MANIPULATION OF RUMINAL FERMENTATION AND NUTRIENT UTILIZATION IN CATTLE FED ENERGY DENSE DIETS

by

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ABSTRACT

Manipulation of Ruminal Fermentation and Nutrient Utilization in Cattle Fed Energy Dense Diets

by

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Experiments were conducted on barren, mature holstein cows in an attempt to stabilize ruminal fermentation and improve nutrient utilization when diets that contain large proportions of cereal grain were fed. Two mehtods were used, increasing the rate of digesta passage and intra-ruminal fungal inoculation.

Digesta passage rates were increased by exogenous additions of mineral salts to the diet and by endogenous addition of mineral salts via artificial stimulation of the saliva glands with parasympathomimetic agents. Rumen fermentaton was altered favoring an increased acetate:propionate ratio. Ruminal pH and number of cellulolytic organisms were also inçreased by treatments. Total tract apparent digestibility of dry matter, crude protein, and dietary fiber was increased by stimulating saliva flow.

In order to measure the physiological effects of the parasympathomimetic agents used to stimulate saliva flow, meadow voles (Microtus pennsylvanicus) were used as animal models. Effects of the agents on blood chemistry and liver, kidney, and parotid function and structure were measured at graded agent dosages. It was concluded there

were no detrimental agent effects at the dosage levels used in the cow studies. At higher doages, however, parotid exhaustion and liver degeneration were noted.

The intra-ruminal fungal inoculations were commercially used strains of Aspergillus oryzea and Saccharomyces cerevisiae. Inoculations were made daily via additions to the diet. Ruminal fermentation patterns were unaffected by treatments, but number of ruminal cellulolytic organisms was increased. Digestibility of dry matter, crude protein, and hemicellulose was increased by fungal inoculants. The effect was most pronounced when the combination of fungi were inoculated.

An in vitro study was initiated to measure the effects of adaptation time to the fungal inoculants. Results indicated that an adaptation period of at least 21 days was necessary to achieve optimum affectiveness of the fungal inoculants

(117 pages)

INTRODUCTION AND LITERATURE REVIEW

The digestive system of cattle is adapted for the fermentation of large quantities of bulky feeds that are low in energy density (1.0-2.5 Mcal/Kg) and high in insoluble carbohydrate polymers such as cellulose and hemicellulose (30-80%). This adaptation is facilitated by an enlargement of the esophageal region of the gastric stomach forming in essence a pregastric, continuous fermentation vat, the rumen (242). Due to the bulkiness of the normal diet, the rumen is relatively large, with an average capacity of 85 grams of digesta /Kg body weight. Ruminal liquid volume can be as high as 150 liters in lactating dairy cattle (193). The ruminal environment is ideal for the growth of cellulolytic bacteria that possess the enzymatic machinery to cleave beta-linked sugars from cellulose and hemicellulose, an adaptation completely lacking in the mammalian species. Since nearly 50% of the solar energy used for biological synthetic purposes is for the production of structural carbohydrates such as cellulose and hemicellulose, cattle have an advantage in being able to utilize these nutrients because they are abundant and are not competive in the human food supply (51).

Advances in agricultural practices and research since World War II have lead to surpluses of cereal grains and livestock animals with increased genetic potential for performance. The increased genetic potential for performance in livestock animals concomitantly increased the dietary nutrient density requirements of those animals (225). Consequently, the practice of feeding cattle cereal grains has increased since World War II due to the fact that they were abundant and therefore relatively inexpensive and due to the fact that they are relatively dense in energy (2.9-3.5 Mcal/Kg).

The feeding of energy dense cereal grains to cattle has allowed them to reach their genetic performance potential, but it has also increased the incidense of several metabolic anomolies such as: lactic acidosis, bovine ketosis, bloat, displaced abomasum, polioencphalomalacia, milk butterfat depression syndrome, and depressed fiber utilization. These problems have arisen mainly because the digestive system of cattle is adapted to a low energy dense diet such as forages and not high energy dense diets such as cereal grains.

Lactic Acidosis

The rapid ruminal fermentation of the soluble carbohydrates in cereal grains reduces the ruminal pH by the production of organic acids. Two main types of organic acids are produced, namely, volatile fatty acids and lactate (74). When cattle are fed low energy dense diets (forages) the production of lactate is almost below detection because the bacteria that produce lactate such as Streptococcus bovis are low in number, the metabolic pathways of those bacteria favor propionic and acetic acid production, and because lactate utilizing bacterial species such as Megasphaera elsdinii and Selenamonas ruminantium are relatively high in number (70, 199, 207).

When cattle are fed diets high in cereal grains (40-90%), the ruminal microbial profiles are changed and the number of lactate producing bacteria increase (119, 212). Total volatile fatty acid production also increases (53). Lactate (pKa 3.87) is a 10X stronger acid in the rumen than volatile fatty acids (pKa 4.79) (116). Uhart and Carroll (236) switched beef steers from an alfalfa hay diet to a high-grain diet and noted the following changes: ruminal pH dropped from 6.98 to 4.81, ruminal lactate increased form 0.10 mM/L to 99.96 mM/L, blood

pH dropped from 7.37 to 7.29, and blood HCO_3/CO_2 ratio dropped from 18.9 to 15.3. The steers were suffering from acute lactic acidosis and exhibited the classical clinical signs: anorexia, diarrhea, rapid respiration, and reduction of skin elasticy. Once the steers adapted to the high grain diet, the ruminal pH, ruminal lactate, blood pH, and blood HCO_3/CO_2 ratio stabilized at 6.30, 0.29 mM/L, 7.36, and 18.2, respectively. Urine pH when the steers were on the alfalfa hay diet was 8.23 and stabilized at 6.93 on the high-grain diet. After the steers had stabilized on the high-grain diet, they suffered from chronic acidosis, expending energy to maintain homeostasis at the expense of production.

The changes in ruminal environment associated with the feeding of high-cereal grain diets results in detrimental changes in the rumen microbial populations: reduction in the number of cellulolytic bacteria, reduction in the number of protozoa, and the growth of pathogenic bacteria.

As mentioned earlier, one advantage cattle have in the livestock industry is their ability to utilize beta-linked carbohydrates. This ability is dependant on the maintenance of a population of cellulolytic bacteria in the rumen. The proportion of cellulolytic bacteria (Ruminococcus albus, Ruminococcus flavafaciens, Bacteroides succugenes, and Butyrivibrio fibrisolvens) can dropped to 5% when cattle are fed high-grain diets (8, 119, 210, 211).

The role of protozoa in the ruminal ecological has not been fully elucidated. Few, if any, protozoa are found in cattle fed high-grain diets ad libitum (70, 211). The absence of protozoa is due to the low ruminal pH and high ruminal tonicity measured in high-grain diets (116, 207). This lack of protozoa could eliminate an important nutrient

storage sink in the rumen since they engulf large amounts of starch, thereby sheilding it from lactate producing bacterial fermentation (71, 178).

The changes in the rumen ecosystem due to high-grain diets allows the proliferation of bacteria and yeasts that are normally in very low numbers (207). The altered rumen ecosystem also elicits changes in the metabolic by-products produced by the bacteria. Lactobacillus sp. have been shown to convert histidine to histamine, which reduced the frequency and amplitude of rumen contraction (189, 190). The same affect was observed with the excessive lactate produced with high-grain diets (45). Allison et al. (7) found an increase in Clostridium perfringens and coliforms after overfeeding cattle and sheep with wheat. These organisms could possibly be involved in producing microbial endotoxins that could result in the diarrhea observed with acidosis.

Another detrimantal affect of lactic acidosis is rumenitis. Kay et al. (132) suggested that the pathological changes in the rumen wall, characteristically found in animals fed barley, may begin with thickening of the epithelium and lamina propria, and that this thickening is a reaction to the fall in the pH of the rumen contents. These pathological changes in the rumen wall tissues, often called ruminal parakeratosis, decrease the absorption of volatile fatty acids (109). Since 70-80% of the energy utilized by cattle is from volatile fatty acid, decreased absorption would reduce performance.

The pathological changes of rumen wall tissue involve depigmentation and loss of papillae, scars, ulcers, pits and nodules. Desquamatized papillae are often observed. Inflamation of the serosal side included petechial and ecchymotic hemorrhages. The rumen tissues

are somewhat necrotic and easily abraided by forage particles and hair (100). Jensen et al. (127) reported that 38% of the stomachs of 1,535 fat cattle examined had lesions of rumenitis. If rumen wall abrasions occur, bacterial invasion can occur. Spherophorus necrophorus colonies are often observed in necrotic rumen wall tissue. These bacteria can be released into the portal blood and lodge in the liver where a condition called necrobacillosis or liver abscess ensues (123). Cattle with liver abscesses have a 5-8% decreased in average daily gain and a 3-5% decrease in feed efficiency compared to cattle without liver abcesses (43, 44, 105, 184).

Bovine Ketosis

Bovine ketosis manifests itself in two forms, clinical and subclinical. Clinical ketosis typically occurs spontaneously in susceptible high-yielding dairy cows between the 2nd and 10th week of lactation (54). The incidence of ketosis in the U.S. is estimated to be between 10 and 15%. The clinical signs are loss of appetite, particularly for grains, decrease in milk production and rapid loss of body condition. The demeanor of most cows is apathetic. The body temperature is normal and milk shows a positive reaction to the Rothera test, indicating acetoacetic (69, 108). Other signs include hyperketonemia, hypoglycemia, elevated nonesterified fatty acid (NEFA) levels in the blood, fatty infiltration of the liver and loss of liver glycogen (18,19). Several intermediates in the gluconeogenic pathway, both at the tricarboxylic acid level and at the Embden-Meyerhof level, are decreased below normal (18).

Most workers agree that the etiology of bovine ketosis is involved

with homeorhetic mechanisms giving the mammary gland preference for glucose at the expense of other body tissues with a concomitant stimulated release of fats from the adipose tissue. The utilization of these fats by extramammary tissues, mainly the liver, in a glucose deficient state results in the production of ketone bodies that can build to toxic levels. Primary bovine ketosis then occurs (28, 41, 204). During early lactation homeorhetic hormones insure that the mammary gland has preference for blood nutrients. Increased prolactin and somatotropin levels inhibit lipoprotein lipase in the adipose tissue and stimulate that same enzyme in the mammary gland (253).

Homeastatic hormones also play a role in insuring that the mammary gland has preference for blood glucose. During early lactation secretion of insulin is much below normal (150). This is due to a high glucose demand in the mammary gland and an inadequate nutrient intake lowering blood glucose. It is a well established fact that most tissues require insulin to utilize glucose. This is not the case with mammary tissue, which can take up glucose in the absence of insulin (142, 188). Low insulin levels also encourage the mobilization of fats from adipose tissue (17).

Homeorhetic and homeostatic mechanism working in concert may insure the mammary gland an adequate supply of nutrients but these same mechanism are, at least in part, the cause of primary ketosis in predisposed animals. As already stated, the low insulin levels in early lactation cows encourage mobilization of fats from the adipose tissues, most of which would pass through the liver. In the liver, NEFA can be processed in three ways: esterification, lipogenesis, or reduction to ketone bodies (249). Lipogenesis is very low in the bovine liver (21).

Insulin has an antiketogenic effect by encouraging esterification of fats (249). With low blood insulin and glucose levels, blood glucogon levels increase. Glucogon not only stimulates adipose tissue mobilization but has a ketogenic effect in the liver by decreasing the rate of esterification in the liver, thus pushing the liver into ketone body production (40).

The above mentioned observations plus the deficient glucose status often measured in early lactation are said to be the major etiological factors in primary bovine ketosis since the lack of liver glucose would lead to a deficiency of oxaloacetate that is needed for the utilization of fats. Thus, the build up of acetyl-CoA groups triggers ketone body production (19).

The thesis of this review is that grain feeding increases the incidence of bovine ketosis. This may be surprising in view of the above explanation of the etiology of bovine ketosis being, in part, caused by a deficiency of glucose since grain diets are glucogenic in nature. Kronfeld (141) sites evidence that highly glucogenic diets in high producing dairy herds may, if fact, increase the incidence of ketosis. Sixteen cows were dosed with 115 grams of propylene glycol twice daily for 2 weeks prior to parturition and then 230 grams twice daily for 2 months postpartum. Of the 16 cows, 10 were treated for clinical ketosis (139, 140).

Kronfeld (141) states that the major factor controlling milk production in dairy cows is the amount of glucose supplied to the mammary gland. High grain diets would therefore increase production since they are glucogenic. Glucose in the mammary gland can be used for lactose production or for an energy sources but it cannot be used as a

precursor for milk fat synthesis. The enzymes needed for this process are lacking in ruminants (21, 101). Three conceivable conditions could therefore arise: 1) the ration may provide sufficient lipogenic nutrients to sustain high production and health; 2) the ration may supply insufficient lipogenic nutrients, and the milk fat content may decline; or 3) the ration may provide insufficient lipogenic nutrients and body fats are mobilized to maintain milk fat. In the latter case, serum free fatty acids are increased with concomitant increases in ketogenesis. Another complication in mammary lipogenesis in early lactation is the fact that acetate uptake by the mammary gland appears to be somewhat insulin mediated (126). Kronfeld (141) proposes that spontaneous ketosis in well fed dairy herds is not simply a matter of negetive glucose balance but an improper glucogenic:lipogenic nutrient ratio in the feed. Feeds that result in elevated blood glucose levels, those with large amounts of cereal grains or those high in protein, result in a stimulation of heavy milk flow that rapidly depletes blood glucose, resulting in decreased blood insulin and increased blood glucogon. The decreased blood insulin results in decreased extra-mammary tissue uptake of glucose, thereby making more glucose available to the mammary gland for milk production. Decreased blood insulin also decreases mammary uptake of acetate, a major precursor for milk fat. Thus, milk fat production is more dependant on the mammmary uptake of serum free fatty acids. If the diet does not supply adequate amounts of fatty acids, some cows will respond by simply decreasing the fat content of the milk. Other cows will mobilize free fatty acids from the adipose tissue. This situation is already augmented by the low blood insulin and high blood prolactin, somatotropin, and glucogon levels. The problem

arises when large amounts of mobilized free fatty acids are processed in a liver primed for ketone body production by a high blood glucogon level. One would think that dietary fats would result in an similar situation, but research has shown that the long chain free fatty acids associated with chylomicrons, the situation with dietary fats in ruminants, are processed to only a limited extent in the bovine liver (180, 221). Thus dietary fats result in a decreased ketone body production compared to free fatty acids mobilized from adipose tissue.

Many cows do not show the classical clinical signs of ketosis but do suffer from elevated blood ketone levels. This type of ketosis, called subclinical ketosis, has not been fully explored. Since these cows do not show clinical signs, they are not treated. The result would be not only a decrease in milk production, but several other health and production factors could be affected. Due to the low blood insulin level many peripheral organs would have impaired uptake of glucose, acetate and ketone bodies. This could not only impair function, but could cause perminant damage. There is some indication the elevated blood ketones are related to infertility (203). Sommer (216) suggests that metabolic stresses such as ketosis alters all organ functions and thus not only affect production and fertility, but also renders the animal less able to resist disease.

Bloat

Bovine bloat (tympanites) occurs in two forms: legume and feedlot. Modern agricultural practices have increased the incidence of both of these maladies with increases in the feeding of both legumes and cereal grains. Losses, including death and morbidity, are estimated to be will over $100 million/year (53).

