THE INFLUENCE OF TREATMENT OF WHOLE FAT SOYBEANS OR SOY FLOUR WITH FORMALDEHYDE TO PROTECT THE POLYUNSATURATED FATTY ACIDS FROM BIOHYDROGENATION IN THE RUMEN

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CHAPTER I

INTRODUCTION

Lipids in plants ingested by ruminants consist largely of triglycerides which are made up chiefly of unsaturated fatty acids. However, upon entering the rumen, the microbes hydrolyze the triglycerides to free fatty acids and hydrogenate the unsaturated fatty acids to more saturated forms. The highly saturated fatty acids thus produced are absorbed and deposited in the fat tissues making ruminant fat characteristically highly saturated. There has been much emphasis lately on the role of polyunsaturated fatty acids in human diets as an aid to the prevention of atherosclerosis. Thus, the production of more polyunsaturated ruminant products would benefit researchers in the field of coronary heart disease and potentially increase the value of ruminant products.

Recently, workers in Australia developed a method for protecting the polyunsaturated fatty acids of vegetable oils (safflower, sunflower, soybeans, etc.) against biohydrogenation in the rumen. Polyunsaturated oil droplets were encapsulated with protein and then the whole complex was sprayed with formaldehyde. This oil-protein complex was resistant to hydrogenation under conditions found in the rumen (pH 6-7); but upon entering the acidic (pH 2-3) conditions of the abomasum, hydrolysis occurred releasing the polyunsaturated fatty acids for absorption in the small intestine. This resulted in substantial changes in the

triglycerides of plasma, milk and depot fats in which there was an increase in the proportion of polyunsaturated fatty acids from two to five percent to 20 to 30 percent of total fatty acids. In addition, an increase has been shown in the milk fat content.

Since the above process for protecting the oil-protein complex may be too costly to make it feasible economically, it would seem conceivable that the same protection could be afforded by treating ground, whole soybeans with formaldehyde since soybeans have a natural oil-protein complex. Therefore, this experiment was conducted using *in vitro* and *in vivo* methods to determine if the polyunsaturated fatty acids of ground, full-fat soybeans could be protected from biohydrogenation in the rumen when fed to growing lambs. Organoleptic studies were conducted to determine if such treatments would result in any flavor differences in the meat.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Although ruminants and non-ruminants may consume the same pasture, striking contrasts are evident in the composition of their adipose tissues. Differences in lipid metabolism lead to the formation of depot fats that are soft and oily in monogastrics and hard in ruminants. These differences are attributable to the substantial amounts of linoleic and linolenic fatty acids in the depot fats of non-ruminants (e.g., horse and rabbit) while only trace amounts are found in depot fats of ruminants (Lough and Garton, 1957a).

The depot fats of ruminants are highly saturated consisting mainly of palmitic, stearic and oleic acids, which sharply contrasts with the fatty acid composition of ingested pasture grasses. Grasses are rich in C_{18} unsaturated fatty acids, especially linolenic acid. Hilditch (1956) reported that 78.5% of the total fatty acids of mixed pasture grasses consist of C_{18} unsaturated components. Of this, 50% is linolenic, 20% linoleic and the remainder largely oleic acid. The lipid intake of a cow grazing pasture is estimated to be approximately 450 grams of lipid daily (Garton, 1960).

The difference between the fatty acid composition of the diet and the fatty acid composition of depot fats and milk fats, which are also

highly saturated in ruminants, is the result of the activity of rumen microorganisms. Rumen microorganisms are responsible for the extensive biohydrogenation of the unsaturated fatty acids of the diet (Reiser, 1951; Willey et al., 1952; Shorland, Weenink and Johns, 1955; Shorland et al., 1957; Reiser and Reddy, 1956; Garton, Hobson and Lough, 1958; Garton, Lough and Vioque, 1959, 1961; Wright, 1959, 1960; Ward, Scott and Dawson, 1964). Consequently, the polyunsaturated fat content of ruminant tissues could be increased if some method could be developed for protecting dietary lipid from ruminal hydrogenation.

Fate of Lipids in Ruminants

Biohydrogenation

Grazing ruminants consume mostly leaves of grasses which are 5 to 10% lipid on a dry matter basis (Garton, 1967). The major components of this lipid are glycerides in the form of mono- and digalactosyl derivatives of 1,2-diglycerides (Weenink, 1961; Shorland, 1961). Other constituents include phospholipids, triglycerides, sulpholipids, sterols, sterol esters, waxes, hydrocarbons and free fatty acids (Hilditch, 1956; Weenink, 1962). Upon entering the rumen, partial or complete hydrolysis of the ester linkages of the dietary glycerides (Garton et al., 1958, 1959) and phospholipids (Dawson, 1959) to their constituent fatty acids and glycerol occurs. The unsaturated fatty acids then undergo hydrogenation. However, some hydrogenation can occur while the fatty acids are still in the glyceride combination although the process proceeds more readily following the hydrolytic release of the fatty acids (Garton et al., 1959, 1961; Hawke and Robertson, 1964). Also,

galactoglycerides liberate galactose in addition to fatty acids (Bailey, 1964). Both glycerol and galactose undergo fermentation to yield volatile fatty acids.

Once the fatty acids are liberated, complete anaerobiosis is necessary for hydrogenation to take place (Polan, McNeill and Tove, 1964). Sklan, Volcani and Budowski (1971) and Sklan, Budowski and Volcani (1972) reported that when rumen liquor from the rumen of immature calves three to five weeks old or from older calves maintained on a milk replacer diet was incubated aerobically, an increase in the proportion of octadecadienoic (linoleic) acid was observed accompanied by a decrease of approximately equal magnitude in stearic and oleic acids. However, if the rumen fluid from the calves was incubated anaerobically or if rumen liquor was incubated from animals accustomed to a diet of roughage and concentrate, the rumen liquor lost this ability to synthesize octadecadienoic acid and instead hydrogenated the lipids. Hydrogenation activity began when the calves reached five weeks of age. This suggests that in young ruminants prior to the development of an active rumen in which an aerobic environment exists, polyunsaturated fatty acids from the diet can be incorporated into fat depots in a manner similar to monogastrics (Hoflund, Holmberg and Sellmann, 1956; Siren, 1962). However, upon development of the rumen, the environment becomes anaerobic and supports hydrogenation. Bryant et al. (1958) reported that counts of aerobic bacteria were highest in the rumen of calves during the early weeks of life, and Lengemann and Allen (1959) found a rapid fall in the number of aerobes whenever calves were transferred from liquid to solid food. Several workers have reported that although the concentrations of the 18-carbon polyunsaturated fatty acids in the various

tissues of the newborn lamb are very low, there is a pronounced increase in concentrations of these acids during the first three to four days after birth in spite of the extremely low concentrations of polyunsaturated fatty acids in the colostrum and milk (Leat, 1966; Noble, Steel and Moore, 1970, 1971a,b, 1972). Also, Noble et al. (1972) reported that the linoleic content, expressed as a percent of total fatty acids, of lamb carcasses at 10 and 20 days after birth were similar to the dietary intake of linoleic acid, but by 30 days the linoleic acid content of the carcasses was less than the dietary intake.

Both bacteria (Polan et al., 1964; Kepler et al., 1965; Wilde and Dawson, 1966; Wright, 1960) and protozoa (Wright, 1959; Gutierrez et al., 1962; Williams, Gutierrez and Davis, 1963; Chalupa and Kutches, 1968) are capable of hydrogenating unsaturated fatty acids. The complete hydrogenation of the C_{18} polyunsaturated fatty acids yields stearic acid while oleic acid or its isomers are products of incomplete hydrogenation.

Unsaturated fatty acids found in plants are almost all of the cis-configuration but Hartman, Shorland and McDonald (1954) reported the presence of trans-C_{18} monoenoic fatty acids in the depot fats of ruminants but not in other herbivorous animals. Shorland et al. (1955, 1957) showed that rumen fermentation was the probable source of these isomers, and this has been confirmed (Wood et al., 1963; Ward et al., 1964; Czerkawski and Blaxter, 1965; Wilde and Dawson, 1966; Harfoot, Noble and Moore, 1973; Noble, Moore and Harfoot, 1974). Polan and coworkers (1964) have provided evidence that the biohydrogenation of linoleic acid involves two systems: one specific for conversion of linoleic to oleic acid and another for hydrogenating oleic to stearic

acid. When low concentrations of linoleic acid were incubated *in vitro*, Polan *et al*. (1964) and Harfoot *et al*. (1973) reported that stearic acid was the major end product of hydrogenation while oleic acid was the major end product when high levels of linoleic acid were incubated. Harfoot *et al*. (1973) suggested that large amounts of linoleic acid prevent hydrogenation of oleic to stearic acid by irreversible inhibition rather than by simply competing for hydrogen as suggested by Polan *et al*. (1964).

Several workers have also shown that the efficiency with which rumen microbes hydrogenate unsaturated fatty acids depends on whether these acids are presented as substrates in the free or esterified form. Biohydrogenation of trilinolein results in an accumulation of stearic acid while biohydrogenation of an equivalent amount of free linoleic acid results in an increase in oleic acid with little or no production of stearic acid in both *in vitro* (Moore *et al*. 1969b) and *in vivo* (Noble, Steele and Moore, 1969) studies. Noble *et al*. (1974) indicated that this pattern is observed irrespective of initial linoleic acid levels. They proposed several explanations: (1) the rate of hydrolysis of trilinolein may be insufficient to saturate the hydrogenating system with free linoleic acid whereas free linoleic acid saturated the system or (2) the rumen microbes are adapted to absorb and metabolize esterified fatty acids to a greater extent than free fatty acids since dietary lipids are composed mainly of esterified fatty acids.

Bacterial Lipids

In addition to the diet as a source of lipids, the rumen contents also include lipids that arise as part of the structural components of

microorganisms. Keeney, Katz and Allison (1962) reported that these structural lipids elaborated de novo by the rumen bacteria include branched-chain fatty acids, n-fatty acids having an odd number of carbon atoms, and considerable amounts of straight-chain fatty acids such as palmitic, stearic and oleic acids. Protozoa also produce these odd fatty acids but to a considerable lesser extent. These authors also stated that the rumen microbes are the source of the odd length and branched chain fatty acids found in the milk and body fats of ruminants. No unsaturated fatty acids containing more than one double bond were detected in rumen bacterial lipids (Cunningham and Loosli, 1954; Garton and Oxford, 1955) which agrees with the conclusion of Scheuerbrandt and Bloch (1962) that polyunsaturated fatty acids do not occur in bacteria. However, Williams and Dinusson (1973) reported bacteria to contain 5.6% linoleic and traces of linolenic acids. Gutierrez et al. (1962) and Williams and Dinusson (1973) found palmitic acid to be the major component of protozoal lipids while Williams and Dinusson (1973) reported stearic acid to be higher in bacteria than in protozoa.

The processes of hydrolytic release of esterified fatty acids, the hydrogenation of unsaturated fatty acids and the fermentation of glycerol liberated during lipolysis and of galactose from galactoglycerides occur concurrently. Thus, digesta, consisting mostly of long-chain unesterified, saturated fatty acids (particularly stearic) along with associated microorganisms pass rather continuously from the rumen through the abomasum to the small intestine where absorption takes place.

Absorption

In studies with sheep Felinski et al. (1964) found that in the

jejunum the proportions of esterified fatty acids increased as did the C_{18} unsaturated fatty acids present in the unesterified and esterified fatty acid fractions. Similar observations with respect to the total C_{18} unsaturated fatty acids present in the digesta of the small intestine as compared with rumen contents have been made in goats (McCarthy, 1962) and sheep (Ward et al., 1964; Leat, 1965). Lennox, Lough and Garton (1968) confirmed these findings and showed that the increase in the esterified fatty acids in the lipids of the upper small intestine was due almost entirely to an increase in the proportion of phospholipids present. They also showed that the phospholipid fraction accounted for the increase in the esterified C_{18} unsaturated fatty acids. Pancreatic juice contains virtually no lipid (Leat, 1965) but bile was shown to be the most important source of these phospholipids and that these biliary phospholipids contained significant amounts of C_{18} unsaturated fatty acids, thus accounting in part, if not entirely, for the increment in the amounts of these acids that was observed in the lipids of the upper part of the small intestine (Adams and Heath, 1963; Leat, 1965; Lennox, Lough and Garton, 1965; Lennox and Garton, 1968). Leat (1965) reported that bile lecithin is degraded by pancreatic juice to free fatty acids and lysolecithin and that this reaction could account for the increased presence of unsaturated fatty acids in the jejunum. Lennox et al. (1968) also reported that the unesterified fatty acid fraction of bile is highly unsaturated.

Fatty acids greater than 10 carbons in length, either saturated or unsaturated, are carried in the lymph as chylomicrons consisting primarily of triglycerides while the larger portion of C_{10} and virtually all of those less than 10 carbons long leave the intestine via the

portal circulation as unesterified fatty acids (Cantarow and Schepartz, 1962). The lymph lipids of sheep (Lough, Felinski and Garton, 1963; Felinski et al., 1964; Heath, Adams and Morris, 1964) and cows (Hartmann and Lascelles, 1966; Wadsworth, 1968) have been investigated using cannulas in either the thoracic or intestinal lymph duct and have been found to consist of 70 to 80% triglycerides, 15 to 20% phospholipids along with small amounts of cholesterol, cholesterol esters and unesterified fatty acids. These authors reported that the triglycerides consist largely of stearic acid along with significant amounts of trans and positional isomers of C_{18} unsaturated acids and fatty acids derived from bacterial lipids. Although Bickerstaffe and Annison (1968) have shown that preparations of sheep intestinal epithelium can desaturate stearic acid to give oleic acid, this process did not seem quantitatively very significant in vivo.

Lennox and Garton (1968) using re-entrant cannulas in different parts of the small intestine showed that when the digesta had reached the ileum, the uptake of fatty acids was almost complete as was the hydrolytic release of esterified fatty acids. Ward et al. (1964) reported that the trans-oleic acid is absorbed almost completely from the ileum of sheep while Heath et al. (1964) showed also that trans-oleic acid appeared in the lymph in considerable amounts following the intraruminal infusion of maize oil into sheep. Although Lennox and Garton (1968) were unable to show a marked selective uptake of the trans-monoenoic acid, it did comprise a smaller proportion of the oleic acid leaving the terminal ileum as compared to the proportion entering the upper jejunum.

