HINDGUT FERMENTATION IN RUMINATING HOLSTEIN CALVES

by

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(ABSTRACT)

The effects of quantity of dietary starch and type of dietary protein on hindgut fermentation were evaluated. Thirty-two Holstein bull calves were fed diets containing variable amounts of orchardgrass hay and a grain mixture. The amount of starch and types of protein were: [L1] low starch, soybean meal (SBM); [L2] low starch, fishmeal plus dried brewers' grains (FBG); [H1] high starch and SBM; [H2] high starch, FBG. The percentages of acid detergent fiber (ADF) and crude protein were: [L1] 19.2%, 15.1%; [L2] 18.0%, 15.6%; [H1] 9.5%, 14.9%; [H2] 9.6%, 15.4%. After calves were fed the diets for 17 days, they were slaughtered to obtain their intestinal tracts. Ileal, cecal, and colonic digesta and feces of calves fed H1 and H2 versus L1 and L2 contained less water and ADF. Concentration of nitrogen in digesta and feces did not differ. Ileal, cecal, and colonic digesta from calves fed H1 and H2 had significantly greater numbers of viable anaerobic bacteria and lower pH. Cecal digesta from calves fed high fiber diets (L1 and L2) had lower total VFA, propionate, and buytrate concentrations than calves fed high starch diets. Colonic and cecal digesta of calves fed diets H1 and H2 contained less ammonia. Acetate and propionate flux across cecal epithelium in vitro was faster for diets H1 and H2. Results indicate that high dietary starch stimulated

anaerobic bacterial growth and fermentation in the hindgut, and enhanced acetate and propionate flux across the cecal epithelium. Acetate and propionate transport across the cecal wall probably is not due solely to passive diffusion, but it may involve a carrier. Replacement of SBM by FBG also altered cecal fermentation to a lesser extent. Calves fed H2 had significantly greater numbers of viable anaerobic bacteria in cecal and ileal digesta and 2 to 10 times the number of bacteria associated with cecal epithelium than calves fed the other diets. Butyrate cecal concentration and production was significantly increased when calves were fed diets containing FBG. Cecal VFA production may account for approximately 3 to 5% of digestible energy intake.

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TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

INTRODUCTION

The entire ruminant gastrointestinal tract has a substantial microbial population. Attention has been focused mainly upon the rumen which comprises approximately 75% of the total tract volume. Study of rumen function has demonstrated the importance of microbial degradation of feedstuffs for ruminants. Recently, studies concerning function of the lower tract indicated that ruminants may benefit from large intestinal fermentation. Volatile fatty acid (VFA) production in the ovine large intestine accounts for 8 to 17% of the total amount of acids produced. Approximately 4.2 to 26% of the digestible energy disappearance in the digestive tract occurs in the hindgut (75).

The significance of hindgut fermentation depends upon the ability of microoganisms to degrade substrates which enter the lower tract and availability of their endproducts for the host. Hindgut fermentation may be especially important when considering the fate of protein and carbohydrate which escape ruminal degradation. Most studies concerning fermentation in the lower tract have used sheep, and few have used cattle. Lower tract fermentation may be especially significant for dairy cattle because of the large amounts of concentrates fed in early lactation and use of less degradable proteins. The objectives of this study were:

1. To characterize the nature of fermentation in the hindgut.

2. To determine the extent to which starch and protein which escape degradation in the rumen are utilized by microorganisms of the hindgut.

REVIEW OF LITERATURE

IMPORTANCE OF MICROBIAL FERMENTATION

Most mammals have microorganisms in their digestive tracts and derive some nutritional benefit from the microbial fermentation. The capacity of the gut and diet composition determines importance of microbial degradation of feedstuffs for mammals.

Mammals may be classified based upon their gastrointestinal anatomy as either pre or post-gastric fermentors. Pre-gastric fermentors have an enlarged foregut which is the site of microbial activity such that fermentation occurs before enzymatic digestion. Diverse species including ruminants as well as nonruminants such as colobine monkeys, hamsters, and voles are classified as pre-gastric fermentors. Two groups of hindgut fermentors exists: cecal digestors (rodents) and colonic digestors. Colonic digestors can be subclassified according to the extent of sacculation of their colon. Carnivores such as dogs and cats have unsacculated colons whereas the horse, man and the pig have sacculated colons (54, 77). Hindgut fermentors outnumber ruminants; there are a greater number of insect and rodent genera and a greater diversity of digestive anatomy among the latter than among foregut fermentors (42).

For ruminants most fermentation occurs in the rumen, but cecal and colonic fermentation may also be important. Conversely, fermentation also occurs in the stomach of horses, hamsters, and rabbits (54).

The proportion of digesta residing in fermentative compartments of the gut indicates the relative importance of microbial degradation of feedstuffs. Ruminants have a large portion of their digestive tracts devoted to microbial fermentation. The fermentative capacity of cattle is 75% of the total digestive tract. Of that, the cecum constitutes 5% and the colon 5-8%. With increasing body size, the relative capacity of the digestive tract increases as a percent of body weight. Small herbivores have a unique problem; they have increased energy requirements and decreased fermentation contents per unit weight. Rats and rabbits, cecal fermentors, have a greater proportion of their gut adapted to fermentation than cattle, but actually digest fiber less well because of their small size. The fermentative capacity of the digestive tract of omnivores and carnivores is smaller than for herbivores (54, 77).

MICROORGANISMS AND THEIR HABITATS

Microorganisms are present throughout the gastro-intestinal tract of most animals, including mammals, reptiles, birds and insects. The microbes in the digestive tract are either autochthonous (indigenous) or allochthonous (temporary residents). Autochthonous organisms may reside in several different habitats in a portion of the gut: intestinal lumen; intestinal epithelium; mucosal crypts. Diet may regulate growth and locale of microbes. Within any given habitat, autochthonous organisms have a niche;they prefer certain substrates and release certain endproducts. Allochthonous microorganisms may be present in the intestines, and cause

detrimental effects only if they proliferate as a result of a physiological disturbance (64).

Indigenous microorganisms possess certain attributes:they are anaerobes; they are present in the tracts of normal adults; they inhabit particular areas of the intestinal tract; they colonize their gut region during population sucession in young animals; when climax communities are established indigenous microbes maintain stable population levels; indigenous microorganisms may also be found intimately associated with the intestinal epithelium in their particular habitat (64).

The microbes in the gut interact with their hosts in many ways. They may benefit their host by digesting polymers such that their host may utilize the endproducts. They may synthesize vitamins which may be available to the host. Also, microorganisms are involved in nitrogen recycling (42). Indigenous microbes may help their host to resist infections but they may be implicated in the etiology of some diseases including cancer (64).

MICROBIOLOGY OF THE HINDGUT

ENVIRONMENT OF THE LOWER TRACT

Environmental conditions, such as temperature, degree of anaerobiosis (Eh), and pH remain fairly constant in the lower tract. The eH of the cecum (54) and colon (1) seems comparable to the rumen. Cecal and colonic bacteria have less tolerance for oxygen than microorganisms in the small intestine and stomach (39). Cecal and colonic pH often is close to neu-

trality but like the rumen, diet affects the pH. For example, when sucrose was infused post-ruminally in sheep, pH of both rumen and cecum dropped (77). Regulation of hindgut pH depends upon absorption of volatile fatty acids (VFA) and diffusion of Na into the lumen. Release of ammonium bicarbonate from microbial ureolysis also helps to buffer the intestinal contents (77).

Microorganisms in the lower gut have a constant food supply, which is less dependent upon dietary pulses than in the rumen (77). For ruminants, nutrients available for lower tract fermentation include any carbohydrate which has escaped ruminal degradation and enzymatic digestion such as starch, cellulose, hemicellulose and oligosaccharides. Nonruminant herbivores digest soluble components of the diet in the foregut, and cecal microorganisms degrade mostly dietary fiber. Other substrates which cecal and intestinal organisms utilize include mucopolysaccharides, endogenous secretions such as bile and pancreatic juices, and celluar debris (54, 77).

NUMBERS AND TYPES OF MICROORGANISMS

Many factors affect the numbers and types of microorganisms which populate the intestinal tract. Giesecke (23) noted that diet, feeding behavior and the animal's physiological state affect ruminal microbial densities. Because of the environment of each particular intestinal habitat, numbers and types of organisms vary from one locale to another. Also, individuals of the same species will have differing microbial populations.

Numbers of viable anaerobic bacteria in the ruminant cecum and colon appear comparable to numbers in the rumen. Cecal contents from sheep fed dried grass pellets contained 2 x 10^7 to 1 x 10^9 bacterial cells/ml (75). In another study in which sheep were fed hay and grain, viable anaerobic bacterial counts ranged from 4.3 x 10^9 to 5.2 x 10^{10}/g of cecal contents. The number of small intestinal bacteria was much lower; viable anaerobic counts ranged from 5 x 10^4 to 7 x 10^6/g (49). The ruminant hindgut may contain a substantial population of cellulolytic bacteria. Sheep fed dried grass pellets, lucerne pellets and fresh pasture had 10^8 cells/g of cecal contents (75).

In addition to a luminal bacterial population, other microorganisms may be associated with the epithelium. The bacteria associated with the intestinal tissue seem more aerotolerant than those in the intestinal contents. Nicoletti et al. (49) cultured intestinal epithelial tissue both anaerobically and aerobically. Under anaerobic conditions, the counts ranged from 2 x 10^2 to 4 x 10^4 cells/g. When cultured aerobically, numbers of viable organisms were 5 x 10 to 6 x 10^4 cells/g. The authors suggested that a higher oxygen tension in the intestinal tissue because of adjacent capillary beds favors a more aerotolerant population.

Numbers of bacteria in the alimentary tract differ between ruminant and nonruminant herbivores. Kern et al. (37) studied the microflora of ponies and steers fed timothy hay. The ponies had a large population of anaerobic bacteria in their stomachs. Contents cultured from the fundic region had 2 x 10^8 cells/g. Abomasal contents obtained from the steers contained 200 fold fewer bacteria. Numbers of viable anaerobic bacteria also were greater in the equine cecum and colon. Cellulolytic bacteria

were present throughout the intestinal tract of both ponies and steers, but counts were very low in most gut regions. Ponies had higher numbers in cecal and colonic contents: 4.3×10^6 and 7×10^6/g versus 6×10^6 and 6.5×10^5/g in steers. Davies (14) reported that the number of cellulolytics ranged from 7×10^2 to 1×10^6/g.

Many studies have focused on large intestinal fermentation of dietary fiber in pigs and rats to learn more about the role of dietary fiber in several diseases. Ominvores have large numbers of microorganisms in their lower tract. Allison et al. (1) anaerobically cultured porcine cecal contents and tissue on rumen fluid based media. Viable anaerobic colony counts were $2.37 \times 10^{10} \pm 10^{10}$/g of wet contents and $2.67 \times 10^7 \pm .81/cm^2$ of tissue. The authors also examined aerobic colony counts on three different media. A higher proportion of the cecal wall populations grew aerobically on two out of three media used, demonstrating a greater aerotolerance. The number of cellulolytics in the porcine cecum is approximately 10^8/g dry weight (1, 78). Amount of dietary fiber affects the number of cellulolytic organisms and cellulase activity (78). Rats too have a large microbial population, especially in their cecum, indicating capability to digest fiber. Macy et al. (44) cultured cecal contents from rats. Viable anaerobic bacterial counts averaged 72×10^8/g of wet contents and number of cellulolytics was 6% of the viable count.

Attempts have been made to identify intestinal microorganisms from many species, but it would be impossible to identify each species present. McBee (42) stated that most of the species make up less than 1% of the culturable population, and probably not more than 10 species are responsible for each contributing more than 1% of the population. The dominant

microflora maintain fairly constant levels in the population and probably only drastic changes of diet will causes major changes in the microflora.

No unique hindgut microflora exists (42). Microorganisms in the lower tract have many similarities as well as differences with rumen species. The most commonly isolated organisms from the ovine cecum and colon are gram negative rods of the genera Bacteroides, Fusobacterium, and Butyrivibrio. Other organisms isolated include streptococci, peptostreptococci, selenomonads, and micrococci (75). In a recent study, Nicoletti et al. (49) isolated bacteria from the small intestine and cecum of sheep. They found only four types of organisms in cecal contents, two of which were starch utilizers and the predominant bacteria isolated from small intestinal contents. The authors were suprised by the lack of species diversity, but noted that under direct microscopic examination of digesta contents there were many more species than were isolated.

Kern et al. (37) morphologically classifed the bacteria seen in the intestinal contents of ponies and steers. Ponies had much greater numbers of gram negative rods in their cecum and colon than did steers. They also observed spirochetes in the ponies terminal colon and none in the steers. Davies (14) stated that the equine cecum contained a greater morphological diversity than did the colon. She isolated three cellulolytic bacteria from the cecum and colon of each animal. Two of the three isolates, gram-negative rods, resembled rumen organisms of the genera Bacteroides. The third had similarities to a bacterium of the genus Bacillus found in the intestines of other herbivores. In another study (13) using a fluorescent antibody technique, Davies demonstrated that rabbits, pigs,

guinea pigs, cattle, sheep and horses had antigentically related cellulolytic bacteria in their intestines.

Robinson et al. (59) characterized the normal cecal flora of pigs. They obtained 192 isolates, only five of which did not correspond to any already identified species. Seventy-eight percent of the isolates were gram-negative organisms and 22.5% were gram positive non-sporing rods. Half of the isolates were identified as Bacteroides rumincola and Selenomonas ruminantium. Other species isolated from the cecal contents of each pig were Lactobacillus acidophilus, Butyrivibrio fibrisolvens, Bacteroides uniformis, and Eubacterium aerofaciens. The cecal and colonic populations differed. A higher proportion of colonic bacteria were gram postive. A greater number of cecal organisms were strict anaerobes.

The population in rat small intestine and cecum was characterized by Macy et al. (44). Lactobacillus spp. and Bifidobacterium spp. were the predominant organisms of the small intestine. Cellulolytic species represented a minor portion of the population. They identified the most predominant organisms in the cecum as species belonging to the following genera: Lactobacillus, Eubacterium, Veillonella, and Bacteroides. Cellulolytic organisms accounted for 6.3% of the total number of cecal isolates, with B. succinogenes the most predominant. Ruminococcus flavefaciens was also a common isolate. In another study (47), the authors showed that the strain of B. succinogenes isolated had different vitamin requirements than the strain isolated from the rumen, indicating that rat cecal organisms are a distinct subgroup.

While bacteria commonly inhabit all parts of the alimentary tract, other microorganisms such as protozoa are not as ubiquitous. Ciliated protozoa live in the large intestines of many nonruminant herbivores such as horses, elephants, rhinoceroses, tapirs, guinea pigs, chimpanzees and gorillas (54). Kern et al. (37) reported that the number of ciliates in the equine cecum (567/g) was much lower than in the bovine rumen (10,203 /g). The protozoal species also differed between the rumen and the cecum. The authors found no protozoa in the cecal contents from steers. In other studies, the number of ciliates in the eqiune cecum was more comparable to that in the rumen, about 10^5 to 10^6/ml (51). Flagellate protozoa have been found in ceca of sheep, cattle, and pigs (35).

DIGESTION AND ABSORPTION IN THE HINDGUT

The benefits of hindgut fermentation for all species depends upon efficiency of microbial degradation and availability of fermentation endproducts, VFA and microbial protein, to the host. If cecal and colonic microorganisms utilize little of the substrates which reach the lower gut, then the host must consume more feed to obtain enough nutrients. Animals must be able to absorb VFA and nitrogen in order to gain any beneits from hindgut fermentation.

CARBOHYDRATE METABOLISM

Ruminants and nonruminants digest soluble sugars and starches equally well. For ruminants, the amount of soluble carbohydrate reaching the

hindgut depends upon diet and intake. Also source of starch determines how much starch will reach the cecum; when barley is fed very little reaches the hindgut, but a large proportion of the starch in uncooked maize does (30).

Approximately 70-100% of starch reaching the large intestine is digested (30). Degregorio et al. (16) fed lambs 3 diets containing 0, 40 or 80% corn. As starch intake increased, amount recovered at the ileum increased and amount digested in the hindgut increased. One percent of starch digestion occurred in the hindgut when lambs were fed no corn and approximately 6.5 and 7.0% when lambs were fed the 40 and 80% corn diets, respectively. In a second experiment (16), the authors compared starch digestion in the rumen, small intestine and large intestine of lambs fed an all forage diet or hay and concentrate. Lambs fed only forage digested less starch in the rumen, small intestine and large intestine. Seventy-six percent of the starch which escaped ruminal degradation was digested in the small intestine of concentrate fed lambs. Ninety-seven percent of starch entering the large intestine of concentrate fed lambs was degraded and about 60% degraded for the forage fed lambs. Comparable results have been reported with steers. Steers fed high corn diets had significantly greater intakes of alpha-glucosides and digested significantly more in the whole tract, rumen-reticulum and large intestine, than steers fed diets containing little corn. Between 8 and 9% of the digestion of alpha-glucosides was in the hindgut (12). There may be a limit for starch digestion in the large intestine. Ørskov et al. (52) reported that when more than 138 g of starch was infused into the ovine cecum, most of the additional amount was excreted.

