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Sharon L. Melton

Sharon L. Melton, Major Professor

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and recommend its acceptance:

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Date May 28, 1990

BIOHYDROGENATION OF SOYBEAN OIL IN SPRAY DRIED CASEIN-OIL EMULSIONS FROM DIFFERENT TREATMENTS

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Susan Dianne Byrd Newberry

August 1990

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DEDICATION

This thesis is dedicated to my husband Daniel, and my daughters, Autumn and Jessica Newberry whose love, devotion, patience and encouragement have enabled me to complete this study.

ACKNOWLEDGEMENTS

The author wishes to express her sincere gratitude to Dr. Sharon L. Melton for all of the assistance, dedication, and encouragement she gave the author during this course of study.

The author would also like to thank Dr. William Backus, Dr. James Riemann, Dr. John Waller, Dr. John Mount, and Dr. Marjorie Penfield for their assistance.

And finally, special thanks to Mrs. Nancy Austin, Mrs. Betty Carver, Mrs. Louise Dalton, Mrs. Valerie Garland, Mrs. Ann Henry, Miss Ruth Hill, Mrs. Emayet Spencer, Mrs. Kay Trigiano, and Mr. Cyrus Borozorgmehr for answering the many questions presented to them by the author.

ABSTRACT

The objectives of this investigation were (1) to produce substrates consisting of spray dried casein-soybean oil emulsions treated with different chemical compounds and (2) to investigate the biohydrogenation of the oil in these substrates *in vitro* using bovine ruminal fluid. Prior to spray drying, substrates, casein:soybean oil, 1:1, w/w, were produced by 7 different treatments: untreated (T1) and treated with acetaldehyde (T2), formaldehyde (T3), peanut skin aqueous extract (T4), tobacco stem aqueous extract (T5), diacetyl (T6) or tannic acid (T7). Two replications were run with a single replication consisting of substrates from the 7 treatments being digested 0 and 23 hr by ruminal fluid. The relative percentages of the following fatty acids: 14.0, 16:0, 16:1, 18:0, 18:1 trans, 18:1 cis, 18:1 isomer, 18:2, 18:3, 20:0, 20:1 and 20:4 were determined in each substrate digest at 0 and 23 hr digestion time. The percentage of each acid was statistically analyzed as a function of treatment, digestion time and their interaction. Micrographs of the spray dried emulsion of each treatment also were obtained by scanning electron microscopy.

When averaged across treatment, the percentages of 18:0 and 18:1 trans increased and the percentage of 18:2 decreased ($P<.05$) during 23 hr digestion showing that biohydrogenation occurred in the substrates. When averaged

across digestion time, the percentages of 18:0, 18:1 trans and 18:1 isomer were higher and the percentages of 18:2 and 20:0 were lower ($P<.05$) in group 1 treatments (T1, T2, and T5) than in group 2 treatments (T3, T6 and T7). This showed that more 18:2 was biohydrogenated by ruminal microbes to 18:1 isomer, 18:1 trans and 18:0 in group 1 treatments than in group 2 treatments. Group 2 treatments protected the unsaturated fatty acids from biohydrogenation better than group 1 treatments. The fatty acid composition of T4 digests was most like group 1 treatments, and T4 also did not prevent biohydrogenation. During 23 hr digestion, the level of 18:1 trans increased in T1, T2, T4, T5 and T6 but not in T3 and T7. This indicated that T6 was not as effective in preventing biohydrogenation as T3 and T7. The microstructure of emulsions from T1, T2, T4 and T5 were similar to those of spray dried casein in the absence of fat, and the microstructure of emulsions from T3, T6 and T7 were similar to spray dried cheese powders with a protein:fat ratio of approximately 1:1.

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I. INTRODUCTION

The relationship between saturated fat and heart disease has been publicized to the point where consumers in the U.S.A. are very concerned with eating animal fat because of its relatively high levels of saturated fatty acids (35-45%) (deMan, 1981). Whether or not saturated fat causes coronary heart disease becomes a moot point. Many consumers are afraid that it does, and the consumption of saturated fats such as animal fats is decreasing yearly (Brock, 1989). Thus, consumption of other animal products such as beef, may also decrease in the future. One way of alleviating this problem is to produce leaner beef with fat that has lower levels of saturated fatty acids. Production of such beef can be done by treating dietary fat for ruminants so that it is protected from biohydrogenation.

Beef and mutton containing high levels of linoleic acid (18:2) and low levels of palmitic acid (16:0) have been produced by feeding formaldehyde-treated casein-oil emulsions or sunflower seeds to ruminants (Ralph, 1989). Oil trapped within the formaldehyde-protein complex was protected from biohydrogenation in the rumen (Scott et al., 1971). This method of producing meat with altered fatty acid composition from ruminants, however, is not allowed in the United States. Formaldehyde is a carcinogen, and it is not allowed in animal diets (Waller, 1990). Therefore,

other ways of producing meat with altered fatty acid composition must be found to decrease the level of saturation in meat fat from ruminants.

The possibility exists that treatment of protein with other chemical compounds could form a protein complex in which to trap oil and protect the unsaturated fatty acids from biohydrogenation. Compounds such as aldehydes other than formaldehyde and tannins have been shown to interact with proteins and protect them from degradation in the rumen (Driedger and Hatfield, 1972; Sodano, 1979; Zelter and Leroy, 1976). These compounds might possibly protect a trapped oil from attack by ruminal microorganisms. Nonenzymatic browning of protein also protects it from degradation in the rumen (Fennema, 1985). Intermediate compounds of the browning reaction such as diacetyl might also react with protein trapping oil within a complex and protecting it from biohydrogenation. Therefore, the objectives of this study were (1) to produce substrates consisting of spray-dried casein-oil emulsions treated with different chemical compounds and (2) to investigate the biohydrogenation of the oil in these substrates, *in vitro*, using bovine ruminal fluid.

II. LITERATURE REVIEW

Rumen Microflora and Reactions

Ruminant animals such as cattle, sheep and goats have rumen microorgansms that break down and digest feedstuffs in their diet. These microorganisms also hydrogenate or saturate the unsaturated fatty acids (Annison and Lewis, 1959; Church, 1983; Garton, 1965; Johnson, 1974; Van Soest, 1984). Thus, the body fat composition of the ruminant animal is not a direct reflection of their dietary fat. However, monogastric animals, such as chickens and pigs as well as humans, tend to possess a body fat that is a true reflection of their dietary fat composition (Johnson, 1974). This is due to the fact that the unsaturated fatty acids ingested by the monogastrics are not hydrogenated before they are deposited by the body.

The rumen provides a near optimium environment for its microorganisms and various enzyme systems. The pH ranges from 5.5 to 7.0,and the temperature range is from 39 to 41C. Food is supplied steadily, and there is a continuous removal of fermentation products and food residues (Church, 1983; Hungate, 1966). The rumen is almost completely free of oxygen which also favors its ruminal microorganisms (Church, 1983; Garton, 1975; Hungate, 1966; Van Soest, 1984).

The rumen contains three major classes of rumen microorganisms: bacteria, ciliated protozoa and fungi (Akkada, 1965; Barnett and Reid, 1961; Church, 1983, and Czerkawski, 1986). The protozoal (fauna) and bacterial (flora) forms are mostly associated with both the liquid and solid contents of the rumen while the fungi or vegetative form is associated with the solid portion of the digesta only (Czerkawski, 1986).

The ciliated protozoa or oligotrichs are responsible for breakdown of starch and cellulose in the diet (Akkada, 1965; Church, 1983; Garton, 1975). The protozoa also ingest bacteria which contribute to the microbial turnover in the rumen (Czerkawski, 1986).

The bacterial microbes are responsible for the breakdown of cellulose, hemicellulose, starch, sugars, lactate and succinate as well as the synthesis of proteins and vitamins (Barnett and Reid, 1961; Church, 1983; Czerkawski, 1986; Garton, 1965). Both protozoa and bacteria are responsible for the hydrolysis of dietary lipids (Church, 1983; Garton, 1965). The fungi work in conjunction with the protozoa and bacteria to break down low quality fibrous feed stuffs (Czerkawski, 1986).

There have been a few studies reported concerning the alteration in the number of the microbes in the rumen. Diet, frequency of feeding, and the incorporation of drugs into the body of the ruminant have been shown to influence

the numbers and kinds of ruminal microorganisms (Warner, 1965).

In order for the ruminal microbes to metabolize the dietary constituents, they must come in direct contact with the component. When fat is trapped in a complex that is not soluble in the rumen, dietary unsaturated fatty acids can not be biohydrogenated nor can protein be broken down (Scott et al., 1971; Park and Ford, 1976). One way to entrap oil is to form a protein network around it.

Blocking Biohydrogenation in the Rumen

Several studies have been reported in which the protection of fatty acids from hydrogenation by microorganisms in the rumen has been investigated. Most of the successful studies have utilized formaldehyde in protecting the fatty acids from ruminal biohydrogenation. Scott et al. (1971) treated an emulsion of casein and polyunsaturated oil with formaldehyde prior to spray drying. Other investigators have introduced sunflower seeds-casein feed supplements treated with formaldehyde into the diets of ruminants (Ford and Park, 1975; Park et al., 1978; Wright et al., 1976). Hood and Thorton (1976) had some success in blocking biohydrogenation by feeding steers a diet containing 30% sunflower seed treated with formaldehyde. The formaldehyde reacted with the protein forming a matrix encapsulating the

oil and protecting it from ruminal degradation and hydrogenation. In their study, the formaldehyde protein complex was eventually carried to the abomasum and small intestines where it was hydrolyzed by the acid pH. After the embedded oil in the formaldehyde-protein complex was released in the small intestines, it was digested and absorbed by the body (Johnson, 1974; Scott et al., 1971; Tracy, 1975).

In a study performed by Dinius et al. (1974), the adipose tissue of steers fed a formaldehyde treated casein-safflower oil complex had 11.1% linoleic acid (18:2) compared to only 2.9% in the adipose tissue of steers fed an umprotected casein-safflower oil complex. Hood and Thorton (1976) reported that the 18:2 deposition differed in the adipose tissue of steers fed formaldehyde treated sunflower seed compared to those fed untreated sunflower seed. Steers fed this treated seed had more 18:2 in their intramuscular lipid than those fed unprotected sunflower oil. They also reported that, in 14-month-old steers, the protected sunflower seed fat was deposited preferentially in the internal depots and tissues of the forequarter of the steers rather than the hindquarter region because the perirenal adipose tissue is less active in synthesizing fatty acids than the subcutaneous adipose tissue from above the last rib. In their study, it was calculated that 71.6% of the formaldenhyde treated sunflower seed oil was protected from

hydrogenation in the rumen.

In the study conducted by Dinius et al. (1974), there was no significant preference among the untrained panelists for beef from the steers fed the protected protein-oil supplement or beef from steers fed the unprotected supplement. In similar studies involving lamb or mutton, there was a marked preference for the meat from animals fed the unprotected supplement. Meat from sheep fed the formaldehyde protected protein-oil supplement was described as being stronger, oilier and more earthy tasting. (Park and Ford, 1975; Park et al., 1978).

It should be noted that researchers in Australia have shown that formaldehyde is rapidly metabolized by the animals and that it does not accumulate in the meat or the milk of the animals ingesting these prepared feed supplements (Johnson, 1974). However, as stated earlier, formaldehyde is not acceptable for use as a feed additive for the production of meat in the United States as it is a car cinogen (Waller, 1990).