The polyunsaturated C_{18} fatty acids that do escape ruminal hydrogenation, possibly by being within protoza (Felinski et al., 1964), appear to be selectively esterified with cholesterol and to a lesser degree with phospholipids. This is particularly marked in the cow (Lough and Garton, 1957b; Duncan and Garton, 1962, 1963; Leat, 1966), though it is also evident in the sheep (Horgan and Masters, 1963; Garton and Duncan, 1964; Leat, 1966; Moore, Noble and Steele, 1968, 1969a) and the bison (Evans, 1964). The cholesterol is probably of endogenous origin since none is found in feeds of vegetable origin or bacteria (Garton, 1960, 1967). Moore, Noble and Steele (1969a) suggested that, after absorption from the small intestine of sheep, linolenic and linoleic acids are transported in the triglyceride form to the liver where the triglycerides are partially or completely hydrolyzed. These C_{18} polyunsaturated acids are then preferentially utilized for the synthesis of phospholipids and cholesterol esters but not for the re-synthesis of triglycerides. In a subsequent study, Noble et al. (1969) found the linoleic acid in the plasma triglycerides of sheep to increase three hours after either maize oil or linoleic acid was infused intraruminally while the linoleic acid in the plasma phospholipids and cholesterol esters did not begin to increase until six to nine hours and 24 to 25 hours, respectively, post-infusion supporting Moore's suggestion.

The unsaturated C_{18} fatty acids remaining in the ileal digesta undergo a further hydrogenation in the cecum and colon so that nearly all the acids in the excreta are saturated (Ward et al., 1964). Ward and coworkers showed that cecal bacteria were only capable of hydrogenating

linoleic to oleic, with the trans isomer being predominant. Oleic acid was then slowly hydrogenated in the colon to stearic acid.

Changes in Adipose Tissues During Growth

The development of an active rumen in the young ruminant is associated with marked changes in lipid metabolism. Although the newborn ruminant has very little depot fat (Body and Shorland, 1964; Masters, 1964), it consists primarily of triglycerides as is the case in adult ruminants. The perirenal triglycerides of sheep at birth have been shown by Noble, Christie and Moore (1971) and Garton and Duncan (1968) to consist primarily of palmitic, stearic and oleic acids. Oleic acid made up 62% of the triglyceride while stearic was present at a low 14%. Similar low values for stearic acid content of depot fat in newborn ruminants have been reported by Dahl (1958), Body and Shorland (1964) and Downing (1964). No linoleic, linolenic or longer chain polyunsaturated fatty acids have been found in the depot fat triglycerides of newborn ruminants (Body and Shorland, 1964; Noble et al., 1971). After birth dietary linoleic acid is assimilated into depot fats until the rumen becomes active. Also, with age there is an increase in stearic and a corresponding decrease in palmitic and oleic acids in the triglyceride fraction of depot fats (Masters, 1964; Noble et al., 1971). However, Waldman, Suess and Brungardt (1968) indicated that the composition of subcutaneous fat may vary during the growing period with unsaturated fatty acids increasing as animals increase in weight and fatness. Link et al. (1970) reported stearic decreased while oleic and linoleic acids increased with age in subcutaneous tissues of yearling steers sampled at 60-day intervals. In contrast, Hornstein, Crow and

Hiner (1967) reported that triglyceride fatty acid composition is unaffected by differences in age.

The location of adipose tissue in the body of mature ruminants influence the fatty acids of the triglycerides. The triglycerides of the internal tissues (e.g., mesenteric or perinephric) contain a much higher proportion of saturated fatty acids (particularly stearic acid) than those of external (subcutaneous) tissues (Hilditch and Williams, 1964; Duncan and Garton, 1967). This pattern of fatty acid distribution is associated with a gradient in body temperature and possibly with a difference in the rate of glyceride deposition (Callow, 1958). Duncan and Garton (1967) reported that in the exposed tissues of the leg and ear, oleic acid accounted for as much as 60 to 70% of the total fatty acids present. They also found that the trans-C_{18} monounsaturated fatty acid was present in greatest concentration in internal tissue triglycerides. They concluded that the long-chain fatty acids absorbed from the intestine has its greatest influence on the composition of the triglycerides of internal adipose tissue. The activity of stearic desaturase in sheep microsomal preparations was much higher in subcutaneous tissue than in perinephric tissue (Wahle and Garton, 1972). This may partially account for the increased oleic content of external tissues.

Protein By-Pass

Introduction

The use of formaldehyde as a protective covering for feeds to enable by-pass of ruminal degradation was developed to improve nitrogen

utilization in ruminants. Dietary proteins are extensively hydrolyzed by rumen microbes to their constituent amino acids. The amino acids are then rapidly deaminated with the resulting ammonia either being converted back to microbial protein or being absorbed into the blood stream for recycling and/or excretion in the urine as urea (McDonald, 1948).

This protein restructuring may be advantageous to the animal when a poor quality protein or a non-protein nitrogenous material such as urea is being fed as the rumen microflora will upgrade the biological value of such a feed. However, if the protein is of high quality, the modification that occurs in the rumen may reduce the biological value to that of the microbial protein. Also, at high dietary protein levels, degradation will exceed microbial protein synthesis resulting in substantial loss of dietary protein. Thus, rendering high quality proteins resistant to rumen microbial attack without greatly reducing their nutritive value in the lower gut could quantitatively and qualitatively enhance the protein status of the ruminant.

Early Methods

Cuthbertson and Chalmers (1950), Chalmers, Cuthbertson and Synge (1954), Reis and Schinckel (1961, 1963, 1964), Little and Mitchell (1967), Schelling and Hatfield (1967, 1968), Reis (1969) and Egan (1970) showed that when casein was administered directly into the abomasum or duodenum, nitrogen retention and/or wool growth of sheep were greatly enhanced. In contrast, the addition of a casein supplement to the diet had little effect on wool growth (Ferguson, Hemsley and Reis, 1967; Reis, 1969; Reis and Tunks, 1969).

Partial protection of proteins was achieved by heating proteins fed

to sheep (Chalmers et al., 1954; Tagari, Ascarelli and Bondi, 1962; Sherrod and Tillman, 1962, 1964; Glimp et al., 1967; Hudson et al., 1969; Nishimuta, Ely and Boling, 1973), goats (Chalmers, Jayasinghe and Marshall, 1964) and to calves (Whitelaw, Preston and Dawson, 1961). Tannins were found also to be effective (Leroy, Zelter and Francois, 1965; Tagari et al., 1965; Driedger, Hatfield and Garrigus, 1969; Driedger and Hatfield, 1970; Zelter, Leroy and Tissier, 1970; Rodriguez, Muller and Schingoethe, 1972). However, the nutritional value of both heated proteins (Danke et al., 1966) and tannin treated proteins (Nishimuta et al., 1973) may be impaired by depressing total digestibility.

Formaldehyde Protected Casein

Ferguson et al. (1967) developed the technique of applying formaldehyde to proteins to overcome rumen microbial degradation. They found that the treatment of dietary casein with formaldehyde increased wool growth in mature sheep by 70%. Their preliminary tests indicated that approximately 80% of the casein nitrogen was digested and absorbed. The mechanism by which protein is protected by formaldehyde is through formation of acid-reversible cross-linkages with amino and amide groups of the protein. Thus, under the neutral conditions of the rumen the protein is rendered insoluble, but the linkages are subsequently cleaved under the acidic conditions of the abomasum (pH 2-3) thereby allowing protein digestion and absorption in the small intestine.

It has been demonstrated that formaldehyde treated casein (F-casein) is equivalent to casein infused into the abomasum for promoting wool growth and nitrogen retention (Reis and Tunks, 1969). Reis and

Tunks (1970) found that F-casein in the diet and casein per abomasum resulted in similar changes in the proportion of amino acids in the plasma. Significant increases in the apparent absorption of amino acids from the small intestine (Macrae et al., 1972) and in the level of certain plasma free amino acids (Carrico et al., 1970) have been shown for F-casein as compared to untreated casein. These findings indicated that the nutritive value of the casein was not being adversely affected by formaldehyde.

Other researchers have confirmed an increase in wool growth (Langlands, 1971a; Hughes and Williams, 1971; Barry, 1972) and nitrogen retention (Faichney, 1971; Barry, 1972; Macrae et al., 1972) in response to formaldehyde treatment of dietary casein. This response may be dependent on other factors. For example, Faichney (1971) reported no response in wool growth of lambs receiving a 10% F-casein diet compared to controls fed untreated casein although nitrogen retention was higher for the F-casein diet. Lambs on the untreated casein diet may have been absorbing sufficient amino acids for maximum wool growth so that the F-casein diet could not elicit any further improvement.

Plant Protein Protection

High quality plant proteins have been treated with aldehydes with varying degrees of success. Peter et al. (1971) tested a number of aldehydes at various levels in vitro and found only formaldehyde, glutaraldehyde or glyoxal to depress soybean meal solubility significantly. Active compounds were all chemically bifunctional, suggesting that crosslinking is involved. They also found formaldehyde and glyoxal treated soybean meal significantly improved gain and feed conversion in

lambs. Dinius et al. (1974a) reported that formaldehyde treatment of a soy protein-safflower oil emulsion reduced ammonia release in vitro. However, protection from ruminal degradation could not be shown for formaldehyde treated soybean meal by Nimrick, Peter and Hatfield (1972), Nishimuta et al. (1973), Schmidt et al. (1973), Schmidt, Benevenga and Jorgensen (1974), or Amos, Burdick and Huber (1974).

Faichney and Davies (1972) reported that formaldehyde treatment of peanut meal in a concentrate diet containing 20% crude protein had no effect on the live-weight gain of early-weaned calves. Nevertheless, the treatment considerably increased the amount of protein digested in the intestines of sheep (Faichney, 1972). This meant that the extra protein reaching the intestines as a result of the treatment was in excess of the calves' requirements. In the 20% crude protein ration, peanut meal made up one-half of the total dietary protein. In a second trial, when a 13% crude protein diet consisting of peanut meal (one-third of total dietary protein) was treated, there was only a small increase in the amount of protein digested in the intestines of sheep and a small improvement in rate of gain in calves. Subsequently, Faichney and Davies (1973) treated all the protein in the diet with formaldehyde to determine if total dietary protein requirements could be reduced with formaldehyde and concluded that this, indeed, was a strong possibility. However, Wachira et al. (1972) reported that formaldehyde treatment of all the dietary protein in a concentrate ration did not significantly affect milk production or milk composition in cows or growth rate in lambs. This could have been due to insufficient protection as rumen ammonia levels were only slightly reduced for the treated ration.

Entwistle (1973) examined the possibility of using formaldehyde treated diets for survival feeding of sheep during droughts. Survival diets composed of either wheat or sorghum grain with meat and bone meal and low quality roughage were fed to sheep in which the grain and the meat and bone meal components were untreated or treated with formaldehyde. The formaldehyde treatment resulted in increased gains but not wool growth over a 20-week period. He also found that a low quality sorghum silage supplement with formaldehyde treated meat and bone meal was no more advantageous than an untreated meat and bone supplement. The lack of response to treatment of meat and bone meal with formaldehyde supports the findings of Rattray and Joyce (1970).

Formaldehyde treatment of ground nut meal (Hughes and Williams, 1971; Miller, 1972) fish meal (Nimrick et al., 1972) linseed meal (Rattray and Joyce, 1970), and cracked safflower seed, cracked sesame seed or sesame meal (Dinius et al., 1974a) have all resulted in successful protection of the protein. Sunflower meal (Dinius et al., 1974a; Amos et al., 1974), cottonseed meal (Saville, Gleeson and McManus, 1971; Langlands, 1971b) and rapeseed meal (Sharma, Ingalls and McKirdy, 1972; Sharma and Ingalls, 1973) when treated with formaldehyde have not resulted in any significant improvements in nitrogen utilization thus far. It is evident from the above discussion that under-protection as well as over-protection can result from formaldehyde treatment. Hemsley, Reis and Downes (1973) reported casein could be rendered virtually indigestible by excessive treatment with formaldehyde. Thus, the concentration of formaldehyde must be determined for each different protein source in order to achieve maximum performance.

Formaldehyde Protected Polyunsaturated Oil-Casein Complexes

Introduction

The development of methods for protecting proteins from degradation in the rumen stimulated the development of a procedure for altering the degree of saturation of milk and body fat in ruminants. Post-ruminal administration of polyunsaturated oils (Ogilvie and McClymont, 1961; Erwin, Sterner and Marco, 1963; Moore et al., 1969a) and intravenous infusion of polyunsaturated oil emulsions (Tove and Mochrie, 1963; Storry and Rook, 1964; Stewart and Irvine, 1970) increased the polyunsaturated fatty acids (PUFA) in milk and/or depot fat of ruminants. Therefore, it seemed likely that if polyunsaturated fats could be protected so as to by-pass the rumen, then the depot and milk fats of ruminants would become more polyunsaturated.

Scott, Cook and Mills (1971) of Australia developed such a technique in response to the concern of some researchers of the possible role of saturated ruminant products upon the incidence of atherosclerosis and coronary heart disease in man. A readily available source of polyunsaturated ruminant products would assist in the formulation of special diets for experimental and therapeutical uses. The technique they developed consisted of encapsulating the PUFA of vegetable oils with protein, such as casein, and then spraying the whole oil-protein complex with formaldehyde. The formaldehyde-protein coat was stable under the neutral conditions of the rumen, but was hydrolyzed upon entering the acidic conditions of the abomasum thereby releasing the PUFA for absorption. The formaldehyde was metabolized and there was no

accummulation of this compound in the carcass or milk (Mills et al., 1972).

In Vitro Evaluations

In vitro incubation of formaldehyde protected linseed oil-casein particles (F-LOC) (1:1 w/w) in which the main PUFA is linolenic resulted in 20% of total fatty acids being linolenic acid at the end of 20 hours compared to only 0.4% for the untreated particles (Scott et al., 1970). Scott, Cook and Mills (1971) reported that polyunsaturated oils coated with casein should contain at least 2% formaldehyde on a protein basis, and that a 1:1 oil-protein ratio is sufficient to achieve marked protection of PUFA in vitro. In all of their in vitro incubations, the formaldehyde treated oil-protein complexes resulted in substantial protection of the PUFA for up to 20 hours. Bitman et al. (1972) found formaldehyde-treated casein-safflower oil (F-SOC) to be completely protected in vitro for up to 24 hours. At 48 hours about 50% of the linoleic acid was converted to either oleic or stearic acids. Untreated SOC was practically all hydrogenated at the end of 24 hours to oleic acid with little change in stearic acid. Dinius et al. (1974b) reported the linoleic content of F-SOC was twice that of the untreated sample. However, at the end of a 21-hour incubation, their values were about two-thirds of those reported by Scott et al. (1971). The reduction in linoleic acid reported by Dinius and coworkers was probably due to the application of heat in preparing the oil-protein complex which resulted in partial oxidation of the linoleic acid. Also, when they treated cracked kernels of safflower seed with formaldehyde in vitro, a 70% increase in linoleic acid was noted for the treated sample. However,

when formaldehyde treatment was applied to cracked sesame kernels and incubation carried out, no difference was noted in linoleic acid content between the treated and untreated kernels even though the protein was shown to have been protected by the levels of formaldehyde used.