Fiber components are also digested in the lower tract. For ruminants, the more readily degradable portions of fiber are digested in the rumen. Hemicellulose and cellulose, which are more refractory, reach the lower tract where they are fermented by cecal and colonic microorganisms. More hemicellulose than cellulose seems to reach the large bowel probably because peptic enzymes alter furanosidic linkages of hemicellulose during digestion (77). There is little digestion of fibrous components in the small intestine (30). Several factors may affect the amount of cell wall constituents reaching the large intestine, such as forage maturity, grinding or pelleting and level of intake (30).

Amount of digestion occurring in the lower tract may be significant, but values reported in the literature vary. Degregorio et al. (16) reported that amount of acid detergent fiber (ADF) entering the large intestine increased as ADF intake increased, but ADF digestion in the lower tract increased with increasing starch in the diets. When lambs were fed an 80% corn diet, approximately 53% of the ADF entering the large intestine was digested accounting for 85% of the total tract ADF digestion. When lambs were fed diets containing 0 and 40% corn, significantly less ADF was degraded in the colon. Studies using sheep fed hay, grass or barley indicated that 2.5 to 46% of hemicellulose flowing into the large intestine was digested accounting for 30 to 40% of the total digested. Of the cellulose reaching the hindgut, 18.5 to 49.5% was degraded accounting for 18-27% of the total digested (30).

Generally, it is assumed that as apparent digestibility of fiber decreases, the proportion digested in the large intestine increases. Results from a recent study (6), contradict this assumption. Researchers

observed that although digestiblity of cellulose was fairly high (73%), 26% of cellulose digestion occurred in the colon. The authors suggested that greater microbial degradation of cellulose in the hindgut compensated for low ruminal digestiblity. Results from another study corroborate this suggestion (12).

Nonruminants digest most of the soluble dietary components in the forestomach. Cecal and colonic microorganisms degrade most of the dietary fiber. Hintz et al. (27) reported that for horses, fiber was fermented only in the cecum and colon, whereas protein and starch were enzymatically digested in pre-cecal regions. Microorganisms in the large intestine degrade hemicellulose more easily than other fibrous components probably because hemicellulose is partially hydrolyzed by gastric pepsin. Ruminants may digest fiber better than small nonruminant herbivores, but some large nonruminants may be as efficient as ruminants. The capybara, a large South American rodent, has a digestive system with fermentative capacity comparable to that of sheep and it digests fiber as efficiently (54). Fonnesbeck (22) reported that between 30 to 50% of dietary cellulose is digested by the horse. Rate of passage through the equine digestive tract is faster than through the bovine tract and may result in less efficient fiber digestion. Also, some studies have indicated that less efficient fiber digestion may be due to factors other than rate of passage. When different forages were incubated with ruminal microorganisms and equine cecal microorganisms, using nylon bag technique and an _in vitro_ method, all forages except for high quality alfalfa hay were more extensively digested by rumen bacteria (28).

In swine, the cecum and colon are the main sites of fiber digestion. Apparent crude fiber digestiblity varies between studies due to the type of dietary fiber, chemical treatment of crude fiber, and proportions of the constituents of crude fiber (58). Ehle et al. (19) reported that cellulose digestiblity ranged from 18.2 to 46.9% and hemicellulose digestibility from 48.6 to 88.3%. Differences in fiber digestiblity due to diet may reflect changes in hindgut microorganisms. In vitro incubations of different fiber substrates indicated that cecal and colonic microorganisms can adapt to diets, which results in degradation of a higher proportion of the fiber reaching the lower tract.

VOLATILE FATTY ACID PRODUCTION AND ABSORPTION.

Major microbial fermentation endproducts are volatile fatty acids (VFA). Intestinal microoganisms usually produce acetic, propionic and butyric acids in the greatest quantities. Concentrations and relative proportions of VFA will depend upon several factors including intake, time after feeding and the physiological condition of the animal (54).

Concentration of VFA in the ruminant hindgut varies along the length of the intestine. In sheep, the amount of acids increases slightly between the duodenum and terminal ileum, peaks in the cecum and proximal colon, and steadily decreases throughout the rest of the colon (75). Concentrations of acids in the cecum and colon are comparable to ruminal concentrations. Faichney (21) and Williams (80) reported that changes in cecal VFA concentration showed no distinct pattern. In both studies researchers used sheep which had rumen fistulas and duodenal cannulas.

Samples were removed immediately before feeding and several times after feeding. Faichney (21) reported no correlation between fluctuations in cecal organic acid concentration and pH and the amount of time since feeding. Fluctuations in cecal organic concentrations may be due to intermittent flow of digesta through the cecum. Ileal digesta containing small amounts of organic acids periodically enters the cecum and cecal digesta containing large amounts of VFA flows into the colon.

Diet affects proportions and concentrations of VFA in intestinal digesta. Degregorio et al. (15) reported that cecal and colonic digesta from lambs fed a diet consisting mostly of ground alfalfa-orchard grass hay contained 38.9 umoles of VFA/ml and from concentrate fed lambs 96.5 umoles/ml. When amount of dietary starch increases, the ratio of acetate to propionate decreases in cecal contents just as in the rumen. Ørskov et al. (52) infused starch into the ceca of two sheep which resulted in higher proportion of butryic acid in cecal digesta. Cecal contents of barley-fed sheep had a higher molar percentage of isobutyric and isovaleric acids than did rumen fluid indicating extensive protein degradation in the cecum (52).

Intestinal microorganisms of nonruminant herbivores also produce a considerable amount of VFA. Kern et al. (37) fed steers and ponies timothy hay. The ponies had geater concentrations of VFA in their ceca (97.4 umoles/g) and colons (25.6 umoles/g) than in their stomaches (14.3 umoles/g). The steers had greater ruminal concentrations (58.6 umoles/g) than cecal (34.4 umoles/g). Steers and ponies had only minute quantities of organic acids in the small intestine. As in the ruminant, diet affects VFA concentration in the equine tract. Ponies fed clover had a greater

total VFA concentration in their ceca than did ponies fed timothy hay, perhaps because of more available carbohydrate or protein. When oats and clover were fed, proportions of both propionic and valeric acids were increased, probably due to more readily fermentable carbohydrate available for microbial degradation (38). Argenzio et al. (5) observed that time after feeding affected VFA concentrations in the equine digestive tract. Twenty-four Shetland ponies were were used in their study. The ponies were assigned to one of two dietary groups and were fed 12 times daily. On the day of the experiment, ponies were slaughtered at 2, 4, 8 or 12 hours after their morning meal. Amount of organic acids in the stomach peaked 4 hours post feeding and was lowest at 8 hours. In the cecum and proximal colon, concentrations increased between 0 and 2 hours after feeding and declined by 4 hours. Molar percentage of acetate increased between 2 and 4 hours. As mentioned previously, time after feeding didn't appear to affect cecal VFA concentration in sheep. Discrepancy between studies may be due to species differences or to differences in feeding practices.

Considerable fermentation occurs in the hindgut of omnivores such as pigs and rats, resulting in signifcant VFA production. Ehle et al. (19) measured the concentration of organic acids in the porcine digestive tract. Total VFA concentration as well as the proportion of the acids differed between the cecum, upper large intestine and lower large intestine. The cecum and upper large intestine had a higher concentration of acids (180 mM and 174 mM) than did the lower large intestine (136mM). Whereas concentration of acetate, propionate, and butyrate were lower in the terminal colon, the concentration of isoacids did not decrease.

Dietary fiber affects amount and proportions of organic acids present in the hindgut. Pigs fed a low fiber diet had higher concentrations of acid and a lower ratio of acetate to propionate in their cecal digesta than pigs on a high fiber diet (78). Demigné and Rémésy (17) reported that rats fed a high fiber diet had a higher concentration of VFA in their ceca than did rats fed a fiber free diet (149.5 mM vs 126.5 mM). Sex of rats may influence amount of cecal VFA produced and absorbed, depending upon source of dietary fiber. When rats were fed diets containing gum arabic, cecal concentrations of total VFA, acetate, propionate, and butyrate did not differ due to sex. Male rats fed cellulose-based diets had significantly greater total VFA, acetate and propionate in their ceca than did females fed the same diet. The authors suggested that differences in rates of fermentation or absorption might explain the effect of sex on cecal VFA concentration (73).

Rates of VFA production may be a measure of fermentative capacity, and provide a way to determine importance of cecal fermentation for ruminants and nonruminants. Rates of production by cecal microorganisms appear equal to or greater than ruminal VFA production rates. In one study (30), researchers wished to determine whether cecal microorganisms digested poor quality nutrients more efficiently than rumen microorganisms. A continuous culture was used with effluent from previous digestions as substrate. Rate of acid production by the cecal organisms was slightly higher than by the rumen microbes (94.1 and 77.3 mM/day, respectively).

Production of VFA in the lower tract of ruminants may contribute a significant proportion of their daily energy requirement. Studies have

indicated that 8 to 16.8% of total VFA's produced may arise from fermentation in the lower tract (20, 75). Faichney (20) estimated production of cecal VFA in two sheep fed dried grass, using three different techniques: continuous infusion of ^{14}C-labelled VFA, single injection of labelled acids and an in vitro incubation technique. He obtained three different production rates: 18 mmoles•liter^{-1}•h^{-1}, 55 mmoles•liter^{-1}•h^{-1}, and 18 ±2 mmoles•liter^{-1}•h^{-1} respectively. Using the rate from continuous infusion experiments, Faichney calculated that cecal VFA accounted for 125 Kcal/day which is approximately 5% of the digestible energy intake. In a more recent study (15), researchers estimated rate of VFA production in lambs fed a forage or concentrate diet, using a single injection technique. Apparent acetate production (mM/day) was 189.2 and apparent propionate production was 180.7 for the forage fed lambs, and 239.4 and 431.4, respectively for the concentrate fed lambs. These production rates accounted for 4.5 and 5.8% of digestible energy intake.

Production of VFA in the hindgut of nonruminant herbivores and omnivores is comparable to ruminants. Bailey and McBee (8) studied cecal fermentation in rabbits. Their results indicated that cecal acid production provides between 5.3 and 18.7 kcal/day which is approximately 3.9 to 12% of daily energy requirements for rabbits. Imoto and Namioka (32) determined production rate of VFA's in porcine large intestine. Rates of VFA production were: 400.6 mM/day of acetate; 123.3 mM/day of propionate; 64.5 mM/day butyrate.

Barcroft (9) was first to demonstrate absorption of organic acids from the ruminant cecum. He reported the concentration of acids in the cecal vein was greater than that in arterial vessels. Williams (80) concluded

that absorption occurred in ovine large intestine because concentration of acids decreased between the proximal and distal colon. Dixon and Nolan (18) studied digestion in the ovine large intestine. Sheep were cannulated at the rumen and cecum and fed either lucerne chaff, pasture or barley-bagasse diets. The authors reported that most organic acid absorption occured in the cecum and proximal colon, but also some in the spiral and descending colon. The authors calculated that the absorption rate for sheep fed lucerne was approximately .1 mole/day. VFA absorption rate may depend upon the substrate available for microbial degradation. Ørskov et al. (52) speculated that large amounts of readily fermentable carbohydrate in the large intestine might cause ineffcient absorption of VFA. Also, cecal epithelium metabolizes some of the absorbed VFA. It oxidizes less total acids, takes up less oxygen and has a greater preference for acetate and less for butyrate than does rumen epithelium (75).

Nonruminants in which extensive hindgut fermentation occurs, also must absorb VFA in their large intestine. Barcroft (9) demonstrated that most absorption of VFA from the rabbit gastrointestinal tract is from the cecum. Argenzio et al. (5) compared production and absorption of VFA in different segments of equine gastointestinal tract. They used an in vitro technique to determine rate of VFA transport across gastric, cecal and large intestinal mucosa. The proper gastric mucosa and pyloric mucosa absorbed VFA but didn't transport them to the blood side of the tissue. Stratified squamous gastric epithelium neither took up nor transported any of the acids. Cecal and large intestinal mucosa absorbed and transported VFA at about the same rate as ruminal epithelium. The authors reported net absorption of organic acids from the large intestine took

place between 8 and 12 hours after feeding. The authors concluded that there was about 1.6 moles/day VFA net gain.

Barcroft (9) also demonstrated considerable absorption of VFA from porcine cecum and colon. Absorption occurred to a lesser extent in the small intestine and stomach. More recently, Imoto and Namioka (32) estimated the rate of absorption of VFA from porcine large intestine, by subtracting amount of acids excreted from amount of acids produced daily. Absorption rates (mmoles/day) were 380.5 of acetate, 116.6 of propionate and 61.0 of butyrate.

McBee (43) stated that many rodent species efficiently absorb VFA from their ceca. He noted that cecal epithelium does not absorb acids preferentially, nor does it metabolize acetate, propionate or butyrate very extensively. However, later studies, mostly done with rats don't support his observation. The discrepancy may result from species differences. Demigné and Rémésy (17) studied cecal VFA absorption from rats fed a high fiber or fiber free diet. Rate of absorption for rats fed fiber was 17.5 umoles/min, approximately seventeen times the rate for rats fed no fiber. Generally, it is assumed that VFA transport rates increase with increased chain length, but due to greater cecal concentrations, acetate flux across the cecal wall was faster than propionate and butyrate. When transport rates were divided by luminal concentrations, adjusted absorption rates were 1:1.07:1.01. The authors suggested that the rate of butyrate absorption may have been lower than expected because cecal epithelium was metabolizing it.

Several interrelationships may exist between absorption of VFA from the hindgut, and secretion or absorption of other nutrients such as Cl,

K, and Na, but conflicting results from several experiments make interpretation difficult. Use of different species and different methodologies may explain, in part, the discrepancies. Argenzio et al. (4) reported that acetate inhibited Na transport by equine cecal mucosa and completely abolished its transport by small intestinal mucosa in vitro. Bicarbonate secretion into the lumen accompanies VFA absorption, and the authors proposed that hydration of carbon dioxide either in the lumen or in the mucosal cell could provide hydrogen to produce the undissociated acid, which would then cross the cecal wall. The influence of intracellular H ion concentration upon a Na-H exchange mechanism might explain the effect of VFA concentration upon Na transport. Results from another experiment contradict these findings. Argenzio et al. (3) studied VFA absorption in the goat colon in vivo. Acetate, proionate and Cl crossed the intestinal epithelium more rapidly than Na or K. Removal of VFA from the perfusate reduced the amount of Na and water which were transported to the blood. Lower absorption of Na and water also resulted from replacement of acetate and propionate with Cl. The authors speculated that organic acids enhance Na absorption because the VFA serve as an energy source. Other studies with rabbits (41) and rats (17) indicated that VFA transport did not stimulate nor inhibit Na transport.

The mechanism for VFA absorption may be passive diffusion or it may involove some sort of carrier. Myers et al. (48) studied absoprtion of VFA from the cecum of sheep. They concluded that VFA are not transported by an active process because acids were not absorbed against a concentration gradient, absorption was not affected by metabolic inhibitors and absorption was not saturated even at eight times physiological concen-

trations of acids. They also reported that pH and rate of absorption of acids had an inverse relationship. As pH increased the rate decreased which indicated that the acid form of the fatty acids were absorbed more readily than the salt forms. They could not rule out the possiblity of a carrier mediated system. Umesaki et al. (76) studied acetate transport in isolated colonic epithelial cells from rats. They suggested the existence of two concurrent transport systems: a carrier mediated system and simple diffusion.

NITROGEN METABOLISM

Nitrogen in the ruminant hindgut includes any feed nitrogen which escapes ruminal degradation and enzymatic digestion, endogenous nitrogen, ammonia nitrogen, microbial nitrogen, and urea. The amount of soluble nitrogen as a percentage of total nitrogen is less in the large intestine than in the small intestine (6). Faichney (21) reported that the ovine cecum had higher concentrations of protein nitrogen, ammonia nitrogen and non-protein nitrogen (NPN) than did the rumen. He also noted that the concentrations of each nitrogenous component fluctuated but no consistent pattern emerged. Later work by Hecker (25) showed that cecal ammonia concentrations in sheep remained relatively constant; Hecker attributed the differing results to feeding practices.

