bControl had no additives; other treatments contained listed compounds.

SE=Standard Error

SFA=Saturated Fatty Acids

MUFA=Monounsaturated Fatty Acids.

PUFAn3=n3-Polyunsaturated Fatty Acids.

PUFAn6=n6-Polyunsaturated Fatty Acids.

APPENDIX K

APPENDIX K. Composition of individual and fatty acids
type in the milk fat from lactating ewes before and
after the feeding trial

K-1. ^aComposition of individual fatty acids in the milk fat from lactating ewes before the feeding trial

Fatty Acids	Animals			
	1	2	3	4
-----Concentrations (%) Lsmean (SE)-----				
6:0	0.85 (.02)	0.95 (.02)	0.72 (.02)	0.96 (.02)
8:0	1.34 (.05)	1.41 (.05)	0.90 (.05)	1.41 (.05)
9:0	0.07 (.68)	0.12 (.68)	1.48 (.68)	0.10 (.68)
10:0	4.41 (.07)	4.41 (.07)	2.84 (.11)	4.43 (.07)
11:0	0.06	ND	ND	ND
12:0	2.32 (.03)	2.42 (.03)	1.73 (.03)	2.47 (.03)
14:0	6.84 (.06)	5.55 (.06)	5.00 (.06)	6.06 (.06)
14:1n9	0.15 (.01)	0.16 (.01)	0.13 (.01)	0.17 (.01)
15:0I	0.34 (.01)	0.31 (.01)	0.29 (.01)	0.29 (.01)
15:0	0.82 (.01)	0.68 (.01)	0.62 (.01)	0.67 (.01)
16:0I	0.18 (.01)	0.21 (.01)	0.18 (.01)	0.18 (.01)
16:0	20.96 (.28)	18.21 (.28)	18.74 (.28)	19.65 (.28)
16:1n7	0.42 (.03)	0.47 (.03)	0.44 (.03)	0.45 (.03)
16:1n9	0.73 (.02)	0.91 (.02)	0.93 (.02)	0.91 (.02)
17:0	0.43 (.02)	0.55 (.02)	0.59 (.02)	0.50 (.02)
Unk1	1.18 (.11)	1.57 (.11)	1.64 (.11)	1.50 (.11)
Unk2	0.50 (.03)	0.69 (.03)	0.95 (.03)	0.68 (.03)
18:0	15.05 (.61)	15.70 (.61)	13.61 (.61)	14.79 (.61)
18:1n9	36.03 (.80)	38.11 (.80)	43.99 (.80)	36.93 (.80)
19:0	0.52 (.07)	0.51 (.07)	0.22 (.07)	0.32 (.07)
18:2n6	3.86 (.06)	3.74 (.06)	3.81 (.06)	4.42 (.06)
18:3n3	1.12 (.06)	1.32 (.06)	0.84 (.06)	1.04 (.06)
20:1n11	0.79 (.02)	1.24 (.02)	1.07 (.02)	1.17 (.02)
20:3n6	0.05 (.01)	0.30 (.01)	0.37 (.01)	0.42 (.01)
20:4n6	0.45 (.03)	0.25 (.03)	0.10 (.03)	0.17 (.03)
20:5n3	0.18	0.19	ND	ND
22:4n6	0.05	ND	ND	ND
22:5n3	0.21 (.01)	0.20 (.01)	0.17 (.01)	0.23 (.01)
22:6n3	0.07	0.09	0.08	0.08

^aFor any one fatty acid means in a row followed by different letters are significantly different (p<.05).

ND=Not Determined.

UNK=Unknown .

K-2. ^aComposition of the type of fatty acids in the milk fat from lactating ewes before the feeding trial

Fatty Acids Type	Animals			
	1	2	3	4
-----Concentrations (%) Lsmean (SE)-----				
SFA	54.19a(.86)	51.04a(.8)	45.52b(.86)	51.86a(.86)
MUFA	38.14b(.86)	40.89b(.86)	46.55a(.86)	39.64b(.86)
PUFA	5.98a(.10)	5.80ab(.1)	5.33b(.10)	6.32a(.10)
PUFAn3	1.59a(.66)	1.66a(.06)	1.05b(.06)	1.31ab(.1)
PUFAn6	4.38b(.07)	4.14b(.14)	4.27b(.07)	5.01a(.07)
UNI	1.68 (.13)	2.27 (.13)	2.60 (.13)	2.18 (.13)

SFA=Saturated Fatty Acids; MUFA=Monounsaturated Fatty Acids; PUFA=Polyunsaturated Fatty Acids; UNI=Unidentified.

^aFor any one fatty acid means in a row followed by different letters are significantly different (p<.05).

ND=Not Determined.

K-3. ^aComposition of the type of fatty acids in the milk fat from lactating ewes fed untreated or treated soybean gel supplements.

Fatty Acids	^b Treatments			
	Control	Formaldehyde	Acetaldehyde	Diacetyl
Type	-----Concentrations (%) Lsmean (SE)-----			
SFA	61.49 (1.6)	58.60 (1.6)	60.99 (1.6)	57.39 (1.6)
MUFA	28.70 (1.7)	26.17 (1.7)	27.28 (1.7)	30.99 (1.7)
PUFA	8.60 (.85)	14.20 (.85)	10.58 (.85)	10.30 (.85)
PUFAn3	2.80 (.35)	2.95 (.35)	2.40 (.35)	2.47 (.35)
PUFAn6	5.75a(.55)	11.18a(.55)	8.10b(.55)	7.32b(.55)
UNI	1.20 (.11)	1.02 (.11)	1.18 (.11)	1.31 (.11)

SFA=Saturated Fatty Acids; MUFA=Monounsaturated Fatty Acids; PUFA=Polyunsaturated Fatty Acids; UNI=Unidentified

^aFor any one fatty acid means in a row followed by different letters are significantly different ($p < .10$).

^bControl had no additives; other treatments contained listed compounds.

ND=Not Determined

VITA

Dje Yobouet was born in Abli (P de Toumodi), the Ivory Coast. He graduated from high school (Lycee Modern de Koumassi) in Abidjan, in 1982 with the high school diploma "Baccalaureat D", with Mathematics, Physics and Biology as major. He was granted a scholarship from the Ministry of Education to study Chemistry Biology and Geology in the College of Science and Technology at the National University of Abidjan, Ivory Coast. Dje Yobouet earned the BS degree in Biochemistry in 1986 and was granted two scholarships from both the French and the Ivorian governments for his graduate studies in Food Science. He earned his MS in Food Science and Technology with Food Microbiology as major at The University of Tennessee, Knoxville in may 1990. In December 1994, Dje Yobouet received his Doctoral degree in Food Science and Technology, with Food Chemistry as major at The University of Tennessee, Knoxville.

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