EFFECT OF SARSAPONIN ON RUMEN FERMENTATION AND PRODUCTION OF LACTATING DAIRY COWS

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CHAPTER I

INTRODUCTION

The anabolic and estrogenic activities of some steroidal hormones stimulate growth rate in ruminants. Since the supply of purified hormones and androgens is limited and cost is usually high, researchers in the past decade have searched for synthetic analogs or natural compounds that would have anabolic properties. Differences in chemical structure, variation in concentration in natural feedstuffs, side effects, cost of production, and quality control have limited the success of this research.

Certain natural steroidal hormones and plant steroids have similar chemical structures (Wall et al., 1954). This has caused researchers to search the plant world for sources of inexpensive and abundant naturally occuring steroids of value to the animal industry.

Plant-derived steroid precursors for animals and man have a long history. In 1863 English, French and German researchers reported from their African expeditions that numerous tropical plants had medicinal properties. For the animal industry, one of the first reports of this kind came in 1929 by Jacobs and Heindelberger in which Stophanthus sarmentusus was reported to contain high levels of sarmentogenin and was potentially a richer source of steroids than

bile acids.

In 1948, Dr. J. J. Baldwin of the College of William and Mary, in conjunction with the Bureau of Plant Industry, Soil and Agriculture Engineering, launched an expensive expedition and research program for plant materials with steroidal qualities (Wall et al., 1954). The medical and pharmaceutical industries benefitted the most from these programs. The animal industry paid little attention since good quality feedstuffs were readily available.

Today, with the world's economy and rapid population growth, the optimum use of high quality protein and energy sources has become a more important determinant of the profitability of a business. Estrogenic effects of known steroidal compounds have prohibited their use in the dairy industry. New methods for evaluating and determining nutritional needs, and the awareness of the complementary interactions among nutrients, hormone actions, and production, make a simple non-estrogenic compound with anabolic action have value for the dairy industry.

The lack of safe and effective anabolic compounds has retarded research to improve efficiency of animal production. To improve efficiency more knowledge is needed concerning the factors and anabolic compounds which regulate metabolism and utilization of nutrients.

Sarsaponin, a naturally occuring saponin of the plant Yucca schidegera, is a steroid which has anabolic action. This research was conducted to increase our knowledge of

modes of action of steroidal saponins of the Yucca plant in feed rations of dairy cattle. Specific objectives included:

a) Determination of the effect of sarsaponin on fermentation and digestion by rumen cultures.

b) Evaluation of the effects of sarsaponin in rations of lactating dairy cows in milk yields and composition, fermentation patterns, body weight changes and dry matter intake.

CHAPTER II

LITERATURE REVIEW

Biochemical Characteristics of Sarsaponin

Sarsaponin is composed of six to eight soluble extracts of glycosidic saponins including smilagenin, smilagenin saponin, and sarsaponin. The name saponin is derived from the Latin word "sapo," meaning soap and is descriptive of its surface active agents and foaming action in aqueous solutions (Robinson, 1963).

The nonalkaline properties of saponins make them useful as soap substitutes. Mixed with aqueous solutions and agitated, saponins supposedly are involved with bloat in cattle on forage crops (Cheeke, 1971). Saponins form hemolytic compounds with cholesterol and other B-hydroxysteroids when injected. Saponins are toxic to poikilothermic animals and have been used for hundreds of years as fish poison. Fish killed with saponins are edible, since saponins are not absorbed through the gastrointestinal tract. However, large quantities of saponins irritate the intestinal mucosa and nasal membranes (Heftmann, 1975). Saponins are the immediate precursors in biosynthesis of steroids (Heftmann, 1970).

Chemical Structure

Saponins are glycosides; that is, they are composed of carbohydrates and noncarbohydrates, or aglycone, portions. Aglycones often are called sapogenins. These glycosides can have either a steroidal or a triperpenoid structure (Farnsworth, 1966; Robinson, 1963). The glycosides described in this review have a steroidal structure.

The steroidal saponins are composed of a nucleus with a spiroketal side chain of one xylose, two glucoses, and two galactoses (Robinson, 1963) and never occur in a free sapogenin form (Farnsworth, 1966). To the basic four ring steroid molecule are added two oxygen heterocycles. These are furan, ring E, ring F and pyrane. The sugar portion of the structure is generally composed of oligosaccharides, usually D-glucose, D-xylose, D-galactose, L-rhamnose and L-arabinose (Figure 1). Many plant steroids differ from cholesterol in having additional methyl or ethyl residues at C-24 and/or a double bond at C-22 (Schutte, 1975).

Synthesis of Steroids by Plants

Originally, plants and small organisms were thought to lack the capacity for sterol production, but these sterols have now been identified in certain plants. Over the past decade, cholesterol has been found to have an important function in plants. In animals, cholesterol is the precursor of all other steroids (Heftmann, 1973). Cholesterol in the past was considered to be present only in animals but

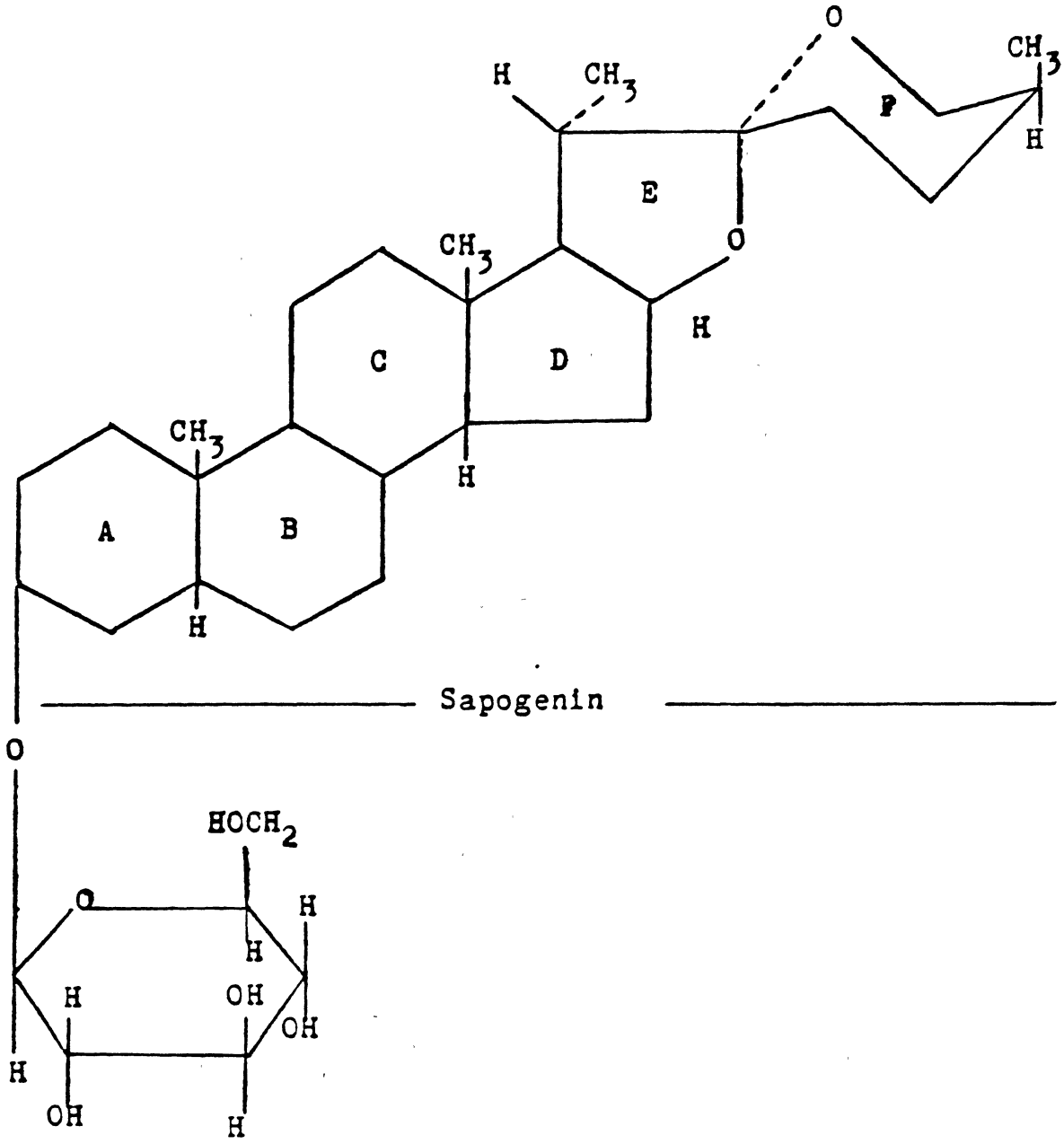


Figure 1.- Sapogenin Steroid

today it has been detected in trace quantities in most plants thanks to modern physical techniques including chromatography and trace methodology. Though biosynthesis and metabolism of steroids is considered to be similar in all organisms, very little is known about the biogenesis or the metabolism of sarsaponin. Certainly sterols are the starting materials for the biosynthesis of all other plant steroids. Intermediates are utilized in assorted ways by various plants to produce characteristic steroids (Heftmann, 1973).

The 27-carbon sapogenins can be used to synthesize steroidal sapogenins causing diosgenin and other valuable materials to accumulate. In a series of studies started in 1961, it was established that cholesterol is the starting material for the biosynthesis of diosgenin (Heftmann, 1973).

Steroidal saponins, which are often found with cardiac glycosides, are synthesized by modifying the side chain of diosgenin. It is believed that this compound is oxidized at C-26 and C-22 and in one of the two terminal carbons of the side chain. The sequence at which they are oxidized is not completely known, but it is believed that the oxygenation of C-26 is the first step in biosynthesis.