Results of several studies indicate that approximately 24-50 g of nitrogen enter the large intestine daily per 100 g ingested. Microorganisms in the ovine large intestine degrade 1-6 g/d of nitrogen and in the bovine hindgut 11-29 g/d (75). In a more recent study of

protein digestion and absorption in the ovine digestive tract, 72.6% of feed nitrogen was digested and large intestinal degradation accounted for 6% (66).

Ammonia is present in the ruminant hindgut as a result of microbial degradation of protein nitrogen and hydrolysis of urea. Cecal ammonia concentrations depend upon diet but generally are comparable to rumen concentrations (18, 25, 21, 37, 80). Large intestine concentrations decrease between cecum and colon. Hecker (26) has demonstrated that cecal contents have considerable proteolytic, ureolytic, and deaminase activities, which accounts for high cecal concentrations of ammonia. Proteolytic activity in large intestinal contents is due to microbial enzymes; trypsin, chymotrypsin, elastase and carboxypeptidase A and B show only minor activity (6).

The large intestine absorbs most nitrogen as ammonia. Absorption occurs along the length of large intestine and may simply diffuse across intestinal epithelium (18, 25, 30). Hecker (25) reported that rate of ammonia absoprtion from ovine large intestine is dependent upon its concentration, indicating that passive diffusion may be the mechanism of transport for ammonia. Williams (80) noted that pH affects ammonia absorption and if it occurs by passive diffusion in nonionic form, then a pH near neutrality would be beneficial (30). Hecker (25) estimated the rate of ammonia absorption from the cecum of a sheep by measuring the rate of production <u>in vitro</u> and ammonia concentration in cecal digesta after the ileal re-entrant cannula of that sheep had been disconnected. Rate of ammonia absorption was 315 mg/100g DM and 130 mg/100 g water during a

seven hour period. Rates of ammonia absorption calculated in a more recent study are similar: 250-350 mg/100 g DM (6).

An important function of the hindgut is transfer of nitrogen to bodily fluids. Ammonia from the hindgut, when converted to urea, may return to the rumen or it may be used in the synthesis of non-essential amino acids. Approximately 39% of total body fluid nitrogen may be contributed by the cecum. Ammonia from the hindgut theoretically may diffuse into all surrounding bodily fluids if there is a concentration gradient. Hoover (30) speculated that because of the proximity of cecum and rumen, perhaps a more direct transfer of nitrogen might be possible, than through the portal vein, to the liver and thence to the peripheral circulation.

Microbial growth occurs in the cecum and large intestine, but microbial protein may be unavailable to ruminants if they are unable to absorb amino acids and peptides from their cecum and colon. Coprophageous animals such as rabbits ingest their night feces and digest any excreted microbial protein. Ørskov et al. (52) suggested that when large amounts of readily fermentable carbohydrate reach the ruminant hindgut, the host excretes most of the microbial protein synthesized. Some studies (36, 75) have indicated absorption of amino acids from the hindgut may occur, but unequivocal proof is lacking (75).

Nitrogen metabolism in nonruminant herbivores is similar to ruminants. Hecker (25) studied ammonia metabolism in the hindgut of herbivores. He observed a relationship between presence of ammonia, urea, and urease in gastrointestinal contents. Ammonia concentration in the equine stomach varied considerably between animals and averaged 11.3 mg/100 g. Small intestinal contents contained little ammonia, but the concentration in-

creased along the length of the large intestine, being greatest at the rectum. Only the small intestine contained significant amounts of urea which were comparable to blood levels. Urease activity on the other hand was only detected in the large intestine. Proteolysis occurs in all segments of the equine gastrointestinal tract, but the proteolytic activity of the ileum was 10-100 times that in the large intestine.

As with ruminants, the major question concerning nitrogen metabolism in the nonruminant hindgut is avaiability of nitrogen to the host. Slade et al. (68) suggested that nitrogen recycling occurs. Prior et al. (55) reported that 200 to 574 mg urea-N/kg $B.W.^{.75}$/d was recycled in the gastrointestinal tract of ponies fed diets containing 6, 9, 13, or 18% crude protein. But another study indicates that urea recycling doesn't occur in horses (57). Nonruminant herbivores which don't practice coprophagy can't benefit from microbial protein synthesized in the lower tract unless they absorb amino acids from their hindgut. Slade et al. (67) reported that mature horses could utilize microbial protein. They prepared a ^{15}N-labelled washed cell suspension from equine cecal digesta and injected the labelled cells into the cecum of a live horse. Blood samples were obtained from the cecal vein every half hour for six hours. The amount of ^{15}N in the blood peaked 1 and 4 hours after administration of cells. The authors suggested that free amino acids and ammonia were absorbed at 60 minutes, causing an increase of labelled nitrogen in blood. The second peak may have resulted from the absorption of amino acids from digestion of labelled proteins. However, a study done by Reitenour and Salsbury (56) indicated that horses may not absorb amino acids from their cecum. They infused fishmeal, soybean meal, or linseed meal into the ceca

of ponies. The ponies also were fed a basal diet. Digestibility of total protein was increased, but nitrogen retention as a percentage of absorbed nitrogen was decreased appreciably when soybean meal and linseed meal were fed. Cecal administration of the protein supplements did not alter plasma protein concentration, but plasma urea nitrogen was increased significantly. The evidence suggests that the infused nitrogen was absorbed probably in the form of ammonia. In their review, Hintz et al. (28) suggested that some amino acids are probably absorbed from the equine cecum, but its extent and significance have not been determined.

Digestion and absorption of nitrogenous compounds in the hindgut of omnivores is similar to herbivores. Rérat (58) suggests that monogastrics efficiently digest carbohydrates in the large intestine, but not protein. In the pig, about 30 to 40% of dietary nitrogen reaches the hindgut, and of this between 20 to 50% disappears in the large intestine, depending upon diet. Holmes et al. (29) reported that when pigs were fed semi-purified diets containing soybean meal or rapeseed meal, 9 and 16% of nitrogen absorption and 4 and 5 % of amino acid disappearance, repectively, occurred in the large intestine. Ammonia production and absorption in the large intestine of pigs and rats is important in nitrogen recyling and synthesis of non-essential amino acids. Hydrolysis of urea by cecal microoganisms is one source of ammonia. Robison et al. (59) isolated and identified porcine cecal bacteria. Fifteen of 192 isolates produced urease. Diet affects amount of ammonia produced and urea utilized by microorganisms in the hindgut. Rats fed a fiber free diet had higher cecal ammonia concentrations but a slower rate of ammonia absorption than did rats fed a high fiber diet. Urea was secreted into the

cecum regardless of diet, but the flow into the ceca of rats fed high fiber was 18 times higher (17). Absorption of amino acids from the rat hindgut may occur according to one study (11), and the mechanism of transport is most likely diffusion.

Hindgut fermentation occurs in ruminants, nonruminant herbivores, and omnivores. Number and types of intestinal microorganisms and the pattern of hindgut fermentation is basically similar for most mammalian species, despite the diversity of digestive strategy. Availability of fermentation endproducts to the host and importance of microbial degradation of feedstuffs in the hindgut does differ between omnivores, ruminants and nonruminant herbivores. Hindgut fermentation for some nonruminant herbivores may be more important than for ruminants, because fiber is digested primarily in their cecum and colon. For example, cecal VFA production provides a greater percentage of the daily energy requirement of a rabbit than of a sheep. Coprophagous animals may derive more benefit from synthesis of microbial protein in their hindgut because they reingest their night feces. Ruminants, omnivores, and nonruminant herbivores which don't practice coprophagy absorb nitrogen from the cecum and colon as ammonia, but their ability to absorb amino acids from the hindgut remains uncertain.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Thirty-two Holstein bull calves aged 6 to 7 months were fed diets containing variable amounts of ground orchard grass hay and a grain mixture. Eight calves were randomly assigned to each treatment group (Table 1). Amount of starch and types of protein were: [L1] low starch, soybean meal (SBM); [L2] low starch, fishmeal and dried brewers' grains (FBG); [H1] high starch, SBM; [H2] high starch, FBG (Table 2). Calves were fed the experimental diets for 17 days prior to slaughter. Calves were penned individually and fed at 8:00 AM and 4:00 PM. Feed intake was recorded for the last 10 days of the experimental period. Weekly samples of the diets being fed were stored at -20 °C until subsequent analysis. Calves were weighed initially and on the day of slaughter.

On the day of slaughter a fecal grab sample was obtained and the calf was transported to the Meats Lab. Calves were killed between 8:00 and 9:00 AM. After removal of the gastrointestinal tract, the gut was divided into segments. The ileum was tied off at the ileo-cecal junction and approximately 1 m proximal to it; the cecum was tied off at the ileo-cecal junction; the colon was tied off at the ileo-cecal junction and approximately 1 m from it. Each gut segment was placed on ice and transported to the laboratory.

Digesta was removed from each portion of the gut, and pH recorded

Table 1. Treatment Design.

	Low starch	High starch
Soybean meal	L1 (8)	H1 (8)
Fishmeal plus dried brewers' grains	L2 (8)	H2 (8)

Table 2. Composition of diets.

	Treatments			
	L1	L2	H1	H2
Ingredient (%)[1]				
Ground orchardgrass hay	40	40	15	15
Ground shelled corn	44	44	69	69
Soybean meal	12.5		12.5	
Dried brewers' grains		7.0		7.0
Fishmeal		6.75		6.75
Molasses	.5	.5	.5	.5
Trace mineral salt	1.0	1.0	1.0	1.0
Vitamin mix[2]	.5	.5	.5	.5
Dicalcium phosphate	.5		.5	
Limestone	1.0	.25	1.0	.25
Composition (%)[1]				
Dry matter	88.1	88.6	88.4	88.8
Crude protein	15.1	15.6	14.9	15.4
Acid detergent fiber	19.2	18.0	9.5	9.6

[1]Dry matter basis.

[2]Vitamin A 7067 IU/kg and vitamin D 739 IU/kg of feed.

(Orion Research Ionanalyzer, model 601A). Contents were then placed in a polyethylene bag [1] filled with anaerobe grade carbon dioxide. After removal of digesta from the cecum, a portion of cecal wall was excised and placed in a Ca-free Kreb's Ringer bicarbonate buffer, maintained at 37°C. The remainder of the cecum was placed in the Atmos bag. The remainder of the contents were frozen at -20°C until analysis. In the Atmos Bag, cecal contents were prepared for in vitro incubations. Cecal epithelium and digesta were prepared for serial dilutions. Both procedures will be described.

RATE OF TRANSPORT

Acetate and propionate transport through cecal epithelium was determined in vitro using parabiotic chambers (Figure 1). The method of Nocek (50) was adapted for use with cecal tissue. Cecal tissue was rinsed with Ca-free Kreb's Ringer Bicarbonate buffer (pH7.4) and blotted between two pieces of gauze. Cecal epithelium was separated from the underlying muscle, clamped between two parabiotic chambers and the incubation vessel was placed in a water bath at 37°C. Ten ml of Kreb's buffer (pH 7.4) containing 80 mM sodium acetate and 20 mM sodium propionate was placed in the luminal chamber. The other chamber contained 10 ml of Kreb's buffer (pH 7.4) which had 2 mM sodium acetate and sodium propionate. Sodium chloride content of the buffer was reduced by the amount of added sodium salts. Immediately after addition buffer, contents of each incu-

[1] Atmos Bag, Aldrich Chemical Co., Milwaukee, WI

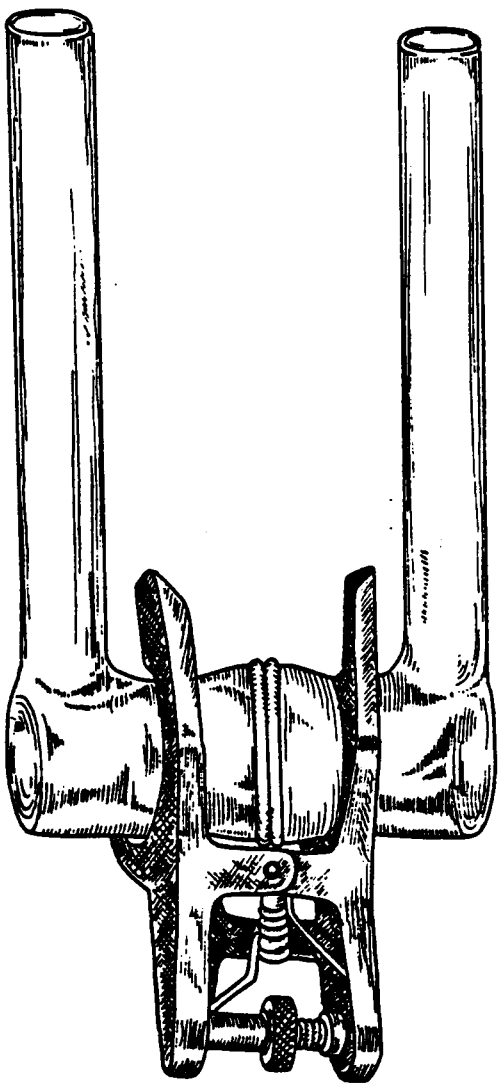

Figure 1. In vitro incubation chamber.

bation vessel were removed to obtain a zero time value. Buffer was then replaced in luminal and serosal chambers of each incubation vessel and the vessels were incubated for 15, 30, 60 or 120 minutes. Chambers were gassed with with a mixture of 5% CO_2/95% O_2 during incubation. A control vessel was prepared also. The luminal chamber contained Kreb's buffer with no added sodium acetate or propionate. Ten ml of Kreb's buffer containing 2 mM of sodium acetate and propionate was added to the serosal chamber. The control was incubated for 0, 15, 30, 60, and 120 minutes. The contents of both lumen and blood chambers were aspirated and stored at -20 °C until analysis. After removal of media, tissue (15 mm in diameter) exposed to buffer was excised, weighed and dried for 24 hours at 105°C. Flow of acetate and propionate to the serosal chamber was calculated by the following formula:

$$\text{Flow} = \{[C_1 - C_0]10\} \div [g \times t]$$

where C_1 = final concentration, C_0 = original concentration, g = weight of dry tissue and t = incubation time. Flow of the acids also were expressed on the basis of area of tissue.

Media samples were analyzed with a Bendix Gas Chromatograph (Model 2600) for acetate and propionate using isocaproic acid as an internal standard (74). Acids were separated on a column packed with 10% SP-1200/1% H_3PO_4 (liquid phase) on 80/20 Chromasorb WAW support[2]. Tem-

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

perature of the injector and detector was 175°C and of the oven 125°C. The nitrogen flow rate was 40 ml/min and sample size was .5 ul.

VOLATILE FATTY ACID PRODUCTION

Rate of VFA production was determined according to the procedure of Whitelaw et al. (79) modified for cecal digesta. In the Atmos Bag, 10 g aliquots of cecal contents were placed in 25 ml erlenmeyer flasks and diluted with distilled water. After capping the flasks with rubber stoppers, the flasks were removed from the Atmos Bag and incubated in a shaking water bath for 15, 30 or 60 minutes in duplicate at 38 °C. Injection of 6 N NaOH through the rubber septa stopped fermentation. Flasks then were placed in an ice bucket to ensure that fermentation was stopped and no acids volatilized. Zero time production rate was estimated by adding NaOH to a pair of flasks immediately and placing them on ice. After NaOH addition, contents were centrifuged at 47,500 x g for 15 minutes, and the supernatant recentrifuged at 12,900 x g for 15 minutes and stored at -20 °C until analysis. Production was expressed as $umoles \cdot ml^{-1} \cdot h^{-1}$.

Samples were assayed for acetate, propionate, isobutyrate, butyrate, isovalerate, and valerate by gas chromatography. Samples were passed through a .45 um Millipore filter[3]. One ml of .25% metaphosphoric acid was added to 4 ml of filtrate and the sample was analyzed for VFA as described previously.

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[3] Gelman Instrument Corporation, Ann Arbor, MI.

MICROBIOLOGICAL ENUMERATION PROCEDURES

In the Atmos Bag 10 g quantities of ileal, cecal and colonic contents were weighed and mixed in a Waring Blender with 90 ml of pre-reduced anaerobic dilution solution (PRAS) (2). The cecum, devoid of contents, was rinsed with approximately 1200 ml of physiological saline and then dried with gauze. Three pieces of cecal tissue (18 mm in diameter) were excised and epithelial and muscle layers separated. A 1 g quantity of epithelium and 9 ml of PRAS were blended. The dilutions (10^1) of digesta and tissue were removed from the Atmos Bag to prepare serial dilutions.