Effect on Milk Lipids, Yield and Fat

It is now well established that the feeding of a formaldehyde protected vegetable oil-casein supplement rich in linolenic or linoleic acid will increase the PUFA of milk fat in cows (Scott et al., 1970, 1971; Plowman et al., 1972; Cook, Scott and Pan, 1972b; Edmondson et al., 1972; Yoncoskie et al., 1972; King et al., 1972; Wrenn et al., 1973; Bitman et al., 1973; Gooden and Lascelles, 1973; Weyant et al., 1974) and goats (Scott et al., 1971) from 2 to 4% for untreated to 14 to 35% for treated supplements. The compensatory effects that usually resulted in the other major long chain fatty acids were a decrease in palmitic and oleic acids and, to a lesser degree, myristic acid. Stearic acid usually remained fairly stable. Also, Scott et al. (1971) reported that the effects of protected lipids upon milk fatty acids occur within 24 to 48 hours of feeding.

The feeding of formaldehyde protected supplements rich in PUFA also resulted in an increase of 1.0 to 1.5 percentage units in the fat content of milk while milk yield was unaltered (Plowman et al., 1972; Pan, Cook and Scott, 1972; Bitman et al., 1973; Weyant et al., 1974). Plowman et al. (1972) reported that the increase in milk fat content occurred within 24 hours after feeding the protected supplement and persisted for 24 to 48 hours after withdrawal of the ration. In a longer term study of the effects of F-SOC diets, Goering et al. (1972)

noted significant increases in linoleic acid of milk fat with no effects on milk fat content, and concluded that milk high in PUFA could be produced by cows over an extended period of time.

Effect on Plasma and Tissue Lipids

Cook et al. (1972a,b) reported an increase of linoleic acid in all plasma lipids as a result of feeding steers or dairy cows a F-SOC diet, but most markedly in the triglyceride and free fatty acid (not included in the cow study) fractions. This effect was apparent in the steers within two weeks of feeding the maximal level of supplement and was maintained throughout the eight-week period. Linoleic acid content for plasma triglycerides (steers and cows) and free fatty acids (steers) at the commencement of feeding was 8 to 10% and this was increased to 30 to 40% by supplementation. Compensatory changes in other plasma triglyceride fatty acids were a decrease in oleic and palmitic acids (only in the steers) with stearic acid remaining unaltered. In the steers, linoleic content as a percent of total fatty acids increased from approximately 3% prior to receiving the supplement to about 20% for subcutaneous and 30% for omental and perirenal fats at the end of eight weeks feeding. This agrees with their later work (Faichney et al., 1972). Weir, Yang and Dunkley (1974) reported that the feeding of a protected sunflower diet to lambs increased linoleic acid from 3% to 14% in six weeks. Cook and coworkers noted that changes in the other major fatty acids are the same as stated above for the plasma triglycerides. They also noted that the subcutaneous fat from both supplemented and unsupplemented steers contained more oleic relative to stearic acid than do the deeper body fats. These results are somewhat

contradictory to those they reported in 1970 in that the feeding of a F-SOC supplement to lambs generally increased oleic and decreased stearic acid in the subcutaneous, perinephric and mesenteric fats. Also, the linoleic content in the fat depots of the steers was higher than that achieved in the lambs. Linoleic content of fat depots continued to increase every two weeks in the steers while there was little difference shown in the fat depots of lambs fed a similar supplement for six weeks compared to those fed for only three weeks. However, Faichney et al. (1972) and Faichney, Scott and Cook (1973) reported linoleic acid increased with continued feeding of a F-SOC diet, but the rate of increase followed a curve of diminishing increments. They noted that the largest increase in linoleic acid was noted at the end of two weeks and only moderate increases occurred thereafter.

Wrenn et al. (1973) fed four four-day-old Holstein calves milk high in linoleic acid (14.1%) for 10 weeks. This was followed by the feeding of a F-SOC or SOC diet to the calves for an additional seven weeks. Both the F-SOC and SOC fed groups contained two calves previously fed polyunsaturated milk and two calves previously fed normal milk. Tailhead fat biopsies taken at the end of 10 weeks showed linoleic acid increased from 3% for the calves receiving normal milk to 12% of total fat for those receiving the polyunsaturated milk, and only slight further increases were shown at slaughter time (18 weeks). This diminishing increment agrees well with Faichney et al. (1972, 1973). Goering et al. (1972) and Weir et al. (1974) have also reported significant increases in linoleic content of tailhead fat as a result of feeding cows protected lipid. At slaughter, omental and perirenal fats showed changes in linoleic content similar to the tailfat

biopsy samples (Wrenn et al., 1973). As the exposure to dietary polyunsaturates increased, linoleic acid in the depot fats also increased. Growth and health of the calves were normal.

Growing-finishing steers (230 kg.) fed a F-SOC diet for 48 days had significantly higher amounts of linoleic acid in biopsy tailhead adipose tissues, subcutaneous rib fat and kidney fat than those receiving an unprotected supplement (Dinius et al., 1974c). In a second experiment, 474 kg. steers received the above treatments. Linoleic acid content of tailhead fat showed only a slight increase for steers fed F-SOC diet. Dinius et al. (1974a) fed a F-SOC diet to finishing cattle for 56 days at levels of 0, 10, 20 and 30% of the diet. Average daily voluntary intake and gain were markedly reduced for the two higher levels. Linoleic acid in the tailhead fat of cattle fed F-SOC diet increased from 3.7% to 5.8% in 56 days. There was no difference among treatments in linoleic percentage in omental and kidney fat, but all casein-oil treatments had significantly higher percentages of linoleic in subcutaneous rib fat and intramuscular loin and round fat than cattle fed no casein-oil.

Formaldehyde Treatment of Whole Soybeans

Hutjens and Schultz (1971) noted no difference in linoleic acid content of plasma or milk lipids of lactating goats when formaldehyde treated ground, whole soybeans (3 ml/100 g) and untreated whole soybeans were compared. No differences in total fecal lipid or fecal fatty acids occurred between treatment groups suggesting overprotection was not the cause of a lack of response. Bitman et al. (1972) compared formaldehyde treated and untreated ground, whole soybeans, soy flour and soy

flakes. Increases in linoleic acid were modest compared to those achieved by feeding F-SOC diets. Linoleic acid increased from 3% to only 7% for the formaldehyde treated whole soybeans and from 2 to 4% for the formaldehyde treated soy flakes with even smaller changes noted for the formaldehyde treated soy flour diet. Formaldehyde treated and untreated full-fat soy flour was fed to lactating cows as a supplement at the level of 3.6 kg. per day by Palmquist and Mattos (1973) and Mattos and Palmquist (1973). They reported linoleic and linolenic acids in milk fat to be double those of cows which received grain in place of soy flour as a supplement while untreated soy flour resulted in a lesser increase. Also, both milk and fat percents were increased in both soy flour diets.

Organoleptic Evaluation of Polyunsaturated Milk and Meat

Some problems have been noted in processed milk products made from polyunsaturated milk. Milk high in linoleic acid has been criticized for having an oxidized off-flavor (Edmondson et al., 1972; King et al., 1972). Wong et al. (1973) noted that cheddar cheese containing up to 30% linoleic acid possessed body and flavor defects. However, if linoleic content is kept to 12% or lower, these defects are negligible (Edmondson et al., 1972; Wong et al., 1973). Yoncoskie et al. (1972) reported that changes are necessary in processing conditions when making butter or whipping creams from polyunsaturated milk fat.

Dinius, Oltjen and Satter (1974d) conducted an organoleptic evaluation on meat produced by steers receiving treatments of no oil, safflower oil given orally or infused into the abomasum. The drippings from the

cooked meat of steers receiving safflower abomasally contained significantly more linoleic acid than either of the other two treatments. The taste panel found no differences in the aroma of meat, flavor intensity or desirability of fat and lean, nor juice quality or quantity among treatments. Cooked meat from oil-infused steers was rated significantly coarser, less tender and less desirable than meat of steers receiving no oil. The Warner-Bratzler shear test also showed the oil-infused steers to have significantly less tender meat. Dinius, et al. (1974c), in a follow-up study, conducted another organoleptic evaluation of polyunsaturated meat. The results were the same as the earlier study except no differences were shown in texture or tenderness of the polyunsaturated meat.

Summary

Grazing ruminants ingest considerable quantities of polyunsaturated fats, but very small quantities are actually incorporated into their milk and adipose tissues. This is due to the biohydrogenation process that takes place in the rumen by the microbes.

If polyunsaturated oils are administered post-ruminally or are protected by a formaldehyde protein coat, milk and adipose tissues show a substantially higher degree of polyunsaturation. To date, the greatest response has been seen for formaldehyde treated casein-oil complexes. Natural protein complexes, such as whole soybeans, show only slight protection of polyunsaturated fats when treated with formaldehyde. Some milk and fat flavor problems arose as a result of increased polyunsaturation, but apparently meat tissues are free of any undesirable defects.

CHAPTER III

TREATMENT OF WHOLE FAT SOYBEANS OR SOY FLOUR WITH FORMALDEHYDE TO PROTECT THE POLYUNSATURATED FATTY ACIDS FROM BIOHYDROGENATION IN THE RUMEN

Summary

Trial 1

Ground, whole soybeans (GSB) were treated in small quantities with one of two levels (5.1 or 10.2 ml/100 gm GSB) of 37% formaldehyde (HCHO) for 30 minutes, two hours, or six hours. Degradation by ruminal bacteria *in vitro* of the polyunsaturated fatty acid (PUFA), linoleic, in the treated meals was measured. Based on these results, two treatments (5.1/30 minutes and 10.2/6 hours) were chosen for use in a tissue composition, organoleptic and growth study on lambs. This involved treating the GSB with HCHO in large, manually mixed batches for comparison with untreated GSB and soybean meal (SBM) supplemented rations.

All GSB treatment levels with HCHO in small quantities showed excellent protection of the PUFA, linoleic, over the 48-hour incubation period. However, the *in vitro* incubations on GSB treated with HCHO in subsequent large batches showed little protection of linoleic acid. Consequently, no significant ($P > .05$) differences were noted in

linoleic acid content of rump or kidney fat between lambs fed the HCHO treated GSB and lambs fed the untreated GSB ration. However, the lambs fed the untreated GSB ration had a significantly ($P < .05$) higher percentage of linoleic acid incorporated into their kidney and rump fat than lambs fed SBM, possibly due to the higher intake of linoleic acid.

In the feeding trial, feed consumption and feed efficiency was significantly ($P < .05$) higher during the last half of the trial for lambs fed all rations. Average daily gain was not significantly ($P > .05$) different between the first and last half of the trial among any of the rations.

Ignoring periods, lambs fed either of the HCHO rations had significantly ($P < .05$) lower daily feed consumption and gain than lambs fed untreated GSB while no significant ($P > .05$) differences were noted in feed efficiency. Lambs fed the untreated GSB had significantly ($P < .05$) lower daily gain than lambs fed SBM.

A six-member taste panel using a triangle test was unable to detect any significant ($P > .05$) differences in flavor of ground loin and no flavor preferences were noted.

Trial 2

In trial 2, full-fat, ground soy flour (GSF) was treated in small quantities with 10.2 ml HCHO/100 gm GSF for two or six hours and the protection of the PUFA, linoleic, from microbial hydrogenation during *in vitro* incubation was measured. The 10.2/2 hour treatment was chosen for the *in vivo* tissue and growth study. The GSF was treated with HCHO in large batches using a mixer.

The HCHO treated GSF was again evaluated using *in vitro* and *in vivo* methods. Samples of kidney knob, rump, shoulder, omental and intramuscular (loin) fat from lambs fed HCHO treated and control (untreated GSF and SBM) rations were compared for linoleic acid incorporation. Digesta samples were also collected at slaughter. Organoleptic evaluations were conducted to examine any effects on the flavor of ground lamb meat.

Excellent protection of the PUFA, linoleic, was observed *in vitro* when GSF was treated with HCHO in small and large quantities. Linoleic acid was significantly increased ($P < .05$) in shoulder, rump, kidney knob and omental fat of lambs fed the HCHO treated as compared to the untreated GSF ration. Intramuscular (loin) fat showed no significant ($P > .05$) increase in linoleic acid in lambs fed the protected GSF ration as compared to the unprotected GSF ration. Lambs fed the untreated GSF ration had significantly ($P < .05$) more linoleic acid in their rump, shoulder, kidney, omental and loin than lambs fed SBM. The digesta samples showed linoleic acid to be equal for the HCHO treated or untreated GSF with some protection against ruminal hydrogenation. No significant ($P > .05$) differences between rations were noted in daily feed consumption, feed/kg gain or average daily gain. No significant ($P > .05$) differences in loin meat flavor or preference was shown among any of the rations.

Introduction

Recently Scott, Cook and Mills (1971) increased the absorption of polyunsaturated fatty acids (PUFA) in ruminants by encapsulating polyunsaturated oils with a protein (casein) coat and subsequently spraying

this complex with formaldehyde (HCHO). This coating rendered the protein-oil complex resistant to rumen microbial hydrogenation. This complex breaks down in the abomasum releasing the PUFA for absorption in the small intestine.

This coating procedure has been shown to increase the PUFA content of milk fat (Scott et al., 1970, 1971; Cook et al., 1972b; Plowman et al., 1972) and adipose tissues (Cook et al., 1970, 1972a; Scott et al., 1971; Faichney and Davies, 1972, 1973) when fed as a supplement to ruminants. Dinius and coworkers (1974c,d) of the USDA compared meat high in PUFA with normal meat in two taste panel trials and found no significant differences between the two types of meat.

Since the above procedure for protecting polyunsaturated oils may be economically infeasible, it was reasoned that whole soybeans might constitute a natural protein-oil complex which could be treated with HCHO in a similar manner. The purpose of trial 1 was to evaluate by in vitro and in vivo criteria the protection against ruminal biohydrogenation afforded by HCHO treatment of ground, whole soybeans fed as a protein supplement to growing lambs. Organoleptic evaluations were conducted to determine if any flavor differences in meat could be detected from such treatments.

Unsatisfactory protection of the PUFA was obtained in trial 1, which was presumably related to particle size and inadequate penetration of HCHO into the ground soybeans. Therefore, in trial 2, full-fat soy flour, which has a much smaller particle size, was treated with HCHO. In vitro tests were conducted to evaluate the effectiveness of HCHO treatment prior to feeding in an in vivo growth and tissue study.

Further, organoleptic tests were conducted to determine if any detectable flavor differences existed.

Materials and Methods

Preparation of Products

In trial 1, ground, whole soybeans were treated with formaldehyde (HCHO) on a small and large batch basis. The small batch preparation consisted of grinding whole soybeans through a 1 mm screen in a Wiley mill and treating approximately 100 gm sub-samples with either 5.1 or 10.2 ml of 37% HCHO along with sufficient water to cover the sample. The soybean-water mixture was allowed to stand for 30 min, 2 hr or 6 hr after which time the excess liquid was poured off. The treated soybeans were placed in a forced air oven overnight (100° C) to dry and reground for *in vitro* evaluation.