Possible Means of Action

From recent work on the biosynthesis and the metabolism of steroids in microorganisms and higher plants, it is apparent that compounds which occur in plants also occur in

animals. Such is the case for cholesterol which has the same fate in all living organisms.

Sterols have three functions in animals. They are precursors for biosynthesis of all other steroids, a stockpile of precursors which are readily convertible to active biological compounds (Heftmann, 1970). Secondly certain sterols exhibit hormonal activity. Thirdly certain sterols are structural cell components (Blunden and Jewers, 1978).

The mode of action and biological effects of steroidal saponins have not been examined. Triperpenoid saponins found in legumes and feedstuffs used in ruminant nutrition have received attention. Even though physical characteristics of steroidal and triperpenoid saponins are similar, their biological activities differ (Cheeke, 1976).

Some biological effects of sarsaponin may relate to the following effects.

(1) Hemolysis of blood cells: Saponins, when associated with cholesterol or 3 β-hydroxysteroids have hemolytic properties (Heftmann, 1970). Saponins supposedly interact with cholesterol in the erythrocyte wall to increase permeability and cause hemolysis (Galanert et al., 1962). Species of animals differ in regard to their susceptibility to erythrocyte hemolysis with ranking as follows: guinea pig, horse > dog, rat, rabbit > man, pig > goat, sheep, cattle (Ewart, 1931). Erythrocytes from ruminants are the least susceptible to hemolysis. This may be due to low absorption of saponins through the intestinal tract.

(2) Effect on blood and tissue cholesterol: Researchers hypothesize that cholesterol in the bile combines with saponins in the gut, and in this manner its reabsorption is prevented. No direct studies on the effect of saponins on cholesterol metabolism in ruminants have been reported, but there is some evidence that the effect may differ from the effect on monogastrics. Binns and Pederson (1964) found no effect of feeding high-saponin alfalfa hay on the blood cholesterol level of calves. If saponins are degraded in the rumen, as it has been demonstrated by Gutierrez (1959b), then the influence of cholesterol reabsorption from the intestinal tract would be slight (Gutierrez and Davis, 1962). On the basis of the limited evidence available, it appears that saponins may have some effect on monogastrics, while in ruminants there is little effect.

(3) Growth effect: While numerous reports have been made on the growth repressing effects triperpenoid saponins have on birds and other monogastrics, the effect of sarsaponin on the growth of ruminants has received little attention. Hale et al. (1961) reported that smilogenin at a level of 17.6 mg/kg of feed, improved the growth rate of fattening lambs. They suggested that the mechanism of the growth stimulating effect was due to a structural similarity of the steroidal saponin and diethylstilbestrol.

It is noteworthy that saponins have been reported to have effects on plant growth, observing that saponins stimulated the growth of wheat and pea embryos (Helmkamp and

Bonner, 1953). It has also been suggested that saponins have a role in the regulation of seed germination (Nord and Van Atta, 1960).

(4) Bloat in ruminants: There is sufficient evidence to assume the significance of the triperpenoid saponins in legume bloat, but no evidence is found indicating these same effects with steroidal saponins. Nevertheless, a distinct characteristic of all saponins is the surface-acting property which will lead to foaming with agitation in aqueous solution.

(5) Inhibitors of smooth muscle activity: Lindhal et al. (1957) examined the effects of alfalfa saponins on the motility of the smooth muscle. Intraruminal administration of saponins resulted in a pronounced reduction in rumen motility. Intravenous administration of triperpenoidal saponins also resulted in reduced rumen motility; the specific site of action was not identified.

(6) Inhibition of enzymes: A possible explanation of the fact that saponins (triperpenoids) may inhibit respiratory enzyme activity, is that saponins inhibit the <u>in vitro</u> oxidation of succinate, an important Kreb's cycle intermediate (Cheeke and Oldfield, 1970).

Besides altering cellular metabolism, sarsaponins may influence digestive enzyme activity. Goodall (Personal Communication, 1984) observed in laboratory trials that Yucca sarsaponins are potent inhibitors of the enzyme

urease. No further studies on the inhibition of enzymes in ruminants are available.

(7) Nutrient absorption: Absorption of sterols from the intestinal tract is highly specific. While cholesterol is readily absorbed, other sterols differing slightly in chemical structure, particularly the phytosterols, are not absorbed (Sperry and Bergman, 1937). Saponins reduce the absorption of cholesterol by forming an insoluble complex. Sarsaponin may combine with other sterols of similar structure such as vitamin D and interfere with absorption. Since steroidal saponins are non-dialyzable (Lindhal et al. 1957), the absorption would be expected to be low. This low level of absorption also can be deduced from toxicity studies as saponins are from 10 to 1000 times more toxic when administered intravenously than when taken orally. The lack of substantial biological effects of oral saponins is presumably due to the low absorption and destruction by bacteria in the digestive tract (Gutierrez et al., 1959). Studies by Gestetner et al. (1972), failed to detect saponins or sapogenins in the blood of chicks, rats or mice fed saponins. While absorption of saponins across the rumen wall has not been demonstrated, the passage of saponins from the blood to the rumen can have systemic effects. Lindahl et al. (1957) found that intravenously administered saponins caused bloating in sheep.

Sources of Sarsaponin

Saponins occur most frequently in the families of the order Liliales. The several families of greatest interest are: Amaryllidaceas, Dioscoriaceas and Liliacea (Lily family) which includes the genera Yucca, the source of most steroidal sapogenins. Yuccas are common in the southwest of the United States (Figure 2).

Botanical Description of the Plant

Yucca schidigera. Mojave Yucca. Spanish Dagger. Datil. This yucca is a shrub 1 to 5 m high. The woody trunk is usually unbranched, with clustered, dagger-like yellow-green leaves from 30 to 50 cm long, 3 to 4 cm wide at the base and displaying curling fibers along the edges. The cream, purple-tinged flowers are 2 to 5 cm long and usually appear from March to May in a single branched cluster about 5 cm long. The fruit is a white oblong capsule 5 to 10 cm long.

Yucca is found with sedge brush and creosote brush on dry slopes and deserts below 1,500 m. Indians used the fibers from the leaves to make sandals, cords, baskets, and rough cloth. Sap from the roots produces a lather if rubbed vigorously in water. A laxative also is derived from the root. The flowers of most species are eaten just before the full grown bud opens. The fleshy fruit is dried and also edible (Coyle and Roberts, 1975).

Geographical Distribution

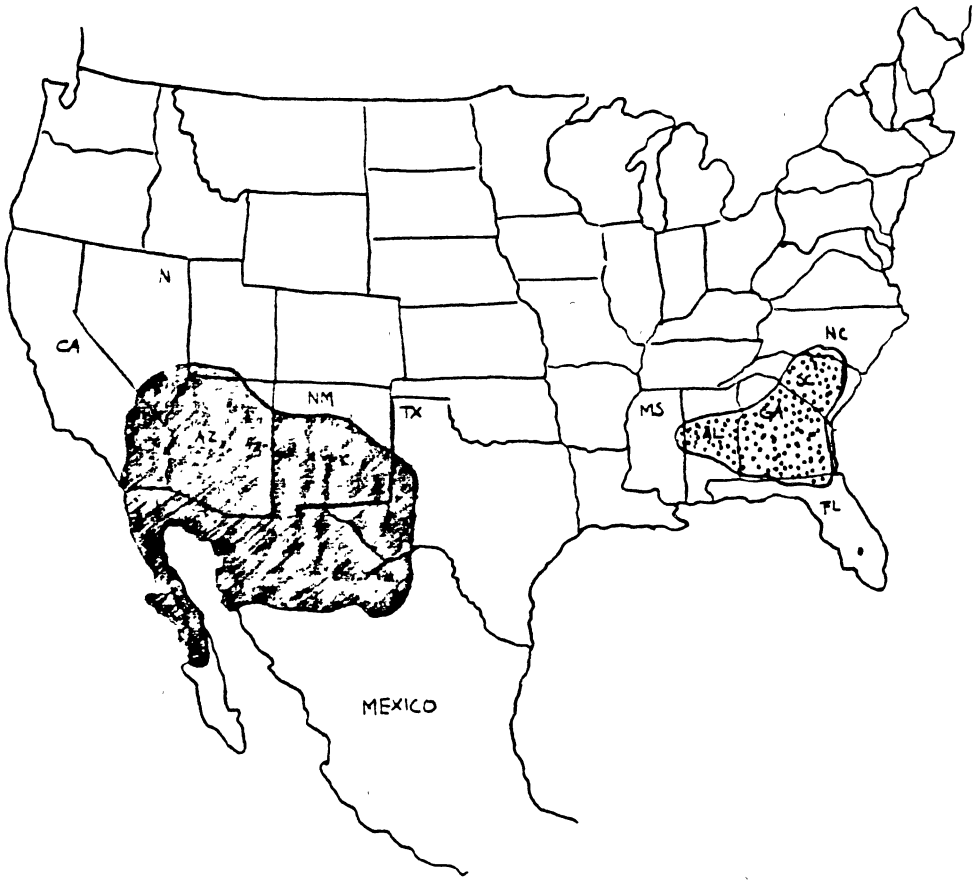
Yucca shidigera is widely spread in most semi-desert areas below 1,500 m. Plant materials have been collected in the southwestern United States, northern Mexico and in the peninsula of Baja California (Figure 2). However, R. K. Godfrey found other Yucca species native east to North Carolina, south to Florida, and as far west as Mississippi as shown on figure 2 (Heardstrom, 1978).