Counts of viable bacteria were made according to procedures in the V.P.I. Anaerobe Laboratory Manual (2). Further serial dilutions were made using the V.P.I. culture system[4]. Three dilutions (10^7, 10^8, and 10^9) of cecal and colonic digesta were inoculated in triplicate into roll tubes of pre-reduced Rumen Fluid Cellibiose Agar (RGCA). Media was prepared according to the manual (2) but 20% of the rumen fluid in RGCA was replaced by clarified cecal fluid (Table 3). Preparation of clarified rumen and cecal fluid for use in media is shown in the appendix. Roll tubes of unmodified RGCA medium were inoculated in triplicate with 10^5, 10^6, and 10^7 of ileal digesta and tissue. All roll tubes were were incubated for 96 hours at 38°C and colonies were counted.

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[4] Bellco Glass, Vineland, NJ.

Table 3. Modified rumen fluid cellobiose-agar medium[1].

Glucose	0.0992	g
Cellobiose	0.0992	g
Ammonium sulfate	0.4	g
Distilled water	80.0	ml
Resazurin solution(.025%)[2]	1.6	ml
Salts solution[2]	200.0	ml
Clarified rumen fluid	96.0	ml
Clarified cecal fluid	24.0	ml
Cysteine-HCl-H_2O	0.2	g
Hemin solution(.05%)[2]	4.0	ml
Vitamin K_1[3] solution[2]	0.08	ml

[1]Pre-reduced media was prepared according to procedures in V. P. I. Anaerobe Laboratory Manual (2). Dispense 10 ml of pre-reduced medium into tubes which contain .2 g agar.

[2]Solutions were prepared as outlined in V. P. I. Laboratory Manual (2).

[3]Final concentration in medium = ca. 1 ug/ml.

DIGESTA ANALYSES

A portion of ileal, cecal, and colonic digesta and feces were lyophilized. Freeze-dried samples were ground[5] through a 1 mm screen and an aliquot dried at 105°C for absolute dry matter determination. Dry digesta and feces were analyzed for ADF (24), nitrogen, and cytosine. Analysis of nitrogen was done by the Kjeldahl procedure (7). Cytosine was analyzed by high pressure liquid chromatography[6] according to the procedure of Koenig (39) as modified in our laboratory. Five hundred mg of dry digesta were placed in 15 ml screw-cap test tubes with 2.5 ml of 70% perchloric acid and incubated overnight at room temperature. Tubes then were placed in a 90°C water bath for 1 hour positioned at 10° from horizontal; tubes were rotated 4 or 5 times during incubation to ensure complete hydrolysis. After cooling, distilled water was added to the samples. Subsequently, contents of the tubes were transferred to 500 ml volumetric flasks, diluted to volume with distilled water and mixed thoroughly. Approximately 20 ml of the hydrolysate was passed through .45 um Millipore filters and the filtrate was used for analysis. Cytosine was separated on a 25 cm Partisal-10 SCXL column[7] at room temperature. The mobile phase was .1M ammonium phosphate buffer adjusted to pH 3.2 with 12M HCl. Flow rate was .6 ml/min and injection volume was 100 ul. De-

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[5] Cyclone Sample Mill., VD Corp., Boulder, CO..

[6] Varian 5000, Palo Alto, CA.

[7] Whatman, Inc., Clifton, NJ.

tection was at 254 nM. Microbial nitrogen as a percentage of the total nitrogen in digesta was calculated as follows:

$$DMN = 100 \times (1.838 \times DC/DN)$$

where DMN = digesta microbial nitrogen (%); DC = digesta cytosine umoles/g DM; DN = digesta nitrogen (mg/g DM); and 1.838 = the ratio of microbial nitrogen to microbial cytosine (65).

Wet cecal and colonic digesta were used for ammonia determination. Samples were centrifuged at 48,400 x g for 15 min and the supernatant respun at 51,500 x g for 15 min. One ml of .25% metaphosphoric acid was added to 4 ml of sample and filtered through .45 um Millipore filters. Samples containing too much particulate matter were diluted with equal volumes of acid and centrifuged again at 51,500 x g prior to fitration. Filtrate was analyzed for ammonia by the phenol-hypochlorite method of Beecher and Whitten (10).

FEED ANALYSES

Weekly feed samples were dried at 50°C and dried samples composited (4 per diet). After grinding through a 1 mm screen (Cyclone sample mill), composites were analyzed for nitrogen by the Kjeldahl method (7) and ADF (24). Another portion of the composite was dried at 105°C for absolute dry matter determination.

STATISTICAL ANALYSES

The experimental design was a completely randomized design with a 2 x 2 factorial arrangement of dietary treatments. The experimental model was:

$$Y_{ij} = u + S_i + P_j + (SP)_{ij} + E_{ij}$$

where Y_{ij} = observed dependent variable; u = mean of Y, S_i = effect of ith factor, starch, i = 1,2; P_j = effect of the jth factor, protein, j = 1,2; $(SP)_{ij}$ = interaction of factors S and P; E_{ij} = random error. Data were analyzed using the General Linear Model of the Statistical Analysis System (63). Because of missing observations, results are presented as least squares means ± standard error. For diets L1, L2, and H1, there were 3 missing fecal samples and for diet H2, 2 missing fecal samples. For 3 calves fed diet H2 and one calf fed diet L2 there was no ileal digesta. In the following tables, treatment means on the same row with unlike superscripts differ, $p < .05$.

RESULTS AND DISCUSSION

DRY MATTER INTAKE AND BODYWEIGHT GAIN

Dry matter intake and weight gain did not differ significantly between treatments during the experimental period (Table 4). Inclusion of fishmeal as a protein source in diets L2 and H2 didn't seem to diminish the calves' appetite or weight gain.

DIGESTA COMPOSITION

Percent ADF, dry matter and nitrogen of digesta and feces are presented in Table 5. Percent dry matter differed due to the amount of starch in diets. Digesta from calves fed high fiber diets (L1 and L2) contained more water perhaps due to increased saliva flow and decreased retention time of digesta in the tract. Percent dry matter increased between ileum and cecum for all diets because the ileum serves as a major site of water conservation. Water is also absorbed in the large intestine as indicated by the increase of dry matter between the colon and rectum. Similarly, fiber content of digesta of calves fed H1 and H2 was significantly lower than that of calves fed L1 and L2.

Nitrogen content of ileal, cecal, and colonic digesta and feces didn't differ due to diet. As mentioned previously, the composition of diets L1, L2, H1, and H2 were formulated to be isonitrogenous. Percent nitrogen

Table 4. Calf bodyweight and dry matter intake[1].

	Treatments[2]				
	L1	L2	H1	H2	SE
Initial bodyweight (kg)	185	184	172	176	11
Final bodyweight (kg)	204	205	196	194	11
Bodyweight gain (kg/d)	1.09	1.28	1.47	1.11	.16
Dry matter intake (kg/d)	5.88	5.45	5.38	5.62	.34

[1]Values are least square means for eight calves per treatment.

[2][L1] low starch, soybean meal (SBM); [L2] low starch, fishmeal plus dried brewer's grains (FBG); [H1] high starch, SBM; [H2] high starch, FBG.

Table 5. Composition of hindgut digesta and feces[1].

	Treatments[2]				
	L1	L2	H1	H2	SE
DM (%)					
Ileum	11.9[a]	14.1[ab]	17.1[b]	19.5[b]	1.5
Cecum	17.0[a]	16.9[a]	21.4[b]	22.9[b]	.9
Colon	15.8[a]	16.1[a]	21.2[b]	22.1[b]	.8
Feces	21.3[a]	22.3[a]	22.8[ab]	25.4[b]	.9
ADF (%)[3]					
Ileum	20.1[a]	21.2[a]	13.1[b]	12.6[b]	2.4
Cecum	20.2[a]	21.1[a]	13.5[b]	15.3[b]	1.0
Colon	21.9[a]	22.6[a]	14.4[b]	16.2[b]	1.1
Feces	21.5[a]	21.6[a]	18.0[b]	18.7[ab]	1.1
Nitrogen (%)[3]					
Ileum	2.1	2.2	2.0	2.1	.1
Cecum	2.1	2.1	2.0	2.1	.1
Colon	2.2	2.2	2.2	2.3	.1
Feces	2.4	2.3	2.6	2.4	.2

[a,b]Means on the same row with unlike superscripts differ ($p<.05$).

[1]Values are least square means. Observations are from eight calves per treatment except as noted in Materials and Methods

[2][L1] low starch, soybean meal (SBM); [L2] low starch, fishmeal plus dried brewers' grains (FBG); [H1] high starch, SBM; [H2] high starch, FBG.

[3]Dry matter basis.

increased slightly between the ileum and rectum regardless of diet but percent of fecal nitrogen wasn't affected by diet.

DIGESTA PH AND BACTERIAL NUMBERS

Digesta pH (Table 6) was significantly lower for calves fed diets H1 and H2. The pH dropped approximately one unit between the ileum and the cecum regardless of diet due to an accumulation of fermentation endproducts in the cecum. Between the colon and the rectum, the pH decreased again for all diets, indicating extensive microbial activity occurs in the large intestine as well. Nicoletti et al. (49) reported that pH of ovine digesta increased between the duodenum and jejunum (pH 7.24) and declined in the ileal region (pH 6.79). The authors suggested that pH may have been lower in the ileum due to backwash of digesta from the cecum. Cecal pH was 6.00, almost 1 unit lower than ileal pH. Fecal pH also differed due to the amount of dietary starch ($p<.1$); Calves fed diets L1 and L2 had higher fecal pH than calves fed diets H1 and H2.

Numbers of viable anaerobic bacteria in ileal, cecal and colonic digesta differed between diets (Table 7). Amount of dietary starch and type of protein affected numbers of ileal and cecal bacteria. Calves fed high starch diets had significantly greater numbers of viable anaerobic bacteria in ileal and cecal digesta, than calves fed diets L1 and L2. Replacement of SBM by FBG and high starch (diet H2) resulted in the greatest number of bacteria in the ileum and cecum. The numbers of colonic bacteria were affected by the amount of starch in the diet; digesta from calves fed H1 and H2 had significantly higher numbers of

bacteria. Colonic pH was lower in calves fed diets H1 and H2 indicating more extensive fermentation in the hindgut. Mann and Ørskov (45) compared effects of conventional versus bottle feeding sucrose, maltose, or glucose to sheep. When the rumen was circumvented, cecal bacteria were more numerous and pH lower than when carbohydrates were fed conventionally. Increased numbers of bacteria in digesta from calves which received H2 may be due to a combination of more readily available carbohydrate and more available amino acids.

Bacterial numbers were lower in the ileum than in the cecum or colon. Other researchers have reported an increase in numbers of viable anaerobic bacteria between the duodenum and rectum (49, 75). Numbers of viable anaerobic bacteria in ileal contents of sheep ranged from 5.0×10^6 to 1.3×10^8 and in the cecum anaerobic counts ranged from 7.9×10^7 to 1.6×10^9 (75). In another study, sheep fed grain and alfalfa hay averaged 2.9×10^{10} bacteria/g of cecal contents (75). Nicoletti et al. (49) noted that direct microscopic counts of digesta from the small intestine were several log units higher than cultural counts, suggesting that numbers of viable organisms actually may be higher than cultural counts indicate. As seen in Table 7, counts varied by as much as two log units. Ileal digesta had the greatest range of colony counts. Extreme variability has been noted in other studies, especially for intestinal bacteria (49, 75). Cultural counts may differ due to animal variability, but methods of culturing intestinal bacteria, especialy from the small intestine, may also partly explain the variation.

Numbers of viable anaerobic bacteria associated with cecal epithelium ranged from 10^5 to 10^8/g of tissue (Table 7). Again, high starch

Table 6. Hindgut digesta and fecal pH[1].

	Treatments[2]				
	L1	L2	H1	H2	SE
Ileum	7.12^{a}	6.89^{b}	6.82^{b}	6.79^{b}	.09
Cecum	6.28^{a}	6.22^{ab}	6.00^{b}	5.77^{c}	.08
Colon	6.30^{a}	6.15^{ab}	6.03^{b}	5.79^{c}	.08
Feces	5.64^{ab}	5.82^{a}	5.64^{ab}	5.36^{b}	.12

^{a,b,c}Means on the same row with unlike superscripts differ ($p<.05$).

[1]Values are least square means. Observations are from eight calves per treatment except as noted in Materials and Methods.

[2][L1] low starch, soybean meal (SBM); [L2] low starch, fishmeal plus dried brewers' grains (FBG); [H1] high starch, SBM; [H2] high starch, FBG.

Table 7. Numbers of viable anaerobic bacteria in ileal, cecal, and colonic digesta and cecal epithelium[1].

	Treatments[2]				
	L1	L2	H1	H2	Range
Ileum[3] ($\times 10^7$)	2.6^{a}	5.2^{a}	13.9^{a}	42.9^{b}	.56-100
Cecum[3] ($\times 10^9$)	2.7^{a}	4.1^{ab}	7.7^{b}	12.1^{c}	.57-21
Colon[3] ($\times 10^9$)	3.4^{a}	3.7^{ab}	13.6^{b}	13.2^{b}	.54-20
Tissue[4] ($\times 10^7$)	2.6^{ab}	1.0^{a}	4.4^{ab}	11.5^{b}	.05-48

^{a,b,c}Means on the same row with unlike superscripts differ ($p<.05$).

[1]Values are least square means. Observations are from eight calves per treatment except as noted in Materials and Methods.

[2][L1] low starch, soybean meal (SBM); [L2] low starch, fishmeal plus dried brewers' grains; [H1] high starch, SBM; [H2] high starch, FBG.

[3]Per g of wet digesta

[4]Per g of wet tissue.

stimulated the growth of cecal microorganisms. Type of dietary protein had a secondary effect, such that the numbers of epithelial bacteria from calves fed H2 were 2 to 10 times as great as L1, L2, or H1. The extreme variability could be a result of culture methods. Similar to their findings with small intestinal bacteria, Nicoletti et al. (49) reported that cultural counts of bacteria adhering to the small intestinal wall were 4 log units lower than direct microscopic counts. They attributed it to the difficulty of culturing these organisms in vitro. The medium used to culture bacteria associated with cecal tissue may not have provided nutrients necessary for growth of all species of bacteria associated with cecal epithelium. If calves fed the same diet had different bacterial species in cecal tissue, variable cultural counts may have been obtained. Variable counts may have been due also to clumping of bacterial cells in the medium or removal of cells during washing of cecal tissue. Occasionally, during preparation of 10^1 dilution of cecal epithelial in the Atmos Bag, tubes became aerobic, which might have resulted in lower counts.

CYTOSINE AS A INDICATOR OF MICROBIAL SYNTHESIS

Cytosine was used as a bacterial marker to estimate the amount of bacterial protein synthesized. Cytosine was quantitated in ileal, cecal, and colonic digesta and feces of four calves per treatment. To estimate amount of microbial nitrogen present in digesta and feces, it is necessary to determine the ratio of nitogen to cytosine in bacterial cells. Because the ratio was not determined for each of the calves in the present study

a book value of 1.838 mg nitrogen/umoles cytosine (65) was used (Table 8). Percentage of total nitrogen as bacterial nitrogen in ileal digesta didn't differ significantly due to diet but tended to be lower for diets L2 and H2 which contained less degradable protein. The lower percentages for diets L2 and H2 probably resulted from a greater amount of undegraded dietary protein reaching the small intestine. For SBM diets (L1 and H1) a greater proportion of the nitrogen might be attributed to microbial cells passing out of the rumen into the abomasum and thus to the small intestine. Zerbini and Polan (81) reported that calves fed diets containing fish meal had a lower percentage of abomasal microbial protein than calves fed a diet containing SBM.

The percentage of nitrogen of microbial origin increased between the ileum and the cecum across all diets. Cecal digesta from all diets had comparable amounts of nitrogen of microbial origin. The cecum contained more viable anaerobic bacteria than did the ileum, which partially accounts for the increase. Also, dietary protein would have been digested to a large extent in the small intestine. Proportion of cecal microbial nitrogen from calves fed diets L2 and H2 tended to be lower than from calves fed diets L1 and H1 which contained SBM. Some fishmeal may have escaped enzymatic digestion and reached the hindgut. Digesta from calves fed high starch diets (H1 and H2) had significantly greater numbers of viable anaerobic bacteria, but starch didn't affect the percentage of cecal nitrogen as bacterial nitrogen. The discrepancy between viable anaerobic counts and estimation of microbial growth by cytosine may be due to several reasons. Determination of microbial growth using cytosine as a marker does not distinguish between live and dead cells. Possibly,

Table 8. Percentage of total nitrogen in digesta and feces as bacterial nitrogen[1].