Grain bloat is usually associated with feedlot or dairy cattle fed large quantities of cereal grain (70-90% of the diet). Increases in rumen fluid viscosity causes fermentation gases to become trapped in a strong foam that impairs eructation (118). The increased viscosity has been correlated with an increased number of encapsulated rumen bacteria (98, 123). Rhamnose in the capsule of a Gram positive bacteria (type D streptocci), found in large numbers in the rumen contents of cattle with bloat, results in the production of ruminal slime (111) that is associated with the increased ruminal viscosity measured in feedlot bloat (95). Meyer and Bartley (159) found that relative viscosities and apparent glucose and rhamnose concentrations were significantly higher ($P<.05$) when cattle were fed concentrate (cereal grains) only, than when they were fed alfalfa hay only. Multiple regression analysis indicated as much as 67% of viscosity variation was associated with apparent rhamnose.

Saliva production may play an important role in feedlot bloat. Cattle consuming diets that contain large amounts of cereal grain and chopped forage produce 3 to 4 times less saliva that cattle consuming longstem hay (15, 20). Mendel and Boda (157) found that bloat prone cattle secreated less saliva than bloat resisant cattle. Injection of atropine sulfate to reduce saliva flow in members of identical twins increased ruminal foaming (252). Bartley and Yadava (22) aerated alfalfa saponin solutions with compressed air and produced large amounts of frothy foam. Additions of bovine saliva and several plant and animal mucins significantly reduced frothing. In the same study, 5 sets of identical twin cows equipped with rumen fistulas were fed bloat promoting diets. Half of the cows were treated intraruminally with

mucins. Treated cows had significantly lower bloat scores than untreated cows. In vitro studies with frothy rumen contents from bloated steers showed that mucin, either from plant sources of bovine saliva, increased the amount of gas released from the froth (233).

Although saliva secretion is decrease with high-cereal grain diets, requirement for saliva is probably increased. Two mucinolytic strains of Streptococcus bovis were studied (238). When these strains were found in high numbers in the rumen, the incidence of bloat increased. The increased numbers of Streptococcus bovis would therefore predispose cattle to bloat in two ways: the increased rhamnose capsules and the destruction of antifrothing mucin in the already limited amount of saliva.

Displaced Abomasum

Abomasal displacement is a disorder normally found in dairy cattle in which the abomasum becomes distended with fluid or gas or both with attendant migration to an abnormal position. The incidence has been increasing as illustrated by Robertson (186) who reported an increase from 3% of the large animal practice in 1960 to 30% in 1963. Economic costs have been estimate at from $150-$200 in lost milk per lactation and medical expenses (187). Most abomasal displacement occurs in older cows and within 30 days of parturition.

Heavy grain feeding prepartum has been implicated in the occurrence of abomasal displacement (4, 186, 213, 250). Svendsen (223) showed that feeding 60% concentrate diets increased the amount of volatile fatty acids in abomasum, which decreased the rate of abomasal contraction. Gas production was also increased in abomasum. These factors resulted in an increased incidence of abomasal displacement.

The practice of feed cereal grains prior to calving (lead feeding) has been used to adapt cows to the high grain diets used following parturition. High energy diets following parturition have been shown to increase milk production (117). Subsequent studies have shown no advantage in lead feeding if cows were fed properly following parturition and if the cows were in good condition in late pregnancy (79, 92).

Polioencephalomalacia

Polioencephalomalacia is a condition in feedlot cattle and sheep being feed diets high in cereal grain. Morbidity can be as high as 25% in groups of feedlot cattle and mortality can be up to 90% in affected cattle (33).

Pathogenesis includes severe cerebral edema with repulsion of the cerebellum into the foreman magnum and flattening of the cerebral cortical gyri. This results in increased cranial pressure. The increased pressure results in compromised blood flow with resultant necrosis of the gray matter (131). Clinical signs include listlessness, circling, muscular incoordination, opisthotonus, head pressing against solid objects, progressive blindness, conculsions, and death (38, 39). Elevated blood pyruvate and favorable response to intravenous injection of thiamine suggest a thiamine deficency.

Since a frank thiamine deficiency is unlikely in ruminants, thiaminase enzymes have been suggested (67). Two types of thiaminase have been suggested, thiaminase II cleaves the methylene bridge between the thiazole and pyrimidine rings and thiaminase I catalyzes a base exchange reaction that substitutes a nitrogen-containing ring, or cosubstrate for the thiazole. Thiaminase I would therefore catalyze the

formation of inactive thiamine analog that would be a competative inhibitor of thiamine.

Rumen bacterial thiaminase I is a membrane bound exoenzyme (1). Sapienza (201) showed that acid shock of rumen bacteria cells at pH 4.5 resulted in thiaminase activity in the cell free rumen fluid. These conditions would exist in the rumen of cattle being fed high grain diets. Thus lactic acidosis may set the stage for polioencephalomalacia (PEM) (37).

Evidence suggests that PEM in feedlot cattle is the result of thiaminase I and not thiaminase II as PEM can be produced in 3 days. A thaimine deficiency would not be expected to manifest itself that quickly while a competative inhibition would (151, 231, 232).

Cosubstrates for ruminal thiaminase I include delta-1-pyrroline, nicotinic acid, pyridine, pyridoxine, histamine, and imidazole (36, 72). Histamine appears in the rumen during lactic acidosos. A thiamine analog containing histamine was detected in the rumen of PEM cattle (201).

Polioencephalomalacia can thus appear in cattle being fed diets that produce acidosis, i.e., those high in cereal grains. The drop in ruminal pH associated with acidosis results in the proliferation of, as yet, undefined species of thiaminase I producing bacteria. Acidotic conditions result in production of cosubstrates of thiaminase I enzymes, histamine being an example. The drop in ruminal pH acid shocks thiaminase I bacteria, releasing thiaminase I activity into the ruminal liquid media. The resultant thiamin analogs inhibit thiamin dependant enzymes, especially those involved in the intermediary metabolism of glucose. Since glucose is the exclusive energy sources in nervous tissue, metabolism is compromised and abnormalities in the gray matter

result in necrosis.

Milk Butterfat Depression Syndrome

The amount of fat in the milk of cattle is controlled by genetics and nutrition. The practice of feeding dairy cows large amounts of cereal grain has lead to a malady known as nutritional bufferfat depression syndrome in which the fat content of the milk drops to 50% of normal (60). Early theories of the etiology suggested that the decreased acetate:propionate ratio measured in cattle being fed diets high in cereal grain resulted in less acetate production (130, 240). Recent studies have shown that acetate production is nearly the same or even slightly higher in high-cereal grain diets (25, 192, 242). The major difference in ruminal volatile fatty acids in cattle fed high-cereal grain diets was a drastic increase in propionate production (192). A diminished acetate production was, therefore, not the cause of decreased butterfat.

Another hypothesis examined was that the combination of an increased propionate production and decreased ruminal vitamin B_{12} synthesis (244) would cause a bottle neck in the metabolism of propionate with a resultant build up of methylmalonic acid. The accumulation of methylmalonic acid was proposed to inhibit milk fat synthesis (90). However, Croom et al. (60) found vitamin B_{12} administration had no effect on bufferfat in cows with low-milk fat syndrome and that blood methylmalonic acid concentrations were not increased.

The most strongly supported theory of milk fat depression is the glucogenic theory of McClymont and Vallance (156). These authors

suggested that enhanced hepatic gluconeogenesis from propionate stimulates insulin secretion, which in turn suppresses the release of fatty acids from the adipose tissue required for milk fat synthesis. This hypothesis is supported by the fact that ruminal propionate infusion (11, 182) and monesin (42), a carboxylic ionophore that increases ruminal propionate production, reduces milk fat concentration in ruminants.

The increased production of ruminal propionate in cattle fed diets high in cereal grain results in an increase in the insulin:glucagon molar ratio either by direct action on the pancreas or indirectly by increased gluconeogenis in the liver. The increased insulin:glucagon ratio elicits an activation of adipose lipoprotein lipase, thus fats are taken up by adipose tissues at the expense of the mammary gland.

Butterfat depression is of prime importance to cattlemen because milk is purchased according to butterfat content, -+ $0.17 per hundred pounds of milk for each 0.1% butterfat above or below 3.5% at the time of this writing. Mammary uptake of lipids is the major criterion determining efficiency of milk production (141). Therefore, the producer receives less monetary return for a product that costs more to produce per unit of volume, if cattle suffer from milk fat depression syndrome.

Depression of Fiber Utilization

Negative associative effects occur when the digestibility of a feed mixture is less than that of the sum of the individual components of the feed (164). A classic example is depressing of fiber digestibility when roughages are supplemented with concentrates (48, 152). Most proposed theories to explain this phenomenon have centered around the effects of increased amounts of non-structural carbohydrate on rumen microbial

populations. One theory postulates that rumen microbes preferentially use non-structural, soluable carbohydrates when they are present (75). Most cellulolytic strains can utilize soluble carbohydrates (118). This would increase the lag time before fiber digestion would begin. Another theory states the decreased ruminal pH associated with feeding high-grain diets depresses the number and/or activity of the cellulolytic bacteria. This theory is supported by the fact that numbers of cellulolytic organism in the rumen drop by a factor of 10^2 as the amount of starch increased in the diet (164). Uden and Van Soest (235) stated that the mean retention time of digesta in vivo is not equivalent to incubation time of digesta in situ or in vitro and proposed that apparent digestibility in vivo could be predicted by solution of simultaneous equations describing rate of fermentation and rate of passage.

In a recent study (161) steers received fescue hay diets containing 0, 20, 40, 60, and 80% cracked corn. Nylon bag technique was used to determine potential extent of fiber digestion, rate of fiber digestion, and lag time of fiber digestion. From the above data, apparent extent of fiber digestibility was determined by conceptualizing fiber disappearence from rumen the as the sum of two first-order processes, digestion and passage, modified by a discrete lag time during which potentially digestible fiber escapes from the rumen undigested before fermentation commences. Results showed that apparent extent of fiber digestion was decreased as the content of cracked corn increased in the diet. Lag effects and competition between passage and digestion had little effect on the differences in fiber digestibility. The major differences were in the potential extent of fiber digestibility in the

diet. Thus, ruminal environment, pH and cellulolytic activity, are the major determinants of how much dietary fiber is digested in diets containing greater than 25% cereal grain.

The recommend level of dietary fiber for dairy cattle is 21% of the dry matter or 4.54 Kg. Apparent extent of fiber digestibility can be doubled if proper ruminal environment can be maintained (135). This would result in an increase of 0.657 megacalories of net energy for lactation, the approxiamate energy needed to produce 1 Kg of 3.4% butterfat milk. At the time of this writing, an increased return of $0.28/head/day would be realized.

Practical Solutions

All of the above mentioned metabolic abnormalities associated with feeding cattle diets high in cereal grain are the result of depressed ruminal pH, altered ruminal microbial profiles, and altered fermentation end production. A common treatment has been the feeding of buffers such as sodium bicarbonate, calcium carbonate, and magnesium oxide. The mode of action of these feed additives is either a direct buffering of ruminal organic acids (47, 80, 83, 114, 158, 168, 185, 193, 215) or an increase in rumen fluid dilution rate (50, 64, 76, 102, 104, 110, 133, 170, 172, 175, 176, 191, 192, 193, 194, 196, 197, 229, 230) or both (228). Calcium carbonate has been shown to have only limited effect in the rumen (81, 177, 193, 194, 195). Sodium bicarbonate and magnesium oxide are effective mainly at the ruminal and tissue level (50, 83, 114, 175, 214, 224, 228). At 1986 prices, the use of dietary buffers will increase ration cost by $0.05-$0.06 per head per day. Production responses have been variable (91). Generally speaking, cattle being fed

corn silage based diets have shown positive production responses with addition of buffers (51, 64, 65, 77, 78, 82, 136, 137, 162, 193, 196, 215). On the other hand, cattle being feed diets that contain alfalfa products have shown variable production response to dietaty buffers (24, 66, 73, 81, 148, 149, 162, 163, 166, 197, 237). The lack of response in diets containing alfalfa is probably due to its inherent buffering capacity (183, 245).

Feeding dietary buffers results in a shift in rumen fermentation end products. Acetate production is increased at the expense of propionate, a fact that aids in recovery from bufferfat depression (65, 85, 103, 112, 115, 122, 137, 191, 192, 193, 194, 209, 215, 229, 230). This affect may be due mainly to increased rumen fluid dilution rate as similar shifts in volatile fatty acids have been measured with supplemental sodium chloride (9, 193) that will increase rumen fluid dilution rate but has no buffering capacity.

Rumen fluid dilution rate and pH are positively correlated (87), suggesting that one method of stabilizing rumen environment in cattle fed high-cereal grain diets would be to increase the rumen liquid outflow rate. High-cereal grain diets result in lower rumen fluid dilution rates than all-forage diets (88, 192, 226). As mentioned above, sodium bicarbonate will increase the rumen fluid dilution rate of cattle being fed high-cereal grain diets. The probable reason is an increase in the osmotic pressure within the rumen (102, 133, 176, 229). Some workers have shown that feeding osmotically active substances such as sodium chloride on an equal milliequivalent basis with sodium bicarbonate have resulted in similar rumen fluid rates, ruminal pH, and production responses (52,61,62,84,106,133, 146, 154, 192, 193, 194).

Cattle being fed high-cereal grain diets produce less saliva than those being fed all forage diets (50 versus 150 liters) (15, 16, 20). This would result in 0.7 Kg versus 2.1 Kg of salivary buffers and osmotically active materials being delivered to the rumen with high-cereal grain versus all-forage diets. Increasing the salivation rate of cattle fed high-cereal grain diets could therefore decrease the incidence of metabolic disorders.

The buffering capacity and osmotically active materials in bovine saliva would aid in maintaining an optimal ruminal pH and rumen fluid dilution rate. Maintaining a ruminal pH between 6.7 and 7.0 is crucial in obtaining maximum fiber digestion (158, 198, 200, 207, 208, 218). In addition, the increased rumen fluid dilution rate would increase the amount of soluble carbohydrates and high quality proteins escaping rumen fermentation (102, 106, 107, 171, 172, 192). This would increase the productive efficiency of cattle since 1 mole of glucose nets 38 moles of adenosine triphosphate (ATP) if completely combusted in an aerobic mammalian system, while glucose is fermented mainly into acetate, propionate, and butyrate by rumen microorganisms. A mole of glucose yields either 2 moles of acetate, 2 moles of propionate, or 1 mole of butyrate in the rumen. If these acids are completely combusted in the mammalian system they net 20, 36, and 27 moles of ATP, respectively. The major energy losses are associated with carbon dioxide, methane, and heat (155).

Increased efficiency of crude protein utilization has also been measured when rumen fluid dilution rate has been increased (136, 197, 198, 209, 234). Efficiency of microbial protein production has been increased with increased rumen fluid dilution rates (56, 143, 220).

hrs and ground in a Wiley mill (1mm screen). Ground digesta samples were analyzed for chromium (89). Particulate rate of passage (PROP) was defined as slope of the natural log of the chromium content versus time.

On day 4, representative samples of mixed rumen digesta were taken at 3.5 hours postfeeding at determine total viable bacteria (VB) and cellulolytic bacteria (CB) in habitat-stimulating, anaerobic roll tubes as described by Hungate (118).