Plant Concentration of Steroidal Saponins

One key factor in classification of plants which contain saponins is the saponin concentration. Concentration of saponins will vary with the site and age of the plant. Biosynthesis of saponins begins when the seeds germinate (Hardman and Sofowora, 1970) and reach a peak with the development of the cotyledons and a second peak when the first true leaves develop (Evans and Crowley, 1972a). As the plant matures, the sapogenin composition changes with the growing root tip and the inflorescence is richest in sapogenin (Evans and Crowley, 1972).

Extraction and Determination of Steroidal Saponins

General procedure: Saponins are easily extracted from plant material with hot water or alcohol and precipitated from solution by the addition of lead salts or ether. Next, fatty materials may be removed with benzene, and the sapo-



Yucca materials from R.K. Godfrey

Area of growth of Yucca shidigera

Figure 2.- Geographical distribution

nins can be extracted with butanol leaving proteins and carbohydrates in the aqueous phase (Heftmann, 1970). Acid or enzyme hydrolysis yields the sapogenins, which may be converted to acetates and purified by alumina chromatography. If hemolysis occurs when this extract is mixed with red blood cells, it can be assumed that sapogenins are present. Triperpenoid and steroidal saponins both act similarly, however, so the two cannot be differentiated on this basis alone. Wall et al. (1954) developed a standard procedure outlined below which has been used for detection and estimation of steroidal saponins, and for differentiation from triperpenoid saponins.

Micro Procedure (Wall et al., 1954). Detection of Saponins: If a 1 ml aliquot of the saponin extract did not hemolyze 10 ml of a saline red blood cell suspension, no significant quantity of steroidal saponins is present. Negative hemolysis automatically means that the sample does not have sufficient amounts of steroidal saponins. If the extract gives a positive test, it is hydrolyzed with a 4 N hydrochloric acid at 75 to 80 C for two hours and the resultant sapogenins are extracted with benzene. After acetylation and alkali purification, the sample is dried and weighed. The sample was classified as negative if the yield of crude acetate was less than 0.1% pure sapogenin. The crude sapogenin acetate can be dissolved in carbon disulfide or chloroform and the infrared spectra determined. This

test gives a positive or a negative test of the existence of steroidal sapogenins.

In most instances, the Liebermann-Burchard (L-B) test has been used to detect this class of compounds and has worked very well for determining triperpenoid and steroidal saponins (Farnsworth, 1966). Blue or green colors are formed in the L-B test with steroidal saponins, and red, pink or purple colors result from triperpenoid sapogenins. However, researchers using powdered materials noted variations in the color produced, depending on the manner in which this test was conducted. Wall et al. (1954) determined which substance caused the interference and pointed out that by using chloroform in the extract and infrared determination, results were more accurate.

Intended Uses of Sarsaponin

Because numerous plant species produce substantial quantities of steroidal saponins, the use of steroidal saponins is quite extensive. This literature review will focus on their use in ruminant nutrition.

Improvement of Growth Rate

The growth-promoting action on ruminants of animal source steroidal extracts, such as estrogens and androgens, has been known for many years. Specific combinations of estrogens with androgens or progesterone have been used as growth stimulants, with the estrogenic action being dimin-

ished by the other hormone so that the anabolic action predominates (Hale et al., 1961). Many of the products of steroid metabolism in plants, such as the C 27 saponins, may have regulatory as well as ecological functions. They are involved in regulating life processes of plants, helping plants survive in a hostile environment (Heftmann, 1970).

Since steroidal saponins are base material for the biosynthesis of other steroids, the economic importance of steroidal saponins is mainly due to their simple conversion to other sterols such as progesterone, rubosterone and estrone. Structural similarities between steroids of animal and plant origin suggest that steroids may be converted to other compounds by ruminants.

Hale et al. (1961) reported weight gain increases of 17.7% and feed efficiencies of 16.2% above controls when fattening lambs were fed 12 mg of smilagenin per lamb per day. Nearly identical growth responses were obtained from 18 g levels (12 mg/lamb/day) of sarsaponin and smilagenin. In general, sarsaponin appeared to increase dressing percent and improve carcass grade. Measurements of the bulbo-urethral gland did not reveal an enlargement from feeding plant steroids as was noted with lambs fed diethystilbestrol.

Hale et al. (1961), observed that, feeding 20 mg of smilagenin daily improved gain in two cattle experiments by an average of 9.6% over controls compared with 9.5% for 10 mg stilbestrol.

Improvement of Feedlot Efficiency

Feed efficiencies and carcass quality are two of the main factors determining the profitability of a feedlot operation. Steroids can improve feedlot efficiencies and carcass quality. Their use has been limited because residual estrogenic compounds in carcasses may endanger the health of the consumer.

Average daily gains and marbling increased when cattle were fed diets containing 100 ppm sarsaponin, while feeding diets with 200 ppm increased rib-eye area (Goodall, 1980) in a 120 day trial with 40 steers. Most of the past research has used steers fed finishing rations high in concentrate. With higher levels of roughage, effects of sarsaponin are reduced. Goodall (1980) fed 180 calves a backgrounding diet consisting primarily of corn silage for 77 days. Average daily gains and feed/gain ratios were depressed slightly when fed diets containing 100 ppm of sarsaponin. In the subsequent 128 day finishing period, average daily gains and feed to gain ratio were improved with added sarsaponin (60 ppm).

Ionophores have had popularity in feedlot operation because of their improvement in feed efficiency. Complementarity with sarsaponin was studied with sarsaponin levels of 0, 500, 700, and 900 mg per head per day fed in combination with 360 mg of the ionophore Losalocid[1] by Stanton et al. (1984). No improvement from added sarsaponin in feed in-

[1]Manufactured by Hoffman-La Roche Inc. Nuttley, N.J. 07110

take, daily gain, feed efficiency or carcass parameters was noted. Zinn et al. (1983) also found no benefit from 66 ppm sarsaponin in rate of gain and feed efficiency of crossbred feedlot calves.

Improvement of Fermentation of Carbohydrates

Sarsaponin was first suggested to stimulate anaerobic fermentation and to function as a stress relief agent (Peekstok, 1979). Altered fermentation in rumen could result in higher levels of desirable end products. Degradation of feed entering the rumen is caused by enzymes from bacteria and protozoa. The rumen is a continuous culture system for anaerobic microorganisms. Foods which enter are partially fermented and yield principally volatile fatty acids (VFA). When fermentation favors propionate and reduces methane, feed-to-gain ratios are generally improved. However, as propionate increases acetate usually decreases, which may reduce percentage of fat in the milk (Maynard et al., 1979). Methods to manipulate fermentation in order to increase efficiency of food production are currently being extensively studied.

Preston (1975) suggested that plant or animal steroids do not exhibit their effect at the tissue level. Because of their molecular structure, steroidal saponins are unable to cross the epithelial membranes in the gastrointestinal tract and should exhibit their effects within the gut.

Grobner et al. (1982) reported that the most consistent effect of sarsaponin, in the presence of monensin on in vitro continuous flow fermenters, was a 10% increase of propionic acid. A similar change was reported by Goodall and Matsushima (1981) with sarsaponin in animals receiving no ionophore. Concentrations of other VFA were not affected in either experiment.

A second indicator of carbohydrate fermentation is the interconversion of acids and gas production. These gases include methane, carbon dioxide and, under some conditions, hydrogen. Grobner et al. (1982) observed that the total gas production averaged 3% higher but that methane production was not decreased by added sarsaponin.

Cellulose and hemicellulose are the most fermented sources of fiber, but both are fermented less rapidly than starches and sugars. An increase in extent of ruminal digestion will increase amounts of available nutrients produced per unit of feed and improve efficiency. Goetsch and Owens (1984) reported that sarsaponin increased starch digestibility in dairy cows. Zinn et al. (1983) observed small increases in ADF digestion with sarsaponin supplementation. Increased ADF digestion may have greater importance since ADF can be digested only by the action of microorganisms and often ADF may exceed 20% of the diet.

Most fermentation in the tract of ruminants is due to ruminal bacteria. Total numbers of bacteria and the relative population of individual species vary with the diet of

the animal (McDonald et al., 1982). Protozoa, though present in lower numbers than bacteria, are larger so total mass may equal that of bacteria. Protozoa are easily killed by low rumen pH, and are generally absent from animals fed diets which promote low pH. Ruminants without rumen protozoa appear normal and healthy, so the benefit of protozoa has been questioned (McDonald et al., 1982). In one experiment, faunated animals grew faster than defaunated animals, possibly because of an effect of protozoa on the relative proportion of rumen VFA.

Effect on Urease and Nitrogen Utilization

In many diets one-third of the performed protein in ruminant diets can be replaced by non-protein nitrogen (NPN). Urea is the most commonly used NPN source because of its chemical characteristics and low price. Urea is also "natural" and is rapidly hydrolyzed in the rumen by bacterial urease. The subsequent use of released ammonia varies with the ability of the rumen microorganisms to utilize it for synthesis of protein. Bloomfield et al. (196Ø) reported that the rate of ammonia released from urea exceeds microbial uptake of ammonia by a factor of four. Hence, one factor limiting the utilization of urea is its rapid hydrolysis. Inhibitoring ureolytic activity could possibly improve urea utilization.

Many urease inhibitors have been studied. Groll (1918) and Shaw (1954) studied single salt compounds or metal ions. Cheeke and Oldfield (1970) reported that saponins may inhibit respiratory enzymes and *in vitro* reduced oxidation of succinate. Inhibition of cellular enzymes by saponins could explain some effects on growth. In addition, saponins may influence activity of digestive enzymes. Isahaaya and Birk (1965) reported that soybean saponins inhibit cholinesterase, chymotrypsin and trypsin activity.