	Treatments[2]				
	L1	L2	H1	H2	SE
Ileum	23	14	24	16	4
Cecum	35	33	36	33	3
Colon	35[ab]	31[a]	40[b]	35[ab]	3
Feces	38[a]	33[a]	46[b]	38[a]	2

[a,b]Means on the same row with unlike superscripts differ ($p<.05$).

[1]Values are least square means for four calves per treatment. To estimate percentage of total nitrogen as microbial nitrogen, a ratio of nitrogen to cytosine in bacterial cells must be determined. A value of 1.838 mg/umoles was used (see 65).

[2][L1] low starch, soybean meal (SBM); [L2] low starch, fishmeal plus dried brewers' grains (FBG); [H1] high starch, SBM; [H2] high starch, FBG.

cytosine may not be sensitive enough to reflect differences in viable counts which are fairly small. Bacteria in the hindgut of calves fed diets H1 and H2 which contained high starch may have synthesized greater amounts of mucopolysaccarhide capsular material due to more available substrate. Therefore, the amount of microbial nitrogen may have been underestimated because the ratio of nitrogen to cytosine would have larger for those bacteria.

Between the cecum and rectum the proportion of nitrogen as bacterial nitrogen increased as a percentage of total nitrogen across all diets except for diet L2. Amount of dietary starch and source of dietary protein significantly affected percentage of total fecal nitrogen as microbial nitrogen. Feces from calves fed H1 had the highest percent of total nitrogen as microbial nitrogen. Though percent fecal nitrogen didn't differ as a result of high dietary starch (H1 and H2), it seems that most of the microbial cells synthesized as a result of the additional substrate were excreted and not utilized by the animal.

CECAL AND COLONIC AMMONIA CONCENTRATION

Cecal and colonic ammonia concentration was affected by dietary starch. As seen in Table 9, calves fed diets H1 and H2 had significantly lower cecal ammonia concentrations, than calves fed diets L1 and L2. Lower ammonia concentration in cecal digesta of calves fed H1 and H2 may have been due to more ammonia nitrogen incoroporation into bacterial cells or from a faster rate of ammonia absorption. Demigné and Rémésy (17)

Table 9. Hindgut digesta ammonia concentration (ug/ml)[1].

	Treatments[2]				
	L1	L2	H1	H2	SE
Cecum	113[ab]	124[a]	65[b]	70[b]	17
Colon	114	122	77	75	18

[a,b]Means on the same row with unlike superscripts differ ($p<.05$).

[1]Values are least squares means for eight calves per treatment.

[2][L1] low starch, soybean meal (SBM); [L2] low starch, fishmeal plus dried brewers' grains (FBG); [H1] high starch, SBM; [H2] high starch, FBG.

reported that high dietary fiber stimulated cecal fermentation in rats, as evidenced by greater cecal VFA concentration, lower cecal pH, and lower cecal ammonia concentration than rats fed no fiber. Ammonia flux across the cecal wall was greater in fiber fed rats, although due to lower pH it might be expected that less ammonia should diffuse across cecal epithelium of fiber fed rats.

CECAL VFA PRODUCTION

Greater intakes of starch resulted in significantly greater total VFA, propionate, and butyrate concentrations (Table 10). Replacement of SBM by FBG in diets L2 and H2 also stimulated butyrate production, such that cecal digesta of calves fed diet H2 had the highest concentration. Increase in total VFA concentration and a change in the molar proportions of acetate, propionate and butyrate, due to increased availability of substrates has been reported in other studies (17, 52). Changes in cecal microflora in response to increased substrates could result in changes in VFA proportions. Kern et al. (38) reported that when ponies were fed either timothy hay or clover hay plus oats, there were greater numbers of Propionibacterium and fewer Bacteroides than when oats were not fed. Ørskov et al. (52) infused starch into the cecum of a sheep and isolated cecal organisms. The predominant organism in the cecum was a Butyrivibrio sp. which might explain the increase of butyrate on high starch diets. Also, Butyrivibrio fibrisolvens digests casein (31) and some strains can liquefy gelatin (51), which might explain why digesta from calves fed diets H2 had significantly higher concentrations of butyrate. Other major

Table 10. Cecal volatile fatty acid concentration (umoles/ml)[1].

	Treatments[2]				
	L1	L2	H1	H2	SE
Acetate	84.8	93.5	98.6	86.1	5.9
Propionate	17.7^{a}	17.9^{a}	27.2^{b}	26.1^{b}	1.9
Butyrate	16.1^{a}	18.9^{a}	26.7^{b}	33.6^{c}	2.2
Total	121.2^{a}	132.2^{ab}	155.0^{b}	148.2^{b}	8.7

^{a,b}Means on the same row with unlike superscripts differ ($p<.05$).

[1]Values are least squares means for eight calves per treatment.

[2][L1] low starch, soybean meal (SBM); [L2] low starch, fishmeal plus dried brewers' grains (FBG); [H1] high starch, SBM; [H2] high starch, FBG.

organisms isolated when starch was infused into the cecum included chains of gram-negative cocci, gram-positive cocci, and gram-negative crescent shaped bacteria presumed to be a Selenomonas species. Gram negative cocci in the genus Veillonella produce propionic and acetic acids. Selenomonas ruminatium also produces propionic acid (2). Megasphera elsdenii another gram-negative bacterium produces butyric acid as well as branched chained acids (2), amd ferments serine, threonine, and arginine (31).

Cecal VFA production was estimated by in vitro incubations of cecal digesta. Change in total VFA, acetate, propionate, and butyrate concentrations over time is presented in figures 2, 3, 4, and 5.
Except for butyrate, the data are curvilinear. Stewart et al. (72) derived an equation to determine rates of ruminal VFA production based on the assumption that increase in VFA concentration is curvilinear. The equation is

$$B = 4y_2 - y_3$$

Where B = the rate of production ($umoles \cdot ml^{-1} \cdot h^{-1}$) at zero-time

y_2 = increase over initial VFA concentration at 30 min

y_3 = increase over initial VFA concentration at 60 min.

Whitelaw et al. (79) determined ruminal VFA production with the above equation using incubations of 15 and 30 min, 30 and 60, and 60 and 120 minutes. To determine rates using these other time points, the coefficients must be changed. If 15 and 30 minute incubations are used, the coefficients are 8 and 2 instead of 4 and 1. They also derived a second equation based upon the assumption that increments in VFA concentration

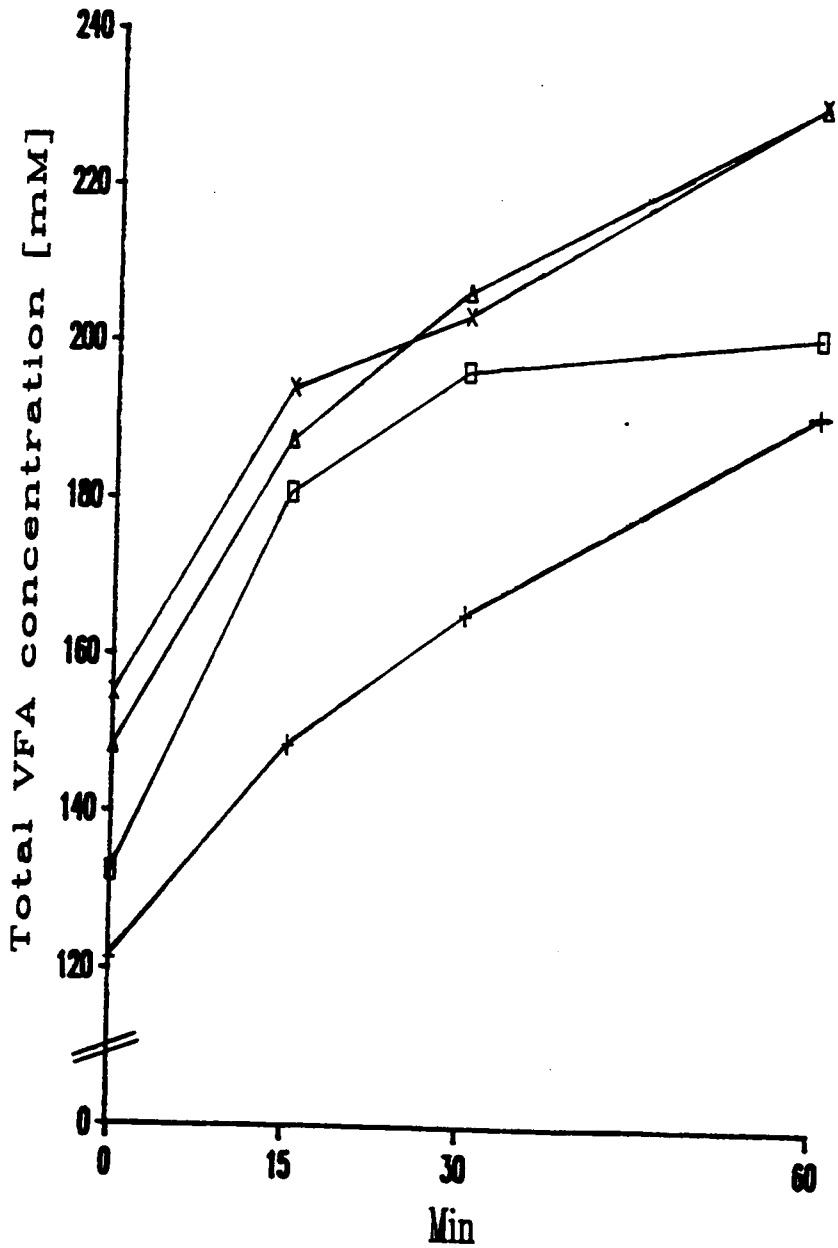

Figure 2. Change in total VFA concentration over time. L1 (+); L2 (□) ; H1 (x); H2 (Δ).

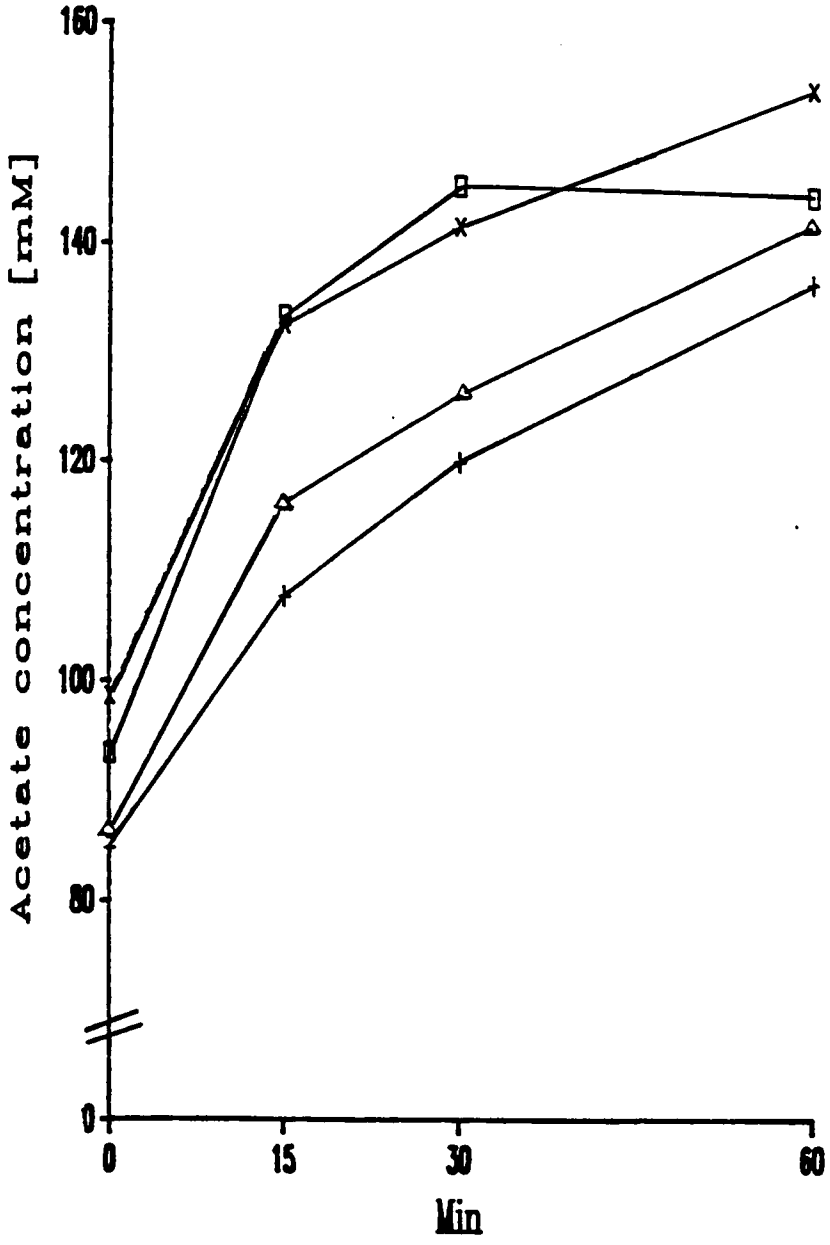

Figure 3. Change in acetate concentration over time. L1 (+); L2 (□); H1 (x); H2 (Δ).

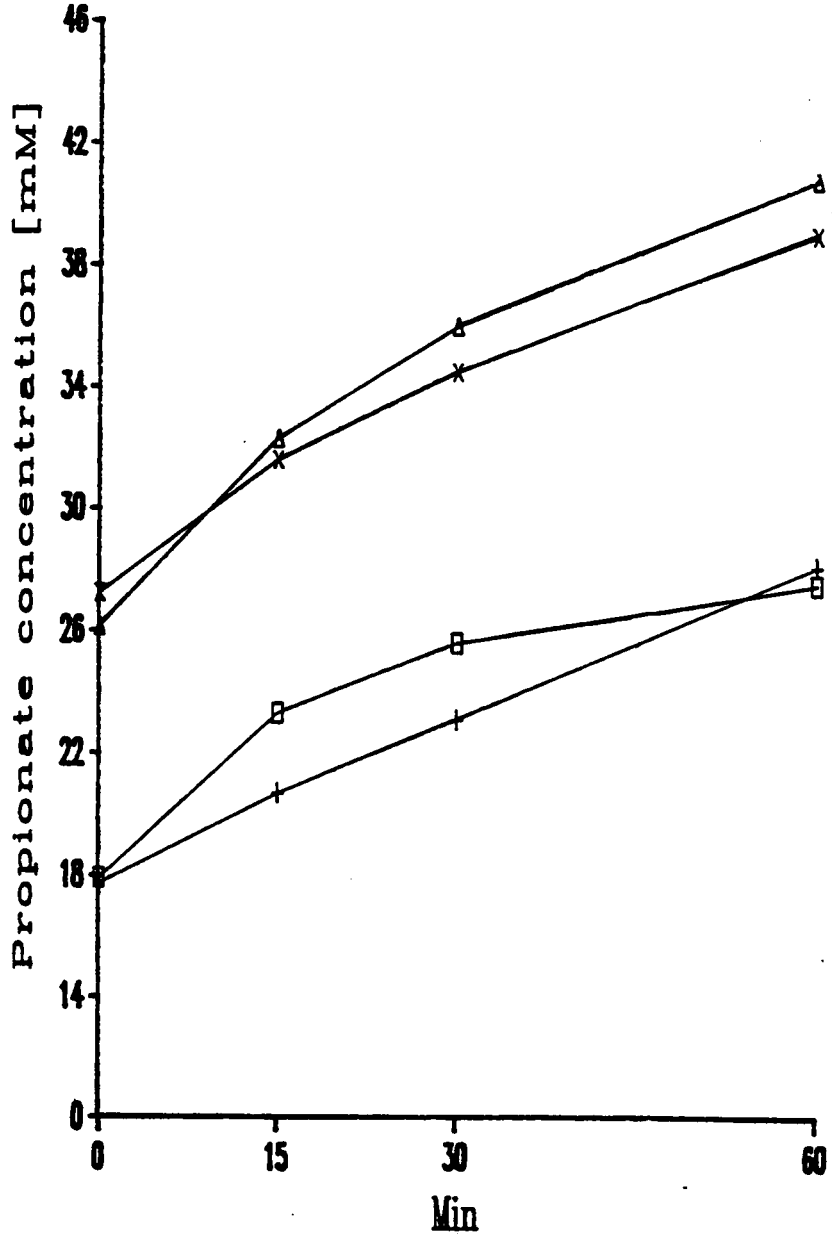

Figure 4. Change in propionate concentration over time. L1 (+); L2 (□); H1 (x); H2 (△).