Data in trial 1 were analyzed using a model for Latin square design. The model included diets, cows, and time periods. When main effect means were significant, treatment means were separated using Duncan's new multiple range test (217).

Trial 2. Six barren Holstein cows fitted with ruminal fistulas were fed the basal diet (C) used in trial 1 and were surgically equipped with osmotically controlled subcutaneous pumps[2] that delivered either 0.01 mg carbachol[3] (CBL)/kg BW/day, 0.10 mg pilocarpine[4] (PCN)/kg BW/day or physiological saline (PS) in a replicated 3x3 latin square design. Treatments were administered for a 14-day adaptation period followed by a 8-day collection period.

On days 1-3 of the collection period, fecal grab samples were taken during the 0400 and 1600 hours feedings. Feed sampling commenced 2 days prior to the collection period and proceeded through the fecal collection period. Feed and fecal samples were subsequently analyzed for DM, acid detergent fiber (ADF), neutral detergent fiber (NDF), crude protein (CP), and acid insoluble ash (AIA). Fecal samples were initially dried in a forced air oven at 60°C for 72 hours. Laboratory

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[2] Alza Co., Palo Alto, CA 94304

[3,4] Sigma Co., St. Louis, MO 63178

462, and 0.44, respectively (122).

One factor that limits production in cattle is feed intake. Dairy cows that produce 9000 Kg of milk in a 305 day lactation must consume 3.8 Kg of dry matter per 100 Kg of body weight. Dairy cows that produce 22,000 Kg of milk in one lactation must consume 6.5 Kg of dry matter per 100 Kg of body weight (55). Intake in cattle is controlled by two factors: rumen capacity and volatile fatty acid concentrations in the rumen. Stretch receptors in the rumen limit intake of roughage diets while chemorecptors in the rumen epithelium and ruminal vein limit intake of high-grain diets (13). Increased acetate and propionate concentrations in the rumen of cattle fed high-grain diets elicit the statiety signal. The increased concentration of volatile fatty acid is due to increased fermentation rate and decreased rumen fluid dilution rate. When the rumen fluid dilution rate of cattle fed high-grain diets was increased by infusion of mineral salts, the concentration of volatile fatty acids was decreased (25, 192). Increased rumen fluid dilution rates are also associated with increased rate of passage of solid digesta allowing increased intake (99, 165, 172, 215, 243). Several workers have measured increased dry matter intakes in dairy cattle, especially when cattle are either switched rapidly from a low to a high-grain diet or when grain exceeds 65% of the diet, if supplemented with mineral salts (82, 136, 137, 195, 215).

One practical method of increasing saliva flow in cattle, thereby increasing the flow of mineral salts to the rumen, is feeding long stemmed forage. The major problem with this method is that it decreases the energy density of the diet and may not compliment modern mechanized feeding systems. Receptors for a salivation arc have been sought by

ruminal infusion of materials such as salt, volatile fatty acids, carbon dioxide, and ammonia. The only consistant finding was that propionate infusion decreased saliva flow (169). Thus feeding cattle high-grain diets would decrease salilva production in two ways: decreased chewing time and and increased propionate production.

The major control of the saliva glands in cattle is elicited via the vagus nerve. Stimulation of the vagus nerve would increase saliva flow in cattle fed high-grain diets. Two parasympathomimitic agents, pilocarpine and carbachol, have been used in cattle, sheep, and laboratory animals to cause profuse salivation (93). Studies with laboratory animals using these two agents have been involved with basic parasympathetic nervous system physiology of associated diseases such as cystic fibrosis (222). Studies with cattle and sheep have been involved with basic parasympathetic nervous system physiology, saliva production, and as a therapeutic agent in the treatment of rumen stasis (14, 26, 27, 31, 32, 68, 181). Little work has been done to find a practical application for these agents. If small doses of these agent could be used to stimulate salivation in cattle fed high-grain diets without causing adverse effects on other organ systems, the incidence of metabolic disorders could be reduced. It is obvious that bovine saliva glands have the capability of producing copious amounts of saliva. Chewing is the major stimulus for salivation in cattle. High-grain diets do not require as much chewing time as forage diets, thus saliva production is depressed. Artificial stimulation of the saliva glands in cattle fed high-grain diets may be a practical means of stabilizing rumen fermentation without the addition of dietary buffers. Increased salivation would also increase the recycling of nitrogen, phosphorus,

sulfur, magnesium, sodium, and trace minerals.

Another way to beneficially stablize the rumen would be to inoculate with microbial species that will proliferate in conditions created by high-grain diets. As mentioned above, several metabolic disorders can result when high-grain diets are fed to cattle. Most of these disorders are the result of changes in microbial populations and/or changes in the metabolic pathways in the microorganisms.

A reduced incidence of metabolic disease and adaptation time has been observed in cattle fasted for 4 days and returned to ad libitum feeding or in cattle abruptly switched to high-grain diets if they were previously inoculated with rumen fluid from cattle previously adapted to the diet (7, 35, 113). One worker used not only rumen fluid inoculum but pure cultures of lactate utilizing rumen bacteria. With one strain of lactate utilizing bacteria and the whole rumen fluid treatment, average daily gain and feed efficiency were increased (58). Jahn et al. (125) grew batches of rumen bacteria in continuous culture and adapted them for specific environments and efficient fermentation and used the cultures as a ruminal inoculum in dairy cattle. Inoculated cattle produced 9% more milk and 17% more fat-corrected milk than controls.

Ruminal inoculation with fungi has had variable results with respect to nutrient digestibility. As early as World War I, Aspergillus sp. were used to increase the protein content of straw in Germany (94). Some studies have shown little beneficial effect of adding fungal cellulase (179) or fungal cultures (173) to ruminant diets. Other studies have shown significant increases in cellulose digestibility (12, 63). Daniels and Hashim (63) found that the dose of cellulase was important. Owen and Appleman (174) added Aspergillus oryzae to corn

silage and the grain mix of dairy cows and found that additions to corn silage increased milk fat percent, had no effect on milk production, and decreased dry matter intake. Addition of the inoculum to the grain mix resulted in similar finding.

Conclusion

The 3 goals of the ruminant nutritionist with respect to the feeding of high-grain diets are: 1) maintain the digestion of the fiberous portion of the diet; 2) improve the efficiency of nutrient utilization; and 3) improve or maintain the general health of the animal. Increasing rumen fluid dilution with mineral salts such as sodium bicarbonate and sodium chloride has been shown to be beneficial in this respect. Mineral salts such as sodium bicarbonate that have buffering capacity are of more benefit in diets that have low inherant buffering capacity such as corn silage base diets, than mineral salts that have no buffering capacity such as sodium chloride. In diets that have adequate inherant buffering capacity, i.e., those containing alfalfa, sodium bicarbonate and sodium chloride are of similar benefit, both increasing rumen fluid dilution rate with the resulting stabilization of ruminal ecology. Artificial stimulation of saliva flow and inoculation of the rumen are other possible ways to attain these goals.

The purpose of this study was to measure the effects of dietary mineral salts, artificially stimulated saliva flow, and dietary ruminal inoculants on nutrient digestibility, ruminal fermentation characteristics, and ruminal microbial profiles in cattle fed high-cereal grain diets.

CHAPTER I

EFFECT OF MINERAL SALTS, CARBACHOL AND PILOCARPINE ON NUTRIENT DIGESTIBILITY AND RUMINAL CHARACTERISTICS IN CATTLE

Introduction

Ruminants are adapted to consume low density, high-forage diets (242). Since World War II the practice of feeding ruminants cereal grains has increased due to an abundant and relatively inexpensive supply (225). This practice has increased the incidence of some metabolic disorders including lactic acidosis (207), liver abscess (184), grain bloat (123), polioencephalomalacia (39), and milk butterfat depression (182). All of these metabolic disorders are associated with changes in the rumen ecosystem due to the rapid fermentation of soluble carbohydrates. These changes include a drop in ruminal pH (53) and cellulolytic activity (164), and an increase in lactate (199) and propionate (192) production.

Dietary buffers are a common remedy for some of these dietary disorders. Dietary sodium bicarbonate ($NaHCO_3$) has been shown to increase ruminal pH (80), fiber digestibility (83), and ruminal liquid dilution rate (LDR) (194). Dietary magnesium oxide has some effect as a ruminal buffer (83) while calcium carbonate is effective postruminally (196). Although increased dietary sodium chloride (NaCl) has been shown to have little effect on ruminal pH, increases in LDR, fiber digestibility, and milk fat have been measured (192, 193, 194). Increased LDR is associated with increased efficiency of microbial protein synthesis (102), reduced propionate production (102), increased milk fat synthesis (193) and increased organic matter utilization (62) in cattle fed high-concentrate diets.

Saliva secretion can be decreased by 4-fold in cattle fed high concentrate-diets (16). Bovine saliva contains HCO_3^- and $HPO_4^=$ buffers (53). Thus a reduction in saliva secretion would reduce buffering capacity of the rumen. Bovine saliva also contains mucins that have been shown to reduce bloat in feedlot cattle (22).

Carbachol (CBL) and pilocarpine (PCN) are parasympathomimetic agents capable of stimulating profuse salivation in cattle and sheep (93). These agents could be used to stimulate saliva secretion in cattle fed high-concentrate diets.

The purpose of this study was to evaluate the effect of $NaHCO_3$, NaCl, CBL, and PCN on ruminal characteristic and nutrient digestibility in cattle fed 50% concentrate diets.

Materials and Methods

Trial 1. Three barren Holstein cows fitted with ruminal fistulas were fed diets containing 50% concentrate (Table 1) plus one of the three following treatments: 2.0% of the dietary dry matter (DM) as $NaHCO_3$; 2.0% DM as NaCl; or no supplemental mineral salts (C) in a 3x3 Latin square design. Cattle were fed 86 g DM/kg body weight $(BW)^{.75}$ per day in two equal portions at 0400 hrs and 1600 hrs for a 14-day adaptation period followed by a 4-day collection period.

On day 1 of the collection period, rumen liquid volume (LV) was determined at 0, 2, 4, and 8 hrs postfeeding using the method of Alexander et al. (3) except 50 g of polyethylene glycol (PEG) was used at each time period instead of 100 g. Approximately 300 ml samples of mixed rumen digesta were removed at each time period. A 50 ml aliquot was placed in an oven at 60°C for 72 hrs to determine dry

matter. A 200 ml aliquot was strained through four layers of cheesecloth and pH was determined on the filtrate with a combination pH electrode. The filtrate was centrifuged at 25,000 x g and 9 parts clarified rumen fluid was acidified with 1 part 6N HCl and frozen at -20°C until subsequent volatile fatty acid (VFA) and ammonia nitrogen NH_3-N analyses. Concentration of VFA was determined by gas chromatography using a 5% PNGS + 1% H_3PO_4 on Anakrom A90/100 column[1] 95°C. Concentration of NH_3-N was determined using a specific ion electrode. A 30 ml aliquot of strained ruminal fluid was diluted to 1:1 with 50% formalin solution. Total rumen bacteria were counted by diluting to 1:500 with 30% glycerol solution and counting and averaging two four-grid counts on a blood hemocytometer at 600x on a phase contrast microscope.

On day 2, a pulse dose of 100 g PEG in 500 mls distilled water was thoroughly mixed with rumen contents by hand just priod to the 0400 feeding. Representative digesta samples were then taken at 0, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, and 24 hrs postdosing. Samples were strained through four layers of cheesecloth, centrifuges at 25,000 x g for 20 mins, and the supernatant stored at -20°C for subsequent PEG analysis (120). Liquid dilution rate (LDR) was defined as the slope of the natural log of PEG concentration versus time.

On day 3, 200 g of the straw portion of the diet was replaced with 200 g of chromium-mordant dietary fiber (99). Representative digesta samples were then taken at 0, 2, 4, 6, 8, 10, 12, 20, 24, 32, 40, and 48 hrs postdosing. Digesta samples were dried in an oven at 60°C for 72

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[1] Analabs GCM-109. Unit of Foxboro Analytical, 80 Republic Drive, North Haven, CT.

hrs and ground in a Wiley mill (1mm screen). Ground digesta samples were analyzed for chromium (89). Particulate rate of passage (PROP) was defined as slope of the natural log of the chromium content versus time.

On day 4, representative samples of mixed rumen digesta were taken at 3.5 hours postfeeding at determine total viable bacteria (VB) and cellulolytic bacteria (CB) in habitat-stimulating, anaerobic roll tubes as described by Hungate (118).

Data in trial 1 were analyzed using a model for Latin square design. The model included diets, cows, and time periods. When main effect means were significant, treatment means were separated using Duncan's new multiple range test (217).

Trial 2. Six barren Holstein cows fitted with ruminal fistulas were fed the basal diet (C) used in trial 1 and were surgically equipped with osmotically controlled subcutaneous pumps[2] that delivered either 0.01 mg carbachol[3] (CBL)/kg BW/day, 0.10 mg pilocarpine[4] (PCN)/kg BW/day or physiological saline (PS) in a replicated 3x3 latin square design. Treatments were administered for a 14-day adaptation period followed by a 8-day collection period.

On days 1-3 of the collection period, fecal grab samples were taken during the 0400 and 1600 hours feedings. Feed sampling commenced 2 days prior to the collection period and proceeded through the fecal collection period. Feed and fecal samples were subsequently analyzed for DM, acid detergent fiber (ADF), neutral detergent fiber (NDF), crude protein (CP), and acid insoluble ash (AIA). Fecal samples were initially dried in a forced air oven at 60°C for 72 hours. Laboratory

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[2] Alza Co., Palo Alto, CA 94304

[3,4] Sigma Co., St. Louis, MO 63178

DM of dried fecal and feed samples was determined by drying in a forced air oven at 100°C for 24 hours. Standard procedures were used to determine ADF and NDF (241) and CP (10). The method of Van Keulen and Young (239) was used to determine AIA. Apparent nutrient digestibilities were determined using AIA as an internal marker.

On day 4, ruminal LV, VFA, NH_3-N, pH and bacterial mass were determined as in trial 1 except chromium ethylenediaminetetraacetic acid (Cr-EDTA) prepared by the method of Binnerts et al. (29) was used as a water soluble marker instead of PEG. To determine chromium (Cr) content, rumen digesta samples were strained through four layers of cheesecloth and centrifuged at 27,000 x g for 20 minutes. Ten milliliters of supernatant was pipetted into a 25 ml volumetric flask and the liquid evaporated in a forced air oven at 100°C for 8 hrs. Chromium standards were prepared in a similar fashion using solutions containing 10, 5, 2.5, 1.25, 0.625 and 0.3125 mg Cr/dl. Chromium content of the residue was then determined by digestion with 5 ml of perchloric-sulfuric acid solution (89). Bacterial mass of the digesta was determined by modification of the method of Mahmoud and Koskows (153). A 20 g aliquot of digesta was blended with 180 ml of acetate-phosphate buffer (APB) (121) at low speed for 60 seconds in a Waring blender. The mixture was centrifuged at 1,000 x g for 20 minutes to remove dietary particulates (121). Ten milliliters of supernatant was then placed on a preweighed 47 mm nylon millipore membrane filter (0.22 micron pore size) with light suction. The filter was rinsed three times with APB, dried at 25°C in a vacuum oven for 8 hours, and weighed.