Other Effects of Dietary Protein Treatment

Formaldehyde treatment of protein supplements for ruminants also increases the level of protein digested in the small intestines since less protein is degraded in the rumen (Barry, 1976; Cotta and Hespell, 1985; Faichney and

White, 1979; Ferguson et al., 1967; Ramshorst and Thomas, 1988; Zelter et al., 1970). This results in increased growth in the animals on a finishing ration (Dinius et al., 1974). Therefore, several treatments of dietary protein to bypass rumen degradation have been studied. In particular, it has been found that tannins and aldehydes, other than formaldehyde, react with protein to protect it from rumen degradation (Sodano, 1979; Zelter et al., 1976). Coating agents and heating of the protein also have shown a potential to protect the protein (Driedger and Hatfield, 1972). The possibility exists that treatment of protein by tannins, other aldehydes or intermediate products of the browning reaction could also trap oil in a complex and protect it from biohydrogenation. However, characteristics of each compound and their reaction with protein and effects on bovine performance need to be considered if the treatment is to be of practical use.

Tannic Acid Characteristics and Interaction With Protein

Tannin or tannic acid is defined as any naturally occurring polyphenolic compound with a molecular weight of 500-3000 which is able to form crosslinks with proteins and other molecules (Kumar and Singh, 1984). Tannins are found in all classes of plants and are divided into two groups, hydrolyzable tannins and condensed tannins (Haas and Hill,

1928; Swain, 1977).

Oak leaf tannin or gallotannic acid is a hydrolyzable tannin (Ramachandra et al., 1977). Feeny (1969) reported that gallotannic acid forms complexes with casein protein at a pH of 5.0. In the latter study, casein was shown to be almost completely protected from hydrolysis by trypsin at a pH of 7.6 after it was complexed with gallotannic acid. Feeny (1969) stated that the degree of complex formation increased with increasing ratio of protein to tannin concentration and longer contact time between the protein and the tannin.

Tannins have the ability to precipitate all proteins by hydrogen and hydrophobic bonding (Goldstein and Swain, 1965; Hagerman and Butler, 1978; Oh et al., 1980). Thus, they also inhibit most proteolytic enzymes. Dietary protein is also unavailable since the proteases are unable to hydrolyze the protein (Swain, 1977). Tannins, therefore have deleterious effects in animals.

Effect of Tannins on Bovine Performance

Tannins have been implicated in growth depression in livestock. Variations in growth depression properties of tannins vary; however, gallotannic acid has been shown to be the most growth depressing tannin (Vohra et al., 1965).

The condensed tannins in leaves and fruits have been

blamed for the loss of weight and the death of ruminants, chicks and rodents (Vohra et al., 1965). Tannic acid has been shown to depress feed intake as well as the utilization of nutrients consumed (Kumar and Singh, 1984; Vohra et al., 1965). In a study by McLeod (1974), it was found that the digestibility and voluntary intake of fodder containing high levels of tannins were reduced thus depressing its nutritive value. Hewitt and Ford (1982) reported similar results involving field peas with high levels of tannins.

Astringency of the tannins seems to be responsible for the reduction in voluntary feed intake of livestock (Kumar and Singh, 1984). Legumes low in tannic acid such as alfalfa, hop, clover and White Dutch clover which have approximately 1.3% tannic acid are consumed in higher quan tities than those with average tannic acid content of approximately 5.0% such as Servicea lespedeza (Hawkins, 1955).

Tannins in ruminant feeds have been reported by Kumar and Singh (1984) to cause low milk yields, intestinal, liver, spleen and kidney damage, mucus in urine, reduction in available sulfur, and fatal constipation. Tannins have also been shown to protect protein from ruminant microbial degradation. Driedger and Hatfield (1972) reported that there was a 90% decrease in the deamination, in the rumen,of soybean meal when the soybean was treated with 10% tannin. They also reported in the same study, that the average daily gains, feed efficiencies and nitrogen balances were

greater in lambs feeding on soybean meal treated with 10% tara tannin. Nitrogen utilization was also shown to be enhanced by the tannin treatment.

Dietary Peanut Skin Effect on Protein

Peanut skins are a by-product of the peanut blanching industry (McBrayer et al., 1983). About 30,000 metric tons of peanut skins are produced in the United States every year (Hale and McCormick, 1981).

Peanut skins comprise about 2 to 3.5% of the weight of peanut kernels (Stansbury et al., 1950). Peanut skins contain a water soluble catchechol type tannin as well as other similar pigments (Stansbury et al., 1950). The tannin content of peanut skins range from 16 to 23.8% (Hale and McCormick, 1981; Hill et al., 1986b; McBrayer et al., 1983). Sanders (1979) reported, however, that the tannin content in peanut skins differed with production area as well as with variety.

Peanut skins contain an average of 17.3% protein and 26.6% ether extract (NAS, 1971) on an as fed basis (McBrayer et al., 1983). Thus, peanut skins could be considered suitable protein supplements in ruminant diets. The feeding value of peanut skins is related also to the amount of tannin consumed relative to the amount of protein eaten. The high tannin content of peanut skins is known to inhibit en-

zymes thus making protein unavailable (McBrayer et al., 1983).

Dietary Peanut Skins Effect on Bovine Performance

Several studies have been conducted involving the use of peanut skins as feed supplements in ruminant animals. It has been shown that peanut skins fed at levels of 10% or less of the total diet had no effect on steer performance; however, at levels of 20%, performance was depressed in feedlot heifers (McBrayer et al., 1983).

Utley and Hellwig (1985) reported greater digestibility of dry matter and calculated total digestive nutrient values for steers fed bermudagrass pellets containing 10% peanut skins than for steers fed bermudagrass pellets without peanut skins. They concluded that peanut skins were a desirable addition to pelleted dehydrated bermudagrass when added in levels up to 10% of the weight of the finished pellet. Although high levels of tannins can cause severe health problems in cattle, McBrayer et al., (1983) reported cattle fed 10% or less peanut skins exhibit no abnormal health behavior. Thus, it is assumed, that since there were no abnormal health problems, peanut skin tannin is not absorbed but performs its action within the lumen of the digestive tract.

In cases where detrimental effects associated with

peanut skin tannins were reported, it was found that increasing the amount of protein in the diet could reverse those effects (Hill et al., 1986a,b; McBrayer et al., 1983). Hill et al. (1986a) reported increasing the dietary crude protein level from 15% to 16% in peanut skin diets using soybean meal or a soybean meal-urea combination was very effective in overcoming the detrimental effects.

Tobacco Polyphenols

Another source of tannins could be tobacco if tannins are considered to be any polyphenolic compound. The major polyphenolic constituents found in tobacco are chlorogenic acid and its derivatives, scopoletin and scopolin, and a flavone called rutin (Reid, 1959; Weaving, 1958).

Chlorogenic acid, (3 caffeoyl-quinic acid), is the primary polyphenol found in the tobacco plant (Reid, 1959; Rosa and Caughill, 1984; Stedman, 1957; Weaving, 1958). Chlorogenic acid, rutin, and many other of the polyphenols present in tobacco are soluble in water (Rosa and Caughill, 1984). In a study conducted by Anderson and Todd (1968), it was reported that the total plant phenols in tobacco ranged from 0.11% to 8.81% of the chlorogenic acid equivalents. The leaves of flue cured tobacco contained 8.81% chlorogenic acid equivalents while the tobacco stem piths contained only 0.11% of the total plant phenols.

Other studies have shown that the amounts of chlorogenic acid, flavones, and other minor polyphenols are directly related to the age, growth, varietal and curing conditions, as well as the leaf location of the plant (Anderson and Todd,1968; Court et al., 1983; Snook and Chortyk, 1982; Stedman, 1957; Weaving, 1958). The possibility also exists that these polyphenols could react with protein forming a complex that would prevent protein degradation in the rumen and also protect oil from biohydrogenation.

Reaction of Carbonyls With Proteins

Carbonyls such as formaldehyde and malonaldehyde bind with proteins (Fennema, 1985). This binding results in partial cross linking of the proteins and the formation of covalent bonds (Fennema, 1985). Damodaran and Kinsella (1981a) reported that the magnitude of the interaction between protein and carbonyls depends upon the chain length and the position of the keto group in the chain. In their work with soy protein, Damodaran and Kinsella (1981a) found that there appeared to be four binding sites on soy protein for carbonyls and that the interaction between the two was hydrophobic in nature. In this particular study, they concluded the interaction between carbonyls and soy protein was relatively weak from a thermodynamic standpoint. In another study, Damodaran and Kinsella (1981b) found that the mag-

nitude of hydrophobic interaction was greater at higher temperatures. Thus, heat treatment increases the intramolecular hydrophobic regions.

The Maillard browning reaction is responsible for the formation of several carbonyl compounds. These carbonyl compounds contribute to the flavor of foods (deMan, 1980). The browning effect of the Maillard reaction will cause proteins, if present, to lose their nutritive value (Fennema, 1985).

Fennema (1985) reported that it is very useful to protect proteins high in nutritional value from ruminal degradation by treating them with small amounts of aldehydes or tannins prior to feeding. Fennema (1985) also reported that casein reacted with malonaldehyde is not readily hydrolyzed by proteases.

Scanning Electron Microscopy

Kerr et al. (1983) conducted a study in which they examined the scanning electron micrographs of 11 different commercially spray dried products which included spray dried cheeses as well as spray dried casein, whey and nonfat milk solids. The study was conducted to show the various morphological characteristics of each product as well as the differences between the products. It was reported in the study that the fat and protein content as well as the ratios

of these components were important factors in the determination of the shape and appearance of these products. The spray dried casein, nonfat dry milk, and whey all had prominant indentions and deep concave areas which characterize spray dried products which are low in fat. The spray dried cheeses such as Cheddar, Blue, Provolone and Swiss which have a higher fat content, and a fat:protein ratio of 1:1,had fewer prominant indentions and involutions and a more globular appearance.

Soybean Oil Composition

Soybean oil contains approximately 9% linolenic, 50% linoleic, and 18% oleic acids when bought as an unhydrogenated salad oil (Weiss, 1983). This oil in soybeans is the most economical source of polyunsaturated fatty acids in the U.S.A.(Weiss, 1983). The possibility exists that the full fat soybean could be treated with a chemical component that would entrap the oil within a protein matrix similar to the formaldehyde treatment of full fat sunflower seeds (Hood and Thornton, 1976; Park et al., 1978). Treated soybeans with entrapped oil possibly could be used as a lipid supplement to increase the unsaturation in meat lipids from ruminant animals. For these reasons and its availability,

soybean oil was selected as part of the substrate to study biohydrogenation in this experiment.

III. EXPERIMENTAL PROCEDURES

Experimental Plan

In this experiment, casein-soybean oil substrates were prepared by different chemical treatments. Treatment 1 substrate was untreated; treatment 2 was treated with acetaldehyde; treatment 3 with formaldehyde; treatment 4 with an aqueous extract of peanut skins; treatment 5 with an aqueous extract of tobacco stems; treatment 6 with diacetyl and treatment 7 with tannic acid. Enough substrate (spray-dried casein-oil) emulsion was prepared for each treatment at one time to complete the experiment. A replication consisted of each treatment mixed with rumen fluid for 0 and 23 hr digestion time. Two replications were run and fresh rumen fluid was collected for each replication.

Materials

The following chemical components were purchased from Sigma Laboratories (St. Louis, MO.), casein, reagent grade formaldehyde, reagent grade diacetyl, reagent grade acetaldehyde, and reagent grade tannic acid. Unhydrogenated soybean oil was purchased from a local supermarket.

The aqueous extract of tobacco stems was prepared as follows. Dried tobacco stems (446.0 g) obtained from Austin

Tobacco Co., Greeneville, TN, were soaked in 1500 ml of deionized water for 16 hrs. The supernant was then filtered and concentrated to approximately 600 ml. The total solids in the concentrated extract was determined by a total solids test (AOAC, 1985).

An aqueous extract of peanut skins was also prepared. Peanut skins (400 g) obtained from JFG Coffee Co., Knoxville, TN, were extracted for 16 hrs by petroleum ether in a Soxhlet apparatus. The solvent was evaporated from the fat free skins,under a hood overnight,and the peanut skins were frozen. Then they were freeze dried. The freeze dried, fat free peanut skins were then soaked in 1200 ml deionized water for 16 hrs. The extract was then filtered and concentrated to approximately 800 ml, and the total solids in the concentrate determined as previously described. Unfortunately at the time of preparation, the freeze drier was inoperable preventing the preparation of a larger extract of peanut skins.