For the animal study, whole soybeans were ground through a 4.76 mm screen in a hammermill and treated with HCHO in approximately 20 lb batches. The treatments consisted of 5.1 ml of 37% HCHO for 30 min (5.1/30 min) and 10.2 ml of 37% HCHO for 6 hr (10.2/6 hr) per 100 gm GSB along with sufficient water to cover the soybeans. The soybeans, HCHO and water were mixed in buckets manually. After removing the excess water, the treated soybeans were placed in a forced air dryer until dry. The soybeans were then reground and subsamples used for *in vitro* evaluation.

In trial 2, full-fat soy flour[1] was treated with HCHO on a small

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[1]Courtesy of Archer Daniels Midland Co., Decatur, Illinois.

scale and large scale basis. Approximately 100 gm samples of soy flour were treated with 10.2 ml of 37% HCHO for either 2 or 6 hr along with sufficient water to cover the flour. The excess water was poured off and the samples were placed in a forced air oven overnight to dry. The dried samples were ground in a Wiley mill through a 1 mm screen and subsamples were tested in vitro for protection against microbial hydrogenation.

Large quantities of soy flour were treated with HCHO for use in the tissue and growth study. Approximately 20 lb batches of soy flour were treated with 10.2 ml of HCHO per 100 gm GSF for 2 hr (10.2/2 hr). The soy flour along with HCHO and sufficient water to cover were mixed in a Hobart mixer. The treated soy flour was dried in a forced air dryer and ground through a 4.76 mm screen in a hammermill. Samples were taken for in vitro evaluation of the protection afforded the polyunsaturated fatty acid, linoleic, against biohydrogenation.

In Vitro Methods

One hundred ml of rumen fluid, obtained from a fistulated steer being fed a high concentrate ration and strained through several layers of cheese cloth, along with 150 ml of "Ohio" (Johnson, 1969) in vitro fermentation media were added to 5 gm samples of HCHO treated or untreated samples. Incubation proceeded under continuous carbon dioxide gasing of flasks in a 39° C water bath according to the procedures outlined by Johnson (1969). In trial 1, 20 ml samples were removed and frozen at 0, 24 and 48 hrs of incubation in the first in vitro study with additional samples removed at 6 and 12 hr in the second in vitro study. In trial 2, 20 ml samples were removed at 0, 12, 30 and 48 hr

of incubation in the first *in vitro* study and 0, 12, 24 and 48 hr in the second *in vitro* study and stored in a freezer.

In Vivo Feeding Trial

In trial 1, twenty Western wether lambs weighing an average of 24.0 kg (range 18.0 to 28.2 kg) were randomly assigned to one of four rations with five lambs per ration. The ration treatments as shown in Table I compared: (1) soybean meal (SBM), (2) ground, whole soybeans (GSB), (3) ground, whole soybeans treated with 5.1 ml HCHO for 30 min (5.1/30 min) and (4) ground, whole soybeans treated with 10.2 ml HCHO for 6 hr (10.2/6 hr) as the supplemental protein source. The SBM and GSB rations served as controls. The rations were 70% concentrate and contained 8% digestible protein. Treated GSB were substituted for untreated GSB on an equal weight basis.

Lambs were assigned to individual pens at random with water available at all times. They were allowed to eat *ad libitum* and feed refused was weighed back periodically. The lambs were weighed every two weeks with feed being withheld that day until weights were taken and all lambs[2] were slaughtered when the majority had reached market weight of approximately 45 kg. Lambs fed the SBM and 5.1/30 min rations were on trial for 84 days while lambs fed the GSB and 10.2/6 hr rations were on trial 91 days.

Omental fat samples were taken at time of slaughter. The carcasses were chilled at 0° C and within a few days samples were taken of the rump, shoulder and kidney knob fat depots and frozen. The loins were

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[2]One lamb on the GSB ration in trial 1 was not slaughtered as it had an abnormal growth rate.

TABLE I

RATION COMPOSITION[1]

Ingredient	SBM	GSB	5.1/30 min	10.2/6 hr
	%	%	%	%
Corn	59.6	57.2	57.2	57.2
Soybean meal	9.2	--	--	--
Ground, whole soybeans[2]	--	11.5	11.5	11.5
Cottonseed hulls	29.8	29.8	29.8	29.8
Calcium carbonate	0.8	0.8	0.8	0.8
Salt	0.5	0.5	0.5	0.5
Vitamin A (30,000 IU/gm), gm/ton	25.0	25.0	25.0	25.0
Vitamin D (15,000 IU/gm), gm/ton	10.0	10.0	10.0	10.0

[1]Dry matter basis.

[2]Treated as shown.

removed, cut lengthwise and stored in a freezer pending organoleptic evaluation.

In trial 2, nine Western rams weighing an average of 33.5 kg (range 27.6 to 36.5 kg) were randomly assigned to one of three rations with three lambs per ration. The ration treatments as shown in Table II compared: (1) SBM, (2) ground soy flour, and (3) ground soy flour treated with 10.2 ml HCHO for 2 hr (10.2/2 hr) as the supplemental protein source. SBM and GSF were the control rations. Substitution of treated GSF for untreated GSF was on an equal weight basis. The rations were 70% concentrate and contained 8% digestible protein. Lambs were on the feeding trial for 50 days.

TABLE II

RATION COMPOSITION[1]

Ingredient	SBM	GSF	10.2/2 hr
	%	%	%
Corn	59.6	57.2	57.2
Soybean meal	9.2	--	--
Soy flour	--	11.5	11.5
Cottonseed hulls	29.8	29.8	29.8
Calcium carbonate	0.8	0.8	0.8
Salt	0.5	0.5	0.5
Vitamin A (30,000 IU/gm), gm/ton	25.0	25.0	25.0
Vitamin D (15,000 IU/gm), gm/ton	10.0	10.0	10.0

[1]Dry matter basis.

All the other procedures involved with the growth study were identical to trial 1. At slaughter, fat samples were collected as in trial 1 with the addition of an intramuscular (loin) fat sample. Also, the gut was sectioned off immediately post-slaughter and samples of the rumen, abomasal and small intestinal contents were collected and frozen. The loin was also removed as described in trial 1 for organoleptic evaluation.

Chemical Methods

The in vitro incubation and tissue samples were lyophilized and extracted in chloroform:methanol (2:1 v/v). Extracts were evaporated to dryness on a rotary evaporator and the lipids were transferred to 50 ml of petroleum ether and placed in a freezer for storage. The triglyceride was hydrolyzed and methyl esters of fatty acids were prepared by transmethylation by placing one ml of lipid extract and 10 ml of 1% sulfuric acid in methanol into a small Erlenmeyer flask and boiling on a steam bath until the volume was reduced to 2 to 3 milliliters. To this reduced volume, 10 ml of glass distilled water, 10 ml of petroleum ether and two drops of a methyl red indicator solution were added. The flask was swirled and allowed to stand overnight.

The following day the petroleum ether layer was poured off into a vial and again placed on a steam bath to reduce the volume to a small quantity. Nitrogen was then used to finish evaporating the sample to dryness. One hundred µl of heptane were added to the lipid as a solvent.

One-half µl samples of the heptane solution were injected into a

Bendix, Series 2500 gas chromatograph[3] for long chain fatty acid analysis. A glass U shaped column 183 cm in length with an inside diameter of 2 mm was packed with 15% DEGS on Chromosorb W, 80/100 mesh.[4] Flow rates of nitrogen (carrier gas), hydrogen and air were maintained at 30, 25 and 1.8 cc/min, respectively. Temperature of the column was 170° C while inlet and detector temperatures were 220 and 250° C, respectively. Peak areas measured by an Autolab 6300 digital integrator[5] were compared to areas recorded for known standards of pure fatty acids. Weight percents of stearic, oleic, linoleic and linolenic fatty acids were calculated on the in vitro samples and myristic, palmitic, palmitoleic (except in trial 1), stearic, oleic, linoleic, and linolenic fatty acids were calculated on the tissue samples. Attempts to separate or quanitate cis-trans and trans-trans isomers were unsuccessful. All levels of fatty acids reported are as a percentage of total C_{18}, and thereby ignore any bacterial lipolysis.

Organoleptic Procedures

One of the loin halves was boned and all excess fat was trimmed. All the loins from lambs receiving the same ration were composited and ground--first coarsely and then finely. Treatment comparisons in trial 1 were made between: (1) SBM--GSB, (2) SBM--5.1/30 min, (3) SBM--10.2/6 hr, (4) GSB--5.1/30 min and (5) GSB--10.2/6 hr and in trial 2 comparisons were made between: (1) SBM--GSF, (2) SBM--10.2/2 hr and (3) GSF--10.2/2

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[3]The Bendix Corporation, Ronceverte, W. Va.

[4]Supelco, Inc., Bellefonte, Pa.

[5]Vidar Autolab, Mt. View, Calif.

hr with four replications per treatment comparison. The ground lamb was baked in 125 gm loaves wrapped in aluminum foil to an internal temperature of 65° C (71° C in trial 2) in a 163° C oven and served to a taste panel of three females and three males using the triangle test. The panel members were asked to check their preference for either the pair or single sample and then rank both of them on a scale from highly unacceptable to highly acceptable. The tests were conducted mid-morning and/or mid-afternoon with two replicates per sitting. Individual booths were used and bread and water was used to refresh the taste buds between samplings. Since evaluation was to be based only on flavor and not color, the room had low intensity, uniform red lighting. Also, in trial 2, samples of the cooked and uncooked meat from each ration treatment were prepared for fatty acid analysis by gas chromatography.

Statistical Methods

No statistical analyses were conducted on the *in vitro* data since duplication within incubations or replication was not made. The analyses of tissue samples in trial 1 were statistically analyzed by regression as described by Snedecor and Cochran (1967). The tissue samples in trial 2 and the growth study data in both trials were analyzed using analysis of variance (AOV) as outlined by Snedecor and Cochran (1967). No statistical analyses were conducted on the fatty acid data of cooked and uncooked meat as composited samples were used. The organoleptic data were analyzed as outlined by Kramer and Twigg (1970). Differences between treatment means on all tissue and growth data were tested for significance using protected least significant difference (LSD) as explained in Snedecor and Cochran (1967).

Results and Discussion

Trial 1

In Vitro Fermentations. A summary of the in vitro data for soybeans treated with formaldehyde (HCHO) on a small scale basis is shown in Table III. The data is also shown in graph form in Figure 1. Linoleic acid, the major C_{18} polyunsaturated fatty acid in soybeans, decreased from an initial value of 48% to 0% within 24 hrs in the untreated GSB while the saturated C_{18} fatty acid, stearic, increased from 8% to 91% during the 48 hr incubation. Such a pattern is expected as the linoleic acid is being hydrogenated to stearic acid.

The GSB treated with 5.1 ml of HCHO showed substantial protection of linoleic acid from the ruminal hydrogenation process. Linoleic acid decreased slightly from 52% to 30% in the 5.1/30 min treatment while stearic increased from 7% to 21% over the 48 hr incubation period. Oleic acid increased only slightly during this period. The 5.1/2 hr and 5.1/6 hr treatments resulted in stearic, oleic and linoleic acids remaining fairly stable during the whole incubation period. Levels of linolenic acid were too low to measure consistently.

GSB receiving 10.2 ml of HCHO for 30 min showed linoleic acid to be completely protected for 24 hrs from hydrogenation by rumen microbes. At 48 hrs, however, about 50% of the linoleic acid was converted, largely to stearic acid. The 10.2/6 hr treatment resulted in a gradual decrease in linoleic acid over the 48 hr period while stearic and oleic acids showed a corresponding gradual increase. Linolenic acid was present only in minute quantities in both treatments.

TABLE III

IN VITRO C_{18} FATTY ACID BIOHYDROGENATION PATTERN FOR TREATED AND UNTREATED GROUND SOYBEANS PREPARED IN SMALL QUANTITIES

Level of Formaldehyde (ml/100 gm)	Length of Exposure (Hrs)	Incubation (Hrs)	Fatty Acid[1] Weight Percents[2,3] 18:0	18:1	18:2	18:3
0	--	00	7.85	40.15	47.65	4.35
		24	77.05	22.95	0.00	0.00
		48	90.60	9.40	0.00	0.00
5.1	½	00	7.15	41.25	51.60	0.00
		24	18.90	50.55	30.60	0.00
		48	20.80	48.05	29.50	1.60
	2	00	15.80	41.80	42.40	0.00
		24	13.75	45.10	39.80	1.35
		48	10.85	45.80	43.35	0.00
	6	00	17.50	47.20	35.30	0.00
		24	17.30	55.75	26.95	0.00

TABLE III (Continued)

Level of Formaldehyde (ml/100 gm)	Length of Exposure (Hrs)	Incubation (Hrs)	Fatty Acid[1] Weight Percents[2,3] 18:0	18:1	18:2	18:3
		48	19.25	47.65	31.90	1.30
10.2	½	00	8.10	41.35	50.55	0.00
		24	0.00	48.85	51.15	0.00
		48	19.95	54.75	25.30	0.00
	6	00	6.90	41.30	48.70	3.10
		24	12.65	44.15	40.60	2.60
		48	21.90	45.60	28.80	3.70

[1]18:0, stearic; 18:1, oleic; 18:2, linoleic; 18:3, linolenic.

[2]Expressed as a percent of C_{18} fatty acids.

[3]Statistical analysis was not possible as single samples were used.