Very little work has examined urease inhibition by the steroidal saponins of *Yucca schidigera*. Goodall (Personal Communication, 1984), in laboratory work observed inhibitory effects of sarsaponin on urease. Sarsaponin inhibited both bacterial (*Bacillus pasteureii*) and plant enzyme (Jack Bean) urease, with slightly more activity against the bacterial form. These inhibitory effects are not due to changes in metal conductivity or detergent disruption. Goetsch (Personal Communication, 1984), fed dairy steers diets with urea 20% of their nitrogen (N) needs, and observed no differences in rumen ammonia-N, suggesting little effect on urease unless recycling to the rumen was reduced.

Considering the importance of a slow hydrolysis of NPN from feed or recycled urea into ammonia in the rumen adequate to meet needs for protein synthesis, ammonia was measured at various times postfeeding. Goetsch (Personal Communication, 1984), observed in another trial that sarsaponin supplementation of heifers fed a 50% concentrate diet had no

effect on rumen ammonia values at four and eight hours post-feeding, but values were higher 12 hours postfeeding. Contrasting findings were reported by Grobner et al. (1982) who noted lower ammonia nitrogen concentrations with sarsaponin added.

In high producing dairy cows in which high protein levels are needed during early lactation, nitrogen digestibility and utilization play very important roles. Zinn et al. (1983) suggested that the major activity of sarsaponin may be to reduce protein losses from the rumen.

The supply of protein available for absorption is presently being researched. Goetsch (Personal Communication, 1984) observed that the total N and feed N entering the duodenum of cannulated steers increased linearly with increasing levels of sarsaponin while ruminal N loss decreased. Zinn et al. (1983) reported that ruminal loss of N decreased by 22% and efficiency of microbial protein synthesis was increased 23% with sarsaponin addition to a high concentrate diet.

To stress test the protein sparing action of sarsaponin, protein limiting diets should be fed. Zinn et al. (1983) suggested that diets containing 12.5% crude protein plus 66 ppm sarsaponin should be the equivalent to diets that contained 15.5% crude protein but no sarsaponin, indicating a possible reduction of the protien needed to meet the animals requirements. If so, addition of sarsaponin to high protein diets would be beneficial.

Influence on Rate of Passage

The rate of passage of starch, nitrogen and microbial nitrogen may limit digestion and absorption of nutrients. A 33% increase in the level of feed intake led to a general decrease in extent of organic matter digestion in the rumen and an increase in ruminal passage of dietary protein (Zinn and Owens, 1980). Slowing rate of passage through the digestive tract could increase nutrient digestibility. Goodall (1980) reported higher digestibilities of nutrients with sarsaponin supplementation and rate of passage was lower. Slower passage should increase extent of protein digestion in the rumen. In contrast, Goetsch and Owens (1984) reported that passage rate of concentrate, silage and fluid from the rumen tended to be increased by sarsaponin addition to rations of dairy cows. Digestibilities of ruminal and total tract organic matter, starch, ruminal and total N were increased, and duodenal flow of total and feed N tended to be lower, while microbial N was not affected. Zinn et al. (1983) reported that addition of sarsaponin (60 g/ton) increased net protein passage to the small intestine by 22% in agreement with the *in vitro* studies of Grobner et al. (1982) who observed increases of 10 to 18% in the effluent outflow of protein with sarsaponin. Since both protein degradation and microbial protein turnover were reduced, it appears that sarsaponin may directly or indirectly interfere with production or activity of protease or deaminase enzymes within the rumen (Zinn et al., 1983). However, sarsaponin

fed at high levels (700 mg/ton), in combination with an ionophore (360 mg of Bovatec/head/day) had no significant effect on average daily gain, feed efficiency or carcass parameters (Stanton et al., 1984).

Lindhal et al. (1957) found that intraruminal and intravenous administration of saponins reduced rumen motility even though phytosteroids should be non-absorbable (Sperry and Bergman, 1937). Hence the increased passage rate observed by Goetsch and Owens (1984) and Zinn et al. (1983) may be related not to increased rumen motility but to an enhanced rate of microbial digestion.

Theories on How Steroids May Affect Microorganisms and Rumen Digestion

The effects or mechanisms of action of phytosteroids as well as animal steroids are poorly understood. Two possible effects or mechanisms of action on microorganisms and ruminant digestion have been proposed. The first was proposed by Rakoff et al. (1944) stating that because estrogens are circulated in an enterohepatic cycle, similar to bile acids, these compounds are available to the lower gastrointestinal tract where they increase digestibility. Because of the limited absorption of steroidal saponins, this theory is not satisfactory. Pfander (1957) proposed that hormones function through mediation of extracellular enzyme production and activity for the rumen microorganisms in a manner similar to the effect of estrogens in mammalian tissues.

Application of Steroids in The Dairy Industry

In the attempt to increase productivity of dairy cows, interest developed in the early 60's for feeding steroids such as diethystilbestrol (DES) to dairy cattle. At this time, very little information was available on the possible anabolic or estrogenic effects that steroids could have on dairy cattle; therefore, whether their use would be beneficial or detrimental to the dairy industry was unknown.

In early research, Bush and Reuber (1963) observed that heifers with DES made more rapid weight gain from 4 to 15 months of age, after which the effect tended to diminish. No reproductive abnormalities in cattle were attributed to DES and no problems in conceiving or producing normal calves was noted. Similar results were observed by Fosgate (1964) who reported increases in rate of gain due to adding DES, alone or in combination with phenothiazine.

Eisenberg and Gordon (1950) found a considerable difference between anabolic and androgenic action steroids. With milk replacement diets, interest was revived in the economics of production of acceptable veal. Satisfactory growth performance was not obtained with milk replacement diets. This was where anabolic steroids could be used in veal production. MacFadden and Belden (1967) reported significant increases in the rate of gain (43%) and in feed conversion (11%) in dairy calves due to DES.

Nevertheless, negative estrogenic effects arose from research of animal steroids with dairy animals. Cupps and

Briggs (1965) supplementing estrogen to bulls reported that a large number of spermatozoa had looped or bent tails. This effect could not be counteracted by supplementary testosterone. It was concluded that estrogen altered the epididimal epithelium and caused these problems. This and other research linked anabolic and estrogenic effects, thereby increasing the demand for the development of guidelines by the Food and Drug Administration to guarantee the safety of edible products subsequent to treatments with steroids. More research on the safety and residual effects of steroidal compounds is needed. Willette et al. (1967) reported that urine was the major route of excretion of steroids, accounting for 90 % of progesterone, 60 % of estradiol-17 B and approximately one-third of estrogen. In this and other experiments, milk has been a minor pathway for excretion of metabolites of steroidal hormones.

Until a few years ago, no compound considered to be safe and effective for growth promotion, milk production or reproduction performance in dairy cattle was known. Sarsaponin, a natural steroid from the Yucca plant, shows evidence of having desirable effects on ruminal digestion. Goodall (1980) suggested that sarsaponin may act in one or several of the following modes:

1) A precursor of other steroids that are subsequently utilized by the organism.
2) A trigger that sets preexisting mechanisms into motion.
3) A regulator of transport across membranes.

Since steroidal saponins are extracts of several different steroids, they might alter several of these functions. Some research has been conducted on these steroidal saponins. Saponins have effects on ruminal anaerobic fermentation by increasing both the digestibilities in certain parameters and the passage rate with medium concentrate diets; thereby, sarsaponin may be useful in dairy operations.

One objective of this trial was to determine the effect of sarsaponin on fermentation and digestion by in vitro rumen cultures. A performance study was conducted to determine whether or not the observed fermentation patterns increased milk production or had any adverse effect.

CHAPTER III

EFFECTS OF DIETARY SARSAPONIN CONCENTRATION ON FERMENTATION IN SEMICONTINUOUS RUMEN CULTURES

Summary

To determine the effect of sarsaponin concentration on ruminal fermentation, sixteen in vitro semicontinuous rumen cultures were fed a basal diet of 45% ground corn, 45% alfalfa hay and 10% soybean meal, with sarsaponin at 0, 33, 55, and 77 ppm. Each day at 0645 and 1845 hours, a portion of effluent was removed and substrate and buffer were added. The experiment lasted 22 days with analysis of effluent from the final 12 days. As sarsaponin was increased, numbers of protozoa decreased linearly while bacterial numbers tended to increase. Culture pH was similar for sarsaponin levels but ADF fiber digestion increased linearly with increasing concentrations of sarsaponin. Disappearance of feed non-ammonia nitrogen tended to be lowest at the highest concentration of sarsaponin. Microbial nitrogen production was similar for all treatments though microbial efficiency tended to be reduced with added sarsaponin.

Introduction

Sarsaponin, a steroidal glycoside, has been used in rations for finishing cattle and may stimulate microbial growth (Peekstok, 1979). Increased anaerobic fermentation can increase digestibility and have beneficial effects on animal performance. Brooks et al. (1954) studied effects of stilbestrol, estrone and cholesterol (animal steroids) on cellulose digestion by sheep. Each of these steroids increased cellulose digestion *in vitro* by 25 to 35% when added at only 10 to 20 micrograms per mililiter of fermenting substrate. Stilbestrol *in vivo* increased cellulose digestion by 16 to 18%. The plant steroid, sarsaponin, at 100 ppm inceased feedlot performance and carcass characteristics of steers (Goodall, 1980). When sarsaponin was added at 30 ppm, Grobner et al. (1982) observed improved digestibility of feed proteins resulting in higher total nitrogen output and increased production of propionic acid by fermenters.