Ruminal fluid was obtained from a 16-hr fasted, fistulated dairy cow on a diet of alfalfa hay at the University of Tennessee dairy farm in Knoxville, TN. The ruminal fluid was strained through 2 layers of cheese cloth into a thermal container. It was then transported to the Food Technology and Science laboratory where it was kept no longer than one hour before use.

Substrate Preparation

For all emulsions, 25 g casein and 25 g soybean oil were dispersed in one liter of 70 C deionized water by blending in a Waring blender on high speed until the oil phase was well dispersed. After blending, each emulsion was held at 70 C and treated by one of seven treatments. Treatment 1 was untreated. For treatment 2, 1.83 g of acetaldehyde was added. For treatment 4, 518 ml of aqueous tobacco stem extract containing 25 g solids were added to the emulsion and for treatment 5, 790 ml of aqueous peanut skin extract containing 6.32 g of solids were added. Diacetyl (1.75 g) was added to an emulsion for treatment 6, and tannic acid (25 g) was added to another for treatment 7. After the chemical component was added to each emulsion, the pH was adjusted to pH 6.8 by addition of NaOH or HCl.

Each emulsion was maintained at 70 C for 20 min. after pH adjustment and was then homogenized through a single stage homogenizer (Wilh. G. Schroder, Type: LAB 100M35130010). The homogenized emulsion was spray dried in a Niro Spray Drier (Series 1600) with an inlet temperature of 160 C and outlet temperature of 95 - 100 C. The spray drier was thoroughly cleaned between each treatment, and the first 0.5- 1.0 g of the spray-dried substrate was discarded to prevent contamination by other substrates. The freeze-dried substrates were stored in sealed polyethylene bags

(Whirl-Pak) inside a jar filled with anhydrous calcium chloride at -18 C until used.

Digestion in Ruminal Fluid

For one replication of the experiment, substrates from each treatment were digested in ruminal fluid in a similar manner as that reported by Scott et al., (1971). For each treatment, 200 mg of the substrate was mixed with 40 ml ruminal fluid in a 250-ml reaction flask which was flushed with nitrogen and stoppered. Duplicates were prepared for each treatment. After the mixture had been stirred well, 30 ml of 2 N NaOH and 30 ml of methanol were added to one of the duplicates to stop microbal action, and this represented 0 hr digestion time. The other duplicate from each treatment was placed in a shaker bath (Itecator Model 1024) at 38 C for 23 hr after which they were treated in the same way as the 0 hr samples. The lipids in the 0 hr and 23 hr samples were then saponified by heating under reflux for 60 min. After cooling, each sample was acidified by adding 6 N HC1 and then extracted with three 30-ml aliquots of hexane. The aliquots were pooled and dried to dryness on a rotatory evaporator at 50C.

Fatty Acid Analysis

Methyl esters were prepared from each dried sample extract by the AOCS (1973) method using boron trifluoride in methanol. The fatty acid methyl esters were analyzed on a 0.25 mm i.d. by 30 m long fused silica SP-2330 column (Supelco, Inc., Bellefonte, PA) in a Shimadzu Model 6AM gas chromatograph equipped with a flame ionization detector, a Shimadzu Model E-1A data processor and a 1-mv recorder. The esters were analyzed from 50 to 220C at two degrees per min with a helium carrier gas flow rate of 1.2 ml/min using a split ratio of 30:1. Individual fatty acid methyl esters were identified by matching their relative retention times with those of known standard fatty acid methyl esters analyzed under exactly the same conditions. The standard fatty acid methyl esters mixtures, RM-1 and RM-6, were obtained from Supelco, Inc. The relative percentages of total peak area for each of the following known fatty acids were determined: myristic (14:0), palmitic (16:0), palmitoleic (16:1), stearic (18:0), elaidic (18:1 trans), oleic (18:1 cis), isomer of 18:1 (18:1 isomer), linoleic (18:2), linolenic (18:3), eicosanoic (20:0), eicosenoic (20:1), and arachadonic (20:4). Also, the relative percentages of total peak area for all unknown peaks were summed and reported as percent Unknowns.

Scanning Electron Microscopy of Substrates

All of the treatment samples were prepared for scanning electron microscopy (SEM) by sprinkling approximately 0.005g treatment powder on the surface of transparent, double adhesive tape secured to standard SEM studs. The mounted powders were placed in a Hummer sputter coater (Technics, Inc., Alexandria, VA) and coated with gold for 3 min at 10mA in an atmosphere of nitrogen gas. The coated specimens were then observed with a Cambridge S 360 equipped with a Link AN 1000 EDS (Cambridge Scientific Instruments LTD., Cambridge, England) and pictures were taken with a polaroid camera.

Statistical Analysis

The percentages of each fatty acid including unknowns were statistically analyzed as a function of the independent variables, treatment and time of digestion, and their interaction using the general linear model in SAS (1981). When time and/or the interaction sums of squares were not significant for any given fatty acid, they were added to the error sum of squares for testing of treatment effect. Significantly different treatment means were separated by orthogonal contrasts (Sokal and Rohlf, 1969) shown in table 1.

Orthogonal contrasts were written to explain the observed differences in biohydrogenation as reflected by the fatty acid composition for each treatment after the 23 hr digestion. Thus, treatment 2 (acetaldehyde) was compared to treatment 5 (tobacco stem extract) for contrast 2, and treatments 2 and 5 were compared with treatment 1 (untreated) for contrast 1. These treatments formed group 1 treatments. Next, treatment 6 (diacetyl) was compared with treatment 7 (tannic acid) for contrast 5, and treatments 6 and 7 were compared with treatment 3 (formaldehyde) for contrast 4. To make a set of orthogonal contrasts, treatment 4 (peanut skin extract) was compared with all treatments.

Table 1. Orthogonal contrasts for treatments of casein-oil emulsions.

	Treatments[a]						
Contrast	1	2	3	4	5	6	7
1	2	-1	0	0	-1	0	0
2	0	-1	0	0	1	0	0
3	0	0	-2	0	0	1	1
4	-1	-1	1	0	-1	1	1
5	0	0	0	0	0	-1	1
6	-1	-1	-1	6	-1	-1	-1

[a] 1 = control (untreated); 2 = acetaldehyde treated; 3 = formaldehyde treated; 4 = peanut skin extract treated; 5 = tobacco stem extract treated; 6 = diacetyl treated and 7 = tannic acid treated.

IV. RESULTS AND DISCUSSION

Digestion Time Effect

The relative percentages of 14:0, 18:0, 18:1 trans and 18:2 of the substrates were affected at the $P<.05$ level by digestion time (Appendix A). The percentages of 14:0 and 18:2 decreased, and the percentages of 18:0 and 18:1 trans increased significantly during the 23 hr digestion time as shown in table 2. There were only 26 total degrees of freedom (df) in the analyses of variance (Appendix A) because one digest of a substrate (treatment 5, 0 hr, rep 2) was lost.

The metabolism of dietary polyunsaturated fatty acids by ruminal microorganisms starts with their hydrolysis from the lipids and hydrogenation by certain bacteria (Van Soest, 1984). In addition, the remaining double bonds in the fatty acids are converted from cis to trans form and are also distributed in all positions in the fatty acid chains. Since trans acids are more difficult to hydrogenate than cis forms, they also accumulate in the ruminal lipids (Van Soest, 1984). These are the reasons for the decrease in the percentage of 18:2 and the increase in the percentages of 18:0 and 18:1 trans fatty acids during the 23 hr digestion of casein-oil substrates by ruminal microorganisms.

Treatment Effects

The percentages of 14:0 and 18:2 were affected by treatment at the $P<.05$ level when treatments were considered as a group (Appendix A). When individual contrasts of treatments (table 1) were made, differences at the $P<.05$ level among treatments were found for the percentages of Unknowns, 16:0, 16:1, 18:0, 18:1 trans, 18:1 cis, 18:1 isomer, 20:0, 20:1 and 20:4 (Appendix A).

The percentages for the fatty acids averaged across digestion time in each treatment are shown in table 3. Table 3 shows the significant difference (Appendix A) in the percentage of 18:1 between diacetyl treated (treatment 6) and tannic acid treated digests (treatment 7). The digests from treatment 6 had a higher level of 18:1 than did treatment 7 digests. No other significant differences (Contrast 5, Appendix A) in fatty acid levels were found between treatments 6 and 7. Neither were significant differences (Contrast 5, Appendix A) found in the percentage of any fatty acid between treatments 2 and 5.

The average relative percentages of fatty acids for the digests of the control or untreated substrates and across the acetaldehyde and tobacco stem extract treated digests are given in table 4. No significant differences were found between untreated digests and those of acetaldehyde and tobacco stem treated substrates (Contrast 1, Appendix A).

Table 2. Mean percentages[a] of fatty acids in digests of spray dried casein-oil substrates averaged across treatments for each digestion time.

Fatty acid	Digestion time 0 hr (n = 13)	23 hr (n = 14)
14:0	.88a	.16b
16:0	13.28	13.15
16:1	.20	.24
18:0	6.42a	17.02b
18:1 trans	.15b	4.76a
18:1 cis	17.06	16.21
18:1 isomer	1.43	1.33
18:2	47.09a	35.95b
18:3	.36	.32
20:0	4.32	3.76
20:1	.04	.08
20:4	.72	.21
Unknowns	8.04	6.82

[a] Means in a row followed by unlike letters are different at the $P<.05$ level.

Table 5 shows the average percentages of fatty acids in the digests of formaldehyde treated substrates and across the digests of diacetyl and tannic acid treated substrates. No significant differences in the percentages of any fatty acids were found for contrast 3 (table 1) as shown in Appendix A; therefore, no differences are given in table 5.

The average percentages across digests of group 1 substrates (untreated, acetaldehyde treated, and tobacco stem extract treated) and across those of group 2 substrates (formaldehyde treated, diacetyl treated and tannic acid treated) are presented in table 6. Significant differences (Appendix A) due to contrast 4 (table 1) are shown also in table 6. Group 1 treated digests had higher percentages of 18:0, 18:1 trans, and 18:1 isomer and lower levels of 18:2 and 20:0 than group 2 treated digests.

Table 7 gives the average percentages of fatty acids for digests of treatment 4 (peanut skins extract) and across digests from all other treatments and shows significant differences defined by contrast 6 (table 1). Compared to all other treatments, the digests of casein-oil emulsion treated with the peanut skin extract had higher percentages of 14:0, 16:0, 16:1, 20:1, 20:4 and Unknowns and lower levels of 18:1 cis and 18:2. In fact, treatment 4 had the highest percentage of Unknowns of all the treatments (table 3). The fatty acid composition in the digests from treatment 4 more closely resembled the fatty acid composition in digests from

Table 3. Mean percentages[a] of fatty acids in digests of spray dried casein-oil substrates averaged across digestion time for each treatment (contrasts 2 and 5[b]).

Fatty acid	Treatments[c]						
	1	2	3	4	5	6	7
14:0	.78	.73	.09	1.41	.03	.21	.20
16:0	12.78	16.07	10.17	19.04	13.38	11.44	9.63
16:1	.00	.09	.05	.73	.45	.23	.06
18:0	18.77	18.01	5.25	17.25	9.93	7.14	6.58
18:1 trans	3.16	4.00	.76	2.92	4.17	1.98	1.19
18:1 cis	15.67	15.04	18.39	13.08	18.25	20.72a	15.59b
18:1 isomer	1.70	1.61	.81	1.94	1.61	1.26	.75
18:2	35.47	33.26	55.26	24.14	38.19	48.95	53.15
18:3	.19	.35	.31	.38	.34	.30	.52
20:0	3.16	3.93	5.86	1.87	4.24	4.37	5.37
20:1	.04	.10	.02	.16	.00	.06	.03
20:4	.49	.14	.20	1.14	.14	.26	.73
Unknowns	7.80	7.21	2.80	15.95	9.26	3.10	6.20

[a] Means (n = 4 except for treatment 5 where n = 3) in any row followed by unlike letters are different at the P<.05 level.