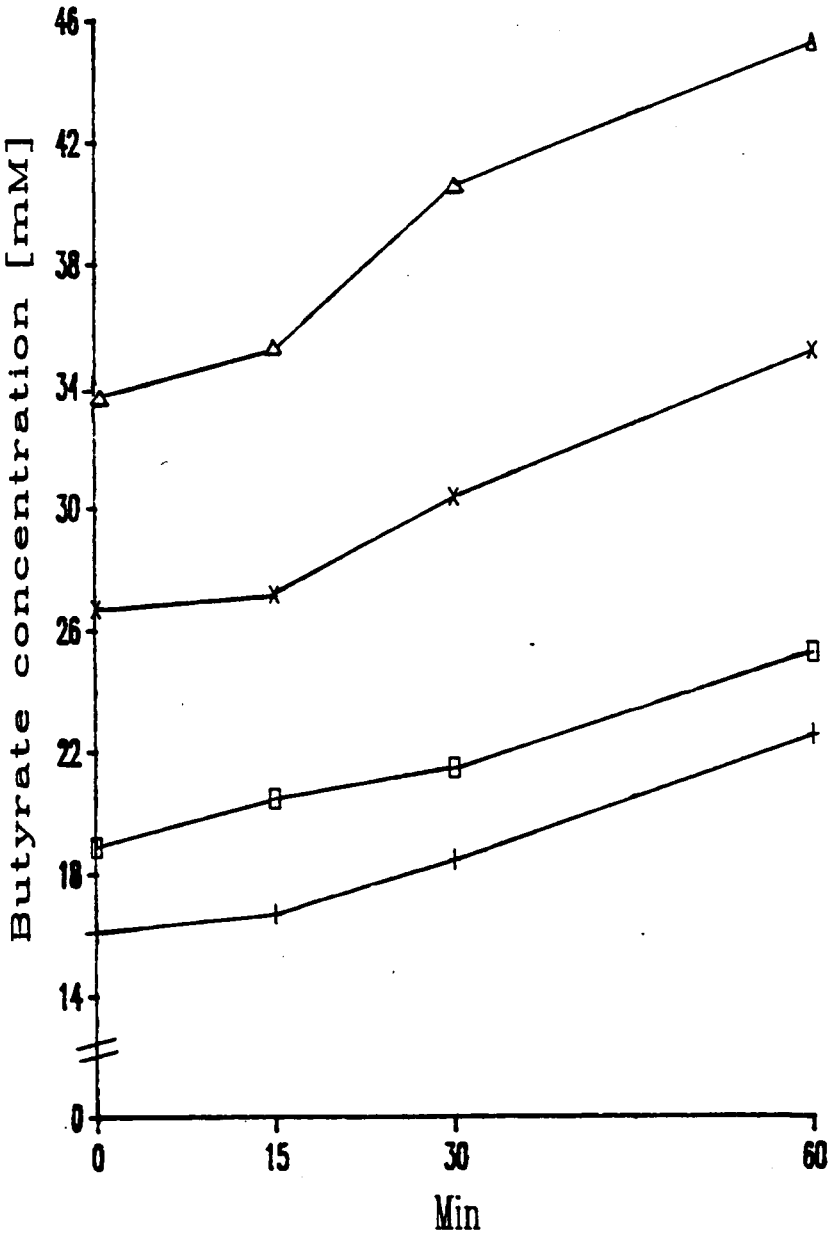

Figure 5. Change in buytrate concentration over time. L1 (+); L2 (□); H1 (x); H2 (△).

decline exponentially with time. They used the same time points as above. The equation is

$$B = 4\{y_1^2 \ln[(y_2-y_1) \div y_1]\} \div \{y_2-2y_1\}$$

where B = rate of production (mmoles$\cdot l^{-1} \cdot h^{-1}$) at zero-time

y_1 = increment at 15 min

y_2 = increment at 30 min

To determine VFA production rate using 30 and 60 minute time points, the coefficient would be changed from 4 to 2. In the present study, cecal production rates were calculated using both equations at 15 and 30 and 30 and 60 min. For clarity, the equation based upon the curvilinear model is labelled A and the other equation B (Table 11 and Table 12). Production rates determined using both equations were higher for the 15 to 30 minute interval, and were higher overall for equation B. Rates determined using equation B were higher because of missing data points. When the increment at 30 or 60 min was lower than at 15 or 30 min, respectively, $y_2 - y_1$ was a negative value. The natural log of the difference was set to zero. But the rate of production obviously wasn't zero, and therefore, a production rate was not entered for these calves. As a result of missing values, standard errors for equation B were much higher. When the increase in concentration over the initial was lower at 30 or 60 min, the production rate was inflated using equation A.

When production rates were determined using equation A, diets L2 and H2 had significantly greater production of propionate (15 to 30 min).

Table 11. Cecal volatile fatty acid production rates (umoles•ml^{-1}•h^{-1}) using equation A[1,2].

	Treatments[3]				
	L1	L2	H1	H2	SE
15-30 minutes					
Acetate	59.5^{a}	107.5^{b}	92.7^{ab}	79.5^{ab}	12.9
Propionate	7.1^{a}	13.8^{ab}	10.3^{ab}	16.0^{b}	2.9
Butyrate	0.7	4.1	-1.1	-0.1	3.8
Total	65.6^{a}	126.^{b}	104.5^{ab}	100.5^{ab}	15.2
30-60 minutes					
Acetate	44.1^{a}	78.0^{b}	57.6^{ab}	53.5^{a}	7.3
Propionate	5.6^{a}	10.6^{ab}	8.7^{ab}	12.4^{b}	2.1
Butyrate	1.4^{a}	2.0^{a}	3.1^{ab}	7.6^{b}	1.7
Total	52.7^{a}	93.9^{b}	70.9^{ab}	79.8^{b}	8.7

^{a,b}Means on the same row with unlike superscripts differ ($p<.05$).

[1]$B = 8y_1-2y_2$ or $4y_2-y_3$, where B = rate of production using increase over initial concentration at 15 (y_1), 30 (y_2), or 60 (y_3) minutes.

[2]Values are least squares means for eight calves per treatment.

[3][L1] low starch, soybean meal (SBM); [L2] low starch, fishmeal plus dried brewers' grains (FBG); [H1] high starch, SBM; [H2] high starch, FBG.

Table 12. Cecal volatile fatty acid production rates (umoles•ml^{-1}•h^{-1}) using equation B[1,2].

	Treatments[3]				
	L1	L2	H1	H2	SE
15-30 minutes					
Acetate	65.9	150.8	138.0	120.5	30.8
Propionate	6.6	17.1	17.5	19.0	5.5
Butyrate	2.1^{a}	4.2^{ab}	4.6^{ab}	9.8^{b}	2.0
Total	77.2	169.0	143.8	139.5	32.1
30-60 minutes					
Acetate	52.0	83.5	83.4	57.9	13.8
Propionate	6.4	8.4	8.5	8.4	1.5
Butyrate	1.8	3.3	7.5	12.8	3.9
Total	58.8	94.2	94.0	82.8	17.4

^{a,b}Means on the same row with unlike superscripts differ ($p<.05$).

[1] $B = 4\{y_1^2 \ln[(y_2-y_1)/y_1]\}/\{y_2-2y_1\}$
or $B = 2\{y_2^2 \ln[(y_3-y_2)/y_2]\}/y_3-2y_2\}$,
where B=rate of production, using increase over initial concentration at 15 (y_1), 30 (y_2), and 60 (y_3) minutes.

[2]Values are least squares means for eight calves per treatment.

[3][L1] low starch, soybean meal (SBM); [L2] low starch, fishmeal plus dried brewers' grains (FBG); [H1] high starch, SBM; [H2] high starch FBG.

When the 30 to 60 min interval was used, L2 and H2 had significantly greater production of propionate and total VFA. Starch stimulated butyrate production at 30 and 60 min. Source of protein ($p<.1$) and starch both infuenced the rate of buytrate production estimated by equation B (15 to 30 min). In every case, diet L1 had the lowest rate of production of total VFA and of acetate, and diet L2 the highest. Digesta from calves fed L1 had significantly lower numbers of viable anaerobic bacteria and significantly lower total VFA concentrations. While acetate production was highest for L2, cecal acetate concentration for L2 was not. Conversion of acetate and butyrate might partially explain the discrepancy. Faichney (20) noted that 27% of butyric acid carbon was derived from acetic acid and 12% of acetic acid from butyric acid. Some Butyrivibrio fibrisolvens strains convert acetic to butyric acid (31, 40). Rate of conversion depends upon several factors including concentration of acetic acid and growth rate (40). Total VFA concentration in digesta of calves fed L2 was lower than that of the high starch diets, though the production rate was greater. Differences in microflora, in part, may account for the higher production rate. As seen in figures 2, 3, and 4, concentration of acetate, propionate, and total acids is not sustained for L2 at the same rate as other groups, indicating different patterns of fermentation.

Butyrate production rate was not adequately estimated by either equation because the data did not correspond to either model. When simple regression was used the rate (umoles$\cdot ml^{-1} \cdot h^{-1}$) and r values for each diet were:[L1] 6.7,.99;[L2] 6.3,.99;[H1] 9.8,.98; [H2] 12.3,.98. Linear increase in butyrate concentration may have resulted from a shift in the

Table 13. Initial cecal volatile fatty acid production rate (umoles•ml^{-1}•h^{-1})[1,2].

	Treatments[3]				
	L1	L2	H1	H2	SE
Acetate	45.9^{a}	79.4^{b}	67.7^{ab}	63.2^{ab}	7.5
Propionate	6.0.	10.7	15.7	12.9	4.4
Butyrate	2.0	3.4	1.3	3.5	2.2
Total	54.9^{a}	95.4^{b}	79.5^{ab}	78.0^{ab}	9.0

^{a,b}Means on the same row with unlike superscripts differ ($p<.05$).

[1]Rate was determined using 15 minute incubations.

[2]Values are least squares means for eight calves per treatment.

[3][L1] low starch, soybean meal (SBM); [L2] low starch, fishmeal plus dried brewers' grains (FBG); [H1] high starch, SBM; [H2] high starch, FBG.

microbial population in response to changes in substrate availability. Also, it is difficult to determine how much conversion between the acetate and butyrate fraction occurred.

Rates of initial VFA production (umoles$\cdot ml^{-1} \cdot h^{-1}$) were determined from the change in concentration after incubation for 15 minutes (Table 13). Rates of cecal VFA production determined from 30 and 60 minute incubations are comparable to inital rates for calves fed L1, L2, and H2. Rate of initial butyrate production was low. As seen in figure 5 the rate of cecal butyrate production seems to increase after 15 minutes at least for calves fed H1 and H2.

Estimates of the amount of energy produced in the hindgut as a percent of digestible energy intake range from 4.2 to 26% (75). Percent of each calf's energy intake accounted for by cecal production of acetate, propionate and butyrate in this study was estimated. Production rates determined using equation A (30-60 min) were used for calculation of the energy intake accounted for by cecal VFA production. Cecal weight is approximately .8% of body weight in cattle (77), and a value was determined for each calf based on final body weight. Digestible energy intake was calculated using TDN values for each diet (Table 14). The estimates ranged from 2.6 to 4.8% of the digestible energy intake. Calves fed diet L2 had a significantly greater percentage of their digestible energy intake accounted for by cecal VFA production. Differences reflect differences in calculated cecal VFA production rates. Faichney (20) estimated that for sheep, cecal VFA production could account for 5.3% of digestible energy intake.

Table 14. Estimates of percent of digestible energy intake of calves accounted for by cecal volatile fatty acid production[1].

	Treatments[2]				
	L1	L2	H1	H2	SE
Digestible energy intake (%)	2.6[a]	4.8[b]	3.6[ab]	3.7[ab]	.5

[a,b]Means on the same row with unlike superscripts differ ($p<.05$).

[1]Values are least squares means for eight calves per treatment.

[2][L1] low starch, soybean meal (SBM); [L2] low starch, fishmeal plus dried brewers' grains (FBG); [H1] high starch, SBM; [H2] high starch, FBG.

FLUX OF ACETATE AND PROPIONATE ACROSS THE CECAL EPITHELIUM

Acetate and propionate transport across the cecal wall was determined *in vitro*. Change in concentration of acetate and propionate over time in the serosal chamber is in figures 6 and 7. Whereas acetate concentration seems curvilinear, propionate concentration increases linearly. Acetate begins to plateau between 30 and 60 minutes, suggesting that the tissue might be dying, but the amount of propionate increases between 30 and 60 minutes. The tissue may be metabolizing more acetate than propionate, especially at 30 and 60 minutes which might explain the non-linearity of the acetate curve. Packett et al. (53) studied metabolism of acetate, propionate and butyrate by ovine cecal and ruminal epithelium *in vitro*. All tissue pieces were incubated with 38 uM acetate, 11.5 uM propionate, and 8.7 uM butyrate. Cecal mucosa oxidized significantly less VFA than ruminal epithelium. Of the total VFA metabolized by the rumen mucosa 51% was butyrate, 38% acetate and 11% propionate. Cecal tissue metabolized acids in the following order: acetate (70%), butyrate (22%) and propionate (8%). Cecal tissue from animals fed concentrate tended to use less acetate and butyrate and more propionate than when fed roughages. The authors suggested that rate of utilization of VFA by the tissue was correlated with the proportion of acids in the intestinal tract. Stevens and Stettler (71) reported that propionate transport *in vitro* across ruminal epithelium was increased when acetate and butyrate were present. They suggested that this may result from a decreased metabolism of propionate by the tissue. Roediger (60) studied the metabolism of short chained fatty acids by isolating colon cells from

rats. He suggested that both butyrate and acetate inhibit glucose oxidation. Acetate and butyrate suppress production of $^{14}CO_2$ from [1-^{14}C] pyruvate, indicating a decreased flux of pyruvate through pyruvate dehydrogenase. The author suggested also that activation of fatty acids in the intestinal wall may be in the order of butyrate > acetate > propionate. Production of $^{14}CO_2$ from butyrate is decreased significantly in the presence of acetate, and decreased only slighty when propionate is added, indicating that colonic cells do not readily metabolize propionate.

Flux of acetate and propionate across cecal epithelium was determined and results are presented in Table 15 and Table 16. When no sodium acetate or sodium propionate was placed in the luminal chamber, there was no change in the serosal acid concentration over time indicating little production of VFA by the cecal wall. Thus, any change in in serosal acetate or propionate concentration was due to flow from the luminal chamber. Rates of appearance of acetate and propionate on the serosal side of the tissue were calculated after 15 and 60 min incubations. The results are expressed as umoles•g of dry tissue^{-1}•min^{-1} and as umoles•cm^2 of tissue^{-1}•h^{-1}. High dietary starch caused a significant increase in flux of acetate at 15 and 60 min ($p<.05$) and propionate ($p<.1$) at 60 min when the results were expressed on the basis of dry tissue weight. Tissue from calves fed H1 had significantly higher acetate flux at 15 min expressed on the basis of tissue weight and diameter than tissue from calves fed L1, L2, or H2. Stevens (69) reported that equine cecal tissue and porcine cecal tissue transported 1.6 ±0.2 and 4.3 ±0.6 VFA

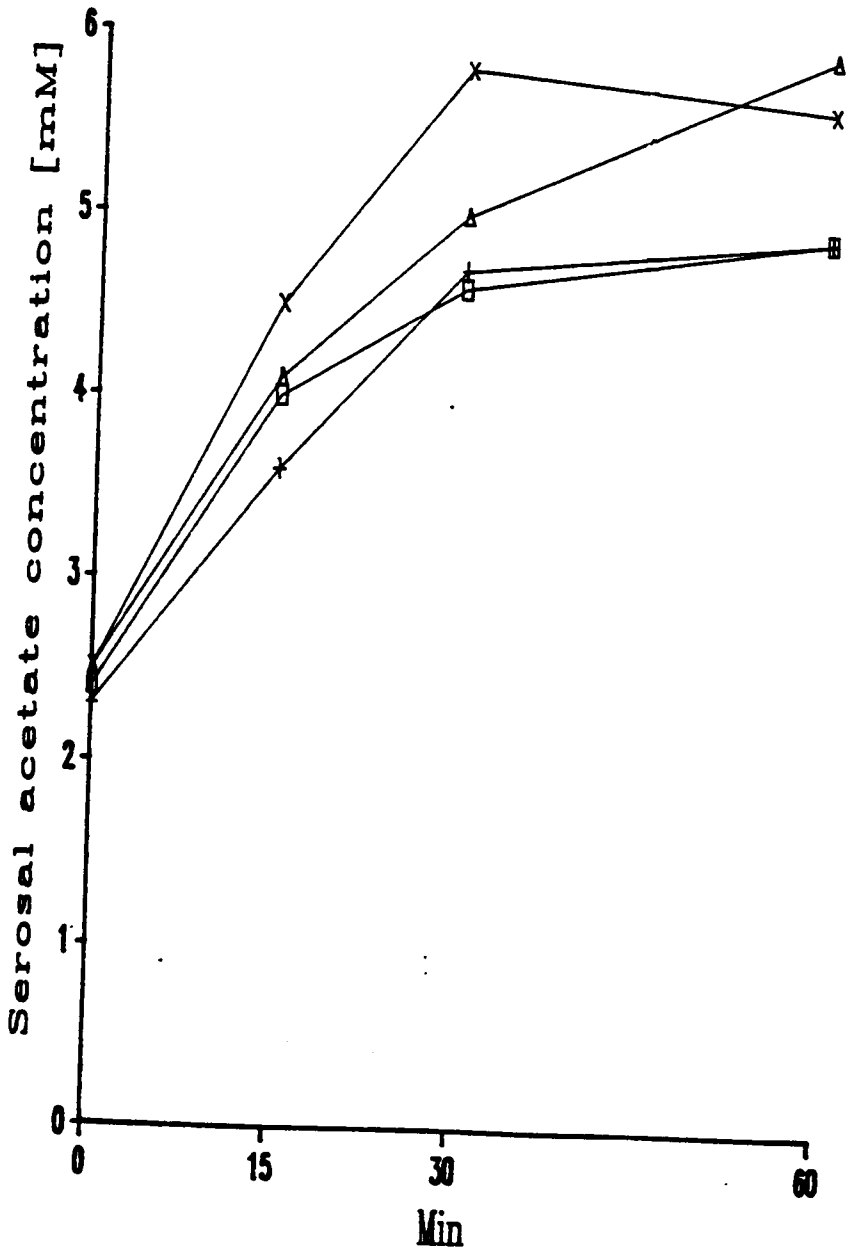

Figure 6. Change in the serosal acetate concentration over time. L1 (+);L2 (□); H1 (x); H2 (Δ).