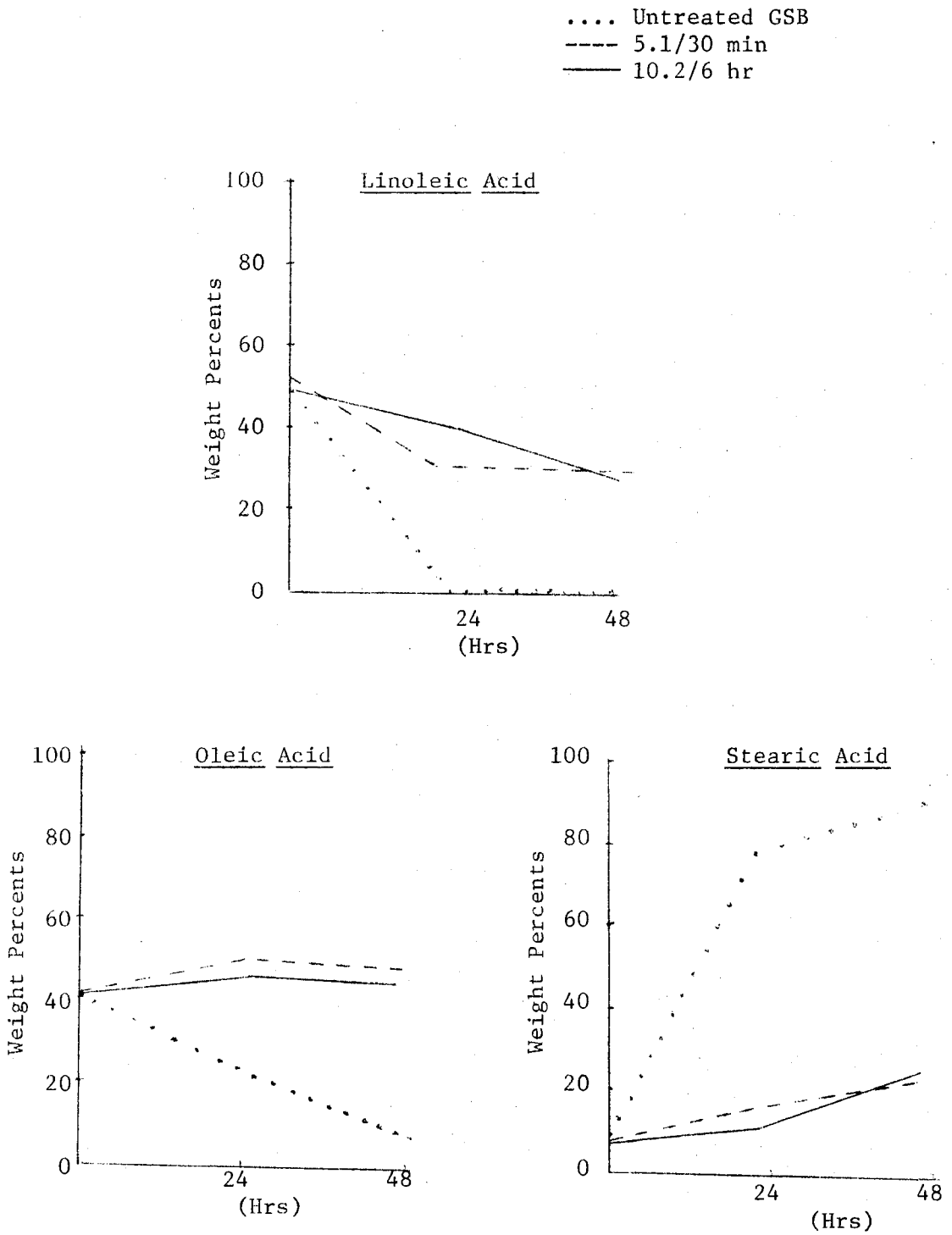

Figure 1. *In Vitro* Biohydrogenation of the 18-Carbon Fatty Acids in Untreated Ground Soybeans and in Ground Soybeans Treated With Formaldehyde in Small Batches

Since linoleic acid was markedly protected from hydrogenation by all the HCHO treatments, the two extreme treatments were chosen for the feeding trial. Table IV and Figure 2 summarize the in vitro incubations conducted on the HCHO treated GSB prepared in large quantities for the in vivo trial. Incubations of the untreated GSB for 48 hrs resulted in a decrease of linoleic acid from 46% to 1% while oleic and stearic increased from 21% to 27% and 29% to 72%, respectively.

GSB receiving the 5.1/30 min treatment showed a decrease in linoleic acid over the 48 hr period from 60% to 10%. Oleic acid remained fairly stable and a sharp increase was shown in stearic acid. Approximately the same pattern of changes in C_{18} fatty acids occurred over the incubation period for the 10.2/6 hr treatment. Again, only small quantities of linolenic acid were detected. Protection of linoleic acid from ruminal biohydrogenation in both of these treatments was quantitatively insignificant as compared with the GSB treated with HCHO in small batches. Failure to protect the linoleic acid in the GSB treated with HCHO on a large scale basis was the result of either: (1) inadequate mixing or (2) excessive particle size for HCHO penetration, resulting in insufficient surface area for adequate HCHO coating.

Tissue Study. The long chain fatty acid composition, expressed as a percentage of the total fatty acids, of the rump fat from lambs fed protected and unprotected GSB is shown in Table V. Rump fat from lambs fed untreated GSB had significantly ($P < .05$) more linoleic acid than rump fat from lambs fed SBM. The lambs on the 5.1/30 min ration had significantly ($P < .05$) less linoleic acid deposited in their rump fat than lambs fed untreated GSB. No significant ($P > .05$) treatment differences were shown for any of the other long chain fatty acids.

TABLE IV

IN VITRO C_{18} FATTY ACID BIOHYDROGENATION PATTERN FOR TREATED AND UNTREATED GROUND SOYBEANS PREPARED IN LARGE QUANTITIES

Level of Formaldehyde (ml/100 gm)	Length of Exposure (Hrs)	Incubation (Hrs)	Fatty Acid[1] Weight Percents[2,3] 18:0	18:1	18:2	18:3
0	--	00	29.20	20.51	46.17	4.12
		06	49.33	15.42	32.76	2.48
		12	67.91	16.20	14.54	1.34
		24	63.42	27.96	6.84	1.78
		48	71.76	26.64	1.38	0.22
5.1	½	00	9.01	25.36	59.86	5.77
		06	43.44	17.31	37.67	1.58
		12	72.36	12.80	14.84	0.00
		24	80.64	12.00	6.56	0.81
		48	66.54	20.72	10.66	2.08
10.2	6	00	11.80	24.72	54.92	8.56

TABLE IV (Continued)

Level of Formaldehyde (ml/100 gm)	Length of Exposure (Hrs)	Incubation (Hrs)	Fatty Acid[1] Weight Percents[2,3] 18:0	18:1	18:2	18:3
		06	44.06	13.75	38.36	3.82
		12	48.22	13.68	34.74	3.36
		24	81.53	12.46	6.01	0.00
		48	79.93	11.08	8.10	0.89

[1]18:0, stearic; 18:1, oleic; 18:2, linoleic; 18:3, linolenic.

[2]Expressed as a percent of C_{18} fatty acids.

[3]Statistical analysis was not possible as single samples were used.

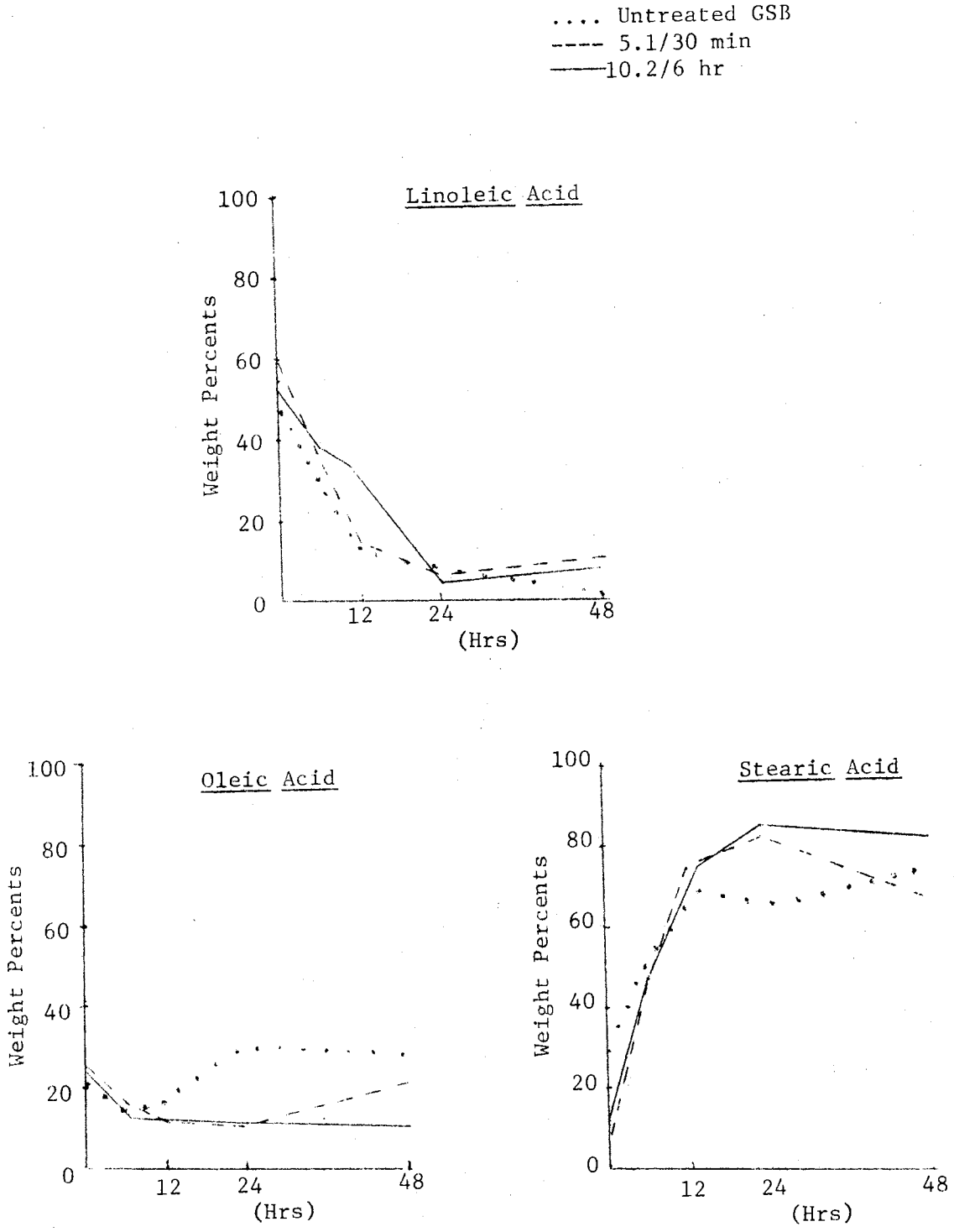

Figure 2. *In Vitro* Biohydrogenation of the 18-Carbon Fatty Acids in Untreated Ground Soybeans and in Ground Soybeans Treated With Formaldehyde in Large Batches

TABLE V

WEIGHT PERCENTS OF THE MAJOR FATTY ACIDS IN RUMP FAT OF GSB TRIAL

Rations	14:0[1,2]	16:0	18:0	18:1	18:2	18:3
SBM	3.52[a]	22.88[a]	17.92[a]	38.21[a]	5.85[a]	0.54[a]
GSB[3]	4.85[a]	21.65[a]	19.16[a]	36.08[a]	10.56[b]	1.30[a]
5.1/30 min	4.08[a]	23.67[a]	16.54[a]	38.12[a]	6.53[a]	1.73[a]
10.2/6 hr	4.46[a]	22.66[a]	18.24[a]	35.46[a]	8.82[a,b]	1.20[a]

[1]14:0, myristic; 16:0, palmitic; 18:0, stearic; 18:1, oleic; 18:2, linoleic; 18:3, linolenic.

[2]ab: Values in the same column with different superscripts differ significantly ($P < .05$).

[3]Values are the means of four lambs while all other treatment means consist of five lambs.

Long chain fatty acid concentrations in kidney fat are shown in Table VI. Lambs on the untreated GSB ration had significantly ($P < .05$) more linoleic acid in their kidney fat than lambs fed SBM. There were no significant ($P > .05$) differences in linoleic acid content of kidney fat from lambs fed either of the HCHO treated GSB and those receiving the untreated GSB indicating failure of protection of linoleic acid in GSB treated with HCHO. No significant ($P > .05$) differences were shown for any of the other fatty acids.

TABLE VI

WEIGHT PERCENTS OF THE MAJOR FATTY ACIDS IN KIDNEY FAT OF GSB TRIAL

	14:0[1,2]	16:0	18:0	18:1	18:2	18:3
SBM	3.21[a]	23.44[a]	28.93[a]	31.00[a]	5.48[a]	1.51[a]
GSB[3]	3.52[a]	19.71[a]	30.66[a]	30.00[a]	9.73[b]	0.88[a]
5.1/30 min	3.65[a]	21.39[a]	23.75[a]	32.00[a]	8.65[b]	2.03[a]
10.2/6 hr	3.16[a]	21.88[a]	28.89[a]	30.18[a]	8.03[a,b]	1.31[a]

[1]See Table V for fatty acid nomenclature.

[2]ab: Values in the same column with different superscripts differ significantly ($P < .05$).

[3]Values are the means of four lambs while all other treatment means consist of five lambs.

The failure of the HCHO treated GSB to increase the linoleic acid content of the rump and kidney fat of lambs is in agreement with the *in vitro* study conducted on the treated and untreated GSB used in these rations. Hutjens and Schultz (1971) fed dairy cows HCHO treated GSB (3 ml HCHO/100 gm of soybeans) and noted no significant differences in milk linoleic acid, plasma triglyceride linoleic acid content, total fecal lipids or fecal fatty acids between SBM, untreated GSB and HCHO treated GSB rations. Bitman *et al.* (1973) mixed GSB with HCHO (10% of GSB weight) in a horizontal feed mixer and when fed to dairy cows, milk linoleic acid doubled from 3% to 6 to 7% of total milk fat. This is a small increase when compared with 20 to 30% increases above the controls

for HCHO protected casein-safflower oil supplements fed to dairy cows (i.e., Scott et al., 1971; Bitman et al., 1973).

Growth Study. Lambs fed the HCHO treated rations consumed significantly ($P < .05$) less feed per day (Table VII) and, consequently, gained significantly ($P < .05$) less per day than lambs fed untreated GSB. No significant ($P > .05$) difference in feed efficiency (feed/kg gain) was noted between lambs fed the HCHO treated rations and lambs fed the untreated GSB ration. No significant ($P > .05$) differences were observed between lambs fed GSB and lambs fed SBM for daily feed consumption or feed efficiency. However, lambs fed the SBM ration gained significantly ($P < .05$) more per day than lambs fed GSB.

TABLE VII

GROWTH PERFORMANCE DATA ON LAMBS IN TRIAL 1 SHOWING TREATMENT EFFECTS

Ration	Daily Feed, kg[1]	Feed/Gain, kg	Daily Gain, kg
SBM	1.38[a]	5.94[a]	0.24[a]
GSB[2]	1.29[a]	6.47[a,b]	0.21[b]
5.1/30 min	1.06[b]	6.84[a,b]	0.16[c]
10.2/6 hr	1.02[b]	7.24[b]	0.14[c]

[1]abc: Values in the same column with different superscripts differ significantly ($P < .05$).

[2]Values are the means of four lambs while all other treatment means consist of five lambs.

Table VIII shows that all lambs consumed significantly ($P < .05$) more feed per day and had a significantly ($P < .05$) higher feed efficiency in the last half of the feeding trial as compared to the first half. No significant ($P > .05$) differences in average daily gain (ADG) were noted between periods 1 and 2.

TABLE VIII

GROWTH PERFORMANCE DATA ON LAMBS IN TRIAL 1
SHOWING FEEDING PERIOD EFFECTS

	Period 1	Period 2
Daily Feed, kg[1]	1.02[a]	1.36[b]
Feed/Gain, kg	5.65[a]	7.62[b]
Daily Gain, kg	0.18[a]	0.18[a]

[1]ab: Values in the same row with different superscripts differ significantly ($P < .05$).