On day 4, blood samples were taken from the coccygeal vein at 3.5 hrs post 0400 feeding. Samples were stored in heparinized tubes on ice

and analyzed for pH, pCO_2, pO_2, HCO_3, total CO_2, and base excess (Corning™ pH/Blood Gas System, Model 165[5]). Plasma calcium and magnesium (Technicon Autoanalyzer II[6]), sodium and potassium (Beckman Model E4A[7]) were determined on the same blood sample. On day 5, samples of mixed rumen digesta were taken at 3.5 hours postfeeding and analyzed for viable and cellulolytic bacteria as in trial 1. On day 6, liquid dilution rate (LDR) was determined as in trial 1 except Cr-EDTA was used as the marker. Chromium was analyzed as mentioned above. On days 7-8, particulate rate of passage was determined as in trial 1.

Data in trial 2 were analyzed using a model for replicated Latin square design. The model included treatments, replicates, cows within replicates and time periods. When main effects means were significant, treatment means were separated using Duncan's new multiple range test (217).

Results and Discussion

Trial 1. The addition of dietary $NaHCO_3$ and NaCl increased rumen volume (RV) by approximately 13% over basal diet ($P<.05$, Table 2). Similar measurements in steers ruminally infused with $NaHCO_3$ and NaCl have been reported (192). The increase was attributed to increased water consumption and increased ruminal hypertonicity. A 10.2 and 13.7% increase in LDR was measured with dietary $NaHCO_3$ and NaCl, respectively. Similar results have been measured in lactating dairy cattle fed $NaHCO_3$ and NaCl (193) and steers infused with the same

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[5] Corning Scientific Instruments, Medfield, MA

[6] Technicon Co., Inc., Tarrytown, NJ

[7] Beckman Co., Inc., Palo Alto, CA

mineral salts (194). Dietary $NaHCO_3$ and NaCl increased the PROP by 13.9 and 11.1%, respectively. Few studies have measured differences in PROP with dietary mineral salts. Kovacik et al. (138) did not observe a difference in PROP when 50% corn diets were supplemented with $NaHCO_3$ using chromium oxide as the solid phase marker. Chromium oxide can be associated with both solid and liquid phases (53). Rogers et al. (197) measured no differences in PROP with dietary $NaHCO_3$. Rare earth markers were used, which have been shown to transfer from particle to particle (242). Haaland and Tyrrell (99) showed that passage of chromium-mordant dietary fiber tended to increase with dietary $NaHCO_3$, however, only 50 g of marked fiber was used in the study. Okeke et al. (172) used 200 g of chromium-mordanted soybean meal particles and measured increased passage rates with dietary $NaHCO_3$. The use of 200 g of marked fiber mixed with the diet in our study may have more evenly distributed the chromium-mordanted fibers in the rumen contents, which appeared to increase the precision of the PROP measurements.

The $NaHCO_3$ treatment tended to increase ruminal pH. The NaCl treatment increased ruminal pH ($P<.05$, Table 3). Similar results have been reported by Rogers et al. (192, 193). In our study the NaCl treatment resulted in the highest LDR ($P<.05$). Estell and Gaylean (87) summarized data from several studies in which LDR was measured and reported a positive relationship between ruminal pH and LDR. Although total VFA and NH_3-N concentrations did not differ between treatments, daily mean pools of VFA, acetate, branched chain VFA, and NH_3-N tended to be increased by dietary $NaHCO_3$ and NaCl as was the Ac/Pr. Harrison et al. (103) measured increased Ac/Pr when LDR was increased with ruminal infusions of $NaHCO_3$, NaCl and PEG. Increased acetate pools and

decreased propionate pools were reported by Rogers and Davis (192) with NaCl and $NaHCO_3$ treatments. Propionate pools were not changed in our study. A negative relationship between LDR and propionate molar proportion was reported by Estell and Gaylean (87). Increased milk fat with an associated decrease in molar percent propionate has been measured in dairy cattle supplemented with $NaHCO_3$ and NaCl (193). The trend toward increased ruminal NH_3-N pools could be associated with increased protein solubility measured as ruminal pH increases (136). Increased protein solubility would result in increased degradability, which would account for the trend toward increased branched chain VFA pools with $NaHCO_3$ and NaCl treatments.

Branched chain VFA have been shown to be stimulatory to ruminal cellulolytic bacteria (46). The combination of increased ruminal pH (164) and branched chain VFA pools measured with dietary $NaHCO_3$ and NaCl would account for the increased % cellulolytic bacteria measured (Table 4). A trend toward increased bacterial cell production was measured with $NaHCO_3$ and NaCl treatments (Table 4). This increase may be associated with increased LDR. Harrison et al. (102) measured increased microbial amino acids at the duodenum of sheep as LDR was increased with ruminally infused artificial saliva and PEG.

Trial 2. Ruminal LV was decreased by both PCN and CBL treatment ($P<.05$, Table 5). Although amplitude and rate of ruminal contractions were not measured in this study, PCN can increase reticulo-rumen muscle tone (96) and CBL is used in veterinary medicine to treat rumen stasis (93). The reduced LV may have been due to increased activity in rumen musculature. Increased LDR and PROP measured with both the PCN and CBL treatments may have resulted from an increased salivation rate (15), an

increase in the amplitude and rate of contractions in rumen musculature, or both. Estimated ruminal fluid outflow rate was increased by the treatments. This was associated with a 13.36% increase in LDR.

Treatments with PCN and CBL tended to increase ruminal pH, while Ac/Pr increased ($P<.05$, Table 5). Pools of VFA and NH_3-N tended to decrease with the PCN and CBL treatments due to a decrease in LV.

Dietary mineral salts increased LV in trial 1. The increased saliva flow with PCN and CBL treatment should have increased the mineral salts in the rumen, and therefore LV. Increased rumen musculature activity may account for the reduced LV. Similar to trial 1, Ac/Pr and pH were increased by PCN and CBL probably due to buffers introduced via increased saliva flow.

Viable bacteria and cellulolytic bacteria cell output tended to increase with PCN and CBL treatments, while % cellulolytic bacteria was increased by the treatments ($P<.05$, Table 7). As in trial 1, cellulolytic bacteria habitat was probably improved by increased ruminal pH. Bacterial mass produced per day tended to increase with PCN and CBL treatment. This is consistent with findings in trial 1 and Harrison et al. (102). Chalupa (49) states that metabolizable energy (ME) content of the diet is the main determinant of bacterial protein production, and used an average factor of 26 g of bacteria crude protein per megacalorie (Mcal) of ME. Cattle in our study received 23.1 Mcal of ME per day and theoretically should have produced 601.4 grams of bacterial crude protein. If rumen bacteria contain approximately 65% crude protein, 925.2 grams of bacterial mass should have been produced. The membrane filter method (153) may underestimate ruminal bacterial cell mass production but is adequate to separate treatment differences.

Apparent digestibility of DM, CP, ADF, and NDF tended to increase with PCN and CBL treatments (Table 8). Digestibility of ADF and NDF increased 6.65 and 7.92%, respectively. This increase was probably due to the increased cellulolytic bacteria numbers measured with the PCN and CBL treatments. Erdman et al. (83) reported a 12% increase in ADF digestibility with supplemental dietary $NaHCO_3$. Rogers et al. (197) fed diets similar to that in our study except the alfalfa portion was either chopped or long stemmed hay. Results showed larger increases in ADF digestibility with chopped hay. There would be less saliva produced with chopped hay, thus dietary $NaHCO_3$ would improve cellulolytic bacteria habitat by maintaining a more optimum ruminal pH.

Blood pH and gas profiles were unaffected by treatments. Similar results have been reported in studies with dietary $NaHCO_3$ (136, 196) (Table 9). Blood electrolyte profiles also were unaffected by treatments. Dietary $NaHCO_3$ treatments have been shown to reduce blood magnesium and potassium levels in dairy cattle but not below normal ranges (196).

The results of our study show that supplemental dietary $NaHCO_3$ and NaCl are equally effective in maintaining a more optimum ruminal pH and LDR and thus increased cellulolytic activity and fiber digestion. Effects of supplemental NaCl are not as dramatic as $NaHCO_3$ in corn:corn silage diets (193) since NaCl has no buffering capacity. The diets used in our study contained alfalfa hay, which has inherent buffering capacity (183), therefore the buffering capacity of $NaHCO_3$ was not an advantage. Supplemental NaCl may be an economical way to stabilize the rumen environment in high-concentrate diets containing alfalfa hay. Results of our study also show that stimulating saliva flow may hold

promise as an endogenous means of stabilizing ruminal environment in high-grain and/or chopped forage diets. Further study on agents capable of stimulating saliva flow and resulting effect on nutritional physiology are needed.

TABLE I.1 Ingredient and chemical composition of experimental diets, trial 1.

	Diets (Dry Matter Basis)		
	Basal	$NaHCO_3$	NaCl
	%		
Ingredient			
Rolled Barley	49.67	48.50	48.50
Chopped Alfalfa Hay	39.67	39.25	39.25
Chopped Barley Straw	9.66	9.25	9.25
TM Salt[a]	0.50	0.50	0.50
$NaHCO_3$	0	2.00	0
Plain White Salt	0	0	2.00
Vitamin Supplement[b]	0.50	0.50	0.50
Estimated Nutrient Analysis			
Crude Protein	12.50	12.30	12.30
Metabolizable Energy, Mcal/kg	2.57	2.51	2.51
ADF	24.60	24.20	24.20
Calcium	0.53	0.53	0.53
Chloride	0.55	0.55	1.75
Magnesium	0.20	0.19	0.19
Phosphorus	0.28	0.28	0.28
Potassium	1.23	1.20	1.20
Sodium	0.27	0.82	1.07
Sulfur	0.23	0.22	0.22

[a] Selenium, 0.002%; manganese, 0.200%; iron, 0.200%; cobalt, 0.005%; copper, 0.30%; iodine, 0.007%, zinc, 0.350%.

[b] Vitamin A, 136.363 I.U./kg; vitamin D_3, 13.636 I.U./kg; vitamin E, 136 I.U./kg.

TABLE I.2 Dietary treatment effects on ruminal digesta flow kinetics, trial 1.

Item	Diet			SE
	Basal	$NaHCO_3$	NaCl	
Volume (Liters)	44.81[a]	51.93[b]	50.60[b]	0.44
Liquid Dilution Rate (%/hour)	6.31[a]	7.03[b]	7.31[c]	0.21
Total Rumen Fluid Outflow[d], (liters/day)	67.86[a]	87.62[b]	88.77[b]	0.67
Particulate Rate of Passage (%/hour)	3.91[a]	4.54[c]	4.40[b]	0.03

[a,b,c] Means in the same row with different superscripts differ ($P<.05$).

[d] Liquid volume x liquid dilution rate x 24.

TABLE I.3 Dietary treatment effects on daily mean ruminal characteristics, trial 1.

Item	Diet			SE
	Basal	$NaHCO_3$	NaCl	
pH	6.19[a]	6.32[ab]	6.57[b]	0.06
Total VFA, mM/L	108.75	96.68	112.03	3.24
VFA Pools[c], moles	4.87	5.02	5.67	0.56
Acetate Pool[d], moles	3.12	3.26	3.74	0.48
Propionate Pool[d], moles	0.93	0.85	0.96	0.13
Ac/Pr Ratio	3.35	3.83	3.90	0.51
Branched Chain VFA Pool[d], moles	0.27	0.29	0.37	0.08
NH_3-N Pool[c], g	55.21	66.11	60.97	3.54
NH_3-N, mg/dl	12.32	12.73	12.05	0.72

[a,b] Means in the same row with different superscripts differ, ($P<.05$).

[c] Concentration x liquid volume.

[d] Molar % x VFA pool.

TABLE I.4 Dietary treatment effects on ruminal microbial profiles, trial 1.

Item	Diet			SE
	Basal	$NaHCO_3$	NaCl	
Total Viable Bacteria, x 10^9/ml	2.37	1.68	2.76	1.08
Viable Bacteria Pools[e], x 10^{12}	10.62	8.72	13.97	1.73
Cellulolytic Bacteria, x 10^8/ml	3.48	2.78	4.29	1.70
Cellulolytic Bacteria Pools[e], x 10^{12}	1.56	1.44	2.17	0.18
Cellulolytic Bacteria, %	14.69[a]	16.51[c]	15.53[b]	0.04
Direct Count Bacteria, x 10^9/ml	7.90	6.69	7.89	0.89
Total Bacteria Cells Produced per day[d], x 10^{13}	5.36	5.86	7.00	0.82

[a,b,c] Means in the same row with different superscripts differ, ($P<.05$).

[d] Direct Count Bacteria x liquid volume x liquid dilution rate x 24.

[e] Concentration x liquid volume.

TABLE I.5 Treatment effects on ruminal digesta flow kinetics, trial 2.

Item	Treatment			SE
	Saline	Pilocarpine	Carbachol	
Volume, (Liter)	57.68[a]	52.76[b]	52.55[b]	1.14
Liquid Dilution Rate, (%/hour)	7.46[a]	8.61[b]	8.61[b]	0.03
Total Ruminal Fluid Outflow[d], (Liters/day)	103.27[a]	109.02[b]	108.59[b]	2.07
Particulate Rate of Passage, (%/hour)	4.77[a]	5.93[c]	5.82[b]	0.14

[a,b,c] Means in the same row with different superscripts differ, (P<.05).

[d] Liquid volume x liquid dilution rate x 24.

TABLE I.6 Treatment effects on daily mean ruminal characteristics, trial 2.

Item	Treatment			SE
	Saline	Pilocarpine	Carbachol	
pH	6.53	6.74	6.67	0.05
Total VFA, (mM/L)	116.80	116.20	119.20	2.52
Total VFA Pool[c], (moles)	6.74	6.13	6.26	0.14
Acetate Pool[d], (moles)	4.05	3.65	3.84	0.08
Propionate Pool[d], (moles)	1.23	1.08	1.12	0.02
Ac/Pr Ratio	3.29[a]	3.38[b]	3.43[b]	0.02
Branched Chain VFA Pool[d], (moles)	0.44	0.44	0.44	0.01
NH_3-N Pool[c], g	85.65	74.66	69.42	4.30
NH_3-N, mg/dl	14.85	14.15	13.21	0.79

[a,b] Means in the same row with different superscripts differ, ($P<.05$).

[c] Concentration x liquid volume.

[d] Molar % x VFA Pool.

TABLE I.7 Treatment effects on ruminal microbial characteristics, trial 2.

Item	Treatment			SE
	Saline	Pilocarpine	Carbachol	
Total Viable Bacteria, x 10^9/ml	3.81	3.97	3.95	0.18
Viable Bacteria Cells/day[c], x 10^{13}	3.93	4.33	4.29	0.13
Cellulolytic Bacteria, x 10^8/ml	4.44	4.82	4.77	0.21
Cellulolytic Bacteria Cells/day[c], x 10^{12}	4.59	5.25	5.18	0.32
Cellulolytic Bacteria, %	11.67[a]	12.14[b]	12.08[b]	0.03
Bacterial Mass[c], g/day	787.9	876.2	837.8	42.3

[a,b] Means in the same row with different superscripts differ, ($P<.05$).

[c] Concentration x total ruminal fluid outflow.