[b] Contrast 2 compares treatment 2 with treatment 5 and contrast 5 compares treatment 6 with treatment 7.

[c] 1 = control (untreated); 2 = acetaldehyde treated; 3 = formaldehyde treated; 4 = peanut skin extract treated; 5 = tobacco stem extract treated; 6 = diacetyl treated; and 7 = tannic acid treated.

Table 4. Mean percentages of fatty acids in digests of spray dried casein and oil substrates for the control treatment and the acetaldehyde plus tobacco stem extract treatments (contrast 1[a]).

	Treatments	
Fatty acid	Control[b] (n = 4)	Acetaldehyde plus tobacco stem[c] (n=7)
14:0	.78	.48
16:0	12.78	14.92
16:1	.00	.24
18:0	18.77	14.55
18:1 trans	3.16	4.07
18:1 cis	15.67	16.42
18:1 isomer	1.70	1.61
18:2	35.47	35.37
18:3	.19	.35
20:0	3.16	4.06
20:1	.04	.06
20:4	.49	.14
Unknowns	7.80	8.09

[a] Contrast 1 compares treatment 1 with treatments 2 and 5.
[b] Control = treatment 1 (untreated).
[c] Acetaldehyde = treatment 2 and tobacco stem = treatment 5.

Table 5. Mean percentages of fatty acids in digests of spray dried casein-oil substrates for the formaldehyde treatment and the diacetyl plus tannic acid treatments (contrast 3[a]).

	Treatments	
Fatty acid	Formaldehyde[b] (n = 4)	Diacetyl+ tannic acid[c] (n = 8)
14:0	.09	.21
16:0	10.17	10.54
16:1	.05	.15
18:0	5.25	6.86
18:1 trans	.76	1.59
18:1 cis	18.39	18.15
18:1 isomer	.81	1.01
18:2	55.26	51.05
18:3	.31	.41
20:0	5.86	4.87
20:1	.02	.05
20:4	.20	.49
Unknowns	2.80	4.64

[a] Contrast 3 compares treatment 3 with treatments 6 and 7.
[b] Formaldehyde = treatment 3.
[c] Diacetyl = treatment 6 and tannic acid = treatment 7.

Table 6. Mean percentages[a] of fatty acids in digests of spray dried casein-oil substrates treated with different chemicals showing differences between groups of treatments (contrast 4[b]).

Fatty acid	Group 1 treatments[c] (n = 11)	Group 2 treatments[d] (n = 12)
14:0	0.56	0.17
16:0	14.14	10.42
16:1	0.16	0.12
18:0	16.08a	6.32b
18:1 trans	3.74a	1.31b
18:1 cis	16.14	18.23
18:1 isomer	1.64b	0.94a
18:2	35.41b	52.45a
18:3	.29	.38
20:0	3.54a	5.20b
20:1	0.05	0.04
20:4	0.27	0.40
Unknowns	7.98	4.04

[a] Means in a row followed by unlike letters are different at the $P<.05$ level.

[b] Comparison 4 compared the mean of treatments 1, 2 and 5 with the mean of treatments 3, 6 and 7.

[c] Group 1 included treatments 1, control (untreated); 2, acetaldehyde treated casein and oil and 5, tobacco stem extract treated casein and oil.

[d] Group 2 included treatments 3, formaldehyde treated casein and oil; 6, diacetyl treated casein and oil, and 7, tannic acid treated casein and oil.

Table 7. Mean percentages[a] of fatty acids in digests of spray dried casein-oil substrates for the peanut skin extract treated and for all of the other treatments showing differences defined by contrast 6[b].

Fatty acid	Peanut skins treated (n = 4)	All other treatments (n = 23)
14:0	1.41a	.36b
16:0	19.04a	12.20b
16:1	.73a	.14b
18:0	17.25	10.99
18:1 trans	2.92	2.47
18:1 cis	13.08b	17.23a
18:1 isomer	1.94	1.27
18:2	24.14b	44.30a
18:3	.38	.34
20:0	1.87b	4.41a
20:1	.16a	.04b
20:4	1.14a	.34b
Unknowns	15.95a	5.92b

[a] Means in a row followed by unlike letters are different at the $P<.05$ level.

[b] Contrast 6 compared the mean of treatment 4 with the means of treatments 1, 2, 3, 5, 6 and 7.

treatments 1, 2 and 5 (group 1 treatments) than that in digests from treatments 3, 6 and 7 (group 2 treatments) also shown in table 3. This indicates that treatment 4 was more like group 1 treatments than group 2 treatments (table 6).

Treatment by Digestion Time Interaction

For most of the fatty acids, the treatment by digestion time (Trt x Time) interaction was not very significant ($P>.25$). However, the interaction for 14:0 had a F-value with a $P<.027$ and for 18:1 trans, a F-value with a $P<.11$ (Appendix A). When the percentages of 14:0 and 18:1 were analyzed as a function of time for each treatment (Appendixes B and C, respectively), the way in which digestion time affected the concentrations of 14:0 and 18:1 trans fatty acids was dependent upon the treatment.

Table 8 shows the percentage of 14:0 in the digests for each treatment and digestion time. The percentage of 14:0 in treatments 1, 2 and 4 decreased significantly from 0 to 23 hr; however, the decrease was greatest in treatment 4 which was the aqueous peanut skin extract treatment. In treatments 6 and 7, there was a trend for the percentage of 14:0 to increase across time. These observations explain the significant interaction between treatment and time of digestion for this acid. Levels of 14:0 greater than 0.1% in the digests must be due to the microbial lipids not the

Table 8. Percentage[a] of myristic acid (14:0) in digests of casein-oil substrates from each treatment and digestion time combination.

Treatment[b]	Digestion time	
	0 hr	23 hr
1	1.43a	.13b
2	1.35a	.11b
3	.16	.03
4	2.52a	.30b
5	.04	.02
6	.09	.35
7	.17	.23

[a] Means in a row followed by different letters are different at the $P<.05$ level.

[b] 1 = control (untreated), 2 = acetaldehyde treated, 3 = formaldehyde treated, 4 = peanut skin extract treated, 5 = tobacco skin extract treated, 6 = diacetyl treated and 7 = tannic acid treated.

soybean oil. Soybean oil normally has less than 0.10% of 14:0 (Weiss, 1983). The reasons why the levels of 14:0 were not similar in each treatment at 0 hr digestion time are not known; however, it could be due to a nonhomogeneous dispersion of microbes in the ruminal fluid added to each treatment.

The percentages of 18:1 trans for each treatment and time are presented in table 9. The percentage of 18:1 trans increased ($P<.05$) during the 23 hr of digestion in the untreated (treatment 1), acetaldehyde treated (treatment 2), peanut skin extract treated (treatment 4), tobacco stem extract treated (treatment 5) and diacetyl treated (treatment 6) casein-soybean oil digests. The level of 18:1 trans in the formaldehyde-treated (treatment 3) and in the tannic acid treated (treatment 7), however, did not change significantly.

Trans unsaturated fatty acids such as 18:1 trans are produced in the rumen via isomerization and partial biohydrogenation of 18:2 and of isomerization of 18:1 cis by certain ruminal bacteria (Van Soest, 1984). However, since the level of 18:1 cis did not change significantly during the 23 hr digestion period (table 2, p.28), and the percentage of 18:2 decreased, 18:1 trans most likely was formed via hydrogenation of 18:2. Trans unsaturated fatty acids are hard to biohydrogenate and accumulate in ruminal lipids. For these reasons, in this study, higher levels of 18:1

Table 9. Percentage[a] of elaidic acid (18:1 trans) in digests of casein-oil substrates from each treatment and digestion time combination.

Treatment[b]	Digestion time	
	0 - hr	23 hr
1	.11a	6.20b
2	.26a	7.74b
3	.09	1.43
4	.18a	5.65b
5	.31a	6.10b
6	.14a	3.83b
7	.05	2.34

[a] Means in a row followed by different letters are different at the $P<.05$ level.

[b] 1 = control (untreated), 2 = acetaldehyde treated, 3 = formaldehyde treated, 4 = peanut skin extract treated, 5 = tobacco stem extract treated, 6 = diacetyl treated and 7 = tannic acid.

trans in the digests should indicate that unsaturated fatty acids are being biohydrogenated. The results in table 9 indicate the formaldehyde or tannic acid treatment of casein-oil emulsions prevented the microbial formation of 18:1 trans, and thus, better protected the oil from biohydrogenation than the other treatments.

Implications of Treatment and Digestion Time Effects

In addition to 18:1 trans being formed from 18:2 fatty acids, 18:0 and 18:1 cis are also formed (Scott et al., 1971). As previously stated, biohydrogenation occurred in the present study as shown by the decrease in the percentage of 18:2 and increase in the percentages of 18:1 trans and 18:0 in the digests during 23 hr digestion with ruminal fluid (table 2, p.28). Since biohydrogenation occurred and a single source of soybean oil was used to produce the substrates in all treatments and a single source of ruminal fluid was used for each replication, differences ($P<.05$) among treatments (Tables 3 through 7) are most likely due to ruminal microbial metabolism. Thus, the results given in table 6, p.33, indicate that more of the 18:2 fatty acid was biohydrogenated to 18:0, 18:1 trans and 18:1 isomer in group 1 treatments (untreated, acetaldehyde and tobacco stem extract) than in group 2 treatments (formaldehyde, diacetyl and tannic acid). Compared to group 1 treatments, group 2

treatments either hindered or prohibited biohydrogenation of the unsaturated fatty acids by ruminal microbes. Since treatment 4 (peanut skin extract) fatty acid composition was more like that of group 1 treatments (table 3, p.30), it also was less effective in preventing biohydrogenation than group 2 treatments.

No significant differences in the fatty acid composition were found among treatments 1, 2 and 5 digests (table 3, p.30 and table 4, p.31). In addition, the fatty acid composition of digests from treatment 4 were very similar to those in treatments 1, 2 and 5. Biohydrogenation, however occurred in each of these treatments as shown by the significant increase in the percentage of 18:1 trans after 23 hr digestion by ruminal fluid (table 9). Since treatment 1 was the untreated substrate, these results indicate that acetaldehyde and the aqueous extract of tobacco stems did not at all protect the fatty acids in the soybean oil-casein substrate from biohydrogenation.

When compared to group 1 treatments, group 2 treatments (3, 6 and 7) either hindered or prevented biohydrogenation. However, the diacetyl treatment was not as effective as tannic acid or formaldehyde treatment. The digests of diacetyl treated substrates (treatment 6) had higher levels of 18:1 cis ($P<.05$) than those of the tannic acid treated substrates (treatment 7) and also tended to have a lower level of 18:2

than tannic acid or formaldehyde treated substrates (table 3). In addition, the percentage 18:1 trans increased ($P<.05$) in the diactyl treated digests, but not in the formaldehyde or tannic acid treated digests (table 9). The percentage of 18:1 trans in treatment 6, however, appeared to be higher than that of treatments 3 and 7 and lower than that of treatments 1, 2, 4, and 5 (table 9). The percentage of 18:0 in treatment 6 also was higher, though not at the ($P<.05$) level, than the percentage of 18:0 in treatments 6 and 7 and lower than that in treatments 1, 2, 4, and 5 (table 3). These results suggest that diacetyl treatment hindered the biohydrogenation of soybean oil more than treatments 1, 2, 4 and 5 but not as much as treatments 3 and 7.