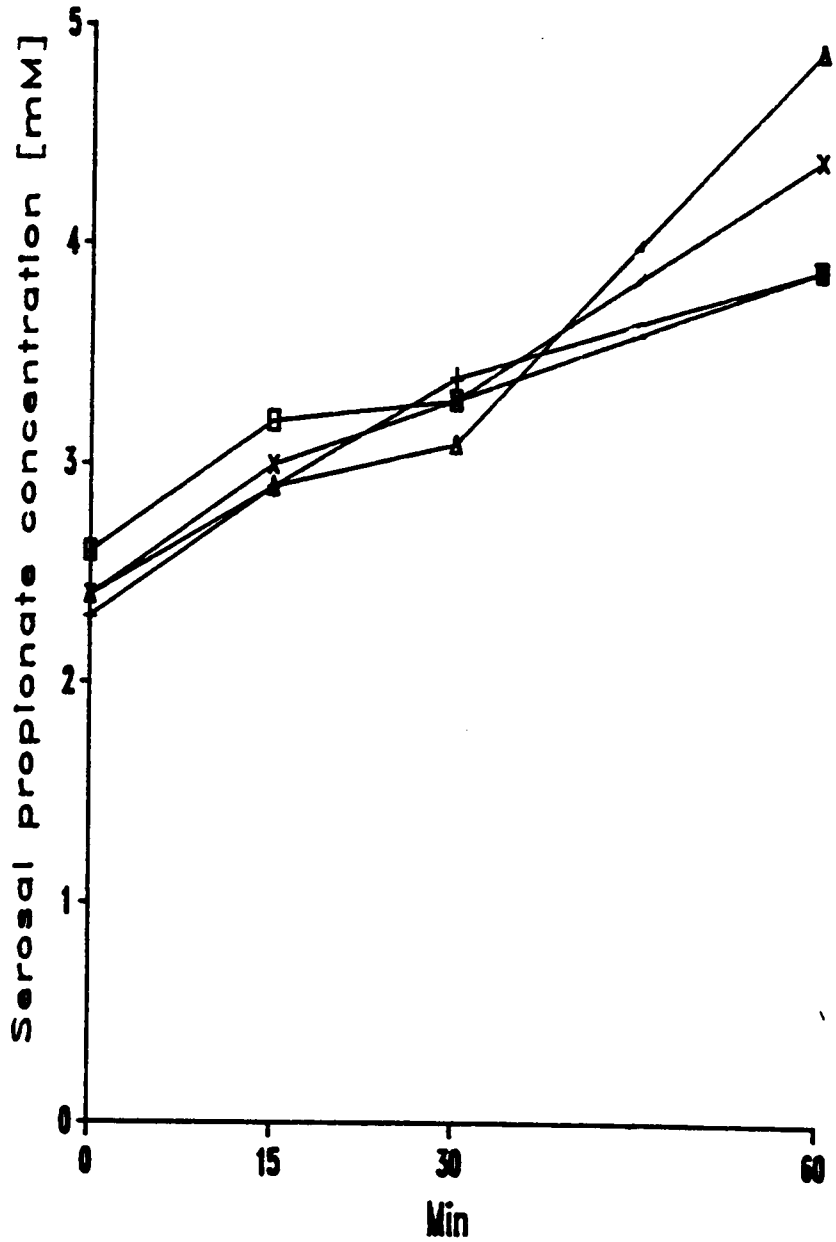

Figure 7. Change in the serosal propionate concentration over time.L1 (+); L2 (□); H1 (x); H2 (Δ).

Table 15. Flux (umoles•g of dry tissue^{-1}•min^{-1}) of acetate and propionate across cecal epithelium *in vitro*[1].

	Treatments[2]				
	L1	L2	H1	H2	SE
Initial rate (15 min)					
Acetate	51.1^{a}	56.5^{ab}	81.8^{b}	72.1^{ab}	10.2
Propionate	23.3	23.4	27.2	22.4	7.8
Equilibrium rate (60 min)					
Acetate	26.8^{ab}	24.3^{a}	29.8^{ab}	41.9^{b}	5.6
Propionate	16.4^{ab}	13.2^{a}	17.9^{ab}	31.2^{b}	5.4

^{a,b}Means on the same row with unlike superscripts differ ($p<.05$).

[1]Values are least square means for eight calves per treatment.

[2][L1] low starch, soybean meal (SBM); [L2] low starch, fishmeal plus dried brewers' grains (FBG); [H1] high starch, SBM; [H2] high starch, FBG.

Table 16. Flux (umoles•cm^2 of tissue^{-1}•h^{-1}) of acetate and propionate across cecal epithelium *in vitro*[1].

	Treatments[2]				
	L1	L2	H1	H2	SE
Initial rate (15 min)					
Acetate	7.3^{a}	8.8^{ab}	10.9^{b}	8.8^{ab}	1.2
Propionate	3.5	3.4	3.4	2.8	1.0
Equilibrium rate (60 min)					
Acetate	3.8	3.6	4.2	4.8	.6
Propionate	2.2	1.9	2.8	3.4	.6

^{a,b}Means on the same row with unlike superscripts differ ($p<.05$).

[1]Values are least square means for eight calves per treatment.

[2][L1] low starch, soybean meal (SBM); [L2] low starch, fishmeal plus dried brewers' grains (FBG); [H1] high starch, SBM; [H2] high starch, FBG.

(um•cm^2 of tissue^{-1}•h^{-1}), respectively. Their results were calculated from a 2.5 hour incubation using Ringer's solution containing equimolar amounts of 90 mM acetate, propionate and butyrate. Flow of VFA across ruminal epithelium was 11 umoles/cm^2 during 2.5 hour incubation when a solution of acetate (60mM), propionate (20mM), and butyrate (15mM) at pH 6.4 was placed in the luminal chamber (71). Results of the present study may differ because tissue was incubated for shorter periods of time. Concentration of acids may have plateaued long before the end of the 2.5 hour incubations of Stevens (70, 71). Also, differences in methodology may also explain the discrepancy. Weight of the equine cecal tissue from the chambers (5) was 7 times greater than the tissue used in the present study, though the circumference of the tissue exposed to the bathing solutions were equal (7.1 cm^2). Demigné and Rémséy (17) measured flux of acetate, propionate, and butyrate in vivo across the cecal wall of rats. They reported rates of 10.35, 4.64, and 2.54 umoles/min of acetate, propionate, and butyrate respectively when rats were fed a high fiber diet. The authors reported that cecal wall weight was 2.48 g. If a tissue dry matter content of 10% is assumed, the rates are 41.7, 18.7, and 10.2 umoles•g of dry tissue^{-1}•min^{-1}. When expressed on a dry weight basis, the above rates are comparable to rates determined in vitro for calves in the present study. Moreover, the rates of VFA production in the ceca of the rats may actually be faster if expressed on the basis of dry weight of cecal epithelium rather than on the weight of cecal wall.

Rates of propionate transport were lower than rates of acetate transport (Table 15 and Table 16). But when rates are adjusted for concentrations of sodium acetate and propionate placed in the luminal cham-

ber, actually a greater proportion of sodium propionate appeared in the serosal chamber. While similar absoprtion mechanisms probably operate in vivo for acetate, propionate, and butyrate, it is thought that rate of VFA absorption increases wih increasing chain length (17). Stevens and Stettler (71) noted that when equimolar mixture of acetate, propionate, and butyrate was used, in vitro rates of transport were acetate > propionate > butyrate, which seems opposite of the process in vivo. They suggested that rates of transport are determined to a great extent by their metabolism in tissue. Also, they suggested conversion of butyrate to acetate in the tissue might affect the rates (70, 71). In the present study, if the tissue was metabolizing acetate, rate of acetate transport across the cecal epithelium might be lower than if another substrate such as butyrate were present.

As mentioned previously, starch did stimulate the flux of VFA's across the cecal epithelium. Rates of acetate and propionate transport were determined using an in vitro system, in which amount of acids, and pH in the luminal chamber were alike for each dietary group. Dietary factors may have stimulated changes in cecal tissue which might explain, in part, the differing rates. Increase in production of fatty acids, (61, 62) especially butyrate, (62) may have a stimulatory effect on proliferation of rumen epithelial cells. Dietary fibers cause a hyperproliferative effect on rat colonic epithelium which may be mediated by VFA production in the hindgut (34). Demigné and Rémésy (17) reported that high fiber diets stimulated the flux of acetate, propionate and butyrate across the cecal wall in rats; absorption of total VFA's was 17.5 umoles/min, 17 times the rate for rats fed no fiber. Fiber fed rats had ceca which

weighed more, had a greater cecal VFA concentration, and had greater cecal blood flow than rats fed the basal diet. They suggested that increased cecal blood flow and increased cecal VFA concentration could not account for the enhanced absorption and suggested that the there was more efficient absorption across the cecal wall of fiber fed rats. Rate of transport of VFA across equine cecal mucosa in vitro was affected by the diet fed to the animals prior to slaughter. Flux of acids across cecal mucosa from horses fed a high fiber, low protein diet was slower than when horses were fed a commercial pelleted hay-grain diet. Perhaps the higher VFA and butyrate concentrations in digesta of calves fed diets H1 and H2 caused changes in the cecal wall.

Weak electrolytes may diffuse passively across intestinal epithelium, but some evidence indicates that the mechanism may be more complicated. Umesaki et al. (76) studied acetate uptake by isolated colonic epitheial cells from rats. They concluded that perhaps a dual system of carrier mediated diffusion and passive diffusion may exist. While acetate was transported more easily at lower pH the ratio of transported acetate to protonated acetate wasn't the same over the range of pH used, but was larger at higher pH, indicating that not all the acetate was transported in protonated form. Some metabolic inhibitors also decreased acetate uptake by the isolated cells. Jackson (33) stated that several factors may influence rate and mechanism of transport of weak electrolytes. He proposed a three compartment model for transport; in addition to the fluid bathing the mucosal and serosal surfaces of the intestinal wall, the extracellular space comprises a third compartment through which nutrients must pass. He suggested that there are two groups of determinants of weak

electrolyte movement. One refers to the driving forces of movement such as concentration and electrical gradients. The second group includes rate limiting factors such as the discriminatory properties of the epithelium, and the pH of the three compartments. A third property which may affect rate of transport is the luminal unstirred layer. Jackson has hypothesized that there is a layer of fluid adjacent to the mucosa which does not mix with the bulk of the contents, and may have different composition than mucosal or serosal fluids. The unstirred layer may be a rate limiting step for transport of weak electrolytes.

In the present study, it is difficult to assess the means of transport of acetate and propionate across cecal epithelium. Because the pH of fluid bathing both surfaces of the tissue was the same, there is not an obvious gradient unless an unstirred layer exists which had a different pH. Probably, if the luminal chamber contained a pH closer to that found in vivo, the acids may have flowed through the tissue more quickly. Transepithelial electrical potential was not measured in this study. Stevens and Stettler (71) reported that the addition of a transepithelial electrical potential to a chemical potential of the same sign did not increase the amount of acetate transported across bovine ruminal epithelium. A concentration gradient existed between the luminal and serosal chambers, and acetate and propionate may have flowed down their concentration gradient. However, though the luminal concentrations of sodium acetate and propionate were alike no matter what diet the calf had received, rates did differ due to treatment.

To determine if the process was passive diffusion, it was necessary to determine if the system were saturable. For four calves, 540, 552,

586, and 588 fed diet L1, additional parabiotic chambers containing varying amouts of sodium acetate and sodium propionate were incubated. Concentration of acid [mM] placed in the luminal chamber was plotted against acid concentration [mM] in the serosal chamber determined after incubation for 15 minutes. The curves indicate that saturation may occur at 40 or 50 times physiological concentration. The evidence suggests that passive diffusion may not be the sole mechanism of transport, and a carrier may be involved. However, more data are neeed to determine if a carrier-operated system may actually exist in vivo.

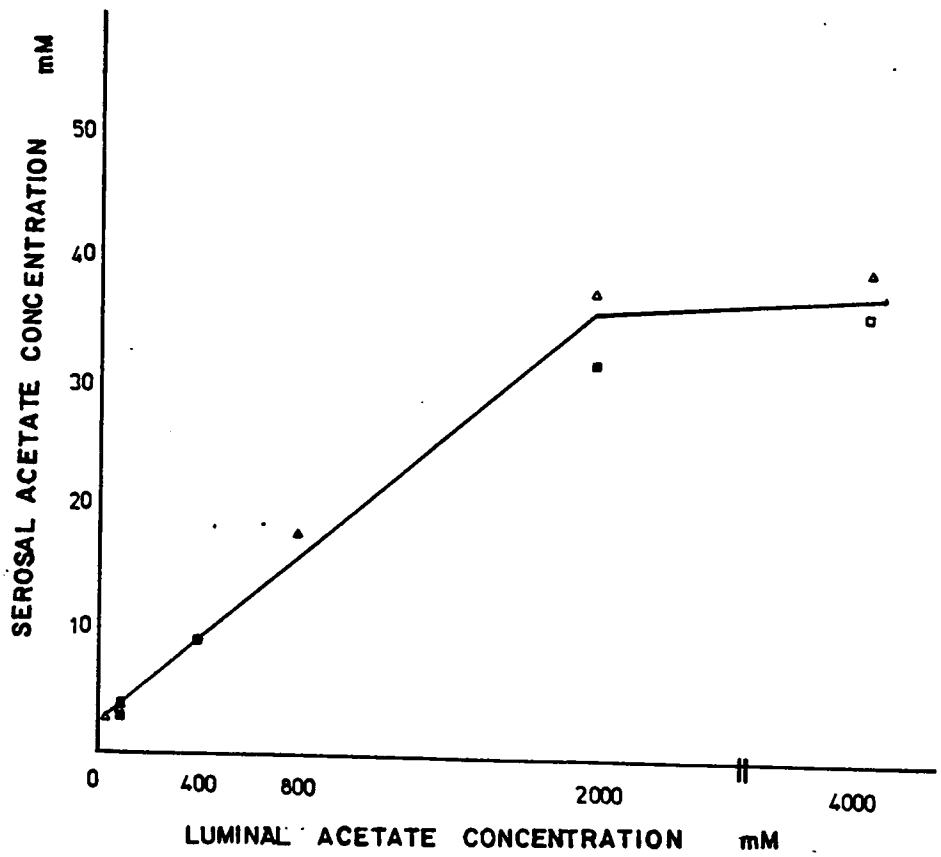

Figure 8. Effect of acetate concentration on its flux across cecal wall in vitro of calves 540 (■); 552 (□); 586 (▲); 588 (△).