TABLE I.8. Treatment effects on apparent total tract nutrient digestibilities, trial 2.

	Treatment			SE
	Saline	Pilocarpine	Carbachol	
Dry Matter, %	73.3	76.2	76.9	1.41
Crude Protein, %	77.3	78.5	79.9	1.31
Acid Detergent Fiber, %	63.2	67.1	68.3	2.23
Neutral Detergent Fiber, %	61.0	65.6	68.3	2.32

TABLE I.9 Treatment effects on blood gas and electrolytes, trial 2.

Item	Treatment			SE
	Saline	Pilocarpine	Carbachol	
pH	7.42	7.42	7.42	0.006
pO_2, mmHg	34.5	32.3	33.85	1.80
pCO_2, mmHg	46.6	47.8	46.5	0.92
HCO_3, mM/L	30.6	30.3	29.5	0.70
Total CO_2, mM/L	32.5	32.1	31.2	0.96
Base Excess, meq/L	5.67	5.77	5.33	0.50
Na, meq/L	143.0	143.0	143.0	0.70
K, meq/L	4.2	4.2	4.1	0.10
Mg, meq/Lg	2.3	2.1	2.1	0.10
Ca, meq/LL	14.1	14.0	14.2	0.20

CHAPTER II

EFFECT OF ORALLY ADMINISTERED PILOCARPINE ON RUMINAL CHARACTERISTICS AND NUTRIENT DIGESTIBILITY IN CATTLE

Introduction

Since World War II the supply of relatively inexpensive cereal grains has increased (225). Genetic selection for cattle with superior performance has increased the dietary energy density requirement. Consequently, dairy cow diets of 70%, and feedlot cattle diets of 80%, cereal grains are not uncommon. Ruminants are adapted to consume low-density, high-forage diets (242). Although the practice of feeding cattle high-cereal grain diets has improved performance, it has also been implicated in an increased incidence of metabolic disorders including: lactic acidosis (207); liver abcess (184); feedlot bloat (123); polioencephalomalacia (39); and milk butterfat depression (182).

The digestibility of structural carbohydrates in 70-90% cereal grain diets is not of paramount importance, because cattle are generally fed diets that are high in cereal grain for only a relatively short time. Diets of 50% cereal grain are the norm for high producing feedlot and dairy cattle (86). Decreased structural carbohydrate digestion in these diets would greatly decrease the net energy for production available to cattle. Dietary concentrate levels as low as 25% have been shown to decrease the utilization of structural carbohydrates (59, 144).

Ruminal cellulolytic bacteria are responsible for structural carbohydrate digestion. They generally account for no more than 25% of total bacterial population, even on all-forage diets (54). The optimum pH for cellulolytic bacteria is 6.7-7.0 (164). The rapid fermentation

of soluble carbohydrates and reduced saliva flow in cereal grain diets result in an accumulation of volatile fatty acids and lactate that drop ruminal pH below this optimum , compromising cellulolysis. The one, real advantage ruminants have in livestock production is their symbiotic relationship with cellulolytic bacteria. Improving cellulolytic bacteria habitat in cattle being fed diets containing cereal grain would, therefore, be beneficial.

This study was initiated to measure effects on nutrient digestion, ruminal digesta flow kinetics, and ruminal fermentation and bacterial characteristics resulting from stimulating saliva flow with pilocarpine (93) in cattle being fed a 45% cereal grain diet.

Materials and Methods

Four barren Holstein cows weighing approximately 637 kg and fitted with ruminal fistulas were assigned the four dietary pilocarpine[1] (PLCN) treatments shown in Table 1 in a 4x4 Latin square design. Cows were fed individually at 0400 and 1600 hours daily, and had free access to clean water. Diets were fed for a 14-day adaptation period followed by an 8-day collection period.

On days 1-3 of the collection period, fecal grab samples were taken during the 0400 and 1600 hour feedings. Diet samples were taken daily starting 2 days prior to the fecal collection period. Fecal samples were initially dried for 72 hours in a forced air oven at 60° C. Laboratory dry matter (DM) was determined in a forced air oven at 100° C for 24 hours. Feed and dried fecal samples were Wiley mill ground (1mm

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[1] Sigma Co., St. Louis, MO 63178

screen) and analyzed for crude protein (CP) (10), acid detergent fiber (ADF) (241), neutral detergent fiber (NDF) (241), acid detergent lignin (ADL) (241), and acid insoluble ash (AIA) (239). Acid insoluble ash was used as an internal marker to determine apparent nutrient digestibility.

On day 4, ruminal liquid volume (LV) was determined at 0, 3, 6, and 9 hours postfeeding (0400 h) using the method of Alexander et al. (3), except 500 ml of chromium ethylenediaminetetraacetic acid solution (Cr-EDTA) (29) was used as the water-soluble marker instead of polyethylene glycol. A 300 ml sample of mixed rumen digesta was taken at each time period. A 50 ml aliquot was used to determine DM by drying for 72 hours in a forced air oven at 60° C. A 200 ml aliquot was strained through 4 layers of cheesecloth and pH measured on the filtrate using a combination electrode. A portion of the filtrate was acidified by placing 9 parts filtrate with 1 part 6N HCl. The mixture was then clarified by centrifugation at 25,000 x g for 20 minutes and analyzed for volatile fatty acids (VFA) using gas chromatography with a 5% PNGS and 1% H_3PO_4 on Anakrour A90/100 column[2] at 115° C and ammonia nitrogen (NH_3-N) using Nessler's reagent (10). Another portion of the filtrate was centrifuged at 25,000 x g for 20 minutes and analyzed for chromium content using a modification of the method of Fenton and Fenton (89). Ten ml aliquots of clarified ruminal fluid was placed in a 25 ml volumetric flask and evaporated in a forced air oven at 100° C. Chromium standards were prepared similarly using chromium chloride and contained 10, 5, 2.5, 1.25, 0.625, and 0.3125 mg chromium/dl. Chromium content of the residue was determined by digestion with 5ml of the

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[2] Analabs GCM-109. Unit of Foxboro Analytical, 80 Republic Drive, North Haven.

perchloric-sulfuric acid solution (89) until yellow color appeared. The residue was diluted to volume with distilled water after cooling and was allowed to stand overnight. Percent transmittance was then read at 430 nm with a spectrophotometer. Difference in chromium content of the ruminal fluid before and after the addition of 500 ml of Cr-EDTA into the rumen was used to determine liquid volume (LV). Bacterial mass of the digesta was determined by modification of the method of Mahmoud and Koskows (153). A 20g aliquot of digesta was blended with 180ml of acetate-phosphate buffer (APB) (121) at low speed for 60 seconds in a Waring blender. The mixture was centrifuged at 1000 x g for 20 minutes to remove dietary particulates (121). Ten ml of supernatant was then placed on a preweighed 47 mm nylon millipore membrane filter (0.22 micron pore size) with light suction. The filter was rinsed 3 times with APB, dried at 25° C in a vacuum oven for 8 hours, and reweighed.

On day 5, a pulse dose of 1000 ml of Cr-EDTA solution (29) was hand mixed with rumen digesta immediately prior to the 0400 hour feeding. Samples of rumen digesta were collected at 0, 1, 2, 3, 4, 6, 8, 10, 12, 16, and 24 hours postdosing and analyzed for chromium as above. Slope of the natural log of chromium content regressed on time was defined as the ruminal liquid dilution rate (LDR).

On days 6-7, 200g of chromium mordanted dietary fiber (99) was mixed with the 0400 hour ration. Rumen digesta samples were collected at 0, 2, 4, 6, 8, 10, 12, 16, 20, 24, 32, 40, and 48 hours postfeeding and dried in a forced air oven for 72 hours at 60° C. Samples were Wiley mill ground (1mm screen) and analyzed for chromium (89). Ruminal particulate rate of passage (PROP) was defined as the slope of the natural log of the chromium content regressed on time.

On day 8, samples of mixed rumen digesta were collected at 3 hours after the 0400 hour feeding and analyzed for total viable bacteria and cellulolytic bacteria using the habitat stimulating media in anaerobic roll tubes described by Hungate (118).

Data were analyzed using a model for the Latin square design. The model included dietary treatments, cows and time periods. When main effect means were significant, treatment means were separated using Duncan's new multiple range test (217).

Results and Discussion

The effect of orally administered PLCN on ruminal digest flow kinetics is presented in table 2. Ruminal LV was unaffected by PLCN treatment. This is in contrast to the effect of PLCN administered continuously via subcutaneous osmotically controlled pumps that reduced LV (247). The proposed reason for the reduced LV was increased activity of the rumen musculature (96). An orally administered pulse dose of PLCN may be rapidly metabolized in the rumen and its effect would therefore be transitory compared to continuous dosing. Ruminal LDR was increased 30% as the dose of PLCN increased from 0 to 4 mg/kg BW ($P<.05$). Previous work has shown that a dose of 0.1 mg/kg BW increased LDR by 13.7% when administered subcutaneously. In the present study, a dose 10 times (1.0 mg/kg BW) that level administered orally did not significantly ($P>.05$) increase LDR. A numerical increase of 3.6% was measured, however. Increased LV, LDR, and ruminal outflow rates were measured in cattle in which the salivation rate was increased by 29% with administration of slaframine (124). Similar to LDR, PROP increased by 31% as the level of PLCN increased from 0 to 4 mg/kg BW ($P<.05$). Similar results have been reported when the rumen of sheep were infused

with artificial saliva and sodium bicarbonate (102, 219).

The effects of orally administered PLCN on ruminal characteristics is presented in table 3. Ruminal pH was increased by 4.2% as the level of PLCN increased from 0 to 4 mg/kg BW ($P<.05$). This effect was probably due to increased salivary buffering. Concentration of VFA was increased at the 1 mg PLCN/kg BW level only ($P<.05$). This observation is difficult to explain, but when VFA concentrations are multipled by daily ruminal liquid outflows, administration of PLCN increased VFA flow compared to controls. Flow of VFA also tended to increase as level of PLCN increased. Flow of VFA is not to be confused with VFA production because absorption from the rumen is not accounted for, but VFA concentrations would be affected by ruminal outflow rates. Molar % of individual VFA was unaffected by treatment. This observation is somewhat surprising in view of the fact that increased acetate:propionate ratios have been measured when LDR is increased with mineral salts (102, 192, 194, 247). Rogers et al. (197) fed a basal diet that contained 54% alfalfa hay (similar to the diet in the present study) and 46% concentrate with or without sodium bicarbonate and measured no differences in molar proportions of VFA. Liquid dilution rate usually affects acetate:propionate ratio only when propionate is greater than 25% (192, 194). Ruminal NH_3-N concentration decreased by 37.7% as the level of PLCN increased from 0 to 4 mg/kg BW ($P<.05$). Increased pH has been associated with increased protein solubility and degradability (251). Ruminal pH increased as level of PLCN increased and an increase in NH_3-N would be expected. The increased liquid and particulate outflow rates measured with PLCN treatment may account for the decreased concentration of NH_3-N. Similar trends were measured when

PLCN was administered subcutaneously (194).

Effects of PLCN treatment on ruminal bacteria are presented in table 4. Concentration of viable bacteria was unaffected by treatment. Concentration of cellulolytic bacteria (CB) and percent CB tended to increase with PLCN treatment. This increase is probably due to improved CB habitat as pH increased (164). Treatment with PLCN tended to increase bacterial mass production (BMP). Harrison et al. (102) measured increased amounts of microbial amino acids at the duodenum of sheep ruminally infused with artificial saliva solution. Efficiency of microbial nitrogen production was also improved (102). Issacson et al. (122) showed that as the dilution rate was increased from 2% to 12%/hour in continuous cultures of mixed rumen bacteria, bacteria cells produced per day and per mole of glucose fermented increased.

Effects of PLCN administration on nutrient digestibility are presented in table 5. Dry matter digestibility (DMD) was increased by 8.5% as the level of PLCN increased from 0 to 4 mg/kg BW. From regression analysis it appeared that DMD = 71.86 + (1.68 x mg PLCN/kg BW), r^2 = 0.64. Crude protein digestibility (CPD) increased 6.1% as the level PLCN was increased from 0 to 4 mg/kg BW. The regression equation was, CPD = 83.99 + (1.42 x mg PLCN/kg BW), r^2 = 0.40. Hemicellulose digestibility was unaffected by treatment. Cellulose digestibility (CLD) was increased by 8.5% as the level of PLCN was increased from 0 to 4 mg/kg BW and was described by the relationship, CLD = 50.37 + (1.17 x mg PLCN/kg BW), r^2 = 0.69. Increased ADF digestibility has been measured in cattle fed high-grain diets when LDR and ruminal pH was increased with sodium bicarbonate (193, 215). Decreased amounts of cellulose and hemicellulose reached the duodenum of sheep that were

ruminally infused with artificial saliva (102). In the present study, cellulolytic bacteria concentrations and proportions tended to increase as LDR was increased with PLCN, accounting for the increased cellulose digestibility. Treatment with PLCN in the present study and treatment with mineral salts (102) increased PROP, which would be expected to decrease retention time of fiber and thus its availability for microbial attack. Improving CB habitat appears to be more important in enhancing digestibility of fiber than ruminal fiber retention time in diets that contain substantial proportions of concentrate.

In conclusion, the reduction in saliva flow in cattle caused by high-cereal grain, chopped-forage diets can be corrected by stimulating salivation with orally administered PLCN. Buffering capacity and LDR are increased and tended to affect the rumen microbial population in 3 ways: 1) increased BMP; 2) increased microbial efficiency; and 3) increased numbers and proportion of cellulolytic bacteria. These trends could have resulted in the increase in the digestibility of cellulose and hemicellulose, which is a major advantage to ruminants. Further study on the effects of PLCN on the performance of cattle when in a productive state is needed to acertain its efficacy in increasing the supply of useful products from cattle to man.

TABLE II.1 Composition and analysis of diets.

Item	Diet, mg Pilocarpine/kg BW			
	0	1	2	4
Chopped Alfalfa Hay, %	53.03	53.03	53.03	53.03
Rolled Barley, %	45.00	45.00	45.00	45.00
Trace Mineralized Salt	0.25	0.25	0.25	0.25
Wheat Bran, %	1.47	0	0	0
Pilocarpine Supplements[1]	0	1.47	1.47	1.47
Metabolizable Energy, Mcal/kg	2.56	2.56	2.56	2.56
Crude Protein, %	15.93	15.93	15.93	15.93
Neutral Detergent Fiber, %	31.57	31.57	31.57	31.57
Acid Detergent Fiber, %	20.08	20.08	20.08	20.08
Calcium, %	0.79	0.79	0.79	0.79
Magnesium, %	0.25	0.25	0.25	0.25
Phosphorus, %	0.29	0.29	0.29	0.29
Potassium, %	1.59	1.59	1.59	1.59
Sulfur, %	0.23	0.23	0.23	0.23
Zinc, mg/kg	30.16	30.16	30.16	30.16
Cobalt, mg/kg	0.37	0.37	0.37	0.37
Manganese, mg/kg	28.67	28.67	28.67	28.67
Copper, mg/kg	12.47	12.47	12.47	12.47

[1] Supplements contained 0.56%, 1.12%, and 2.24% pilocarpine, respectively, the remainder being wheat bran to equal 100%.

TABLE II.2 Effect of pilocarpine on ruminal digesta flow kinetics.