Prevention of biohydrogenation by formaldehyde treatment of casein-oil emulsions was reported by Scott et al. (1971). However, the prevention of biohydrogenation in such emulsions treated by diacetyl or tannic acid has not been reported previously. Diacetyl, $CH_3COCOCH_3$, is a dicarbonyl compound similar to malonaldehyde ($COCH_2CO$) which binds tightly with proteins (Fennema, 1985). No reports were found of studies in which diacetyl had reacted with protein. Because of the similarity of diacetyl and malonaldehyde, it would seem that diacetyl could react with protein and entrap oil in a protein network preventing its biohydrogenation as shown in the present study. On the other hand, the fact

that plant tannins and tannic acid react with proteins is well known (Oh et al., 1980), and entrapment of oil in a tannin-protein complex with subsequent protection from biohydrogenation was expected. However, the success of tannins at hindering or preventing biohydrogenation of oil entrapped in a protein complex may well depend upon their concentration ratio to protein as well as their source.

It is surprising that acetaldehyde treatment did not prevent biohydrogenation of the oil. The level (.041 moles) of acetaldehyde used in treatment 2 was approximately the same as that (.042 moles) of formaldehyde used in treatment 7. Therefore, it is unlikely that the concentration was the reason for its failure. Damodaran and Kinsella (1981a) reported that the magnitude of interactions between proteins and aldehyde depended upon the chain length of the aldehyde. Perhaps the acetaldehyde was not reactive enough towards casein to form a protein network in which to trap the oil.

One of the reasons that diacetyl might not be as effective as tannic acid or formaldehyde is the level used to treat the casein-oil emulsion. Scott et al. (1971) reported that the level of protection against biohydrogenation of 18:2 in a spray dried casein-oil emulsion by ruminal microbes increased as the percentage of formaldehyde increased from 0 to 4.8% of the weight of casein. In the study done for this thesis, formaldehyde was added at 5% of the weight of casein in substrates and diacetyl in at 7%.

The molar ratio between the levels of formaldehyde in treatment 3 and diacetyl in treatment 6 was approximately 2 to 1. Using diacetyl at the same molar concentration as formaldehyde in the treatment of protein-oil emulsions might increase the effectiveness of diacetyl for preventing biohydrogenation to that of formaldehyde. Further research is needed to optimize the concentration of diacetyl for prevention of biohydrogenation of oil in a substrate.

The poor performance against biohydrogenation by the aqueous extract treatments of both the peanut skins and tobacco stems also could be concentration related. In the case of the peanut skin extract, only 6.32 g solids were used to treat the emulsion. At the time of preparation of the peanut skin extract substrate, it was impossible to extract additional solids from peanut skins because the freeze dryer broke that was needed for preparation of the skins for the extract. Even if the solids from the extract were 100% tannins, the level in treatment 4 was much less than the 25 g of tannic acid used in treatment 7.

Tannins also were probably lower in concentration in the tobacco stem extract treatment than the 25 g of tannic acid added in treatment 7. The total level of tannins in the whole tobacco plant has been reported to range from 3.0 to 8.8% (Anderson and Todd, 1968; Reid, 1979), but the tobacco stems have been reported to contain only .11% of the total tobacco plant phenols (Anderson and Todd, 1978). Therefore,

the level of tannins in the 25 g of aqueous extract solids could range from less than .015 g(.0011 x .03 x 450 g tobacco stems extracted) to the total 25 g of the solids if the stems contained 8.8% tannins which were water soluble. However, it is unlikely that tannins were the only components that were extracted from the tobacco stems since they also contain water soluble carbohydrates and amino acids (Reid, 1959).

As stated previously, the tannic acid treated substrate resisted biohydrogenation of the fatty acids by ruminal microbes. At the same level as tannic acid, tannins from tobacco stem and peanut skin extracts might also protect unsaturated fatty acids from biohydrogenation. However, the level of tannic acid in treatment 7 was 100% the weight of the casein. Perhaps lower levels of tannic acid would also prevent biohydrogenation of unsaturated fatty acids in a casein oil emulsion. These possibilities need to be investigated.

As to the source of tannins, peanut skins are cheap and readily available since they are a by-product of the peanut industry, (Waller, 1990). However, tobacco stems are now used to make reconsitutued products in the tobacco industry and would not be an economical source of tannins (Colby, 1990). Further research is needed to determine the optimum ratios of diacetyl to protein and of peanut skin tannins to protein for the prevention of biohydrogenation of oils. In

addition, treatment of oilseeds by tannic acid, diacetyl and peanut skin tannins instead of the carcinogen, formaldehyde, to protect oil from biohydrogenation needs to be investigated. Treatments evaluated successfully *in vitro*, also need to be investigated in the live animal. Such research could result in economical, palatable lipid supplements for ruminants that could produce meat with increased levels of unsaturated fatty acids for consumption in the United States.

Scanning Electron Microscopy

Scanning electron micrographs of spray dried casein-oil (1:1, w/w) emulsions from treatments 1 (untreated), 2 (acetaldehyde-treated), 4 (peanut skin extract treated) and 5 (tobacco stem extract treated) are shown in figure 1. The hollow and shrunken appearance of casein spheres in these treatments are similar to those in the spray dried casein scanning electron micrographs published by Kerr et al. (1982). These researchers reported:

> "the hollow appearance of these casein particles resulted from the inability of entrapped internal air to diffuse through the protein skin during thermal expansions and subsequent inability of the casein to shrink uniformly during cooling and vacuole implosion."

The shape of the casein particles in treatments 1, 2, 4 and

5 closely resembles the particle shape of casein spray dried without oil or fat present reported by Kerr et al. (1982).

The scanning electron micrographs of the spray dried casein-oil (1:1 w/w) emulsions from treatments 3 (formaldehyde treated), 6 (diacetyl treated) and 7 (tannic acid treated) are shown in figure 2. The structure in these spray dried emulsions closely resembles the globular matrix of spray dried cheeses containing ratios of fat to protein of approximately 1:1, w/w, also reported by Kerr et al. (1982). The casein spheres in figure 2 also appear less shrunken with fewer holes than those in figure 1.

The differences in the particles and appearance of the spray dried emulsions between treatments shown in figure 1 and those in figure 2 can be related to the protection of the oil from biohydrogenation. The shape and appearance of the particles in figure 2 suggest that oil is present, but the shape and appearance of the particles in figure 1 do not. Only one other study was found in which the microstructure of spray dried casein-oil emulsion was given. Scott et al. (1972) published a transmitting electron micrograph of a spray dried casein:oil (1:1, w/w) emulsion which had been treated with formaldehyde. This formaldehyde treated emulsion also protected the oil from biohydrogenation by ruminal microbes. These latter researchers reported that the particles consisted of a casein matrix with entrapped oil droplets and they contained an air space in

the center. These particles resembled spray dried milk powders.

In figure 2(a) and (c), there are large fibers. These most likely are not from the spray dried emulsion, but from cotton fibers that were left in the spray dryer from cleaning with a cotton rag.

The microstructure of the spray dried casein-oil emulsions from treatments 1, 2, 4 and 5 (figure 1) suggests that no oil was trapped in a protein matrix, and therefore, it was available for biohydrogenation by ruminal microorganisms. The more globular appearance of the microstructure of the spray dried emulsions from treatments 5, 6 and 7 (figure 2) suggests that the casein particles contained entrapped oil, protecting it from ruminal microbial attack.

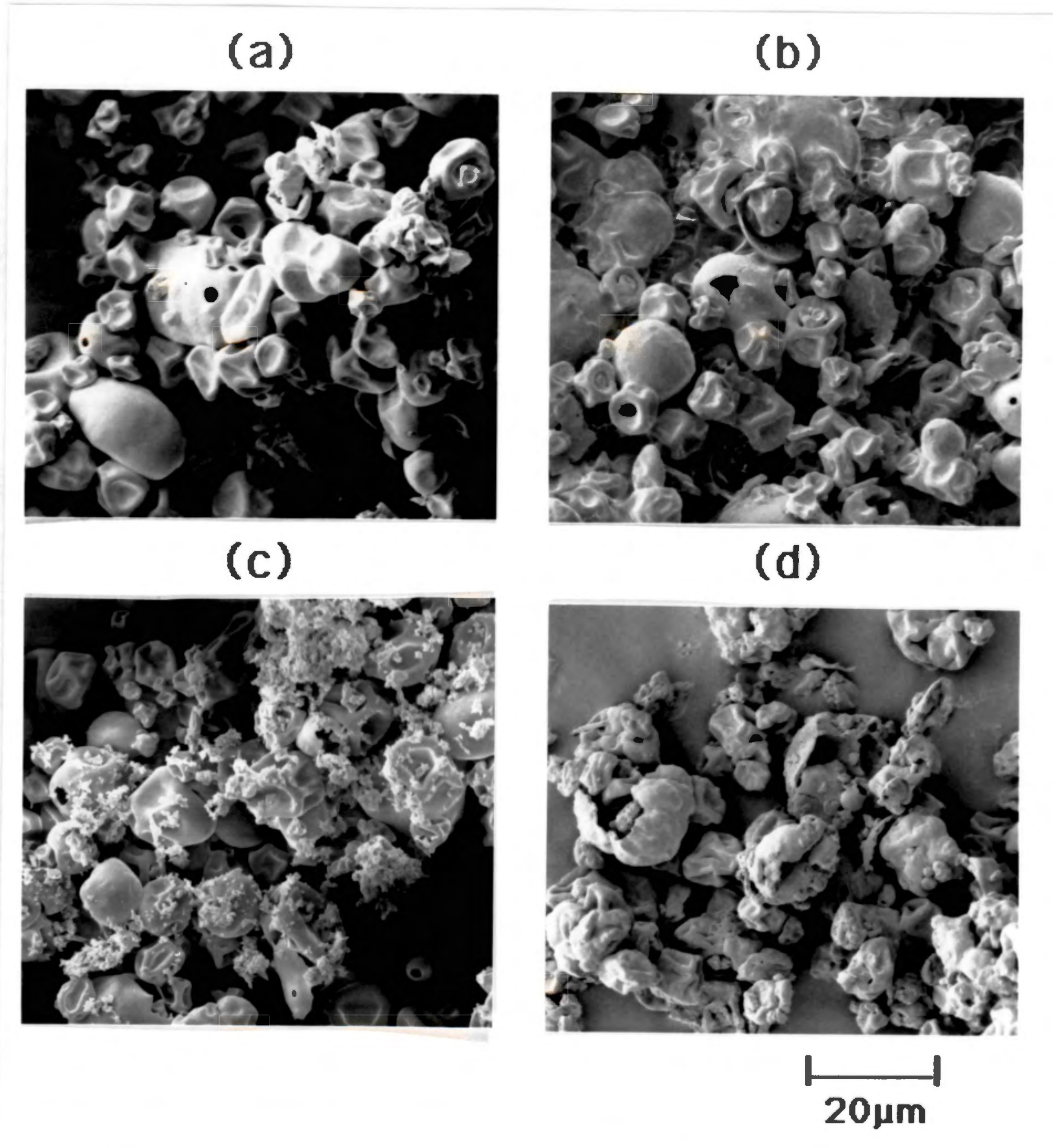
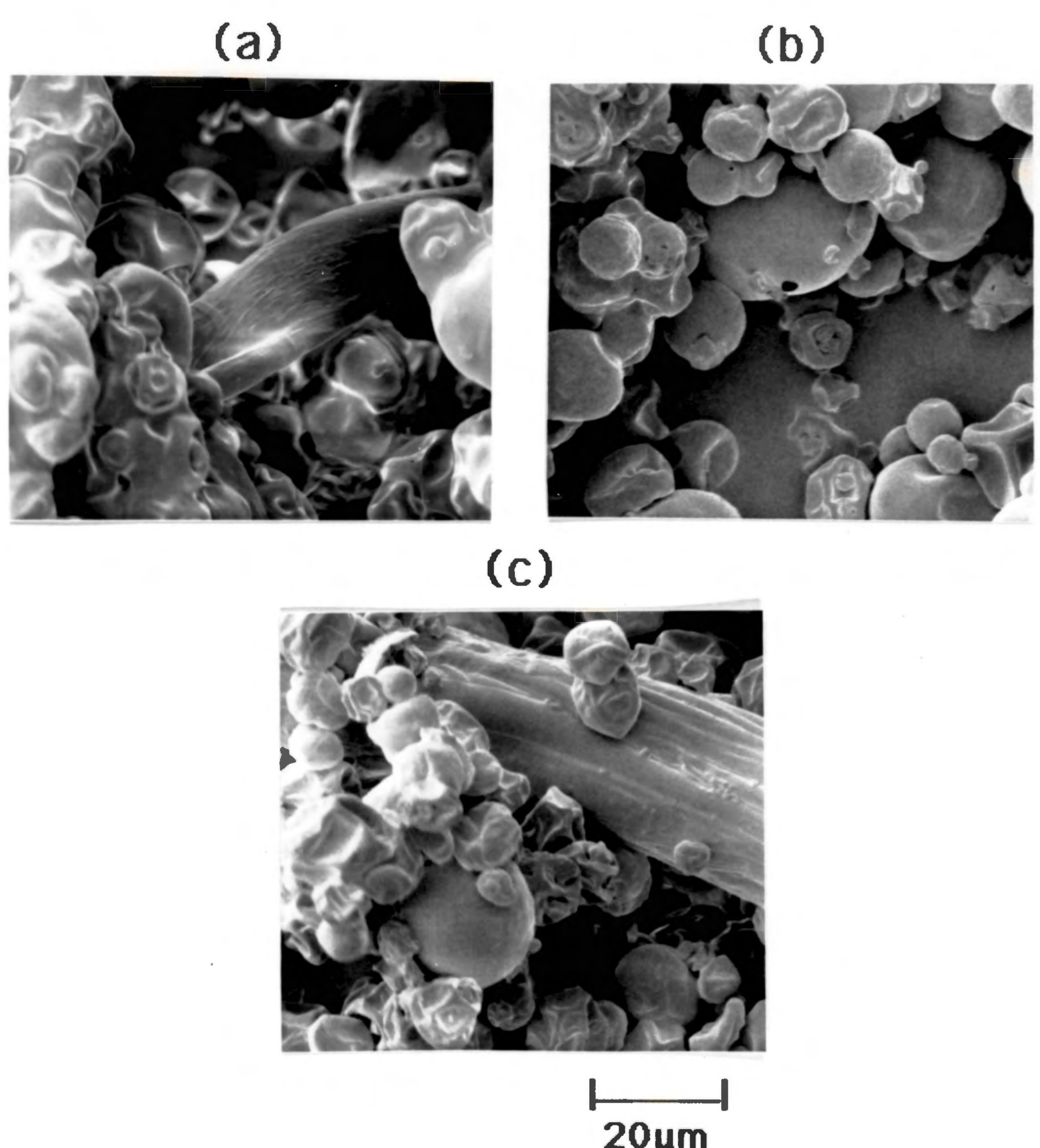