Thus, it appears that the HCHO treated rations were discriminated against by the lambs over the whole feeding trial. Faichney et al. (1972, 1973) have also reported a tendency for both lambs and steers to discriminate against HCHO treated supplements. However, Faichney et al. (1972) reported Mcal intake per kg gain above maintenance in steers to be unchanged by HCHO treatments.

Organoleptic Evaluation. The results of the organoleptic comparisons are shown in Table IX. Flavor intensity did not differ ($P > .05$)

between any of the treatment comparisons. The taste panel members rated the desirability of the flavor for both single and pair samples of the triangle test on a sliding scale from highly unacceptable to highly acceptable. The results compiled only on correctly identified treatment comparisons are shown in Table X. No significant ($P > .05$) differences were shown in flavor preferences among any of the treatments. Similar results have been reported by Dinius et al. (1974c,d).

TABLE IX

FLAVOR INTENSITY OF LOINS FROM LAMBS ON TRIAL 1

Test	# Comparisons	# Correct	Significance
SBM vs GSB	22	10	ns[1]
SBM vs 5.1/30 min	22	8	ns
SBM vs 10.2/6 hr	24	11	ns
GSB vs 5.1/30 min	24	11	ns
GSB vs 10.2/6 hr	24	6	ns

[1]Not significant ($P > .05$).

Trial 2

In Vitro Fermentations. The results of the in vitro fermentations on GSF treated with formaldehyde (HCHO) on a small scale basis are shown in Table XI and Figure 3. Linoleic acid, the major polyunsaturated

fatty acid in soy flour, decreased from 73% at the start of the incubation period to 0% after 48 hrs of incubation. Oleic acid was the chief end product of linolenic and linoleic hydrogenation during the first 30 hrs of incubation after which time stearic acid increased sharply due to the hydrogenation of oleic acid to stearic.

TABLE X

FLAVOR DESIRABILITY OF LOINS FROM LAMBS ON TRIAL 1

Rating	Frequency[1]			
	SBM	GSB	5.1/30 min	10.2/6 hr
Highly Unacceptable	1	0	1	1
Unacceptable	3	1	1	0
Slightly Unacceptable	2	1	2	0
Slightly Acceptable	8	9	9	5
Acceptable	13	15	4	9
Highly Acceptable	2	1	2	2

[1]No significant ($P > .05$) differences among rations for desirability of meat flavor.

GSF treated with 10.2 ml of HCHO for 2 hr showed complete protection of linoleic acid during the 48 hr incubation. Stearic, oleic and linolenic acids also remained stable during this same period.

GSF treated with 10.2 ml of HCHO for 6 hr showed complete protection of linoleic acid for the first 12 hr of incubation. All the other

TABLE XI

IN VITRO C_{18} FATTY ACID BIOHYDROGENATION PATTERN FOR TREATED AND UNTREATED FULL-FAT SOY FLOUR PREPARED IN SMALL QUANTITIES

Level of Formaldehyde (ml/100 gm)	Length of Exposure (Hrs)	Incubation (Hrs)	Fatty Acid[1] Weight Percents[2,3] 18:0	18:1	18:2	18:3
0	--	00	2.88	15.94	72.96	8.22
		12	12.31	83.86	3.83	0.00
		30	18.24	81.28	0.47	0.00
		48	43.67	56.33	0.00	0.00
10.2	2	00	3.20	16.06	73.41	7.32
		30	3.08	19.82	70.54	6.56
		48	2.20	18.15	73.29	6.37
10.2	6	00	4.05	16.13	72.26	7.56
		12	3.37	16.47	73.32	6.84

TABLE XI (Continued

Level of Formaldehyde (ml/100 gm)	Length of Exposure (Hrs)	Incubation (Hrs)	Fatty Acid[1] Weight Percents[2,3] 18:0	18:1	18:2	18:3
		48	4.13	23.36	62.07	10.44

[1]18:0, stearic; 18:1, oleic; 18:2, linoleic; 18:3, linolenic.

[2]Expressed as a percent of C_{18} fatty acids.

[3]Statistical analysis was not possible as single samples were used.

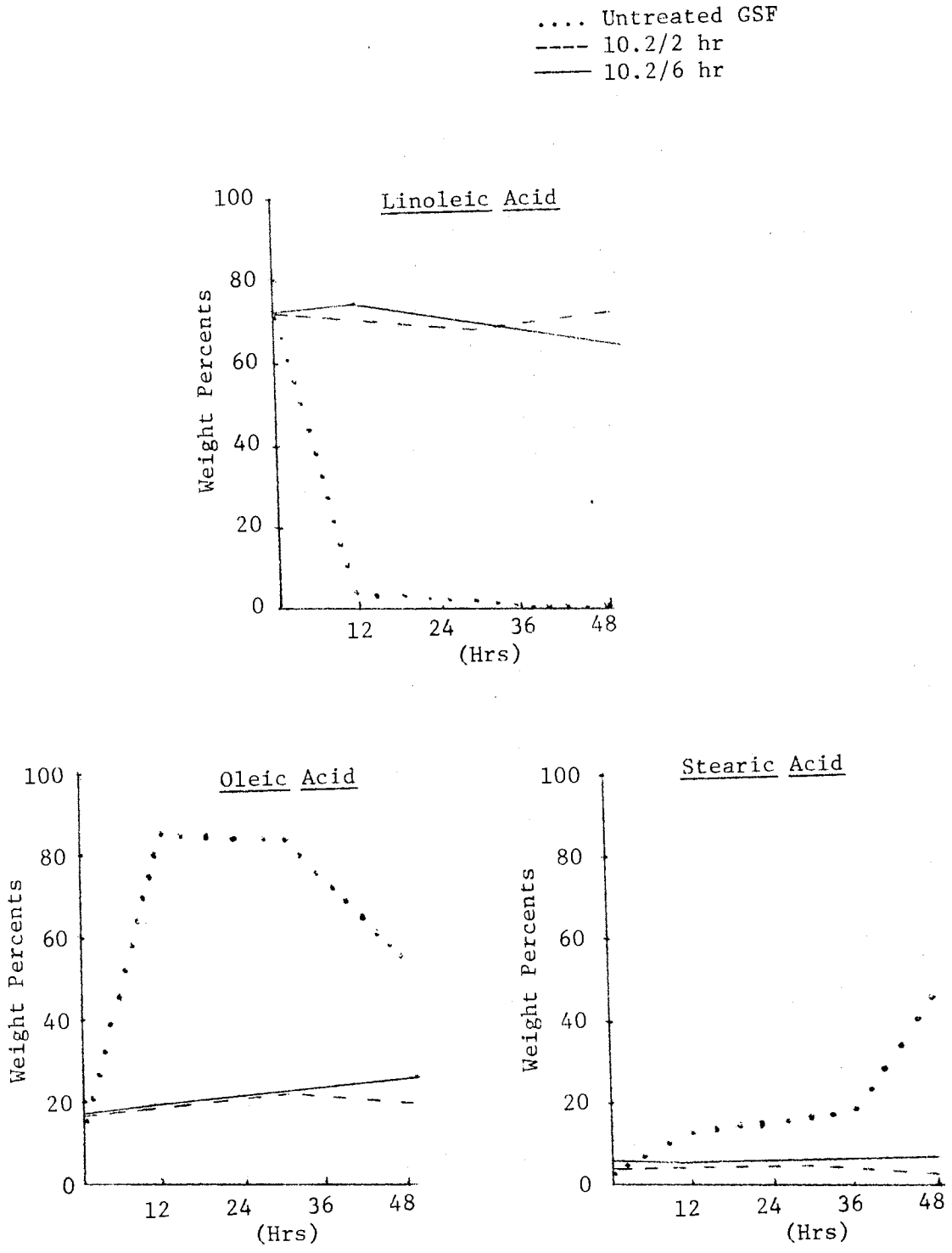

Figure 3. In Vitro Biohydrogenation of the 18-Carbon Fatty Acids in Untreated Ground Soy Flour and in Ground Soy Flour Treated With Formaldehyde in Small Batches

fatty acids remained fairly constant during this period, also. At the end of 48 hrs, linoleic acid showed a slight decrease and oleic a slight increase.

Both HCHO treatments showed excellent protection of linoleic acid from microbial hydrogenation, but the 10.2/2 hr treatment was chosen for the tissue and growth study since no advantage was observed for the longer exposure period.

The in vitro fatty acid results for the GSF treated in large batches are shown in Table XII and Figure 4. The untreated GSF showed linoleic acid to decrease to 2% by the end of 48 hrs while stearic showed a sharp increase over the same period. Oleic acid decreased as did linolenic acid.

However, GSF treated with HCHO (10.2/2 hr) showed linoleic acid to decrease from 65% to only 52% during the first 24 hrs of incubation indicating excellent protection of linoleic acid from microbial hydrogenation. Since 24 hrs represents about the average time that the feed would be expected to stay in the rumen, marked increases in linoleic acid of tissues were anticipated. Linolenic acid also remained stable during the 48 hours. Stearic and oleic acids showed only slight increases during the incubation period.

Tissue Study. Table XIII shows the fatty acid composition of the rump fat from lambs fed the HCHO protected and unprotected GSF for 50 days. Lambs fed the HCHO treated GSF (10.2/2 hr) had significantly ($P < .05$) more linoleic acid than those fed untreated GSF, which in turn, had significantly ($P < .05$) more than those fed SBM. No significant ($P > .05$) differences were noted in myristic, palmitic, palmitoleic, stearic, oleic or linolenic fatty acids in the rump fat of any

TABLE XII

IN VITRO C_{18} FATTY ACID BIOHYDROGENATION PATTERN FOR TREATED AND UNTREATED FULL-FAT SOY FLOUR PREPARED IN LARGE QUANTITIES

Level of Formaldehyde (ml/100 gm)	Length of Exposure (Hrs)	Incubation (Hrs)	Fatty Acid[1] Weight Percents[2,3] 18:0	18:1	18:2	18:3
0	--	12	30.76	49.70	17.62	1.92
		24	61.76	34.84	3.40	0.00
		48	71.38	26.19	2.43	0.00
10.2	2	00	5.61	21.56	64.94	7.90
		12	14.00	27.12	49.87	9.00
		24	13.04	28.74	51.54	6.68
		48	23.52	32.23	37.98	6.28

[1] 18:0, stearic; 18:1, oleic; 18:2, linoleic; 18:3, linolenic.

[2] Expressed as a percent of C_{18} fatty acids.

[3] Statistical analysis was not possible as single samples were used.

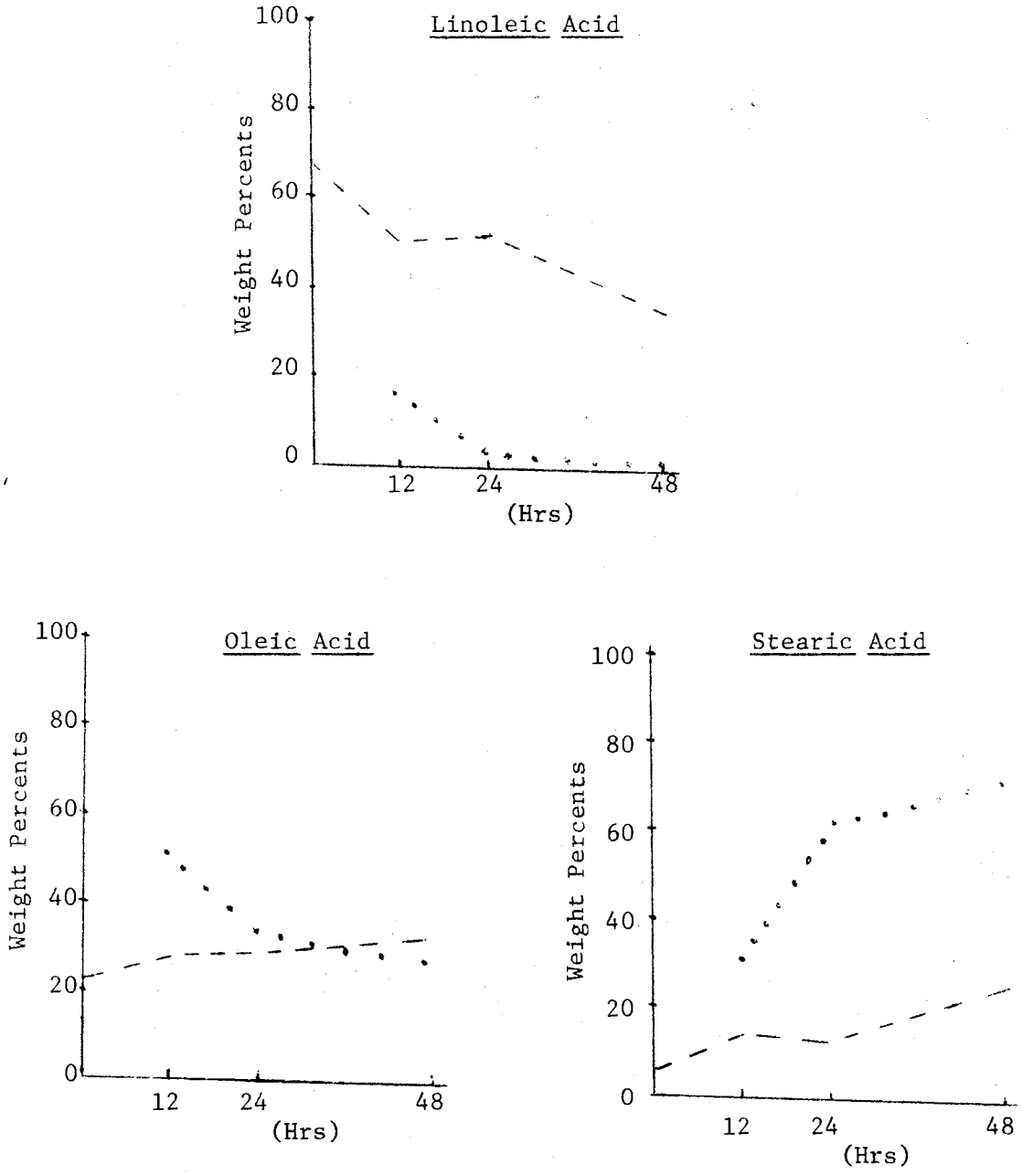

Figure 4. In Vitro Biohydrogenation of the 18-Carbon Fatty Acids in Untreated Ground Soy Flour and in Ground Soy Flour Treated With Formaldehyde in Large Batches

TABLE XIII

WEIGHT PERCENTS OF THE MAJOR FATTY ACIDS
IN RUMP FAT OF GSF TRIAL

Rations	14:0[1,2]	16:0	16:1	18:0	18:1	18:2	18:3
SBM	8.53[a]	23.44[a]	4.38[a]	18.65[a]	40.54[a]	4.31[a]	1.92[a]
GSF	10.96[a]	22.19[a]	5.43[a]	19.17[a]	35.10[a]	6.82[b]	0.33[a]
10.2/2 hr	5.91[a]	21.29[a]	3.65[a]	19.83[a]	35.98[a]	11.86[c]	1.48[a]

[1]14:0, myristic; 16:0, palmitic; 16:1, palmitoleic; 18:0, stearic; 18:1, oleic; 18:2, linoleic; 18:3, linolenic.