Item	Pilocarpine, mg/kg BW					
	0	1	2	4	SE[d]	P>F[e]
Liquid Volume, liters	61.95	61.20	64.48	58.21	4.72	0.83
Liquid Dilution Rate, %/hr	8.76[b]	9.09[b]	10.79[ab]	12.62[a]	0.63	0.02
Liquid outflow rate, liters/hr	5.42	5.66	7.00	7.48	0.62	0.14
Particulate rate of passage, %/hr	6.54[c]	6.88[cb]	8.28[ab]	9.55[a]	0.42	0.01

[a,b,c] Means in the same row with different superscripts differ, (P<.05)
[d] Standard error of mean
[e] Probability >F

TABLE II.3 Effect of pilocarpine on ruminal characteristics.

Item	Pilocarpine, mg/kg BW					
	0	1	2	4	SE[d]	P>F[e]
pH	6.34[c]	6.38[cb]	6.47[b]	6.62[a]	0.04	0.0001
Volatile fatty acids, mmole/L	73.69[b]	93.87[a]	76.53[b]	76.78[b]	3.11	0.0001
Volatile fatty acid flow[f] moles/day	9.47[b]	12.84[a]	12.93[a]	13.82[a]	0.84	0.04
Molar %						
Acetate	65.10	65.24	65.22	64.46	0.32	0.96
Propionate	15.42	15.44	15.33	16.68	0.56	0.28
Butyrate	14.51	14.36	14.18	13.65	0.43	0.53
Isobutyrate	1.16	1.24	1.29	1.28	0.08	0.65
Isovalerate	1.76	1.73	1.86	1.73	0.12	0.84
Valerate	2.02	1.98	2.11	2.23	0.15	0.68
Acetate:Propionate ratio	4.26	4.26	4.29	3.88	0.24	0.61
% Branched Chain Volatile fatty acids	4.95	4.95	5.26	5.23	0.25	0.707
Ammonia Nitrogen	12.25[a]	10.31[b]	10.18[b]	7.63[c]	0.54	0.0001

[a,b,c] Means in the same row with different superscripts differ, (P<.05)
[d] Standard error of mean
[e] Probability >F
[f] Ruminal outflow rate x 24 x volatile fatty acid concentration

TABLE II.4 Effect of pilocarpine on ruminal bacteria.

Item	Pilocarpine, mg/kg BW					
	0	1	2	4	SE[a]	P>F[b]
Viable bacteria x 10^9/ml	14.6	14.9	15.2	15.9	0.69	0.642
Cellulolytic bacteria x 10^9/ml	1.3	1.5	1.6	2.1	0.22	0.161
% Cellulolytic bacteria	8.56	9.95	10.84	14.31	1.49	0.139
Bacterial cell mass production[c], grams/day	716.6	795.2	868.2	1005.2	89.40	0.223
Efficiency of bacterial mass production[d], grams/Mcal M.E.	30.3	33.6	36.7	42.5	3.78	0.223

[a] Standard error of mean
[b] Probability >F
[c] Ruminal outflow rate x 24 x concentration of bacterial mass
[d] Bacterial mass production ÷ megacalories of dietary metabolizable energy.

TABLE II.5 Effect of pilocarpine on nutrient digestibility.

Item	Pilocarpine, mg/kg BW					
	0	1	2	4	SE[d]	P>F[e]
Dry matter	71.24[c]	72.80[c]	75.37[b]	77.85[a]	0.70	.002
Crude protein	83.08[b]	83.46[b]	85.51[b]	88.49[a]	0.81	.011
Hemicellulose	76.66	75.80	81.20	81.51	2.90	.43
Cellulose	49.87[c]	50.96[cb]	52.51[b]	54.59[a]	0.47	.002

[a,b,c] Means in the same row with different superscripts differ, (P<.05).
[d] Standard error of mean
[e] Probability >F

CHAPTER III

EFFECT OF ORALLY ADMINISTERED PILOCARPINE ON SERUM LIVER ENZYMES, LIVER, KIDNEY AND PAROTID GLAND WEIGHTS, AND HISTOPATHOLOGY IN MEADOW VOLES.

Introduction

Pilocarpine (PLCN) is a parasympathomimetic alkaloid capable of stimulating salivation in most mammals (93) Wiedmeier (246) have shown that PLCN is capable of correcting the reduced saliva flow in cattle fed high-cereal grain, chopped-forage diets, thereby stabilizing rumen ecology and increasing nutrient digestibility. The effect of PLCN on the histopathology of liver, kidney, and parotid glands was not investigated in these studies. Before PLCN can be used on a practical basis to improve cattle performance these effects must be ascertained. The use of cattle or smaller ruminant species such as sheep and goats would be expensive. Meadow voles (Microtus pennsylvanicus) have been used successfully as models for ruminants in feed quality, digestibility, and production studies (205). Meadow voles have also proven to be useful animal bioassays for feed palatability (134) and toxicity studies (97), correlating well to ruminants. Although they are monogastric, they have an esophageal, pregastric pouch with twice the capacity of the gastric stomach, the pH of which is 6.5 to 7.0 (135). The native diet is grass but they are capable of consuming diets consisting of up to 70% non-structural carbohydrates without ill effects (206).

The purpose of this study was to use meadow voles as a model for cattle to measure and observe the effects of orally administered PLCN on liver, kidney, and parotid gland weights and histopathology.

Materials and Methods

Fifteen mature, female meadow voles (Microtus pennsylvancius) were assigned to each of the four diets in Table 1 in a completely randomized design. Diets were fed as a total mixed meal, the ingredients being ground to pass through a 1mm screen. Voles were individually housed in a 60 cage stainless steel battery with mess floors. Cages were equipped with food consumption jars, water tubes and cotton bedding. The voles were housed in a room with relative humidity of 38-40%, temperature of 18° C, and day length of 12 hours.

Voles and food consumption jars were weighed at 0, 7, and 14 days after initiation of the study. On day 14, voles were euthanized using CO_2. Blood samples were immediately withdrawn by cardiac puncture. Blood samples from two voles were pooled to provide adequate volume for serum glutamic oxaloacetate transaminase (SGOT), glutamic pyruvate transaminase (SGPT), and alkaline phosphatase (ALKP) analysis, (Gilford System 103)[1]. Kidneys, liver, and parotid glands were then removed, blotted, weighed and fixed in 50% formalin solution. Fixed samples were in paraffin and sliced with a microtome at 6 microns. Slices were stained with hematoxylineasin and examined for histopathology.

Data were analyzed using a model for a completely randomized design for unbalanced data with 4 treatments and 12 to 15 observations per treatment. Means were separated using the t-test (202).

Results and Discussion

Levels of SGOT, SGPT, and ALKP were not affected by PLCN treatment (Table 2). Since it was necessary to obtain as large a blood sample as

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[1] Gilford Scientific, 132 Artino St., Oberlin, OH, 44074.

possible from each vole, there was considerable hemolysis that resulted in elevated serum levels of these enzymes with the analytical method used. However, there appeared to be no extensive tissue damage in the voles as a result of PLCN treatment.

The effects of PLCN treatment on liver, kidney, and parotid gland weights and feed intake are presented in Table 3. Liver weight tended to decrease with PLCN treatment but there was not a dose response. Liver weight was reduced compared to controls only at the 1 mg/kg BW treatment. Kidney weight was unaffected by PLCN treatment versus controls but was reduced by the 10 mg/kg body weight (BW) compared to the 1 and 100 mg/kg BW treatment. Parotid gland weight tended to decrease with the 1 and 10 mg/kg BW PLCN treatment and was reduced by 31% by the 100 mg/kg BW treatment. The reason why some doses of PLCN had an affect on organ weights while others did not is difficult to deduce from the data collected in this study, but there was no apparent tissue damage indicated by SGOT, SGPT, or ALKP levels. Treatment with PLCN has been shown to initially increase pancreas weight, but prolonged treatment resulted in the opposite effect (222). But, PLCN treatment initially decreased thymus weight, while prolonged treatment showed normal thymus weights (222). Treatment with PLCN obviously has different effects on different glands through time. In animals treated with PLCN, glands that have primarily a secretory function such as the pancreas and parotids have shown initial increases in weight due to hypertrophy but continued treatment has shown reduction in weight probably due to synthesis not keeping up with secretion (23, 222). The effects of PLCN on nonsecretory glands such as thymus, liver and spleen have been variable. Nagai et al. (167) showed that carbachol, a

parasympathomimetic agent similar in effect to PLCN, depressed liver gluconeogenesis while activating glycolysis. Sturgess and Reid (222) measured no effect of PLCN on liver or spleen, but, as already mentioned, thymus weight was initially reduced and later returned to normal. The mechanism for the initial drop in weight and eventual return to normal is not known, but a similar effect on liver and kidney was observed in the present study. Feed intake was increased when PLCN was administered at the 1 mg/kg BW level. Increased saliva flow and increased rate of gastrointestinal contractions on the 1 mg PLCN/kg BW treatment could account for increased intake. Increased flow rates of digesta have been measured in cattle administered PLCN orally (248). Higher levels of PLCN had no effect on intake, possibly due to overstimulation of the parotid gland with eventual exhaustion. Parotid weights were decreased at the higher levels of PLCN.

Data on the effects of PLCN on mortality and liver, kidney, and parotid gland histopathology are presented in Table 4. No effect of PLCN can be demonstrated conclusively. Effects on histopathology that were observed were non-specific and reversible in nature.

In conclusion, orally administered PLCN at levels of from 0 to 100 mg/kg BW did not indicate any tissue damage in the organs studied in the meadow vole. Levels of PLCN from 10-100 mg/kg BW resulted in a decrease in parotid gland weight but no changes in histopathology. At the 1 mg/kg BW level, PLCN increased feed intake. Data presented in this study indicate that levels of orally administered PLCN up to 10 mg/kg BW can be used without damage to liver, kidney or parotid tissues.

Table III.1 Composition of meadow vole diets.

	Pilocarpine, mg/kg BW			
Item	0	1	10	100
Corn	48.50	48.499	49.49	49.40
Soybean meal	30.00	30.000	30.00	30.00
Alfalfa meal	13.00	13.000	13.00	13.00
Sucrose	5.00	5.000	5.00	5.00
Mineral mix[1]	3.00	3.000	3.00	3.00
Vitamin mix[2]	0.50	0.500	0.50	0.50
Pilocarpine[3]	0	0.001	0.01	0.10

[1] NaCl, 13.9325%; KH_2PO_4, 38.8967%; $MgSO_{4P}$, 5.7302; $CaCO_3$, 38.1442%; $FeSO_4 \cdot 7H_2$), 2.6960%; KI, 0.0790%; $MnSO_4 \cdot 2H_2O$, 0.4453%; ZnCl, 0.0259; $CuSO_4 \cdot 5\ H_2O$, 0.475%; $CoCl_2 \cdot 6\ H_2O$, 0.0022%.

[2] vitamin A, 200 K.I.U/Kg; vitamin E, 6 K.I.U./Kg; Vitamin D_3, 20 K.I.U./Kg; vitamin K, 10 mg/Kg; thiamine, 250 mg/Kg; riboflavin, 250 mg/Kg; pyridoxine, 120 mg/Kg; niacin, 1500 mg/Kg; calcium pantothenate, 800 mg/Kg; vitamin B_{12}, 1 mg/Kg; choline chloride, 75 g/Kg.

[3] Sigma, Co., St.Louis, MO, 63178.

Table III.2 Effect of orally administered pilocarine on blood, SGOT[1], SGPT[2], alkaline phosphatase.

Item	Pilocarpine, mg/kg BW				SE	P>F
	0	1	10	100		
SGOT[1], i.u./L	1317	647	996	935	330.5	0.328
SGPT[2], i.u./L	490	379	399	386	95.8	0.685
ALKP[3], i.u./L	244	302	254	224	35.5	0.254

[1] Serum glutamic oxaloacetic transaminase

[2] Serum glutamic pyruvic transaminase

Table III.3 Effect of orally administered pilocarpine on feed intake and liver, kidney, and parotid weights (Fresh wt).

Item	Pilocarpine, mg/kg BW				SE	P>F
	0	1	10	100		
Liver, mg/gBW	40.28[a]	34.28[b]	37.61[ab]	38.81[ab]	3.04	0.069
Kidney, mg/gBW	10.56[ab]	11.38[a]	9.94[b]	11.30[a]	0.72	0.035
Parotid, mg/gBW	3.22[a]	3.18[a]	2.86[a]	2.21[b]	0.42	0.014
Feed Intake, mg/gBW/day	136.31[a]	160.86[b]	139.49[a]	148.42[ab]	14.68	0.124

[a,b] Means in the same row with different superscripts differ (P<.05).

Table III.4 Effects of orally administered pilocarpine on vole mortality and histopathology of liver, kidney and parotid tissue.

Level of Pilocarpine mg/Kg BW	Number of Voles	Mortality	Number of Voles Showing Tissue Alterations		
			Liver	Kidney	Parotid
0	15	1	0	2[b]	0
1	15	1	1[a]	2[b]	0
10	15	0	4[a]	1[b]	0
100	15	3	3[a]	0	0

[a] Slight hydropic degeneration of hepatocytes

[b] Occasional segmental cystic dilation of a convoluted tubule in the cortex and degeneration of tubular epithelial cells.

CHAPTER IV

EFFECT OF YEAST CULTURE AND/OR *ASPERGILLUS ORYZAE* FERMENTATION EXTRACT ON RUMINAL CHARACTERISTICS AND NUTRIENT DIGESTIBILITY[1]

Introduction

Feeding cereal grains to increase the energy density of cattle diets has resulted in increased performance, but has also resulted in a negative associative effect with regard to structural carbohydrate digestion. The negative effect is presumably due to alteration in cellulolytic bacteria habitat (164). One solution has been the feeding of buffers to maintain a more optimum ruminal pH (83). A pH of 6.7 - 7.0 has been determined to be the optimum range for ruminal cellulolytic activity (218). Another method of maintaining structural carbohydrate digestion in high-cereal grain diets would be daily ruminal inoculation with organisms capable of maintaining cellulolytic activity in the ruminal habitat produced by high cereal grain diets. Jahn et al. (125) adapted rumen bacteria in batch cultures to specific habitats and ruminally inoculated lactating dairy cattle with the cultures. Inoculated cattle produced 17% more fat-corrected milk. The addition of a fungal culture to the diet of lactating dairy cows improved milk fat content (174). Huber (113) reported increased fat-corrected milk production when the diet of dairy cows was supplemented with the same *Aspergillus oryzae* fermentation extract used in this study.

The purpose of this study was to measure the effect of supplemental viable fungal cultures on ruminal fermentation characteristics and

[1] Supported in part by grants from Diamond V Mills, Inc. and BioZyme Enterprises, Inc.

nutrient digestibility in cattle fed 50% concentrate diets.

Materials and Methods

Four barren Holstein cows fitted with ruminal fistulas were assigned each of four dietary treatments (Tables 1 and 2). Cows were fed individually at 0400 and 1600 hours daily, and had free access to clean water. Diets were fed for a 14 day adaptation followed by an 8 day collection period.