Figure 1: Scanning electron micrographs of spray dried casein- oil emulsions (a) untreated (treatment 1), and treated by (b) acetaldehyde (treatment 2), (c) aqueous extract of peanut skins (treatment 4), and (d) aqueous extract of tobacco stems (treatment 5).


(a)
(b)
(c)
20μm

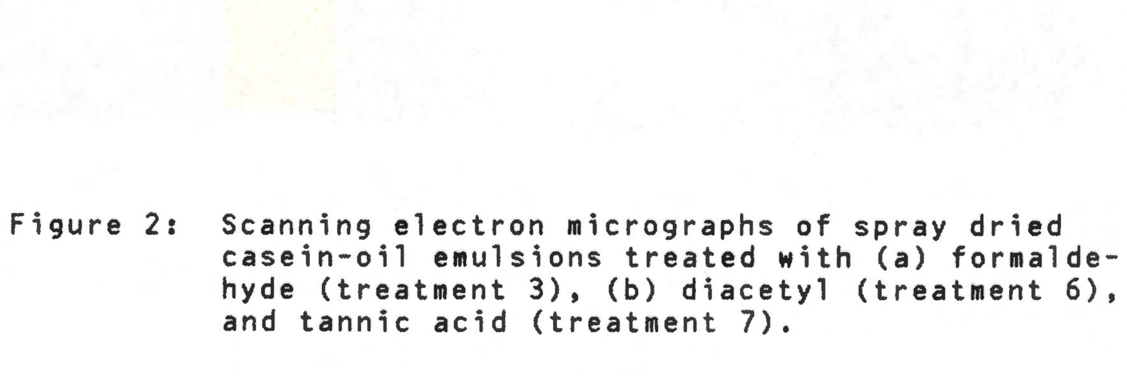


Figure 2: Scanning electron micrographs of spray dried casein-oil emulsions treated with (a) formaldehyde (treatment 3), (b) diacetyl (treatment 6), and tannic acid (treatment 7).

V. SUMMARY

In this experiment, spray dried emulsions containing casein: soybean oil, 1:1, w/w (substrates) were prepared by seven different treatments and were digested with ruminal fluid at 38C for 23 hr. The seven treatments were untreated (treatment 1), acetaldehyde treated (treatment 2), formaldehyde treated (treatment 3), peanut skin aqueous extract treated (treatment 4), tobacco stem aqueous extract (treatment 5), diacetyl treated (treatment 6) and tannic acid treated (treatment 7). The extracts of the peanut skins and tobacco stems also contained tannins. The relative percentage fatty acid composition of each substrate and ruminal fluid mixture (digest) were determined after 0 and 23 hr digestion by gas chromatographic analysis of the methyl esters of the fatty acids. The fatty acids analyzed included myristic (14:0), palmitic (16:0), palmitoleic (16:1), stearic (18:0), elaidic (18:1 trans), oleic (18:1 cis), an isomer of 18:1 (18:1 isomer), linoleic (18:2), linolenic (18:3), arachidic (20:0), gadoleic (20:1), arachadonic (20:4) and a group of unidentified fatty acids (Unknowns). Two replications were run with a single replication consisting of digests from all seven treatments at 0 and 23 hr digestion time. In addition, the microstructure of the spray dried emulsion from each treatment was determined by scanning electron microscopy.

When averaged across all treatments, percentages of 18:0 and 18:1 trans fatty acids increased (P<.05) from 0 to 23 hr digestion by ruminal microorganisms. These results indicated that 18:2 was being biohydrogenated to 18:0 and 18:1 cis, with isomerization of the 18:1 cis to 18:1 trans. This type of biohydrogenation by ruminal microbes is supported by Van Soest (1984).

Since only one source of soybean oil was used to prepare the substrates in all treatments and the ruminal fluid in each replication was from a single source, differences among treatments in fatty acid percentages averaged across digestion time are due to ruminal microbial metabolism. The following results were found when levels of fatty acids were expressed in this manner. Digests of spray dried casein-oil emulsions untreated or treated with acetaldehyde or tobacco stem extracts (group 1 treatments) had lower percentages of 18:2 and 20:0 and higher percentages of 18:0, 18:1 trans and 18:1 isomer than digests of emulsions that were treated with formaldehyde, diacetyl or tannic acid (group 2 treatments). The fatty acid composition of digests of emulsions treated with peanut skin extract was closer to that of group 1 treatments than of group 2 treatments. In addition, the percentage of 18:1 trans did not increase from 0 to 23 hr in digests of emulsions treated with formaldehyde or tannic acid but increased (P<.05) in those of emulsions from all other treatments. These results showed that treatment of

casein-oil emulsions with formaldehyde, diacetyl or tannic acid protected the soybean oil 18:2 acid from biohydrogenation better than treating the emulsions with acetaldehyde or tobacco stem extract or leaving them untreated.

Within group 2 treatments, however, the fact that 18:1 trans increased in the digests of emulsions that had been diacetyl treated but not in those treated with formaldehyde or tannic acid suggests that the diacetyl was not as effective as treatments 3 and 7 at preventing biohydrogenation. Also supporting this fact, digests of emulsions treated with diacetyl had higher levels of 18:1 cis than those treated with tannic acid. However, the percentages of 18:0, 18:1 trans, 18:1 cis, 18:1 isomer and 18:2 when averaged across the diacetyl and tannic acid treatments were not significantly different from those in the formaldehyde treatment indicating that the diacetyl treatment did protect against biohydrogenation.

The microstructure of the spray dried casein-oil emulsions which were untreated or treated with acetaldehyde, peanut skin extract or tobacco stem extract was different from that of the emulsions treated with formaldehyde, diacetyl or tannic acid (group 2 treatments). The casein spheres in emulsions from group 2 treatments (figure 2) were not as shrunken and had fewer holes than the casein spheres in emulsions from the other treatments (figure 1). The microstructure in emulsions from group 2 treatments also ap-

peared globular in nature similar to the microstructure of spray dried cheeses containing protein:fat ratios of approximately 1:1 (w/w) published by Kerr et al. (1982). The globular nature of the microstructure and the well rounded casein spheres without holes in micrographs of emulsions from group 2 treatments indicate that the oil is entrapped within a casein matrix, and thus, would be protected from biohydrogenation by ruminal bacteria. The casein spheres in emulsions from treatments other than group 2 appeared to be more like the microstructure of casein without fat also reported by Kerr et al. (1982). The shrunken casein spheres containing holes in the micrographs of emulsions from treatments other than group 2 suggest that the oil is not entrapped within a casein matrix and would be available to attack by ruminal microbes.

The protection of different chemical treatments of casein-soybean oil emulsions may be dependent upon the level of chemicals used in the treatment. The levels of tannins in the extracts which did not protect against biohydrogenation were much less than the level of tannic acid (25g) used in treatment 7 which protected the oil from biohydrogena tion. Based on molar concentration, formaldehyde which protected against biohydrogenation was used at twice the level of diacetyl which was less effective. However, the molar concentration of acetaldehyde (.041 moles) used in

treatment 2 was nearly equal to that of formaldehyde (.042 moles) used in treatment 3. Therefore, the lack of prevention of biohydrogenation by acetaldehyde, compared to formaldehyde is not because of concentration. Further research is needed to determine if higher levels of tannins from plant extracts would protect oil in a protein matrix against biohydrogenation. Optimum levels of diacetyl and tannic acid in treatments of protein-oil emulsions to prevent biohydrogenation also need to be determined.

REFERENCES

REFERENCES

Akkada, A.R. 1965. "Physiology of Digestion in the Ruminant," Butterworths, Washington p. 335.

Anderson, R.A. and Todd, J.R. 1968. Estimation of total tobacco plant phenols by their bonding to polyvinylpyrrolidone. Tobacco Sci. 12:107.

Annison, E.F. and Lewis, D. 1959. Metabolism in the Rumen, A.R.C. Institute of Animal Physiology, John Wiley and Sons Inc., New York. p. 23.

AOAC. 1985. "Official Methods of Analysis," 14th ed. Association of Official Analytical Chemists, Washington, DC.

AOCS. 1975. "Official and Tenative Methods," 4th ed. American Oil Chemists' Society, Champaign IL.

Barnett, A.J.G. and Reid, R.L. 1961. "Reactions in the Rumen," Edward Arnold Publishers LTD, London. p. 16.

Barry, T.N. 1976. The effectiveness of formaldehyde treatment in protecting dietary protein from rumen microbial degradation. Proc. Nutr. Soc. 35:221.

Brock, R.A. 1989. Oil consumption continues upward trend. Snack World Sept. p. 14.

Church, D.C. 1983. Digestive Physiology and Nutrition of Ruminants. 2nd. ed. O & B Books, Inc. Corvallis, Ore.

Colby, D.A. 1990. Personal communication. Brown and Williamson Tobacco Co., Louisville, Ky.