In vitro fermentation systems are useful to study fermentation and digestion by ruminal microbes (Estell et al., 1982), and allow strict control of experimental conditions. Tests with these systems require less labor, facilities and expense than do animal trials.

The objective of this experiment was to determine the effect of sarsaponin concentrations on rumen fermentation by rumen microbes in semicontinuous *in vitro* fermenters.

Materials and Methods

A modification of a semicontinuous *in vitro* digestion system described by Tilly and Terry (1963) was used. Erlenmeyer flasks, 250 ml were sealed with rubber stoppers. Each stopper had three ports -- one long glass tube (12 cm) extending to near the bottom of the flask for effluent extraction, a short tube (6 cm) for substrate injection, and a third containing a bunsen valve for release of gas. Sarsaponin (SARS) was added at concentrations of 0, 33, 55, and 77 ppm to a substrate consisting of 55% concentrate on an air dry basis; (Table I). A total of 16 rumen cultures were used in a completely randomized experiment with four cultures per treatment. The experiment lasted 22 days with samples collected on the final 12 days. Sarsaponin was administered as Sevarin[1], a commercial product containing steroidal saponins extracted from the plant *Yucca shidigera* including sarsaponin at 30%.

Rumen fluid was obtained from two mature Hereford heifers fed a 58% concentrate diet of ground corn and chopped alfalfa. The two collections were composited, strained through cheese cloth, divided and placed into 16 fermenter flasks. Flasks were sealed and flooded with carbon dioxide to purge existing oxygen from the gas phase. Flasks were placed in a 39 C water bath.

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[1]Manufactured by Distributors Processing Inc., 17656 Ave. Porterville, CA

TABLE I

COMPOSITION OF BASAL SUBSTRATE[a]

ITEM	%
GROUND CORN	45
GROUND ALFALFA	45
SOYBEAN MEAL	10

[a]Analyzed 92.6% dry matter, 2.4% N, 56.6% starch, 15.5% ADF, and 10.1% ash on a dry matter basis.

Initially, each fermenter flask contained 150 ml buffer (McDougall, 1948) preheated at 39 C, to which 50 ml rumen fluid, a small (1.5 cm) stir bar and 2 g of substrate were added initially. At 12-hour intervals (0645 and 1845), flasks were placed on a stir plate to slurry contents into a homogeneous mixture for sampling. A sample (50 ml) was removed using the long tube with a plastic syringe, and .5 g substrate and 50 ml buffer was introduced into each fermenter through the short tube. Cultures were then replaced in the 39 C water bath and gassed with CO_2 for 15 seconds.

The initial 10 days of the experiment were used for stabilization. After stabilization, samples obtained daily at 1845 hours were used for analysis. The pH was measured

immediately. A 1 ml aliquot of the sample was removed with a wide-bore 1 ml pipet and mixed with 2 ml .85% NaCl (W/V) containing 10% formalin and .5 ml of a 1:10,000 (W/V) methylene blue solution.

After the methylene blue solution had the opportunity to stain the bacteria cells (about 12 h), 7 ml of .85% NaCl (W/V) and 10% formalin solution was added. Samples were diluted and numbers of bacteria and protozoa were enumerated in duplicate in a counting chamber. Protozoal numbers were determined with phase contrast microscopy at a magnification of 100 X. Bacteria were enumerated with a Petroff-Hauser chamber at a magnification of 600 X.

For ammonia analysis, 20 ml of effluent were acidified with 0.5 ml of 20% (V/V) sulfuric acid and frozen. Concentrations of ammonia nitrogen (NH_3-N) were later determined by the phenol-hypochlorite assay of Broderick and Kang (1980). Remaining effluent (29 ml) was composited within cultures into vessels containing 250 ml of .85% saline:10% formalin solution. Samples were dried at 55 C and analyzed for dry matter (DM), ash, nitrogen (N by AOAC 1975), acid detergent fiber (ADF; by Goering and Van Soest 1970), starch (MacRae and Armstrong, 1968), and nucleic acids to calculate microbial N (Zinn and Owens, 1982).

Data were analyzed by analysis of variance, using the Statistical Analysis System (SAS,1983). Treatment means were tested for linear and quadratic effects and one contrast, control diet versus sarsaponin diets.

Results and Discussion

Addition of sarsaponin overall increased (P<.Ø5) bacterial numbers (Table II) though the linear effect was not significant (P=.69). In contrast protozoal numbers decreased (P<.Ø5) linearly with added sarsaponin, from 36,ØØØ/ml to 29,ØØØ/ml as concentration of SARS increased from Ø to 77 ppm (Table II).

Generally, numbers of bacteria and protozoa are inversally related (Van Soest, 1982) presumably due to competition for energy or nutrients. Protozoa ingest and presumably digest bacteria (Hungate, 1966). Protozoa and bacteria numbers seemed lower than in natural rumen conditions, probably due to the fermentation system used, as observed previously (Crawford, et al., 198Ø). Species of bacteria or protozoa were not identified though cultures mantained at pH 6.7 have previously contained most of the types of bacteria found at large numbers in the rumen of cattle (Slyter, 1966). The pH values for our fermenters at 12 hr post-feeding varied from 6.76 to 6.78 (Table III) which should be adequate for fiber digestion (Stanely and Kesler, 1959). Digestion of ADF was obtained reflecting anaerobic and pH conditions of the fermenters. Previously in <u>in vitro</u> studies, pH has decreased with addition of sarsaponin (Grobner et al., 1982; Goodall, 198Ø). Strength of the buffering system was much lower in their studies than in ours.

TABLE II

INFLUENCE OF SARSAPONIN ON BACTERIAL AND PROTOZOAL NUMBERS

ITEM	SARSAPONIN CONCENTRATION, ppm Ø	33	55	77	SE
BACTERIA, X 10^{10}/ml	1.24[a]	1.24	1.30	1.33	.09
PROTOZOA, X 10^{4}/ml[b]	3.6	3.3	2.8	2.9	.20

[a] Control differs from other means (P<.05).
[b] Linear effect of sarsaponin (P<.05).

Ammonia nitrogen (NH_3-N) values for all treatments were similar ranging from 4.6 to 4.9 mg/dl (Table III). This concentration of rumen ammonia is generally regarded as adequate for protein synthesis (Satter and Slyter, 1974). Previously, Grobner et al. (1982) reported that sarsaponin at 60 ppm decreased NH_3-N values. Presence or absence of protozoa can influence ammonia concentrations.

Organic matter digestibility was increased (P<.05) by sarsaponin in the diet (Table IV), similar to observations by Goetsch and Owens (1984).

TABLE III

CULTURE pH AND NH_3-N CONCENTRATION

	SARSAPONIN CONCENTRATION, ppm			
ITEM	Ø	33	55	77
pH	6.77	6.77	6.78	6.76
NH_3-N, mg/dl	4.7	4.8	4.9	4.6

Starch digestibility (P<Ø.Ø5) was increased with sarsaponin added but was quadratically related (P<Ø.1) to sarsaponin level, with increased digestion to concentration of 55 ppm and a decrease with 77 ppm (Table IV). No explanation for this effect is apparent though Zinn et al. (1983) observed a depression in ruminal starch digestion with addition of 66 ppm sarsaponin to a high concentrate diet. Increased starch digestibility may be associated with the increase in bacterial numbers. Grobner et al. (1982) reported that sarsaponin increased propionic acid (P<.Ø4) production in vitro but no significant change in starch digestion. This could be due to the shift in the microbial population. Crawford et al., (198Ø) observed that microorganisms in semicontinuous flow fermenters usually have higher efficiencies than measured in vivo.

Apparent digestibility of ADF tended to increase ($P<.10$) linearly with level of sarsaponin approaching significance ($P<.10$) (Table IV). Similar effects in vivo have been reported by Goetsch and Owens (1984) and Zinn et al. (1983).

No significant change ($P>.05$) in feed nitrogen digestion was apparent though numerically loss was lowest at the highest level of sarsaponin. Similar non-significant differences were observed for microbial-N output though microbial efficiency tended to decline with sarsaponin added (Table IV). In contrast, Zinn et al. (1983) reported that microbial protein synthesis increased and feed N digestion decreased in vivo with 66 ppm of sarsaponin in high concentrate diets.

In summary, sarsaponin altered fermentation in the semicontinuous fermenters increasing digestibility of organic matter, ADF and starch. Sarsaponin decreased numbers of protozoa and increased numbers of bacteria.

TABLE IV

NUTRIENT UTILIZATION WITH ADDED SARSAPONIN

	SARSAPONIN CONCENTRATION, ppm			
ITEM	Ø	33	55	77
APPARENT DIGESTIBILITY, %				
ORGANIC MATTER[a]	52.9	68.7	6Ø.7	59.7
STARCH[b]	96.4	97.6	98.2	97.4
ADF[c]	1Ø.2	21.5	3Ø.6	47.7
NITROGEN INTERCHANGE				
N OUTPUT, g/day				
TOTAL	Ø.4	Ø.4	Ø.4	Ø.4
MICROBIAL	2.1	2.2	1.9	1.8
FEED	Ø.23	Ø.24	Ø.33	Ø.23
FEED NITROGEN	67.4	67.4	63.9	49.8
MICROBIAL EFFICIENCY, (g MN/KG OM FERM.)	44.7	32.7	3Ø.1	33.5

[b] Significant difference between control and sarsaponin supplemented diets (P<.Ø5).
[c] Linear effect (P<.1Ø).
[a] Linear effect (P<.Ø5).