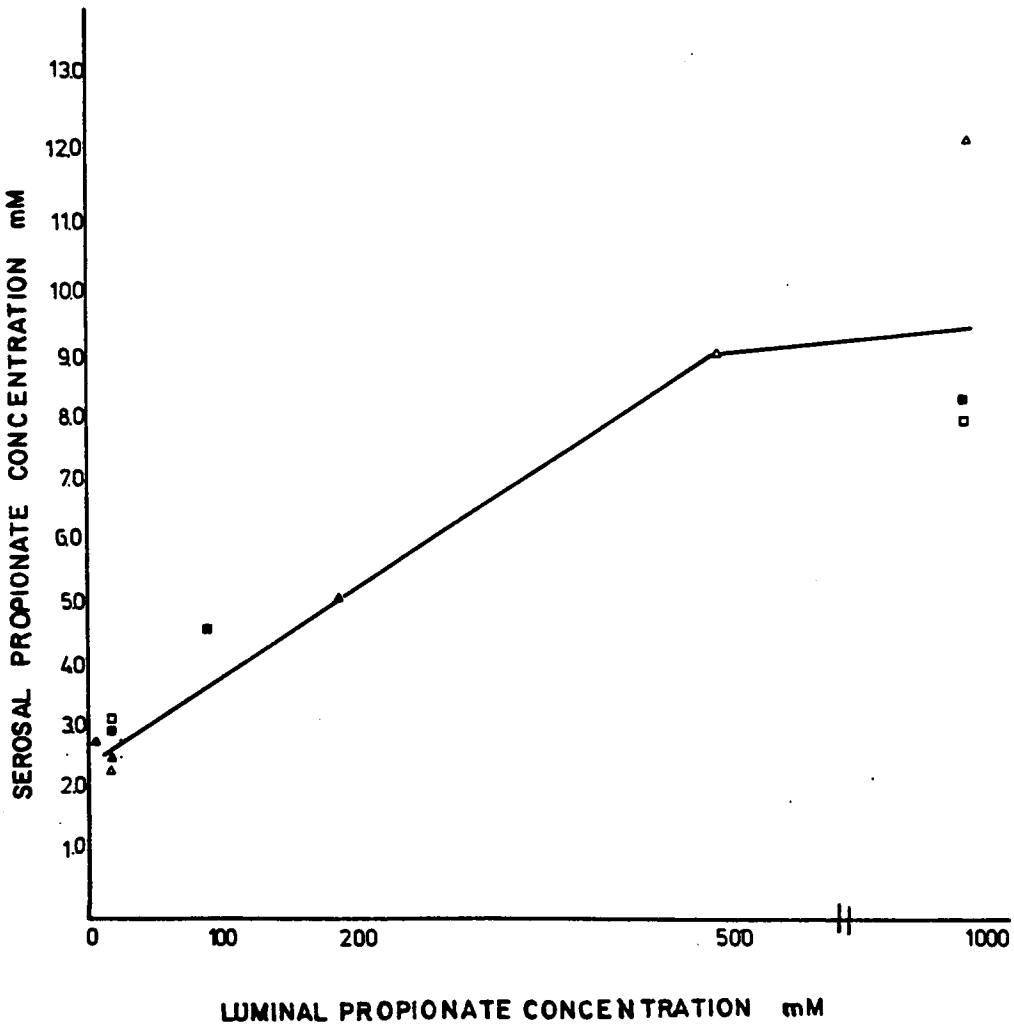

Figure 9. Effect of propionate concentration on its flux across cecal wall in vitro of calves 540 (■); 552 (□); 586 (▲); 588 (△).

SUMMARY AND CONCLUSIONS

The objectives of the study were to characterize the nature of fermentation in the hindgut and to determine the extent to which the protein and starch which bypass the rumen are utilized by microorganisms in the hindgut. Thirty-two ruminating Holstein bull calves, aged 6 to 7 months, were fed diets containing variable amounts of orchardgrass hay and grain. Amount of starch and types of protein were: [L1] low starch, SBM ; [L2] low starch, FBG; [H1] high starch, SBM; [H2] high starch, FBG.

High dietary starch stimulated hindgut fermentation, as indicated by greater numbers of viable anaerobic bacteria and lower pH in ileal, cecal and colonic digesta from calves fed diets H1 and H2. Also, total cecal VFA concentration was greater for calves consuming diets H1 and H2. With increasing starch intake, concentration of propionate and butyrate in cecal digesta also increased probably as a result of changes in hindgut microflora. Cecal and colonic digesta from calves fed H1 and H2 also had lower ammonia concentrations. Percent of total nitrogen as bacterial nitrogen in ileal digesta didn't differ due to diet, but tended to be lower for diets L2 and H2 which contained FBG. Percentage of nitrogen of microbial origin increased 50% from ileum to cecum for SBM diets and 100% for FBG diets. From cecum to rectum the percentage continued to increase for all diets except L2. Feces from calves fed diet H1 had the highest percent of total nitrogen as microbial nitrogen. Though percentage of fecal nitrogen didn't differ due to high dietary starch (H1 and H2), it seems that most of the microbial cells synthesized as a result

of the additional substrate were excreted and not utilized by the animal. High dietary starch also caused more flow of of acetate and propionate across the cecal epithelium in vitro. Increased transport rates may have resulted from changes in cecal tissue in response to greater cecal VFA concentration. Acetate and propionate flux across cecal epithelium probably is not due solely to passive diffusion; transport may involve some sort of carrier. Replacement of SBM by FBG also altered cecal fermentation, to a lesser extent. Calves fed diet H2 had significantly greater numbers of viable anaerobic bacteria in cecal and ileal digesta than calves fed the other diets. Numbers of anaerobic bacteria associated with cecal epithelium for calves fed H2 were 2 to 10 times greater than for L1, L2, or H1. Greater numbers of anaerobic bacteria in digesta of calves fed diet H2 may have been due to increased availablity of amino acids and more readily available carbohydrate. Butyrate concentration and production in the cecum also was significantly increased when calves were fed diets containing FBG. Cecal VFA production may account for approximately 3 to 5% of digestible energy intake, which is a small but perhaps important contribution to the animals' overall energy balance.

Very little research has been done on hindgut fermentation in cattle. Most research on hindgut fermentation in ruminants has been done with sheep. Results of the present study indicate that fermentation in the hindgut of cattle is similar to fermentation in the ovine hindgut. Numbers of viable anaerobic bacteria in the ovine gastrointestinal tract (49, 75) seem comparable to the numbers cultured from intestinal contents of the calves in the present study. Amount of digestible energy intake accounted for by cecal VFA production in calves is comparable to values for

sheep (15, 20). Hindgut fermentation differs to some extent between sheep and cattle. The concentration of total VFA and butyric acid in the cecum of calves in this study were higher than values reported for sheep in several studies (18, 15, 16, 45). Also, the ammonia concentration in cecum and colon was less than reported for sheep (18, 26, 80).

Fermentation in the hindgut of nonruminant herbivores and omnivores has similarities and differences to fermentation in the bovine hindgut. Concentration and proportions of VFA (19) and numbers of viable anaerobic bacteria in the hindgut of pigs (1) and rats (44) may be similar to the bovine hindgut. Fermentation characteristics may differ because of alternative digestive strategies. For example, high dietary fiber stimulated fermentation in the hindgut of rats (17) whereas in the present studdy, high dietary starch stimulated microbial activity in the bovine hindgut.

The mechanism of transport for VFA across the cecal epithelium has been studied in sheep and some nonruminants (48, 53, 76). Results from the present study indicate that absorption of VFA from the bovine cecum is probably similar to other species. The mechanism of transport remains unclear, but it could be carrier mediated. Results from this study suggest that the system is saturable.

Fermentation in the bovine cecum may be more important than previously thought. Cattle obviously benefit from the production of VFA and ammonia in the cecum and colon, but several questions about the significance of fermentation in the hindgut remain unanswered. Further research is needed to determine if cattle can absorb amino acids from their cecum and colon. If amino acids are absorbed from the bovine hindgut, then microbial ni-

trogen will be more effectively utilized. Also, mechanisms of transport for nutrients across the cecal and colonic epithelium need to be elucidated.

APPENDIX A. CECAL FLAGELLATES

Eight additional Holstein bull calves were used for this analysis. Two calves were assigned to each diet and fed for 17 days prior to slaughter. On the day of slaughter the cecum was excised as described previously. Rumen fluid was obtained by squeezing rumen contents through 8 layers of cheesecloth. The pH of cecal contents and rumen fluid was determined using a glass electrode (Orion Research Ionanalyzer Model 601A). Cecal contents were diluted with PRAS using the V.P.I. Culture System.[8] One ml of the 10^2 dilution was placed in a Sedgewick Rafter Counting Chamber. Living flagellate protozoa within the area of the microscope delineated by a grid were counted. Number of flagellate protozoa/ml were calculated as follows:

$$\frac{[\text{Total \# of protozoa} \div \text{\# of slides}] \times [\text{Dilution Factor}]}{[\text{\# of areas counted on one slide}] \times [\text{Volume above area}]}$$

Results (not analyzed statistically) are presented in Table 17. Flagellate protozoa were not found in digesta of two calves, possibly because of an extremely low pH. Photographs of a flagellate protozoan from a sheep's cecum and steer's cecum, are shown in figures 10 and 11.

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[8] Bellco Glass, Vineland, NJ

Table 17. Numbers of flagellate protozoa and pH of cecal fluid and pH of rumen fluid[1].

Treatment[2]	Protozoa[3]	Cecal pH	Ruminal pH
L1	1.9×10^5	6.05	6.11
L1	1.7×10^5	5.90	6.10
L2	4.8×10^5	6.35	6.75
L2	2.2×10^4	6.20	6.30
H1	0	—	5.45
H1	1.3×10^5	5.73	5.85
H2	0	5.05	6.18
H2	1.7×10^5	5.62	5.74

[1]Results were not analyzed statistically. Each row contains observations from one calf. There were two calves for each treatment.

[2][L1] low starch, soybean meal (SBM); [L2] low starch, fishmeal plus dried brewers' grains (FBG); [H1] high starch, SBM; [H2] high starch, FBG.

[3]per ml

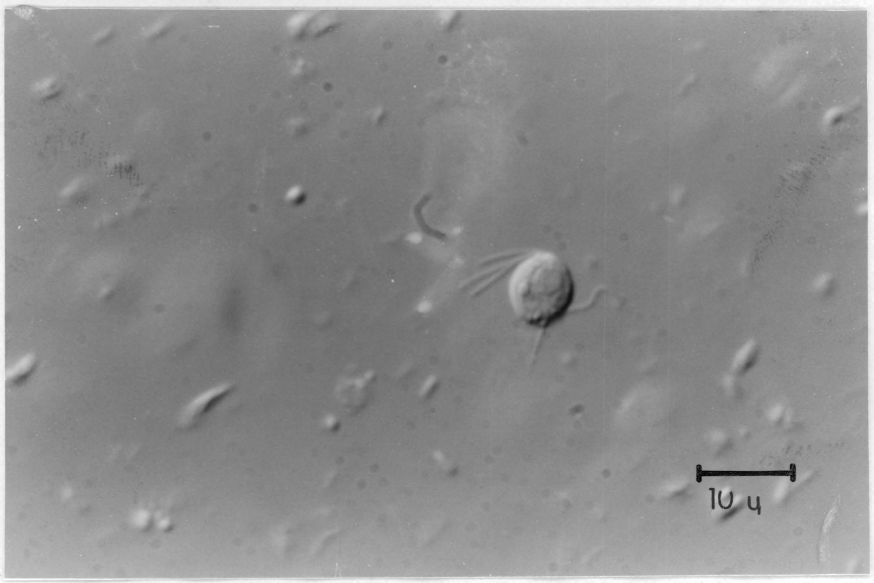

Figure 10. Flagellate protozoan from a sheep's cecum. x 1828

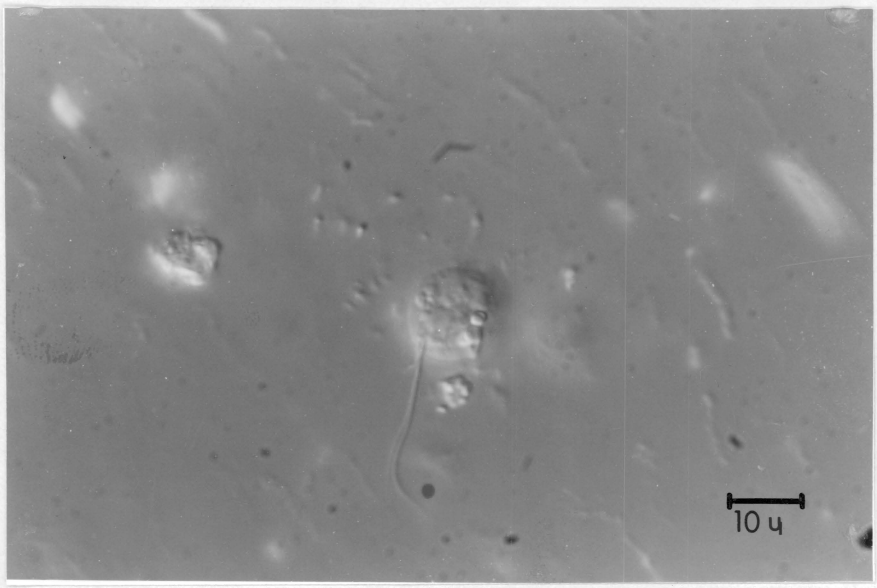

Figure 11. Flagellate protozoan from a steer's cecum. x 1828

APPENDIX B. CYTOSINE AS A MICROBIAL MARKER

Bacteria from rumen fluid collected from the additional calves were harvested to calculate the ratio of microbial nitrogen to microbial cytosine. A separate value for each dietary treatment was calculated using the data from two calves per treatment. The microbial fraction of rumen fluid was separated using the procedure of Meyer et al. (46). After separation bacterial cells were resuspended in distilled water and frozen at -20°C. Frozen cells were lyophilized, ground through a 1 mm screen [9], and analyzed for nitrogen (7) and cytosine as described previously.

Percentage of total nitrogen in digesta and feces as microbial nitrogen was calculated as described in Materials and Methods using ratios for each diet, instead of 1.838. Results are in Table 18. Because of larger ratios, the values for all diets are higher than when the factor 1.838 was used. Replacement of SBM by FBG resulted in signifcantly lower microbial nitrogen fractions in ileal digesta, colonic digesta, and feces of calves fed high starch diets. Percentage of fecal microbial nitrogen was also significantly greater due to high dietary starch.

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[9] Cyclone sample mill, VD Corp., Boulder, Co.

Table 18. Percentage of total nitrogen in digesta and feces as bacterial nitrogen using factors[1] calculated for each diet[2].

	Treatments[3]				
	L1	L2	H1	H2	SE
Ileum	26	18	29	17	5
Cecum	39^{ab}	41^{ab}	45^{b}	33^{a}	3
Colon	40^{a}	39^{a}	50^{b}	36^{a}	3
Feces	43^{a}	40^{a}	57^{b}	38^{a}	2

^{a,b}Means on the same row with unlike superscripts differ ($p<.05$).

[1][L1] 2.065; [L2] 2.294; [H1] 2.278; [H2] 1.875.

[2]Values are least square means for four calves per treatment.

[3][L1] low starch, soybean meal (SBM); [L2] low starch, fishmeal plus dried brewers' grains (FBG); [H1] high starch, SBM; [H2] high starch, FBG.

Table 19. Preparation of clarified cecal and rumen fluid.

1. Rumen and cecal contents were collected from dairy bulls and steers at the meats lab.

2. Rumen and cecal contents were squeezed through cheesecloth into 1 liter flasks which had been flushed with anaerobe grade CO_2

3. Rumen fluid was allowed to stand for 20-30 min until the particulate matter had settled out. Then the clear fluid was siphoned off into another 1 liter flask being flushed with CO_2.

4. The rumen fluid was transferred to 500 ml round bottom flasks and autoclaved in a press at 15 p.s.i. for 25 min.

5. Cecal fluid was centrifuged at 39,200 x g, and the supernatant was transferred into 100 ml dilution bottles being flushed with anaerobe grade CO_2. The dilution bottles were autoclaved in a press at 15 p.s.i. for 15 min.

6. Autoclaved rumen and cecal fluid were stored at 4 °C until use. Before being added to media, both were centrifuged at 48,400 x g for 15 min.

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