Cotta, M.A. and Hespell, R.B. 1985. "Physiology of Digestion in the Ruminant," Newcastle-upon-Tyne, U.K.:Oriel Press LTD. p. 122.

Court, W.A., Binns, M.R., and Hendel, J.G. 1983. Estimation of the influence of curing and stalk position on the phenolic constituents of flue-cured tobacco. Tobacco Sci. 27:51.

Czerkawski, J.W. 1986. "An Introduction to Rumen Studies," Pergamon Press, New York. pp. 7-13.

Damodaran, S. and Kinsella, J.E. 1981a. Interaction of carbonyls with soy protein: thermodynamic effects. J. Agric. Food Chem. 29:1249.

Damodaran, S. and Kinsella, J.E. 1981b. Interaction of carbonyls with soy protein: conformational effects. J. Agic. Food Chem. 29:1253.

de Man, J.M. 1985. "Principles of Food Chemistry," Revised 5th Printing. The AVI Publishing Co., Inc. Westport, CT. pp. 35 and 109.

Dinius, D.A., Oltjen, R.R., Lyon, C.K., Kohler, G.O., and Walker, H.G. 1974. Utilization of a formaldehyde treated casein-safflower oil complex by growing and finishing steers. J. Anim. Sci. 39:124.

Driedger, A. and Hatfield, E.E. 1972. Influence of tannins on the nutritive value of soybean meal for ruminants. J. Anim. Sci. 34:465.

Faichney, G.J. and White, G.A. 1979. Formaldehyde treatment of concentrate diets for sheep. III* Absorption of amino acids from the small intestine. Aust. J. Agric. Res. 30:1163.

Feeny, P.P. 1969. Inhibitory effect of oak leaft tannins on the hydrolysis of proteins by trypsin. Phytochem. 8:2119.

Fennema, O.R. 1985. "Food Chemistry," 2nd ed. Marcel Dekker, Inc., New York.

Ferguson, K.A., Hemsley, J.A., and Reis, P.J. 1967. The effect of protecting dietary protein from microbial degradation in the rumen. Aust. J. Sci. 30:215.

Ford, A.L. and Park, R.J. 1975. Effect of a protected lipid supplement on flavor properties of sheep meats. J. Food Sci. 40:236.

Garton, G.A. 1965. "Physiology of Digestion in the Ruminant," Butterworths, Washington. p. 390.

Goldstein, J.L. and Swain, T. 1965. The inhibition of enzymes by tannins. Phytochem. 4:185.

Haas, P. and Hill, T.G. 1928. "An Introduction to the Chemistry of Plant Products," Longmans, Green and Co., New York. p. 266-306.

Hagerman, A.E. and Butler, L.G. 1978. Protein precipitation for the quantitative determination of tannins. J. Agric. Food Chem. 26:809.

Hale, O.M., and McCormick, W.C. 1981. Value of peanut skins (testa) as a feed ingredient for growing-finishing swine. J. Anim. Sci. 53:1006.

Hawkins, G.E. 1955. Consumption and digestibility of lespedeza sericea hay and alfalfa hay plus gallotannin. J. Dairy Sci. 38:237.

Hewitt, D. and Ford, J.E. 1982. Influence of tannins on the protein nutritional quality of food grains. Proc. Nutr. Soc. 41:7.

Hill, G.M., Utley, P.R., and Newton, G.L. 1986a. Influence of dietary crude protein on peanut skin digestibility and utilization by feedlot steers. J. Anim. Sci. 62:887.

Hill, G.M., Utley, P.R. and Newton, G.L. 1986b. Digestibility and utilization of ammonia-treated and urea-supplemented peanut skin diets fed to cattle. J. Anim. Sci. 63:705.

Hood, R.L. and Thorton, R.F. 1976. Site variation in the deposition of linoleic acid in adipose tissue of cattle given formaldehyde-treated sunflower seed. Aust. J. Agric. Res. 27:895.

Hungate, R.E. 1966. "The Rumen and Its Microbes," Academic Press, New York. pp. 8-81.

Johnson, A.R. 1974. Biomanipulation of ruminant fats. Food Manufacture 49:19.

Kerr, T.J., Washam, C.J., Evans, A.L., and Rigsby, W.E. 1983. Developments in Industrial Microbiology. Society for Industrial Microbiology., Arlington, Va. 24:475.

Kumar, R., and Singh, M. 1984. Tannins: Their adverse role in ruminant nutrition. J. Agric. Food Chem. 32:447.

McBrayer, A.C., Utley, P.R., Lowrey, R.S., and McCormick, W.C. 1983. Evaluation of peanut skins (testa) as a feed ingredient for growing-finishing cattle. J. Anim. Sci. 56:173.

McLeod, M.N. 1974. Plant tannins-their role in forage quality. Nutr. Abstr. Rev. 44:803.

NAS. 1971. Atlas of Nutritional Data on United States and Canadian Feeds. National Academy of Sciences. Washington, D.C.

Oh, H., Hoff, J.E., Armstrong, G.S., and Haff, L.A. 1980. Hydrophobic interaction in tannin-protein complexes. J. Agric. Food Chem. 28:394.

Park, R.J., and Ford, A.L. 1975. Effect of meat flavor of period of feeding a protected lipid supplement to lambs. J. Food Sci. 40:1217.

Park, R.J., Ford, A.L., and Ratcliff, D. 1978. Use of protected lipid supplement to modify the flavor of mutton. J. Food Sci. 43:874.

Ralph, W. 1989. Making lamb more desirable. CSIRO Aust. Quarterly. Feb. p. 9.

Ramachandra, G., Virupaksha, T.K., and Shadaksharaswamy, M. 1977. Relationship between tannin levels and in vitro protein digestibility in finger millet (Eleusine coracana Gaertin.). J. Agric. Food Chem. 25:1101.

Ramshorst, H., and Thomas, P.C. 1988. Digestion in sheep of diets containing barley chemically treated to reduce its ruminal degradability. J. Sci. Food Agric. 42:1.

Reid, W.W. 1959. Some aspects of polyphenols of tobacco. Tobacco Sci. 12:103.

Rosa, N. and Caughill, C.W.H. 1984. Effect of aqueous ethanol, particle size, and temperature on extractibility of soluble acids from tabacco. Tobacco Sci. 28:1.

Sanders, T.H. 1979. Effect of variety, location and year on tannin content of peanut seed coats. Peanut Sci. 6:62.

SAS. 1985. "SAS User's Guide," SAS Institute Inc., Cary, NC.

Scott, T.W., Cook, L.J. and Mills, S.C. 1971. Protection of dietary polyunsaturated fatty acids against microbial hydrogenation in ruminants. J. Amer. Oil Chem. Soc. 48:358.

Snook, M.E. and Chortyk, O.T. 1982. An improved extraction-HPLC method for tobacco polyphenols. Tobacco Sci. 26:25.

Sodano, C.S. 1979. Animal Feeds and Pet Foods, Recent Developments. Noyes Data Corp., Park Ridge, NJ. p.17.

Sokal, R.R. and Rohlf, F.J. 1969. "Biometry," W.H. Freeman and Co., San Francisco.

Stansbury, M.F., Field, E.T., and Guthrie, J.D. 1950. The tannin and related pigments in the red skins (testa) of peanut kernels. J. Amer. Oil Chem. Soc. 27:317.

Stedman, R.L. 1957. Polyphenol constituents of tobacco. Tobacco Sci. 1:155.

Swain, T. 1977. Secondary compounds as protective agents. Ann. Rev. Plant Physiol. 28:479.

Tracey, M.V. 1975. Altering fatty acid composition of ruminant products. Cereal Foods World 20:77.

Utley, P.R., and Hellwig, R.E. 1985. Feeding value of peanut skins added to bermudagrass pellets and fed to growing beef calves. J. Anim. Sci. 60:329.

Van Soest, P.J. 1984. " Nutritional Ecology of the Ruminant," Corvallis: O & B Books, Inc.

Vohra, P., Kratzler, F.H., and Joslyn, M.A. 1965. The growth depressing and toxic effects of tannins to chicks. Poultry Sci. 44:135.

Waller, J.C. 1990. Personal Communication. University of Tennessee, Knoxville.

Warner, A.C.I. 1965. "Physiology of Digestion in the Ruminant," Butterworths, Washington, DC. p. 346.

Weaving, A.S. 1958. The polyphenols of flue-cured tobacco separation and identification of the major polyphenols. Tobacco Sci. 2:1.

Weiss, T.J. 1983. Food Oils and Their Uses., 2nd ed., AVI Publishing Co., Inc. Westport, CT.

Wright, D.E., Payne, E., Pyle, C., Aitken, W.M., and Kirton, A.H. 1976. Polyunsaturated fat in young ruminants. Anim. Feed Sci. and Tech. 2:93.

Zelter, S.Z., Leroy, F., and Tissier, J.P. 1970. Protection of proteins in the feed against bacteria deamination in the rumen. I. Studies in vitro: Behavior in the rumen of some proteins tanned with tannin from chestnut wood of certain aldehydes (formaldehyde, gluteraldehyde, glyoxal). Ann. Biol. Anim. Biochem. Biophys. 10:111.

APPENDIXES

APPENDIX A. ANALYSES OF VARIANCE FROM COMPUTER PRINT OUT.

CLASS LEVEL INFORMATION

CLASS	LEVELS	VALUES
TRT	7	1 2 3 4 5 6 7
TIME	2	1 2

NUMBER OF OBSERVATIONS IN DATA SET = 27

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: UNKNOWNS

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	13	707.75633220	54.44279478
ERROR	13	442.47355100	34.03642700
CORRECTED TOTAL	26	1150.22988320	

MODEL F = 1.60 PR > F = 0.2041

R-SQUARE	C.V.	ROOT MSE	UNK MEAN
0.615317	78.7499	5.83407465	7.40835355

SOURCE	DF	TYPE III SS	F VALUE	PR > F
TRT	6	461.50036415	2.26	0.1027
CONTRASTS[a]				
C1	1	0.47904148	0.01	0.9069
C2	1	7.19446264	0.21	0.6511
C3	1	8.69274016	0.25	0.6193
C4	1	93.32198606	2.73	0.1138
C5	1	19.37125836	0.57	0.4600
C6	1	332.83433914	9.75	0.0054
TIME	1	8.57049440	0.25	0.6242
TRT*TIME	6	227.45848399	1.11	0.4059

[a]Error mean square (33.93) for contrasts included sums of squares from time and trt*time with DF=20.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: 14:0

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	13	14.07629043	1.08279157
ERROR	13	3.10180981	0.23860075
CORRECTED TOTAL	26	17.17810025	

MODEL F =	4.54		PR > F = 0.0052
R-SQUARE	C.V.	ROOT MSE	P14 MEAN
0.819432	95.8658	0.48846776	0.50953289

SOURCE	DF	TYPE III SS	F VALUE	PR > F
TRT	6	5.75645920	4.02	0.0169
CONTRASTS[a]				
C1	1	0.40881828		
C2	1	0.85272212		
C3	1	0.03378131		
C4	1	0.67225931		
C5	1	0.00028808		
C6	1	3.87240480*		
TIME	1	2.84271186	11.91	0.0043
TRT*TIME	6	5.04324401	3.52	0.0270

[a]Error mean square for contrasts did not include time and trt*time sum of squares (DF=13).