CHAPTER IV

EFFECT OF SARSAPONIN ON RUMEN FERMENTATION AND PRODUCTION OF LACTATING DAIRY COWS I

Summary

Twelve cows in their first and second lactation eight to ten weeks postpartum were fed diets with Ø or 77 ppm sarsaponin in a switchback trial. Milk production (15.Ø and 15.4 Kg/day), fat test (4.56 and 4.53 %) and milk protein percentage (3.57 and 3.52 %) were similar for cows fed sarsaponin and control diets, respectively. Concentrations of rumen ammonia, volatile fatty acid concentrations and ratios, and blood urea were not changed by sarsaponin feeding, nor did sarsaponin alter dry matter intake or body weight change.

Introduction

Certain feed additives have shown promise to improve utilization of forages, grains and their by-products by ruminants. Most such additives are not employed in dairy rations due to the potential of residues in milk. Sterols of many types, including cholesterol, estrone, testosterone, cortisone, and hexesterol have consistently increased digestion in vitro (Pfander, 1957).

One steroid found in plants and not absorbed is sarsaponin. This plant steroid from the Yucca plant, has been classified as a natural feed flavoring, but sarsaponin also is used to stimulate anaerobic fermentation of organic matter in biological waste treatment systems (Peekstok, 1979). Sarsaponin may alter protein utilization as well. Zinn et al (1983) indicated that sarsaponin at 66 ppm increased protein supplied to the animal making a 12.5% crude protein high concentrate equivalent to 15% crude protein. Goetsch and Owens (1984) reported that 44 ppm sarsaponin increased digestion and passage rate with medium concentrate diets. Hence, when ruminal fill limits feed intake, sarsaponin addition may increase feed consumption.

No production trials with sarsaponin for lactating dairy cows have been reported. The objective of this feeding trial was to evaluate the effect of dietary sarsaponin on milk yield and composition and ruminal fermentation by lactating dairy cows.

Materials and Methods

Twelve cows (6-Jerseys, 2-Ayrshires and 4-Holsteins) on their first or second lactation, were started on trial 8 to 10 weeks post-partum. A switchback design was used with three four-week periods. Cows were assigned at random to the two treatments: 1) 77 ppm of Sarsaponin (SARS) and 2) no sarsaponin (control). Complete rations consisting of 55:45 concentrate:silage (dry basis) were divided into three

approximately equal parts and fed to cows in individual pens at 1100, 1900 and 0300 hr. Cows had free access to water. Protein was limited to 80% NRC requirements for each each cow and urea was added to provide protein equivalent to 10% of the total protein requirements of each cow. DM and crude protein (CP) of the basal grain ration, silage and SBM were analyzed weekly. Milk was weighed twice daily and samples for analysis taken at four consecutive milkings each week. Analysis was for fat percentage using a Milko Tester MK III F-3140, and for protein percentage using a Pro-milk MK II F-12500. Feed weighbacks were taken daily and orts were not refed to determine weekly DM intake. Cows were weighed individually two times a week to determine an average weekly body weight. Blood and rumen fluid samples were taken three hours after the 1100 AM feeding on the last day of each period. Blood was withdrawn from the tail vein into vacutainer-vacuum tubes, and 0.2 ml oxalic acid (12.98 g/200 ml .9% saline) was added immediately per 20 ml of blood. Samples were cooled in ice water bath and later centrifuged (2000 xg) for 30 minutes. The supernatant solution was then frozen and later analyzed for blood urea nitrogen by adding 200 microliters of urease buffer, 5 ml phenol reagent, and 5 ml hypochlorite reagent to 100 microliters of sample supernatant and read at 625 nm as described by Fawcett el al. (1960).

TABLE V

BASAL RATION COMPOSITION

INGREDIENT	%, AS FED
Corn, ground	40.0
Sorghum grain, ground	36.5
Oats, crimped	6.0
Cottonseed hulls	6.0
Molasses, liquid	5.0
Limestone	2.0
Dicalcium Phosphate	1.5
Sodium Bicarbonate	1.25
Salt	1.0
Magnesium Oxide	0.75

Rumen fluid samples were taken by stomach tube three to four hours after the 1100 AM feeding the last day of each period. Approximately 250 to 300 ml of rumen fluid were collected from each animal. Fluid was strained with cheese cloth, and 50 ml were withdrawn, mixed with 0.5 ml of a sat-

urated solution of mercuric chloride and frozen. These samples were subsequently thawed and centrifuged (1000 xg) for 10 min and 5 ml of the supernatant solution was combined with 1 ml of 25% metaphosphoric acid and recentrifuged (25,000 xg) for 20 min. Samples were subjected to gas-liquid chromatography for VFA analysis. The sample (2 microliters) was injected onto a 240 cm, 6 mm O.D. glass column packed with 100/120 chromosorb WAW 15% SP-1220 1% H_3PO_4 on fire brick and nitrogen was the carrier gas at a flow rate of 10 ml/min, the column detector temperature was 120 C, and attenuation settings were 10 and 60.

The remainder of the rumen fluid was acidified with 8 ml of 50% HCl and centrifuged (2000 xg) for 10 min. Rumen ammonia nitrogen (NH_3-N) concentration was determined on 50 microliters of the supernatant by addition of 2 ml phenol reagent, 2.5 ml hypochlorite reagent and read at 625 nm as described by Broderick and Kang (1980).

Treatment differences were determined by analysis of variance with method described by Brandt (1938), and an example of the mathematical procedure is illustrated in the appendix.

Results and Discussion

Milk production increased slightly (3%; P>.05) with addition of sarsaponin to the diet (Table II). Protein intake was not as restricted as planned since dry matter intake was slightly higher than expected. Goetsch and Owens (1984)

suggested that possibily of sarsaponin may have an adverse effect on milk fat concentration. In this trial sarsaponin had no significant effect on fat or milk protein percentage (Table VI). Sarsaponin had no effect on DM intake or body weight change.

Rumen NH_3-N was very low in this experiment though diet protein concentration was only 15.5%. No effect of sarsaponin on ammonia was noted. Grobner et al (1982) reported that ammonia level decreased (P<.Ø8) when 6Ø ppm sarsaponin was added. Blood urea concentrations (1Ø.Ø and 9.8 mg/dl) were similar for cows on both treatments (Table VII).

TABLE VI

MILK PRODUCTION AND COMPOSITION

ITEM	SARS.	CONTROL
Milk production, Kg.	15.9	15.4
Fat test, %	4.56	4.53
Milk protein, %	3.57	3.52

TABLE VII

BLOOD UREA AND RUMEN-NH_3

ITEM	SARS	CONTROL
	-------(mg/dl)-------	
Rumen NH_3	Ø.91	Ø.9Ø
Blood urea	1Ø.ØØ	9.8Ø

Total concentration and the molar proportions of acetic, propionic, butyric, and valeric acids were similar for both treatments (Table VIII). In contrast, Grobner et al. (1982) observed that 6Ø ppm sarsaponin increased (P<.Ø4) propionic acid percentage.

In summary, sarsaponin supplementation resulted in no change in feed intake, milk production or composition, VFA levels or pattern, ruminal ammonia or blood urea. Further studies with cows at a higher level of production and with more restricted protein intakes are needed to test the effect of sarsaponin under more stressed conditions.

TABLE VIII

RUMEN VOLATILE FATTY ACIDS

ACID	SARS	CONTROL
Total VFA concentration, mM/l	50.0	54.4
Individual VFA, Molar %		
Acetic	66.8	66.2
Propionic	19.0	19.5
Butyric	10.5	10.5
Valeric	3.7	3.8

CHAPTER V

EFFECT OF SARSAPONIN ON IN SITU DIGESTION BY LACTATING DAIRY COWS

Summary

Disappearance of nitrogen (N), organic matter (OM) and acid detergent fiber (ADF) of a grain supplement (Gr), hay, soybean meal (SBM) and a complete dairy diet without (NS) or with added sarsaponin (S) was measured in situ. Two rumen cannulated animals were used in a cross-over design and were fed a complete ration of concentrate:silage (55:45) three times daily. The diet contained 15.5% crude protein and sarsaponin at Ø or 77 ppm. Duplicate dacron bags containing the 5 feeds were suspended in the rumen for 4, 8, 12, 24 and 72 hr. Periods were ignored for statistical analysis. Feeding sarsaponin had no effect on digestion rates. Disappearance of OM, N, and ADF was greater for soybean meal than for other feedstuffs. ADF disappearance from grain was greater than from S and NS, and disappearance from S tended to be lower than the NS ration. N disappearance was not significantly different for Gr, hay, S and NS. OM disappearance from S was less than NS and hay ($P<.Ø5$).

Introduction

Sarsaponin has been reported to reduce rate of passage of liquids and solids from the rumen and the total digestive tract. Slowing the rate of passage could increase extent of digestion and efficiency of animal performance, especially when fed mixed diets containing both starch and fiber.

Peekstok (1979) observed that sarsaponin stimulated anaerobic fermentation of biological waste in commercial treatment systems. If rate of anaerobic fermentation in the rumen were increased it could affect fermentation pattern. Goodall (1980) observed that total digestive tract nutrient digestibility averaged 6% higher when rations contained 100 ppm sarsaponin. Particulate and liquid flow rates to the abomasum where 15.6 and 17.0 percent lower when diets contained 100 ppm sarsaponin.

The objective of this study was to evaluate the rate of disappearance from dacron bags of various components of a protein supplement, roughage, grain and a complete ration with and without sarsaponin suspended in the rumen of cattle fed or not fed sarsaponin.