[2]abc: Values within columns with different superscripts differ significantly ($P < .05$).

of the lambs.

The effects of feeding lambs HCHO treated GSF upon shoulder fat is shown in Table XIV. Shoulder fat from lambs fed the HCHO treated ration (10.2/2 hr) was significantly ($P < .05$) higher in linoleic acid than those receiving the untreated GSF. Linoleic acid was significantly ($P < .05$) higher in shoulder fat from lambs fed untreated GSF than from those fed SBM. Palmitic acid was significantly ($P < .05$) lower in both the treated and untreated GSF as compared to the SBM. No significant ($P > .05$) differences were observed in myristic, palmitoleic, stearic, oleic, or linolenic fatty acids.

The treating of GSF with HCHO was effective in increasing the polyunsaturated fatty acid (PUFA), linoleic acid, in the subcutaneous depot fats of lambs. The values obtained from the two subcutaneous sites in lambs fed SBM and GSF rations correspond closely to those reported by Cook et al. (1970) for lambs fed a control diet of chopped lucerne hay and wheaten hay. Also, the fatty acid values for the lambs fed the HCHO protected ration agree with the values they reported for lambs fed a HCHO treated casein safflower-oil diet for a period of six weeks. The subcutaneous linoleic acid values for the lambs fed the HCHO treated GSF ration also agree with those reported by Weir et al. (1974) from lambs fed a HCHO treated casein-safflower seed diet for six weeks. However, some other workers have reported even greater increases in subcutaneous linoleic acid of sheep. Faichney et al. (1973) in lambs and Scott et al. (1971) in mature sheep reported increases in subcutaneous fat from 3% for controls to 20 to 30% by feeding HCHO treated casein safflower-oil supplements over a six week period.

TABLE XIV

WEIGHT PERCENTS OF THE MAJOR FATTY ACIDS
IN SHOULDER FAT OF GSF TRIAL

Rations	14:0[1,2]	16:0	16:1	18:0	18:1	18:2	18:3
SBM	7.86[a]	25.81[a]	4.90[a]	14.43[a]	40.83[a]	4.59[a]	1.58[a]
GSF	6.02[a]	21.34[b]	3.54[a]	16.20[a]	41.91[a]	9.28[b]	1.70[a]
10.2/2 hr	5.69[a]	21.57[b]	3.94[a]	15.28[a]	39.60[a]	11.51[c]	2.42[a]

[1]See Table XIII for fatty acid nomenclature.

[2]abc: Values within columns with different superscripts differ significantly ($P < .05$).

The fatty acid composition of kidney fat is shown in Table XV. Linoleic acid was significantly ($P < .05$) higher in kidney fat from lambs fed the 10.2/2 hr ration as compared to lambs fed the GSF ration. However, the lambs fed the GSF ration had significantly ($P < .05$) more linoleic acid than those fed SBM. Kidney fat from lambs fed the 10.2/2 hr ration also had significantly less ($P < .05$) myristic, palmitic and linolenic acids than lambs fed the untreated GSF ration. Palmitic acid was significnatly lower ($P < .05$) and linolenic sigaificantly ($P < .05$) higher in kidney fat from lambs on the GSF ration as compared to those on the SBM ration. Although palmitoleic acid in kidney fat from lambs on the GSF ration was significantly ($P < .05$) lower than for lambs on the other two rations, palmitoleic acid is present in ruminant tissues in such small quantities that consistent analysis on the gas chromatograph is difficult. No significant ($P > .05$) differences were noted in oleic and stearic acids.

Table XVI shows the fatty acid composition of omental fat. Lambs fed the 10.2/2 hr ration had significantly ($P < .05$) more linoleic acid in their omental fat than lambs fed the untreated GSF ration. However, lambs fed the GSF ration had significantly ($P < .05$) more linoleic acid than lambs fed SBM. Palmitic acid was significantly ($P < .05$) lower in both the HCHO treated and untreated GSF rations as compared to SBM. No significant ($P > .05$) differences were observed in myristic, palmitoleic, stearic, oleic or linolenic acids of omental fat.

Bitman et al. (1973) treated GSF with HCHO (15% by weight of soy flour) using a liquid solid blender and noted only about a 1% increase in linoleic acid of milk fat when fed for five days as compared to a 20 to 30% increase obtained from casein safflower oil supplements fed for a

TABLE XV

WEIGHT PERCENTS OF THE MAJOR FATTY ACIDS
IN KIDNEY FAT OF GSF TRIAL

Rations	14:0[1,2]	16:0	16:1	18:0	18:1	18:2	18:3
SBM	5.92[a]	22.41[a]	2.95[a]	30.57[a]	31.97[a]	4.94[a]	1.23[a]
GSF	5.52[a]	20.14[b]	0.00[b]	25.72[a]	31.93[a]	12.08[b]	4.60[b]
10.2/2 hr	3.25[b]	17.17[c]	2.43[a]	28.78[a]	31.57[a]	15.26[c]	1.55[a]

[1]See Table XIII for fatty acid nomenclature.

[2]abc: Values in columns with different superscripts differ significantly ($P < .05$).

TABLE XVI

WEIGHT PERCENTS OF THE MAJOR FATTY ACIDS
IN OMENTAL FAT OF GSF TRIAL

Rations	14:0[1,2]	16:0	16:1	18:0	18:1	18:2	18:3
SBM	8.84[a]	24.44[b]	4.52[a]	23.58[a]	32.55[a]	5.96[a]	0.52[a]
GSF	7.57[a]	20.38[a]	4.33[a]	24.17[a]	29.56[a]	11.50[b]	2.50[a]
10.2/2 hr	6.45[a]	18.86[a]	4.01[a]	22.54[a]	28.76[a]	15.07[c]	4.30[a]

[1]See Table XIII for fatty acid nomenclature.

[2]abc: Values in the same column with different superscripts differ significantly ($P < .05$).

similar length of time. Mattos and Palmquist (1973) treated GSF with 5% HCHO and noted linoleic acid to increase from 10% in milk fat from cows fed untreated GSF to 16% in milk fat from cows fed the protected supplement. Bitman and coworkers concluded that either the soy flour linoleic acid was overprotected or that the physical nature of the oil and protein in the soybean structure prohibited protection comparable to that achieved using the Australian coating technique.

The subcutaneous fat from all groups of lambs contained more oleic relative to stearic acid than did the deeper body fats (kidney and omental) which agrees with the work of others (Garton and Duncan, 1967). However, the deeper body fats tended to reach higher levels of linoleic acid incorporation than the subcutaneous sites. This is consistent with the work of Faichney et al. (1972), Cook et al. (1972a) and Wrenn et al. (1973) in steers. Duncan and Garton (1967) have suggested that the internal tissues preferentially assimilate the fatty acids of chylomicron triglycerides which, in ruminants, are normally high in stearic acid. Cook et al. (1972) reported that a large amount of linoleic acid absorbed from the small intestines of supplemented steers is incorporated into chylomicron triglycerides. Thus, preferential uptake of these triglycerides by internal tissues is consistent with the differences observed between tissue locations in incorporation of linoleic acid.

Associated with increases in linoleic acid as a result of feeding HCHO protected GSF were overall trends for decreases in myristic and/or palmitic acid in all tissues analyzed. Other workers who have increased linoleic acid in fat depots by feeding a HCHO protected casein safflower complex have also noted consistent decreases in these two fatty acids (Cook et al., 1970, 1972a; Scott et al., 1971; Dinius et al., 1974c).

The decrease in palmitic acid can be explained by the higher intakes of fat by the lambs fed the treated and untreated full-fat CSB or GSF rations as compared to lambs fed SBM. Since palmitic acid is the major fatty acid synthesized by adipose tissues when fat is low in the diet, higher fat intakes would result in a decrease in the amount of fatty acids synthesized (mainly palmitic) in the adipose tissues.

The feeding of protected or unprotected GSF rations to lambs produced no consistent trends in stearic or oleic acid content in any of the depot fats. Variable responses have been shown by other workers for these two fatty acids as a result of depot fat containing a high content of PUFA. Stearic acid remained fairly stable in depot fats with normal and high linoleic acid content in studies by Cook et al. (1970) and Wrenn et al. (1973) while Cook et al. (1970) and Scott et al. (1971) showed stearic acid to decrease with high levels of linoleic acid. Scott et al. (1971) and Cook et al. (1972a) reported oleic acid to decrease while Cook et al. (1970) and Wrenn et al. (1973) reported an increase in oleic acid when depot fats had a high content of linoleic acids as compared to controls.

Table XVII shows the fatty acid composition of intramuscular fat taken from the loin chops. There were no significant ($P > .05$) differences between lambs fed the HCHO treated or untreated GSF rations for any of the fatty acids. Lambs fed the untreated GSF ration had significantly ($P < .05$) less palmitic and palmitoleic acid and significantly ($P < .05$) more linoleic acid than lambs fed SBM. Dinius et al., (1974c,d) have reported 5% of lipid was linoleic acid in the longissimus muscle of steers fed either a HCHO (4 gm/100 gm casein) protected casein safflower oil supplement for 48 days or had

TABLE XVII

WEIGHT PERCENTS OF THE MAJOR FATTY ACIDS
IN LOIN CHOPS OF LAMBS ON GSF TRIAL

Rations	14:0[1,2]	16:0	16:1	18:0	18:1	18:2	18:3
SBM	4.88[a]	26.08[b]	4.30[b]	14.29[a]	43.46[a]	5.65[a]	1.34[a]
GSF	4.58[a]	23.68[a]	3.72[a]	16.08[a]	38.61[a]	12.12[b]	1.21[a]
10.2/2 hr	4.11[a]	22.29[a]	3.52[a]	15.95[a]	39.09[a]	13.56[b]	1.48[a]

[1]See Table XIII for fatty acid nomenclature.

[2]abc: Values in columns with different superscripts differ significantly ($P < .05$).

safflower oil injected abomasally for 86 days. Intramuscular linoleic acid values as high as 20 to 25% (compared to 5% in controls) have been reported by Faichney et al. (1972) in steers fed a HCHO casein safflower oil supplement for eight weeks, by Wrenn et al. (1974) in calves fed polyunsaturated milk for 10 weeks followed by seven weeks of a HCHO casein safflower oil supplement and again by Wrenn et al. (1974) in lambs fed a HCHO casein sunflower oil diet for six weeks.

Digesta Study. Table XVIII and Figure 5 show the weight percent of the major fatty acids in the fat of digesta in the rumen, abomasum and small intestines for the various rations. Palmitic acid was significantly ($P < .05$) lower in the abomasum and small intestine of lambs fed SBM as compared to the rumen palmitic acid content. Stearic acid was significantly ($P < .05$) higher in the abomasum of lambs fed SBM as compared to the stearic acid content of the rumen indicating hydrogenation of the linoleic and linolenic acids. Linolenic acid was significantly ($P < .05$) higher in the abomasum and small intestine of lambs fed the HCHO treated ration as compared to the rumen content of linolenic acid. This shows linolenic acid to be effectively protected from hydrogenation. No significant differences ($P > .05$) were observed in ration or location effects for myristic, palmitoleic, oleic or linoleic acids.

Figure 5 shows for all rations a sharp increase in linoleic acid as the digesta passes from the abomasum to the small intestine. Ward et al. (1964) reported both linoleic and oleic acids to be much higher in the ileum section of the small intestine as compared to what it was in the rumen of sheep fed hay diets due to the influx of unsaturated fatty acids via the bile or intestinal mucosa. However, Wrenn et al. (1973) reported a decrease in linoleic acid in the intestinal digesta as

TABLE XVIII

FATTY ACID WEIGHT PERCENTS IN FAT OF DIGESTA OF LAMBS IN TRIAL 2

Ration	Rumen[1]	Abomasum	Small Intestine
		14:0[2]	
SBM[3]	2.96[a,r]	1.40[a,r]	1.58[a,r]
GSF	0.79[a,r]	0.43[a,r]	1.26[a,r]
10.2/2 hr	0.90[a,r]	1.67[a,r]	1.59[a,r]
		16:0	
SBM	18.85[a,r]	10.62[b,r]	12.77[b,r]
GSF	13.71[a,r,s]	10.97[a,r]	11.31[a,r]
10.2/2 hr	11.51[a,s]	11.26[a,r]	10.25[a,r]
		16:1	
SBM	2.17[a,r]	0.33[a,r]	2.70[a,r]
GSF	0.97[a,r]	0.30[a,r]	2.03[a,r]
10.2/2 hr	0.62[a,r]	0.72[a,r]	2.38[a,r]
		18:0	
SBM	37.80[a,c,r]	63.89[b,r]	31.40[c,r]
GSF	26.01[a,r]	25.47[a,r]	17.92[a,r]
10.2/2 hr	28.10[a,r]	27.34[a,r]	18.57[a,r]
		18:1	
SBM	16.18[a,r]	12.48[a,r]	13.86[a,r]
GSF	39.20[a,r]	42.34[a,r]	22.09[a,r]
10.2/2 hr	30.11[a,r]	32.63[a,r]	16.38[a,r]

TABLE XVIII (Continued)

Ration	Rumen[1]	Abomasum	Small Intestine
		18:2	
SBM	$22.03^{a,r}$	$11.28^{a,r}$	$36.93^{a,r}$
GSF	$17.30^{a,r}$	$19.11^{a,r}$	$44.08^{a,r}$
10.2/2 hr	$25.84^{a,r}$	$25.00^{a,r}$	$49.12^{a,r}$
		18:3	
SBM	$0.00^{a,r}$	$0.00^{a,r}$	$0.78^{a,r}$
GSF	$2.00^{a,r,s}$	$1.38^{a,r}$	$1.32^{a,r}$
10.2/2 hr	$2.91^{a,s}$	$1.39^{b,r}$	$1.70^{b,r}$

[1]rst: Values in the same column of each fatty acid with different superscripts differ significantly ($P < .05$).

[2]See Table XIII for fatty acid nomenclature.

[3]abc: Values in the same row with different superscript differ significantly ($P < .05$).

compared to the abomasum in calves fed the HCHO protected and unprotected polyunsaturated diets indicating intestinal absorption of linoleic acid. This inconsistency is not explainable.