*P<.05.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: 16:0

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	13	430.13079943	33.08698457
ERROR	13	228.62413833	17.58647218
CORRECTED TOTAL	26	658.75493776	

MODEL F =	1.88		PR > F = 0.1338
R-SQUARE	C.V.	ROOT MSE	P16 MEAN
0.652945	31.7413	4.19362280	13.21188441

SOURCE	DF	TYPE III SS	F VALUE	PR > F
TRT	6	270.55388917	2.56	0.0730
CONTRASTS[a]				
C1	1	9.59851800	0.49	0.4902
C2	1	12.39937007	0.64	0.4338
C3	1	0.35495750	0.02	0.8938
C4	1	76.24200068	3.92	0.0615
C5	1	6.53167631	0.34	0.5685
C6	1	156.98148803	8.08	0.0101
TIME	1	0.28018407	0.02	0.9015
TRT*TIME	6	159.84013367	1.51	0.2487

[a]Error mean square (19.44) for contrasts included sums of squares from time and trt*time with DF=20.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: 16:1

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	13	2.04235282	0.15710406
ERROR	13	1.28126754	0.09855904
CORRECTED TOTAL	26	3.32362036	

MODEL F =	1.59		PR > F = 0.2058

R-SQUARE	C.V.	ROOT MSE	P161 MEAN
0.614496	140.2106	0.31394114	0.22390687

SOURCE	DF	TYPE III SS	F VALUE	PR > F
TRT	6	1.58661607	2.68	0.0641
CONTRASTS[a]				
C1	1	0.18467964	2.25	0.1491
C2	1	0.22135529	2.70	0.1161
C3	1	0.02348003	0.29	0.5985
C4	1	0.02313599	0.28	0.6012
C5	1	0.05628773	0.69	0.4172
C6	1	1.16581057	14.21	0.0012
TIME	1	0.01099488	0.11	0.7437
TRT*TIME	6	0.35651362	0.60	0.7239

[a]Error mean square (.082) for contrasts included sums of squares from time and trt*time with DF=20.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: 18:0

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	13	1993.58928569	153.35302198
ERROR	13	1575.68860874	121.20681606
CORRECTED TOTAL	26	3569.27789443	

MODEL F =	1.27		PR > F = 0.3389

R-SQUARE	C.V.	ROOT MSE	P18 MEAN
0.558541	92.3811	11.00939672	11.91736826

SOURCE	DF		TYPE III SS	F VALUE	PR > F
TRT	6		848.49640738	1.17	0.3807
CONTRASTS[a]					
C1		1	58.23062181		
C2		1	111.93304661		
C3		1	6.94206161		
C4		1	485.86857435*		
C5		1	0.61800742		
C6		1	135.00545221		
TIME	1		716.26514500	5.91	0.0303
TRT*TIME	6		361.95873549	0.50	0.7992

[a]Error mean square (102) for contrasts included sums of squares from trt*time with DF=19.
*P<.05.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: 18:1 TRANS

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	13	205.79769652	15.83059204
ERROR	13	28.50570608	2.19274662
CORRECTED TOTAL	26	234.30340260	

MODEL F = 7.22 PR > F = 0.0005

R-SQUARE	C.V.	ROOT MSE	P181I MEAN
0.878338	58.3275	1.48079257	2.53875707

SOURCE	DF	TYPE III SS	F VALUE	PR > F
TRT	6	32.70176625	2.49	0.0796
CONTRASTS[a]				
C1	1	2.19320826		
C2	1	0.05012974		
C3	1	1.83934262		
C4	1	34.59328677*		
C5	1	1.24300696		
C6	1	0.48531406		
TIME	1	137.78353049	62.84	0.0001
TRT*TIME	6	28.97370434	2.20	0.1097

[a]Error mean square for contrasts did not include time and trt*time sum of squares (DF=13).
*P<.05.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: 18:1 cis

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	13	239.32126453	18.40932804
ERROR	13	143.93304682	11.07177283
CORRECTED TOTAL	26	383.25431135	

MODEL F =	1.66		PR > F = 0.1855
R-SQUARE	C.V.	ROOT MSE	P181 MEAN
0.624445	20.0249	3.32742736	16.61647588

SOURCE	DF	TYPE III SS	F VALUE	PR > F
TRT	6	151.03111788	2.27	0.1011
CONTRASTS[a]				
C1	1	2.38299933	0.21	0.6521
C2	1	17.65556500	1.55	0.2272
C3	1	0.14433953	0.01	0.9114
C4	1	20.74379498	1.82	0.1919
C5	1	52.73529172	4.64	0.0437
C6	1	60.01087439	5.28	0.0325
TIME	1	4.08190807	0.37	0.5542
TRT*TIME	6	77.06293741	1.16	0.3838

[a]Error mean square (11.25) for contrasts included sums of squares from time and trt*time with DF=20.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: 18:1 ISOMER

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	13	8.14968517	0.62689886
ERROR	13	7.42029158	0.57079166
CORRECTED TOTAL	26	15.56997675	

MODEL F = 1.10 PR > F = 0.4342

R-SQUARE	C.V.	ROOT MSE	P181I2 MEAN
0.523423	54.9274	0.75550755	1.37546472

SOURCE	DF	TYPE III SS	F VALUE	PR > F
TRT	6	4.80747097	1.40	0.2849
CONTRASTS[a]				
C1	1	0.01783545	0.03	0.8565
C2	1	0.00000000	0.00	0.9999
C3	1	0.10202493	0.19	0.6659
C4	1	2.76267839	5.20	0.0337
C5	1	0.52240417	0.98	0.3332
C6	1	1.42883378	2.69	0.1166
TIME	1	0.04050350	0.07	0.7941
TRT*TIME	6	3.10965299	0.91	0.5186

[a]Error mean square (.529) for contrasts included sums of squares from time and trt*time with DF=20.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: 18:2

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	13	4147.74798193	319.05753707
ERROR	13	1587.17002194	122.09000169
CORRECTED TOTAL	26	5734.91800387	

MODEL F =	2.61		PR > F = 0.0476

R-SQUARE	C.V.	ROOT MSE	P182 MEAN
0.723245	26.7455	11.04943445	41.31331365

SOURCE	DF	TYPE III SS	F VALUE	PR > F
TRT	6	3148.53575144	4.30	0.0132
CONTRASTS[a]				
C1	1	0.16048292		
C2	1	41.83341357		
C3	1	47.21458943		
C4	1	1606.54422516*		
C5	1	35.13159313		
C6	1	1348.54595722*		
TIME	1	808.68504799	6.62	0.0231
TRT*TIME	6	159.03844749	0.22	0.9645

[a]Error mean square (91.1) for contrasts included sums of squares from trt*time with DF=19.

*P<.05.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: 18:3

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	13	0.41265980	0.03174306
ERROR	13	0.74785661	0.05752743
CORRECTED TOTAL	26	1.16051641	

MODEL F = 0.55 PR > F = 0.8518

R-SQUARE	C.V.	ROOT MSE	P183 MEAN
0.355583	70.3735	0.23984877	0.34082234

SOURCE	DF	TYPE III SS	F VALUE	PR > F
TRT	6	0.23594624	0.68	0.6664
CONTRASTS[a]				
C1	1	0.06026742	1.30	0.2678
C2	1	0.00004658	0.00	0.9750
C3	1	0.02707716	0.58	0.4538
C4	1	0.03704559	0.80	0.3822
C5	1	0.09683556	2.09	0.1640
C6	1	0.00596639	0.13	0.7236
TIME	1	0.01217928	0.21	0.6530
TRT*TIME	6	0.17173577	0.50	0.7994

[a]Error mean square (.047) for contrasts included sums of squares from time and trt*time with DF=20.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: 20:0

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	13	50.33010155	3.87154627
ERROR	13	48.16536593	3.70502815
CORRECTED TOTAL	26	98.49546748	

MODEL F = 1.04   PR > F = 0.4690

R-SQUARE	C.V.	ROOT MSE	P20 MEAN
0.510989	47.7767	1.92484497	4.02883815

SOURCE	DF	TYPE III SS	F VALUE	PR > F
TRT	6	44.81462674	2.02	0.1362
CONTRASTS[a]				
C1	1	1.10947625	0.41	0.5288
C2	1	1.23997406	0.46	0.5058
C3	1	2.62840831	0.97	0.3356
C4	1	14.56063158	5.39	0.0309
C5	1	2.01647799	0.75	0.3977
C6	1	21.73168734	8.05	0.0102
TIME	1	2.48894814	0.67	0.4272
TRT*TIME	6	3.54407862	0.16	0.9833

[a]Error mean square (2.71) for contrasts included sums of squares from time and trt*time with DF=20.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: 20:1

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	13	0.12954656	0.00996512
ERROR	13	0.10579854	0.00813835
CORRECTED TOTAL	26	0.23534510	

MODEL F =	1.22		PR > F = 0.3602
R-SQUARE	C.V.	ROOT MSE	P201 MEAN
0.550454	146.5442	0.09021280	0.06156014

SOURCE	DF	TYPE III SS	F VALUE	PR > F
TRT	6	0.06641802	1.36	0.3005
CONTRASTS[a]				
C1	1	0.00016877	0.02	0.8886
C2	1	0.01833144	2.19	0.1548
C3	1	0.00218192	0.26	0.6155
C4	1	0.00084491	0.10	0.7542
C5	1	0.00109840	0.13	0.7212
C6	1	0.04525517	5.40	0.0308
TIME	1	0.01280095	1.57	0.2319
TRT*TIME	6	0.04744847	0.97	0.4812

[a]Error mean square (.0083) for contrasts included sums of squares from time and trt*time with DF=20.

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: 20:4

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	13	7.03203678	0.54092591
ERROR	13	6.56984167	0.50537244
CORRECTED TOTAL	26	13.60187845	

MODEL F =	1.07		PR > F = 0.4522
R-SQUARE	C.V.	ROOT MSE	P204 MEAN
0.516990	156.6808	0.71089552	0.45372206

SOURCE	DF	TYPE III SS	F VALUE	PR > F
TRT	6	3.23279305	1.07	0.4299
CONTRASTS[a]				
C1	1	0.30606602		
C2	1	0.00004639		
C3	1	0.22053202		
C4	1	0.11201080		
C5	1	0.44771743		
C6	1	2.27787233*		
TIME	1	1.51126631	2.99	0.1074
TRT*TIME	6	2.07745225	0.69	0.6653

[a]Error mean square (.455) for contrasts included sums of squares from trt*time with DF=19.
*P<.05.

APPENDIX B. SUMS OF SQUARES FOR DIGESTION TIME IN EACH TREATMENT FOR 14:0 FROM COMPUTER PRINT-OUT.

DEPENDENT VARIABLE: 14:0

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
TIME	1	2.84271186	
TRT*TIME	6	5.04324401	
TIME FOR EACH TRT			
TRT 1	1	1.69173462	1.69173462*
TRT 2	1	1.53031446	1.53031446*
TRT 3	1	0.01771898	0.01771898
TRT 4	1	4.93048613	4.93048613*
TRT 5	1	0.00043820	0.00043820
TRT 6	1	0.05436915	0.05436915
TRT 7	1	0.00440618	0.00440618
ERROR	13	3.10180981	0.23860075

*P<.05.

APPENDIX C. SUMS OF SQUARES FOR DIGESTION TIME IN EACH TREATMENT FOR 18:1 TRANS FROM COMPUTER PRINT-OUT.

DEPENDENT VARIABLE: 18:1 trans

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
TIME	1	137.78353049	137.78353049*
TRT* TIME	6	28.97370434	28.97370434
TIME FOR EACH TRT			
TRT 1	1	37.07638223	37.07638223 *
TRT 2	1	55.85438878	55.85438878 *
TRT 3	1	1.79213642	1.79213642
TRT 4	1	29.97643120	29.97643120 *
TRT 5	1	22.38598119	22.38598119 *
TRT 6	1	13.58751600	13.58751600 *
TRT 7	1	5.23912912	5.23912912
ERROR	13	234.30340260	2.19274662

*P<.05

VITA

Susan Dianne Byrd Newberry was born on February 25, 1954, to B.T. and Annie Neal Byrd. In 1972, she graduated from Scotland High School, in Laurinburg, NC. Dianne entered the University of Tennessee in Knoxville in September, 1982, and received her BS in Animal Science in June, 1986.

In September of 1987, she returned to the University of Tennessee in Knoxville to begin work toward a Master of Science degree in the Food Technology and Science Department. In August of 1990, Dianne received her Master of Science degree in Food Technology and Science.