Materials and Methods

Two rumen-cannulated cattle were fed a complete ration suitable for lactating dairy cows. It contained 15.5% crude protein (CP) and consisted of a concentrate:silage (55:45, dry basis) and was fed three times daily (1100, 1900 and 0300 hours). In the cross-over design, two 12 day period

were used. Dietary treatments were: (1) No sarsaponin and (2) 77 ppm sarsaponin. The initial eight days of each period were used for adjustment to diets. During the following 72 hr, different substrates were incubated in situ. Substrates were placed in 5 x 10 cm bags with a pore size of 55 microns. Substrates included: 1) complete diet with 77 ppm of Sarsaponin (S) or 2) without sarsaponin (NS), 3) a roughage source (hay), 4) soybean meal (SBM) and 5) the basal grain mix (Gr). These were attached to a 50 cm string with a weight at the end so that they would stay submerged in the ventral part of the rumen and have more complete and uniform exposure to microbial action. Five different incubation times (4, 8, 12, 24, and 72 hr) were used during each period. After incubation, the bags and contents were washed and dried for 72 hours at 55 C and analyzed for DM, organic matter (OM), nitrogen (N), and acid detergent fiber (ADF).

Residue from bags at each incubation time were regressed on the natural log, 0 time period was ignored to avoid confoundment with solubility and particle size. Rate of disappearance (slope) was subjected to the analysis of variance using Satistical Analysis System (1983). Means were compared by Duncan's multiple range test.

Results and Discussion

Feeding sarsaponin had no significant effect on the rate of disappearance of the substrates from the dacron bags during incubation (Table IX). Therefore, values for each

treatment obtained from the two animals in both periods were averaged.

Rate of disappearance of OM, N, and ADF were greater for SBM than for all other substrates (Table IX). ADF disappearance for NS tended to be greater than for S. This contrasts with in vivo results of Goetsch and Owens (1984) who observed greater total ADF digestibilities with 66 ppm added sarsaponin. Zinn et al. (1983) also reported small increases in ADF digestion due to sarsaponin supplementation. Rate of disappearance of N tended to be less for the substrate with than with out sarsaponin. Zinn et al. (1983) observed that duodenal flow of feed N increased when Sarsaponin was supplemented. In contrast, Goetsch and Owens (1984) reported that ruminal N digestion tended to be greater for cows fed 66 ppm of sarsaponin. OM disappearance for S and hay was lower than for grain and NS ($P<.05$). Reduced ruminal digestion of nitrogen and OM with 77 ppm sarsaponin added should increase by-pass to the small intestine. More research is required to explain the conflicting results from this and other trials.

TABLE IX

RATE OF DISAPPEARANCE FOR NITROGEN, ACID DETERGENT FIBER AND ORGANIC MATTER (%/h)

Substrates	N	OM	ADF
Soybean meal	2.53[a]	2.60[a]	2.44[a]
Hay	0.40[b]	0.50[d]	0.45[c]
No Sarsaponin	1.00[b]	0.80[c]	0.34[c]
Sarsaponin	0.54[b]	0.60[d]	0.12[c]
Grain	0.75[b]	1.20[b]	0.72[b]

[abcd]Means in a column with different superscripts are different (P<.05).

CHAPTER VI

EFFECT OF SARSAPONIN ON RUMEN FERMENTATION AND PRODUCTION BY LACTATING DAIRY COWS II

Summary

Effect of sarsaponin on milk production was tested using 16 Holstein cows in their first lactation. Cows were started on experiment 6 to 10 weeks postpartum. The switch-back design used three 4-wk periods. Treatments were: 1) no sarsaponin (control) and 2) 77 ppm sarsaponin. Complete rations were prepared daily in a proportion of 55:45 concentrate:silage (dry basis), and equal quantities were fed to each cow three times each day. No significant differences in milk production (20.8 and 20.6 kg/day), fat test (3.5 and 3.5%) and milk protein percentage (2.8 and 2.8%) were observed between sarsaponin and control rations. Rumen ammonia (4.8 and 4.7 mg/dl) and blood urea (10.4 and 10.5 mg/dl) were similar for the respective treatment groups. Molar percentage of individual rumen VFA's and total VFA concentration were also similar for the two treatment groups.

Introduction

Sarsaponin, a steroidal glycoside, stimulates anaerobic fermentation of organic matter in biological waste treatment systems (Peekstok. 1979). Enhanced anaerobic fermentation in the rumen could increase utilization of many livestock feeds. Altered fermentation could improve utilization of nutrients by the animal. Grobner et al. (1982) observed a higher nitrogen output ($P<.05$) from continuous flow fermenter systems treated with 30 ppm sarsaponin. In contrast, Goetsch and Owens (1984) observed increased ruminal and total tract N digestion with addition of 44 ppm sarsaponin to the diet of cannulated dairy cows. Ruminal and total tract digestibilities of organic matter also were increased.

The objective of this experiment was to determine the effect of sarsaponin supplementation on feed intake and production of lactating dairy cows under conditions where intake of natural protein was limited in relation to NRC requirements.

Materials and Methods

Sixteen Holstein cows in their first lactation were started on experiment 6 to 10 weeks postpartum. A switchback design was use with two blocks (eight cows per block) and three 4-wk periods. Cows were assigned to blocks on the basis of season of calving and at random within blocks to treatment sequences. The treatments were: 1) no sarsaponin

(control) and 2) 77 ppm sarsaponin (SARS). Complete rations of 55:45 concentrate:silage (dry basis) were prepared fresh daily and approximately equal quantities were fed to cows in individual pens at 1100, 1900 and 0300 hours. Cows had free access to water. Intake of natural protein was restricted by including only enough soybean meal in the ration for each cow so that the total amount of intact protein was sufficient to meet 80% of estimated NRC requirements. Urea was added to provide protein equivalent value equal to 15% of the total protein requirement for each cow. Rations were recalculated every week based on weekly dry matter (DM) intake, body weight, and milk production. DM and crude protein (CP) of the basal grain mix, silage, and SBM were determined weekly. Chromic oxide (Cr_2O_3) was included 30g/day as an indigestible marker to calculate DM digestibility. Milk weights were recorded daily and milk samples for analysis were taken at four consecutive milkings each week. These were analyzed for fat (Milko Tester MK III F3140), and protein percentage (Pro-milk II F-12500). Feed weighbacks were obtained daily and composited weekly to determine protein and DM intake. Each cow was weighed individually twice each week to obtain an average body weight for the purpose of calculating diet allowance.

Blood and rumen fluid samples were taken 3 to 4 hr after the 11:00 AM feeding the last day of each period. Blood was withdrawn from the tail vein into vacutainer tubes, and 0.2 ml of oxalic acid (12.98 g/200 ml in a .9%

saline solution) was added immediately per 20 ml of blood. Samples were cooled by placing them in an ice bath and later centrifuged (2000 g) for 30 min. The supernatant solution was frozen and later analyzed for blood plasma urea by adding 200 microliters of urease buffer, 5 ml phenol reagent, and 5 ml hypochlorite reagent to 100 microliters of sample, and read at 625 nm as method described by Fawcett et al (1960). Rumen fluid was sampled by stomach tube 3 to 4 hours after the 11:00 AM feeding on the last day of each period. Approximately 250 to 300 ml of rumen fluid were collected from each animal. Fluid was strained through cheese cloth. Fifty ml of filtrate were withdrawn and mixed with 0.5 ml of a saturated solution of mercuric chloride and frozen. These samples were subsequently thawed and centrifuged (2000 xg) for 10 min; the supernatant solution (5 ml) was combined with one ml of 25% metaphosphoric acid, recentrifuged (25,000 xg) for 20 min. and subjected to gas-liquid chromatography for rumen VFA analysis. Two microliters of each sample was injected onto a 240 cm 6 mm O.D. glass column, packed with 100/120 chromosorb WAW 15% SP-1220 1% H_3PO_4 on firebrick. Nitrogen was used as the carrier gas at a flow rate of 40 ml per min, the column detector temperature was 120 C and range and attenuation settings were x10 and x100. The remainder of the rumen fluid was acidified with 8 ml of 50% HCl/200 ml of rumen fluid and then frozen. Samples were later thawed and centrifuged (2000 xg) for 10 min. Ammonia concentrations

were determined on 50 microliters of supernatant fluid by first adding 2 ml of phenol reagent and 2.5 ml of hypochlorite reagent. Samples were then incubated in a 39 C water bath for 15 min. and read immediately at 625 nm with a DMS 90 Varian spectrophotometer as described by the method of Broderick and Kang (1980).

Fecal samples were taken two times each day and composited over five days the last week of every period. Chromium in the sample was estimated by atomic absorption spectrophotometry to calculate ration digestibility as described by Kotb and Luckley (1972).

Treatment differences were determined by analysis of variance by the method decribed by Brandt (1938). An example of the mathematical calculations are illustrated in the appendix along with tables for analysis of variance.

Results and Discussion

Sarsaponin addition had no significant effect ($P>.05$) on milk production, fat percentage or milk composition (Table IX). Intake of natural protein was restricted to 80% of the NRC requirements for total protein (Table X), so if sarsaponin increased bypass of protein or increased microbial synthesis of protein in the rumen, the cows should respond by increasing production. This is in agreement with results of Goetsch and Owens (1984) who reported that rumen nitrogen digestion tended to be greater for cows fed diets supplemented with 44.1 ppm of sarsaponin than for cows fed a

control diet. Whether or not a response to sarsaponin supplementation would be obtained with older cows is not known. In three separate trials, one each by Goetsch and Owens (1984), Valdez (1985) and this study, milk production increased fron 1 to 3% with addition of sarsaponin. Though the difference was not significant in any trial, consistency of the direction suggests that sarsaponin may have a positive effect. Roffler et al. (1978) observed that milk yield of first lactation cows did not respond to protein concentration of the diet whereas multiparous cows responded positively. Sarsaponin feeding did not alter DM intake, ration DM digestibility or body weight change.