On days 1-3 of the collection period fecal grab samples were taken during the 0400 and 1600 hour feeding. Diet samples were taken daily starting 2 days prior to the fecal collection period. Fecal samples were initially dried for 72 hours in a forced air oven at 60°C. Laboratory dry matter (DM) was determined in a forced air oven at 100°C for 24 hours. Feed and dried fecal samples were Wiley mill ground (1mm screen) and analyzed for crude protein (CP) (10), acid detergent fiber (ADF) and neutral detergent fiber (NDF) (241), and acid insoluble ash (AIA) (239). Acid insoluble ash was used as an internal marker to determine apparent nutrient digestibility.

On day 4, ruminal liquid volume (LV) was determined at 0, 3, 6, and 9 hours postfeeding (0400 h) using the method of Alexander et al. (3) except 500 ml of chromium ethylenediaminetetraacetic acid solution (Cr-EDTA) (29) was used as the water soluble marker instead of polyethylene glycol. A 300 ml sample of mixed rumen digesta was taken at each time period. A 50 ml aliquot was used to determine DM by drying for 72 hours in a forced air oven at 60°C. A 200 ml aliquot was strained through four layers of cheesecloth and pH measured on the filtrate using a combination electrode. A portion of the filtrate was acidified by placing 9 parts filtrate with 1 part 6N HCL. The mixture was then

clarified by centrifugation at 25,000 xg for 20 minutes and analyzed for volatile fatty acids (VFA) using gas chromatography using a 5% PNGS and 1% H_3PO_4 on Anakrour A90/100 column[2] at 115°C and ammonia nitrogen (NH_3N) using specific ion electrode. Another portion of the filtrate was centrifuged at 25,000 x g for 20 minutes and analyzed for chromium using a modification of the method of Fenton and Fenton (89). Ten ml aliquots of clarified ruminal fluid was placed in a 25 ml volumetric flask and evaporated in a forced air oven at 100°C. Chromium standards were prepared similarly and contained 10, 5, 2.5, 1.25, 0.625, and 0.3125 mg chromium/dl. Chromium content of the residue was determined by digestion with 5ml of the solution described by Fenton and Fenton (89) measuring percent transmittance at a wavelength of 430 nm with a spectrophotometer. The difference in chromium content of the ruminal fluid before and after the addition of 500 ml of Cr-EDTA into the rumen was used to determine liquid volume (LV).

On day 5, a pulse dose of 1000 ml of Cr-EDTA solution (29) was hand mixed with rumen digesta immediately prior to the 0400 hour feeding. Samples of rumen digesta were collected at 0, 1, 2, 3, 4, 6, 8, 10, 12, 16, and 24 hours postdosing and analyzed for chromium as above. Slope of the natural log of chromium content versus time was defined as the ruminal liquid dilution rate (LDR).

On days 6-7, 200g of chromium mordanted dietary fiber (99) was mixed with the 0400 hour ration. Rumen digesta samples were collected at 0, 2, 4, 6, 8, 10, 12, 16, 20, 24, 32, 40, and 48 hours postfeeding

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[2] Analabs GCM-109. Unit of Foxboro Analytical, 80 Republic Dr., North Haven, CT.

and dried in a forced air oven for 72 hours at 60°C. Samples were Wiley mill ground (1mm screen) and analyzed for chromium (89). Ruminal particulate rate of passage (PROP) was defined as the slope of the natural log of the chromium content versus time.

On day 8, samples of mixed rumen digesta were collected at 3 hours after the 0400 hour feeding and analyzed for total viable bacteria (VB) and cellulolytic bacteria (CB) using habitat stimulating media in anaerobic roll tubes described by Hungate (118).

Data were analyzed using a model for the Latin square design. The model included dietary treatments, cows and time periods. When main effect means were significant, treatment means were separated using Duncan's new multiple range test (217).

Results and Discussion

Ruminal digesta flow kinetics are presented in Table 3. Ruminal LV, LDR, PROP, and liquid outflow rate were not affected by treatment. However, LDR and PROP tended to be reduced when the fungal culture was present in the supplement. There is not enough information in the present study to explain the reduced rate of digesta flow from the rumen. Metabolic by-products from AO may have affected the rate and amplitude of rumen contraction. Lemenager et al. (147) reported reduction in ruminal digesta flow that could not be explained by reduced feed intake when steers were supplemented with monensin, a fermentation by-product. Histamine produced in the rumen by Lactobacillus sp. in cattle suffering from lactic acidosis decreased the rate and amplitude of ruminal contractions (189).

No differences were measured in ruminal pH, VFA, or NH_3-N (Table 4). However, acetate:propionate ratio (Ac/Pr), percent branch chain

VFA, and NH_3-N pool tended to increase with all fungal treatments. Milk butterfat depression in dairy cattle is associated with a reduction in Ac/Pr primarily due to an increase in propionate production (25). Although production of acetate and propionate were not measured quantitatively in the present study, Ac/Pr calculated from total rumen pools indicate that fungal treatments, especially those containing AO, tended to either increase acetate production, decrease propionate production, or both. A decrease in propionate production would aid in correcting milk butterfat depression (156). Increased milkfat percent and production have been measured when high-concentrate dairy cow diets have been supplemented with AO (113, 174).

Increased ruminal pH has been associated with increase solubility and degradability of dietary protein (251). Ruminal NH_3-N pool and pH tended to increase in cattle supplemented with AO. The increased pH is surprising since rumen LDR tended to decrease (87) with the addition of the AO and AO/YC treatments. Metabolites of AO may have slightly stimulated salivation, adding buffers to the rumen, while another metabolite may have decreased the rate and amplitude of ruminal contractions. Alterations in rumen motility have been measured with aflatoxin administration (57) and fungal metabolites have been associated with increased salivation (124). The tendency toward increased branched chain VFA would be asssociated with increased dietary protein solubility and degradability as they are produced from the deamination and decarboxylation of branched chain amino acids (6).

Effect of the fungal treatments on rumen bacteria are presented in Table 5. Fungal treatments tended to increase viable bacteria numbers. Cellulolytic bacteria numbers were increased by nearly 40% by fungal

treatments (P<.05). The percent of bacteria that were cellulolytic tended to increase by YC treatment, while treatments that contained AO increased percent cellulolytic bacteria by 27% (P<.05). Since YC is not cellulolytic, the yeast may have provided stimulatory factors for cellulolytic bacteria, such as B-vitamins or branched chain VFA (46). Only 2.63 g of AO supplement was fed per day, therefore stimulatory factors probably would not explain the increased number and proportion of cellulolytic bacteria. Cellulolysis by Aspergillus oryzae is a more probable explanation. The AO/YC treatment may have provided both stimulating factors and cellulolytic organisms. The AO/YC was also fortified with vitamins and minerals (Table 2) that may have stimulated cellulolytic bacteria (46).

Nutrient digestibility data is presented in Table 6. Dry matter digestibility tended to increase by YC treatment and was increased by 4.2% with supplements containing AO (P<.05). Digestibility of crude protein was increased (P<.01) by all fungal treatments, with the AO/YC treatment resulting in the largest increase. The YC treatment probably provided factors stimulatory toward proteolytic bacteria while the AO treatment provided Aspergillus oryzae that have been shown to be actively proteolytic (34). The AO/YC treatment provided both factors. The digestibility of ADF appeared to increase by fungal treatments but hemicellulose digestibility was increased 6.5% with the fungal treatments (P<.05). Increased digestibility of structural carbohydrates was observed concurrently with an increased number and proportion of cellulolytic organism measured (Table 5) and increased ruminal retention time (Table 3).

The addition of viable fungal supplements containing AO and YC to

the diets of cattle fed high-concentrate diets is a means of increasing the digestibility of the structural carbohydrate portion of the diet. The combination of YC, which probably provided stimulatory factors for rumen bacteria and AO, which produces cellulase enzyme was most beneficial. Although AO does not produce the enzymatic machinery to completely depolymerize structural carbohydrates to simple sugars, it does produce enzymes that will cause partial depolymerization (34). This aids rumen cellulolytic bacteria in complete depolymerization of cellulosic material to simple sugars (12). Further research on dose response at different forage:concentrate ratios and characterization of metabolites of AO are needed to elucidate mode of action.

TABLE IV.1 Composition of diets.

Item	Diets (dry matter basis)				
	IFN	Basal	Basal + YC[1]	Basal + AO/YC[2]	Basal + AO[3]
	%				
Chopped alfalfa hay	1-00-063	33.38	33.38	33.38	33.38
Rolled barley	4-07-939	25.87	24.88	24.88	24.88
Beet pulp w/molasses	4-00-672	17.00	17.00	17.00	17.00
Chopped barley straw	1-00-498	15.00	15.00	15.00	15.00
Wheat bran	4-05-190	8.50	8.50	8.50	8.50
TM-salt		0.25	0.25	0.25	0.25
YC	7-05-520	-	0.99	-	-
FC/YC		-	-	0.99	-
AO	5-06-152	-	-	-	0.03

[1] Yeast culture, Diamond V Mills, Inc., Cedar Rapids, IA.

[2] Vitaferm (Amaferm plus yeast culture, vitamins/minerals). BioZyme Enterprises, Inc., St. Joseph, MO.

[3] Amaferm (Aspergillus oryzae fermentation extract). BioZyme Enterprises, Inc., St. Joseph, MO.

TABLE IV.2 Estimated nutrient content of diets.

Item	Diets (dry matter basis)			
	Basal	Basal + YC[1]	Basal + AO/YC[2]	Basal + AO[3]
Metabolizable energy, Mcal/Kg	2.41	2.41	2.41	2.41
Crude protein, %	13.32	13.30	13.32	13.32
Neutral detergent fiber, %	42.75	42.66	42.68	42.75
Acid detergent fiber, %	25.03	25.01	25.04	25.03
Calcium, %	0.64	0.64	0.69	0.64
Magnesium, %	0.26	0.26	0.29	0.26
Phosphorus, %	0.32	0.32	0.35	0.32
Potassium, %	1.75	1.75	1.80	1.75
Sulfur, %	0.26	0.26	0.27	0.26
Cobalt, mg/kg	0.33	0.33	0.55	0.33
Copper, mg/kg	12.41	12.38	14.41	12.41
Manganese, mg/kg	36.77	36.65	40.91	36.80
Zinc, mg/kg	33.61	33.60	49.82	33.64

[1] Yeast Culture, Diamond V Mills, Inc., Cedar Rapids, IA.

[2] Vitaferm (Amaferm plus yeast culture, vitamins/minerals). BioZyme Enterprises, Inc., St. Joseph, MO.

[3] Amaferm (Patent No. 3043748). BioZyme Enterprises, Inc., St. Joseph, MO.

TABLE IV.3 Effect of treatments on ruminal digesta flow kinetics.

Item	Diets				SE
	Basal	Basal + YC[1]	Basal + AO/YC[2]	Basal + AO[3]	
Liquid volume, L	50.92	51.31	52.37	52.60	0.55
Liquid dilution Rate, %/hr	8.90	9.52	6.67	7.31	0.67
Liquid outflow Rate, L/hr	4.53	4.88	3.49	3.85	0.35
Particulate rate of passage, %/hr	4.52	5.11	3.39	3.62	0.39

[1] Yeast culture, Diamond V Mills, Inc., Cedar Rapids, IA.

[2] Vitaferm (Amaferm plus yeast culture, vitamins/minerals), BioZyme Enterprises, Inc., St. Joseph, MO.

[3] Amaferm (Aspergillus oryzae fermentation extract), BioZyme Enterprises, Inc., St. Joseph, MO.

TABLE IV.4 Effect of treatments on ruminal fermentation characteristics.

Item	Diets				SE
	Basal	Basal + YC[1]	Basal + AO/YC[2]	Basal + AO[3]	
pH	6.34	6.34	6.38	6.44	0.04
Total VFA, mM/L	64.60	66.40	63.40	67.90	3.00
VFA pool[4], moles	3.37	3.42	3.27	3.59	0.13
Acetate, molar %	69.30	68.80	69.50	69.30	0.68
Acetate pool, moles	2.35	2.39	2.27	2.49	0.10
Propionate, molar %	14.00	13.80	13.40	13.10	0.31
Propionate pool, moles	0.47	0.46	0.44	0.47	0.02
Butyrate, molar %	12.40	13.00	12.70	13.30	0.33
Butyrate pool, moles	0.41	0.43	0.41	0.48	0.02
Acetate:propionate, ratio[5]	4.96	5.08	5.20	5.30	0.15
Branched chain VFA[6] pool, moles	0.13	0.14	0.14	0.15	0.01
% Branched chain VFA	4.25	4.46	4.43	4.31	0.27
NH_3-N, mg/dl	18.21	18.72	19.32	19.27	0.35
NH_3-N pool, g	9.27	9.61	10.19	10.14	0.21

[1] Yeast culture, Diamond V Mills, Inc., Cedar Rapids, IA.

[2] Vitaferm (Amaferm plus yeast culture, vitamins/minerals), BioZyme Enterprises, Inc., St. Joseph, MO.

[3] Amaferm (Aspergillus oryzae fermentation extract), BioZyme Enterprises, Inc., St. Joseph, MO.

[4] Concentration x rumen volume

[5] Acetate pool / Propionate pool

[6] Isobutyrate + Isovalerate + Valerate

TABLE IV.5 Effect of treatments on ruminal bacteria.

Item	Diets				SE
	Basal	Basal + YC[a]	Basal + AO/YC[b]	Basal + AO[c]	
Total viable bacteria, X 10^8/ml	196.2	255.0	257.3	223.5	16.5
Cellulolytic bacteria, X 10^8/ml	25.0[d]	39.8[e]	45.6[e]	39.1[e]	3.8
% Cellulolytic bacteria	12.9[d]	15.4[de]	18.0[e]	17.5[e]	0.9

[a] Yeast culture, Diamond V Mills, Inc., Cedar Rapids, IA.

[b] Vitaferm (Amaferm plus yeast culture, vitamins/minerals), BioZyme Enterprises, Inc., St. Joseph, MO.

[c] Amaferm (*Aspergillus oryzae* fermentation extract), BioZyme Enterprises, Inc., St. Joseph, MO.

[d,e] Means in the same row with different superscripts differ (P<.05).

TABLE IV.6 Effect of treatments on nutrient digestibility.

Item	Diets				SE
	Basal	Basal + YC[a]	Basal + AO/YC[b]	Basal + AO[c]	
	%				
Dry matter	77.0[d]	79.1[d]	81.0[e]	79.8[e]	0.62
Crude protein	79.5[f]	82.2[gh]	84.4[h]	81.6[g]	0.63
Acid detergent fiber	69.3	70.0	72.6	71.0	0.89
Hemicellulose	76.3[d]	80.5[e]	83.5[e]	80.8[e]	1.03

[a] Yeast culture, Diamond V Mills, Inc., Cedar Rapids, IA.

[b] Vitaferm (Amaferm plus yeast culture, vitamins/minerals), BioZyme Enterprises, Inc., St. Joseph, MO.

[c] Amaferm (Aspergillus oryzae fermentation extract), BioZyme Enterprises, Inc., St. Joseph, MO.

[d,e] Means in the same row with different superscripts differ (P<.05).

[f,g,h] Means in the same row with different superscripts differ (P<.01).