Although Figure 5 shows the untreated GSF to be resistant to ruminal hydrogenation, the tissue data showed significantly more linoleic acid to be incorporated into the tissues of lambs fed the HCHO protected supplement as compared to tissues from lambs fed the unprotected supplement. Since no internal marker was used, it is not known

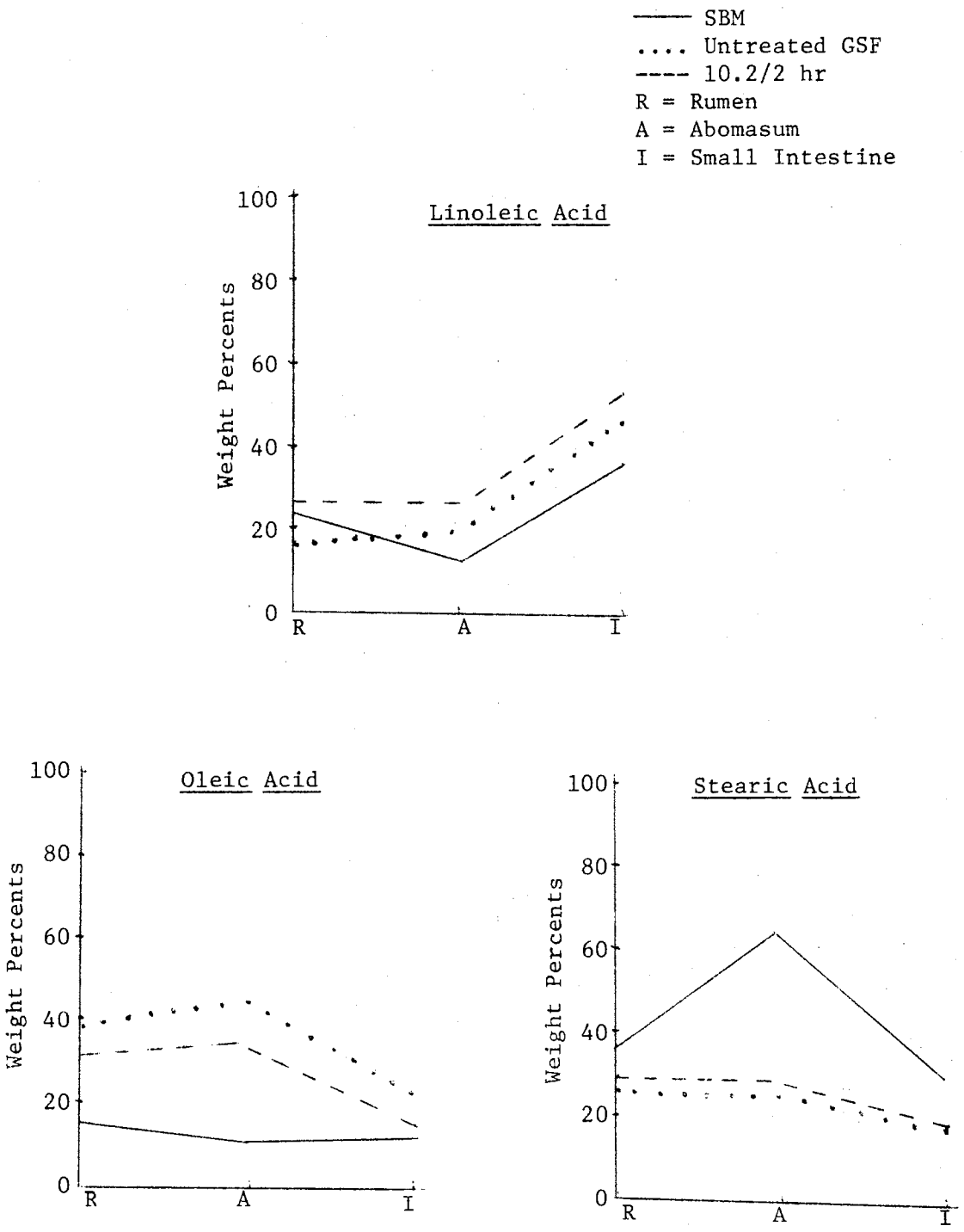

Figure 5. Changes in the 18-Carbon Fatty Acids of the Digesta With Respect to Digestive Tract Sites in Trial 2

how long the digesta has been in these various sections of the gut when the samples were collected. Some lambs may have eaten just prior to slaughter while others may not have eaten for some time. Thus Table XVIII and Figure 5 only give a rough picture of what is actually occurring.

Growth Study. No significant ($P > .05$) differences were observed between the rations for daily feed consumption, feed efficiency (feed/kg gain) or average daily gain (ADG) as is shown in Table XIX. There was a tendency for the lambs on the 10.2/2 hr ration to have a higher feed efficiency and lower gains compared to the controls. Faichney et al. (1972) in steers and Weir et al. (1974) in lambs reported a tendency for animals fed a HCHO treated diet to have better feed efficiencies than animals on the control diet. Faichney et al. (1972) found a tendency for lower gains in steers fed a HCHO protected ration while Weir et al. (1974) found gains to be similar in lambs fed a HCHO protected diet as compared to the controls.

Organoleptic Evaluation. No significant ($P > .05$) differences were found by the taste panel in flavor intensity or desirability among treatments as is shown in Tables XX and XXI. Table XXII shows the fatty acids in cooked and uncooked ground loin for each of the rations. Table XXII shows a tendency for linoleic acid to be higher in the loins from lambs fed the HCHO protected ration as compared to lambs fed the untreated GSF ration. However, Table XVII showed no significant differences in linoleic acid content of intramuscular loin fat between these two rations, and, therefore, the taste panel results would not necessarily be expected to show significant difference between these two rations in flavor intensity due to degree of polyunsaturation. The

HCHO used in treating the feed seemed to have no adverse effects upon flavor. Cooking the meat had no effect upon the fatty acid composition. Dinius et al. (1974c,d) also found essentially no difference in fatty acid composition between cooked and uncooked loin.

TABLE XIX

GROWTH PERFORMANCE FOR LAMBS ON GSF TRIAL

Rations	Daily Feed, kg[1]	Feed/Gain, kg	Daily Gain, kg
SBM	1.38[a]	6.02[a]	0.24[a]
GSF	1.57[a]	5.65[a]	0.28[a]
10.2/2 hr	1.40[a]	8.34[a]	0.18[a]

[1]abc: Values in columns with different superscripts differ significantly ($P < .05$).

TABLE XX

FLAVOR INTENSITY OF LOINS FROM LAMBS ON TRIAL 2

Test	# Comparisons	# Correct	Significance[1]
SBM vs GSF	24	9	ns
SBM vs 10.2/2 hr	24	11	ns
GSF vs 10.2/2 hr	20	10	ns

[1]Not significant ($P > .05$).

TABLE XXI

FLAVOR DESIRABILITY OF LOINS FROM LAMBS ON TRIAL 2

Rating	Frequency[1]		
	SBM	GSF	10.2/2 hr
Highly Unacceptable	1	0	0
Unacceptable	0	2	1
Slightly Unacceptable	1	1	0
Slightly Acceptable	3	7	4
Acceptable	8	4	9
Highly Acceptable	7	5	7

[1]No significant ($P > .05$) differences among rations for desirability of meat flavor.

TABLE XXII

FATTY ACID WEIGHT PERCENTS OF COOKED AND UNCOOKED GROUND LOIN FROM LAMBS ON GSF TRIAL

Fatty Acids[1]	SBM		GSF		10.2/2 hr	
	Uncooked[2]	Cooked	Uncooked	Cooked	Uncooked	Cooked
14:0	5.68	5.80	4.66	4.52	4.31	4.34
16:0	24.18	24.79	22.08	21.50	20.16	20.94
16:1	4.00	3.80	3.45	3.22	3.07	3.15
18:0	16.85	16.51	15.64	16.77	17.89	17.48
18:1	41.98	42.42	42.93	41.77	39.26	39.09
18:2	5.94	5.32	9.56	10.68	13.66	13.05
18:3	1.39	1.35	1.67	1.52	1.65	1.96

[1]See Table XIII for fatty acid nomenclature.

[2]No statistical analysis was conducted as loins were composited according to rations.

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APPENDIX

TABLE XXIII

TRIAL 1: AOV FOR TISSUE FATTY ACIDS

Source	df	MS					
		14:0	16:0	18:0	18:1	18:2	18:3
(Rump)							
Total	37						
Trt[1]	3	2.97	6.11	10.78	19.21	41.84[a]	2.42
Anim/Trt[2]	15	3.51	22.92	44.10	19.14	12.70	1.99
Dup/Anim/Trt	19	0.04	0.53	0.16	0.45	0.19	0.39
(Kidney)							
Total	37						
Trt[1]	3	0.55	21.05	84.50	7.90	30.32[a]	2.07
Anim/Trt[2]	15	2.08	13.44	83.95	12.04	8.65	4.15
Dup/Anim/Trt	19	0.06	0.52	0.53	0.47	0.22	0.31

[1]Treatment MS with superscript is significant ($P < .05$).

[2]Error term used in testing for significant treatment effects.

TABLE XXIV

TRIAL 1: F AND LSD VALUES FOR TISSUE FATTY ACIDS

Fatty Acid	F-Value[1]		LSD[2]			
	Rump	Kidney	Rump[3]		Kidney[3]	
14:0	0.84	0.26	1.89	1.79	1.46	1.37
16:0	0.27	1.56	4.84	4.56	3.70	3.49
18:0	0.24	1.01	6.71	6.33	9.26	8.73
18:1	1.00	0.66	4.42	4.17	3.50	3.31
18:2	3.29[a]	3.50[a]	3.60	3.40	2.97	2.80
18:3	1.21	0.50	1.43	1.34	2.06	1.94

[1]Superscript a denotes significance ($P < .05$).

[2]Least significant difference ($P < .05$).

[3]The first column is to test for significant ($P < .05$) treatment differences between SBM-GSB, GSB-5.1/30 min and GSB-10.2/6 hr while the second column is used to test for treatment differences between SBM-5.1/30 min, SBM-10.2/6 hr and 5.1/30 min-10.2/6 hr.

TABLE XXV

TRIAL 1: AOV FOR GROWTH DATA

Source	df	Daily Feed	Feed/Gain	ADG
Total	37			
Trt[1]	3	0.29^{b}	3.01	$1.94 \times 10^{-2,b}$
Anim(Trt)[2]	15	0.04	1.09	1.11×10^{-3}
Per[1]	1	1.14^{b}	36.93^{b}	4.38×10^{-7}
Trt x Per[1]	3	3.37×10^{-3}	1.58	1.27×10^{-3}
Anim x Per(Trt)[3]	15	0.02	1.51	1.97×10^{-3}

[1]Superscript b denotes significance ($P < .05$).

[2]Error term used to test for treatment effects.

[3]Error term used to test for period and period x treatment interaction effects.

TABLE XXVI

TRIAL 1: F-VALUES FOR GROWTH DATA

	Daily Feed[1]	Feed/Gain[1]	ADG[1]
Treatment	7.42^{a}	2.76	17.52^{a}
Period	54.32^{a}	24.44^{a}	0.0002
Treatment x Period	0.16	1.05	0.64

[1]Superscript a denotes significance ($P < .05$).

TABLE XXVII

TRIAL 1: LSD[1] VALUES FOR GROWTH DATA

	Period	Treatment[2]	
Daily Feed	0.10	0.20	0.19
Feed/Gain	0.85	1.06	0.99
ADG	0.02	0.03	0.03

[1]Least significant difference ($P < .05$).

[2]The first column is to test for significant ($P < .05$) differences between SBM-GSB, GSB-5.1/30 min and GSB-10.2/6 hr while the second column is used to test for differences between SBM-5.1/30 min, SBM-10.2/6 hr and 5.1/30 min-10.2/6 hr.

TABLE XXVIII

TRIAL 1: AOV FOR TASTE PANEL DATA

Source	df	Variance	MS	F-Value
Total	91	110.5543		
Treatments	3	3.9900	1.33	1.10[1]
Residual	88	106.5643	1.21	

[1]Not significant ($P > .05$).

TABLE XXIX

TRIAL 2: AOV FOR TISSUE FATTY ACIDS

Source	df	MS						
		14:0	16:0	16:1	18:0	18:1	18:2	18:3
(Rump)[1]								
Total	17							
Trt[2]	2	38.27	7.02	4.83	14.52	51.22	88.69[a]	4.06
Anim/Trt[3]	6	12.18	2.38	2.59	7.37	15.40	0.83	1.87
Dup/Anim/Trt	9	0.03	0.42	0.02	0.08	0.26	0.21	0.24

[1]AOV for shoulder, kidney, omental and intramuscular (loin) were done in the same manner.

[2]Treatment MS with superscript is significant. a: ($P < .05$).

[3]Error term used in testing for significant treatment effect.

TABLE XXX

TRIAL 2: F AND LSD VALUES FOR TISSUE FATTY ACIDS

	Rump		Shoulder		Kidney		Omental		Loin	
	F[1]	LSD[2]	F[1]	LSD[2]	F[1]	LSD[2]	F[1]	LSD[2]	F[1]	LSD[2]
14:0	3.14	4.93	2.07	2.81	7.01[a]	1.88	0.84	3.77	3.42	0.72
16:0	2.95	2.18	13.71[a]	2.35	43.10[a]	1.38	17.18[a]	2.40	9.29[a]	2.17
16:1	1.86	2.27	2.10	1.65	108.67[a]	0.53	0.10	2.74	8.46[a]	0.48
18:0	1.97	3.84	0.72	3.60	2.83	5.04	0.46	4.21	1.10	3.29
18:1	3.33	5.54	1.07	3.86	0.10	2.41	0.88	7.38	2.60	5.73
18:2	107.35[a]	1.29	127.46[a]	1.08	43.41[a]	2.77	39.61[a]	2.53	6.23[a]	5.84
18:3	2.17	1.93	0.98	1.58	194.79[a]	0.47	4.91	2.96	0.69	0.58

[1]Superscript a denotes significance ($P < .05$).

[2]Least significant difference ($P < .05$).

TABLE XXXI

TRIAL 2: AOV FOR GROWTH DATA

Source	df	MS		
		Daily Feed	Feed/Gain	ADG
Total	8			
Trt[1]	2	0.032	6.38	6.68×10^{-3}
Anim(Trt)[2]	6	0.033	3.75	4.72×10^{-3}

[1]No significant differences ($P > .05$).

[2]Error term used to test for significant treatment effects.

TABLE XXXII

TRIAL 2: F AND LSD VALUES FOR GROWTH DATA

	F-Value[1]	LSD[2]
Daily Feed	0.97	0.24
Feed/Gain	1.70	2.74
ADG	1.42	0.10

[1]No significant ($P > .05$) effects present

[2]Least significant difference ($P < .05$).

TABLE XXXIII

TRIAL 2: AOV FOR TASTE PANEL DATA

Source	df	Variance	MS	F-Value
Total	59	81.60		
Treatments	2	3.06	1.53	1.11[1]
Residual	57	78.54	1.38	

[1]Not significant (P > .05).

VITA

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Master of Science

Thesis: THE INFLUENCE OF TREATMENT OF WHOLE FAT SOYBEANS OR SOY FLOUR WITH FORMALDEHYDE TO PROTECT THE POLYUNSATURATED FATTY ACIDS FROM BIOHYDROGENATION IN THE RUMEN

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