TABLE X

MILK YIELD AND COMPOSITION

ITEM	SARS	CONTROL
Milk production, kg/day	20.8	20.6
Fat test, %	3.5	3.5
Milk protein, %	2.8	2.8

The level of SARS supplementation used in this trial at 77 ppm did not reduce feed intake. It is doubtful that energy intake limited production in this trial since the cows were allowed as much dry matter as they would consume and protein content was adjusted weekly to maintain the desired restrictions of this component in the ration.

TABLE XI

PROTEIN INTAKE AND DRY MATTER CONSUMPTION

ITEM	SARS	CONTROL
Total protein Req., kg	2.3	2.3
Total protein consumed, kg	2.3	2.3
Percent total protein Req.,%	99.7	99.7
Dry matter intake, kg/day	20.0	19.8
Dry matter digestibility, %	77.1	77.4
Body weight change, kg/period	1.4	-.45

Rumen ammonia and blood urea concentrations were similar for both treatments (Table XII). In contrast, Grobner et al. (1982) reported that 60 ppm sarsaponin decreased

ammonia levels of *in vitro* fermenters. Rumen ammonia levels in this feeding trial were much higher than the previous feeding trial (Valdez, 1985; 4.7 to 4.8 vs .90 to .91 mg/dl). Blood urea concentrations in this feeding trial were similar to those observed in our previous feeding trial (Valdez, 1985; 10.0 to 9.8 mg/dl).

TABLE XII

BLOOD UREA AND RUMEN AMMONIA LEVELS

ITEM	SARS	CONTROL
	-------(mg/dl)-------	
Rumen ammonia	4.8	4.7
Blood urea	10.4	10.5

Molar percentages of acetic, propionic, butyric and valeric acids as total VFA concentration were similar for both treatments (Table XII). Previously Goodall (1980) reported that sarsaponin at 60 to 250 ppm increased propionic acid and decreased acetic acid ($P<.05$) in steers.

In summary, supplementation of rations of lactaing dairy cows with 77 ppm sarsaponin did not significantly alter milk production, fat percentage, dry matter intake or body weight.

TABLE XIII

RUMEN VOLATILE FATTY ACIDS

ITEM	SARS	CONTROL
Total VFA concentration, mM/l	153.5	144.7
Individual VFA, molar %		
Acetic	63.1	63.4
Propionic	22.6	22.2
Butyric	13.Ø	13.1
Valeric	1.3	1.3

CHAPTER VII

CONCLUSIONS AND RECOMMENDATIONS

To increase our knowledge about the mode of action of sarsaponin, a steroidal saponin from the Yucca plant, lactating dairy cows were fed diets containing sarsaponin in two trials to determine its effect on milk yield, and fermentation was studied in vitro, in situ, and in vivo.

In the in vitro rumen cultures, sarsaponin decreased numbers of protozoa and tended to increase bacterial numbers. The increase in bacterial numbers may alter rumen fermentation, reduce protein digestion in the rumen and change fermentation parameters. Increases in apparent digestibility of starch, ADF and organic matter were detected with addition of sarsaponin to the diet. Similar results have been reported previously by Peekstok (1979) and Grobner et al. (1982). However, nitrogen digestibility and microbial efficiency was not affected by sarsaponin supplementation in our in vitro study.

In situ, rate of disappearance and organic matter tended to be lower when substrates had sarsaponin added. Such a change should increase by-pass and flow of nitrogen and organic matter to the small intestine.

The protein sparing action of sarsaponin would be economically important in rations for lactating dairy cows.

However, in two trials, additions of sarsaponin caused no significant change in milk production, fat percentage and milk composition. Though cows should benefit from increased protein bypass or increased microbial protein synthesis, production responses were detected in this study.

Although increases in starch digestibility and bacterial numbers were observed in vitro with added sarsaponin, no changes in vivo in fermentation products such as ruminal ammonia and individual and total rumen volatile fatty acids were detected. The high level in these diets may have prevented a change. In feedlot diets, propionate increases have been observed (Goodall, 1980).

Overall, sarsaponin had greater effects on fermentation in semicontinuous rumen cultures and on in situ disappearance than in the rumen on production. Possible beneficial effects were not observed in the two production performance experiments due to variations or other limitations. More research is needed to test sarsaponin for lactating dairy cows at higher concentrations, a more purified form, or with dairy cows which are producing more milk where changes in ruminal fermentation is more likely to alter milk production.

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APPENDIX

ANALYSIS OF VARIANCE

SWITCH-BACK OR DOUBLE-REVERSAL TRIAL

The basic switch-back or double-reversal trial may be defined as one in which two treatments are studied, and each cow receives both treatments, one treatment twice, in either of the sequence 1, 2, 1 or 2, 1, 2.

BASIC PATTERN

COMPARISON PERIOD	SEQUENCE	GROUP
1	1	2
2	2	1
3	1	2

Numerical Example

For the present example data of Milk Yield was used from the feeding trial in Chapter IV. This was a switch-back trial with twelve cows, six cows on each sequence, with two treatments and three periods. Treatment were: (1) 77 ppm sarsaponin, and (2) no sarsaponin.

CONPARISON PERIOD	TRT	DATA FROM INDIVIDUAL COWS						SUM
				SEQUENCE GROUP 1				
		1,1	1,2	1,3	1,4	1,5	1,6	
1	1	40.5	43.4	30.9	35.2	45.6	41.7	237.3
2	2	32.1	28.4	26 9	32.4	44.9	43.8	208.2
3	1	31.5	29.5	26.9	33.4	49.0	43.9	211.2
DIFFERENCE		7.8	16.1	4.5	3.8	1.8	-2.0	32.1 G1
				SEQUENCE GROUP 2				
		2,1	2,2	2,3	2,4	2,5	2,6	
1	2	35.0	34.1	29.6	35.2	36.2	38.7	208.8
2	1	28.7	27.4	26.8	30.7	34.8	42.7	191.1
3	2	26.6	26.5	24.9	28.2	35.6	40.0	181.8 G2
DIFFERENCE		4.2	5.8	0.9	2.0	2.2	-6.7	8.4

Treatment sums of squares:

$$SS\ TRT = \frac{(G1 - G2)^2}{12\ n} = \frac{(23.7)^2}{72} = \frac{561.69}{72} = 7.8$$

d.f.=1

Error sums of squares:

$$SS\ Error = 1/6\ [(4.2)^2 + (7.8)^2 + ... + (-6.7)^2] - 1/6n\ (G1^2 + G2^2)$$

$$= 78.12 - 30.58$$

$$= 47.54$$

d.f.=10

AOV SUMMARY

SOURCE	d.f.	SS	M.S.[a]	F[b]
TRT	1	7.8	7.8	1.64
ERROR	1Ø	47.54	4.75	

[a] Mean Squares (M.S.) = $\frac{SS}{d.f.}$

[b] F value = $\frac{M.S.\ TRT}{M.S.\ ERROR}$

Treatment Means:

TRT 1 = Y + tn

TRT 2 = Y - tn

$Y = 1/6n \times P_{ik} = 1/36\ (1238.4) = 43.4Ø$

$tn = \pm \frac{G1 - G2}{8\ n} = \frac{23.7}{48} = \pm Ø.49$

TRT 1 (Sarsapnin) Y = 34.4 + .49 = 34.84

TRT 2 (Control) Y = 34.4 - .49 = 33.91

TABLE 1. MEAN SQUARES IN ANALYSIS OF VARIANCE FOR VARIABLES IN FEEDING TRIAL I, CHAPTER IV

SOURCE	d.f.	MILK YIELD	FAT %	PROT %	RUMEN NH_3	BLOOD UREA
TRT	1	7.8Ø	3.97	1.4Ø	.15Ø	Ø.48
ERROR	1Ø	4.75	1.11	Ø.37	.131	5.Ø2

TABLE 2. MEAN SQUARES IN ANALYSIS OF VARIANCE FOR VARIABLES IN FEEDING TRIAL 1, CHAPTER IV

SOURCE	d.f.	TOTAL VFA	INDIVIDUAL VFA, MOLAR % ACETIC	PROPIONIC	BUTYRIC	VALERIC
TRT	1	2.31	3.39	7.34	3.21	9.21
ERROR	1Ø	1.54	Ø.98	6.39	5.32	Ø.53

TABLE 3. MEAN SQUARES IN ANALYSIS OF VARIANCE IGNORING BLOCKS FOR VARIABLES IN FEEDING TRIAL II, CAPTER VI

SOURCE	d.f.	MILK YIELD	FAT %	PROT %	DM INTAKE	BLOOD UREA	RUMEN NH_3
TRT	1	40.93	.313	.132	14.35	12.05	9.32
ERROR	10	5.79	.029	.033	3.22	5.45	3.41

TABLE 4. MEAN SQUARES IN ANALYSIS OF VARIANCE IGNORING BLOCKS FOR VARIABLES IN FEEDING TRIAL II, CHAPTER VI

SOURCE	d.f.	TOTAL VFA	INDIVIDUAL VFA, MOLAR % ACETIC	PROPIONIC	BUTYRIC	VALERIC
TRT	1	4.53	31.45	10.24	3.21	9.32
ERROR	10	7.31	21.39	5.32	0.53	16.41

VITA 2

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