CHAPTER V

INFLUENCE OF DONOR ANIMAL ADAPTATION TO ADDED YEAST CULTURE AND/OR *ASPERGILLUS ORYZAE* FERMENTATION EXTRACT[1] ON *IN VITRO* RUMEN FERMENTATION[2]

Introduction

In recent years researchers have used fungal cellulase preparations to increase dry matter (63, 246), fiber (12, 246), and protein (246) digestion in ruminants. However, Leatherwood et al. (145) indicated that fungal cellulase addition had no effect on feed utilization. Tegbe and Zimmerman (227) demonstrated that incorporation of yeast single cell protein into diets of growing pigs improved nitrogen digestibility and retention. However, Johnson and Remillard (129) indicated that the addition of brewers yeast decreased overall diet dry matter, energy and carbohydrate digestibility. Results from in vitro experiments have also varied. Daniels and Hashim (63) demonstrated that in vitro dry matter digestibility (IVDMD) was highest with addition of 375 mg of a cellulase preparation per kilogram of substrate. IVDMD decreased when more than 375 mg/kg was added, perhaps due to metabolite accumulation. Michalowski (160) also attributed the failure to increase IVDMD to metabolite accumulation.

This study determined whether rumen fluid inoculum from ruminants adapted to yeast and/or fungal cultures affected in vitro fermentation.

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[1] Amaferm (Patent No. 3043748). BioZyme Enterprises, Inc., St. Joseph MO.

[2] Supported in part by grants from Diamond V Mills, Inc. and BioZyme Enterprises, Inc.

Materials and Methods

In a series of in vitro fermentation trials, a rumen-fistulated heifer (weighing approximately 590 kg) was fed each of four treatments as follows: 1) basal ration (ad libitum alfalfa hay); 2) basal ration plus 90 gm yeast culture(YC)[3]/day; 3) basal ration plus 2.63 gm Aspergillus oryzae fermentation extract (AO)[4]/day; 4) basal ration plus 90 gm AO PREMIX[5]/day. The heifer was adapted to each treatment 21 days prior to collection of rumen fluid inoculum. Rumen fluid was obtained 3.5 h after the morning feeding from just below the hay mat in the ventral sac and was strained through four layers of cheesecloth.

Alfalfa hay ground through a 1 mm mesh screen was the substrate in the in vitro fermentation experiment. Each treatment was tested on two alternate days. To 2.0 gm of substrate previously weighed into 250 ml centrifuge bottles was added 40 ml rumen fluid inoculum and 160 ml mineral buffer (233). Centrifuge bottles were flushed with CO_2, capped with Bunsen valves and incubated for 12 and 24 h at 39°C. The direct addition of YC, AO and AO PREMIX was at levels of 1.8, 0.053 and 1.8 mg/ml of the in vitro fermentation solution, respectively. At the end of 12 and 24 h, in vitro dry matter disappearance (IVDMD) was determined by centrifugation at 1,200 x g for 10 minutes followed by decantation of the supernatant and drying of residue at 60°C for 72 h. An aliquot of the supernatant from the 24 h fermentation sample was acidified by

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[3] Diamond V Miles, Inc., Cedar Rapids, IA

[4] Amaferm (Aspergillus oryzae fermentation extract), BioZyme Enterprises, Inc., St. Joseph, MO

[5] Vitaferm (Amaferm + YC + vitamins/minerals), BioZyme Enterprises, Inc., St. Joseph, MO

placing 9 parts supernatant with 1 part 6N HCE. The mixture was then clarified by centrifugation at 25,000 x g for 20 minutes and analyzed for volatile fatty acids (VFA) by gas chromatography using 5% PNFS and 1% H_3PO_4 on Anakrour A90/100 column[6] at 110°C and ammonia nitrogen (NH_3-N) using Nessler's reagent (10).

All data were analyzed by two-way analysis of variance with treatment means being tested by least significant difference if F tests were significant.

Results and Discussion

The in vitro dry matter disappearance (IVDMD) of alfalfa hay is shown in Table 1. Overall, IVDMD's were unaffected by YC and/or AO/AJ PREMIX addition. It is doubtful that lack of difference was a result of metabolite accumulation, since there was no treatment by time interaction. However, for AO treatments, donor animal adaptation appeared to slightly increase IVDMD.

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[6] Analabs GCM-109. Unit of Foxboro Analytical, 80 Republic Dr., North Haven, CT

TABLE V.1 Effect of donor animal adaptation to supplemental yeast culture and Aspergillus oryzae fermentation extract on in vitro dry matter disappearance.

Treatment	IVDMD
	%
Control	46.9
YC[a]	
Unadapted	43.9
Adapted	47.0
AO[b]	
Unadapted	46.6
Adapted	51.9
AO PREMIX[c]	
Unadapted	43.9
Adapted	51.3
SE[d]	2.1

[a] Yeast Culture. Diamond V. Mills, Inc., Cedar Rapids, IA.

[b] Amaferm (Aspergillus oryzae fermentation extract). BioZyme Enterprises, Inc. St. Joseph, MO.

[c] Vitaferm (Amaferm + YC + vitamins/minerals). BioZyme Enterprises, Inc., St. Joseph, MO.

[d] Standard error.

Donor adaptation to both yeast and AO increased ($P<.05$) NH_3-N levels (Table 2). The yeast culture probably provided factors that stimulated proteolytic bacteria. The fungal culture treatment provided Aspergillus oryzae, which has been shown to be actively proteolytic (34). Further verification of increased rumen protein degradation is provided by significant increases ($P<.05$) in branch chain volatile fatty acids. Allison and Bryant (6) observed that increased rumen branched chain volatile fatty acid (VFA) concentration was a result of deamination and decarboxylation of branched chain amino acids. The acetate:propionate (Ac/Pr) ratio tended to increase in rumen fluid collected from the donor animal adapted to the fungal culture. Similar trends were observed in vivo (246).

Results indicate that at least a 21-day adaptation to yeast and AJ fermentation extract for ruminants is necessary to properly evaluate rumen fermentation characteristics in vitro. More research is needed to confirm the validity of this hypothesis.

TABLE V.2 Effect of donor adaptation of supplemental fungal cultures on *in vitro* ammonia nitrogen and volatile fatty acid concentration.

Item	Treatment							SE[d]
	Control	YC[a]		AO[b]		AO PREMIX[c]		
		Unadapted	Adapted	Unadapted	Adapted	Unadapted	Adapted	
NH3-N (mg/dl)	18.7^{gh}	14.2^{h}	44.6^{e}	15.7^{h}	21.6^{fg}	14.7^{h}	24.8^{f}	4.2
VFA (mole/ml)	43.9^{g}	44.10^{g}	47.3^{fg}	40.1^{g}	59.6^{e}	41.3^{g}	56.3^{ef}	17.6
Acetate (Molar%)	68.4	66.8	67.9	65.4	70.3	66.6	66.9	2.2
Propionate	18.8^{ef}	19.4^{ef}	17.1^{fg}	19.6^{ef}	16.0^{g}	20.1^{e}	17.9^{efg}	.9
Isobutyrate	.8	.9	1.0	1.0	1.0	.8	.9	.01
Butyrate	8.7	9.0	9.5	9.9	8.8	8.9	10.0	.3
Isovalerate	1.2^{f}	1.4^{ef}	1.5^{e}	1.5^{ef}	1.3^{ef}	1.2^{f}	1.4^{ef}	.01
Valerate	2.2^{f}	2.6^{ef}	3.0^{e}	2.6^{ef}	2.6^{ef}	2.4^{ef}	3.0^{e}	.1
Acetate/Propionate	3.6^{f}	3.4^{f}	4.0^{ef}	3.3^{f}	4.4^{e}	3.3^{f}	3.7^{f}	.1

[a] Yeast Culture. Diamond V. Mills, Inc., Cedar Rapids, IA.

[b] Amaferm (*Aspergillus oryzae* fermentation extract). BioZyme Enterprises, Inc., St. Joseph. MO.

[c] Vitaferm (Amaferm + YC + vitamins/minerals). BioZyme Enterprises, Inc., St. Joseph, MO.

[d] Standard error.

[e,f,g,h] Means in the same row with different superscripts differ ($P<.05$).

CONCLUSIONS

Infusion of mineral salts into the rumen of cattle fed high-cereal grain diets either exogenously by addition to the diet or endogenously by increasing saliva flow are both affective methods of stabilizing fermentation and increasing nutrient utilization. The mode of action is the increased hypertonicity within the rumen resulting in an increased flow of digesta and therefore acidic metabolites out of the rumen. This allows a more optimal environment for cellulolytic bacteria and thus increased dietary fiber digestion. The addition of these material to the diet seems wasteful in light of the fact that the cow is perfectly capable of producing copious amounts through saliva production. Saliva production is greatly reduced when high-cereal grain diets are fed. This study showed that this reduction in saliva flow can be successfully corrected with the use of agents capable of stimulating saliva flow. Additionally, this study showed that these agents have no harmful physiological effects at the dosages used.

Although intra-ruminal fungal inoculation did not effect rumen fermentation characteristic, nutrient digestion was increased. The addition of Saccharomyces cerevisiae probably added growth factors for rumen cellulolytic bacteria since this fungus is not known to be cellulolytic. The Aspergillus oryzea inoculant was probably actively cellulolytic under the rumen conditions produced by the high-cereal grain diets. These conditions are inhibitory for most inherant ruminal cellulolytic species of bacteria. Thus, these organisms may have filled a niche voided by other species. The facts that such a minute amount of Aspergillus oryzea was inoculated and the effects of a combination of Aspergillus oryzea and Saccharomyces cerevisiae were additive suggests

different modes of action of the two species.

Both methods of rumen manipulation used in this study, addition of mineral salts and fungal inoculants, are viable method of improving nutrient utilization in cattle fed high-cereal grain diets. The practical application these findings is yet to be explored.

IDEAS FOR FURTHER RESEARCH

1. The study with agents to increase the saliva flow of cattle is quite unique. The feeding of these agent requires large dosages and contamination of other feed would be a constant concern. An economical, efficient vehicle of subcutaneous administration at a constant rate would be an advantage. Further research is needed in this area before practical applications can be realized.
2. These studies were done on non-productive animals. The ultimate goal of the research would of course be to increase productive efficiency in livestock. These agents should be tested on cattle either producing meat or milk.
3. There are other agents besides the ones used in this study that are capable of stimulating saliva flow in cattle. The efficacy of these agents should be tested.
4. There are probably thousand of fungal species that could be introduced into the rumen cattle that could aid in nutrient digestion, especially dietary fiber digestion. This area definitely deserves more research. Composite organism using new techniques in bio-technology may prove to be the ultimate new frontier in ruminant nutrition.

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Erick - son
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Background

My younger years were spent on a dairy farm in northern Utah and beef cattle-cereal grain farms in northern Utah and central Montana where I was active in the daily chores involved with those enterprises. High school years were in western Montana where I was active in sports, Future Farmers of America and church activities. Undergraduate years were spent at Western Montana College and Montana State University where I was active in sports, biological science curriculum and church activities. Graduate study years were at Washington State University where I studied the utilization of cellulosic wastes in beef cattle and at Utah State University where I studied the manipulation of rumen fermentation to increase the efficiency of nutrient utilization in dairy cattle.

Education

Utah State University, Logan, Utah, Ph.D., expected completion date, August 1986, Animal Science - Nutrition.

Washington State University, Pullman, Washington, M.S., December 1982, Animal Science - Nutrition.

Montana State University, Bozeman, Montana, B.S., March 1975, Zoology-Physiology.

Major Research Interests

Animal Nutrition, Nutrition-Health, Utilization of cellulosic materials, Rumen manipulation, Rumen microbiology.

Ph.D. research thesis - Manipulation of rumen fermentation by altering rumen fluid dilution rate and by addition of microbial innoculants.

M.S. research thesis - Effects of level of crude protein on the digestibility of wheat straw in beef cattle.

Academic Honors

Recipient of Dean's Summer Fellowship Award, 1984.

Recipient of Scholastic Tuition Waiver, 1983, 1984 and 1985.

Society Affiliations

American Society of Animal Science, 1979-Present.

American Society of Dairy Science, 1983-Present.

Religious Activities

Member of Church of Jesus Christ of Latter-Day Saints

Seminary president, 1967
Institute vice president, 1967-1969
Youth Advisor-Teacher, 1971-present
Sunday school presidency, 1975-1978
Elder's quorum presidency, 1979-1981

Teaching and Related Experience

Undergraduate:
Endocrine Physiology-Assistant Lab Instructor, 1975 (1 quarter)
Graduate:
Principles of Artificial Insemination-Lab Instructor, 1976 (1 quarter).
Beef Cattle Management-Teaching Assistant, 1979-1980 (2 semesters).
Dairy Production Practices-Teaching Assistant, 1982 (1 quarter).
Feeds and Feeding-Teaching Assistant, 1983 (1 quarter).
Beef Cattle Management-Instructor, 1984-1985 (2 quarters).
Animal Nutrition-Teaching Assistant, 1985 (1 quarter).
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Ruminant Nutritional Physiology-Teaching Assistant, 1985 (1 quarter).

Scientific Publications

Abstracts:

Wiedmeier, R.D. and J.R. Males. 1980. Effect of dietary crude protein on the dry matter digestibility of wheat straw rations in cattle. J. Animal Sci. 1980 meeting abstract 410.

Wiedmeier, R.D. and M.J. Arambel. 1985. Manipulation of rumen fermentation using mineral salts. J. Dairy Sci. 68 (Supplement 1):50,131.

Arambel, M.J., D.H. Clark, R.D. Wiedmeier, R.C. Lamb, J.L. Walters and R. L. Boman. 1985. Evaluation of sodium bicarbonate and magnesium oxide in a total mixed ration fed to lactation dairy cattle. J. Dairy Sci. (Supplement 1):18, 117-118.

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Wiedmeier, R.D. and J.R. Males. 1983. Effect of dietary crude protein on the dry matter digestibility of wheat straw diets in cattle. J. Animal Sci. 57:1568.

Wiedmeier, R.D., D.H. Clark, M.J. Arambel and R.C. Lamb. 1985. Esophageal tube vs rumen fistula sampling on the ruminal pH, volatile fatty acids and ammonia nitrogen. Nutr. Rpt. Intl. (In press).

Manuscript Form:

Wiedmeier, R.D., M.J. Arambel, R.D. White and R.C. Lamb. 1985. Effect of mineral salts, pilocarpine and carbachol on rumen fermentation and digesta kinetics.

Wiedmeier, R.D., M.J. Arambel, R.D. White and R.C. Lamb. 1985. Effect of orally administered pilocarpine on rumen fermentation and digesta kinetics in cattle on liver, kidney and parotid glands of the meadow voles.

Wiedmeier, R.D. and M.J. Arambel. 1985. Effects of graded amounts of processed poultry exreta on rumen fermentation and digesta kinetics in cattle.

Wiedmeier, R.D., M.J. Arambel and R.C. Lamb. 1985. Millipore membrane filter technique vs diaminopimelic acid or riboneucleic acid for determining microbial mass production in rumen digesta.

Popular Publications:

Arambel, M.J. and R.D. Wiedmeier. 1986. Jan. U.S.U. Nutrition Research Update. Utah Dairy Newsletter, Vol. 9, No. 1.

Males, J.R., R.H. Pritchard and R.D. Wiedmeier. 1980. Fiber sources for beef in the Pacific Northwest. Pacific Northwest Nutrition Conference.

Males, J.R. and R.D. Wiedmeier. 1980. Protein Requirements of beef cows. Proceedings of Beef Information Day, Washington State University.