THE EFFECT OF CARBOHYDRATES ON THE CARBOHYDRASE ACTIVITIES OF THE YOUNG BOVINE

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
DANNY G. BRITT
1972

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ABSTRACT

THE EFFECT OF CARBOHYDRATES ON THE CARBOHYDRASE ACTIVITIES OF THE YOUNG BOVINE

By

Danny G. Britt

A carbohydrate-free (CHO-free) ration was formulated to determine the effect of dietary carbohydrates on digestive enzymes in the young calf. This diet was composed of 30% fat and 70% of a protein premix which contained 92% protein, 1% vitamins, 4.5 % dicalcium phosphate, 1.5% trace mineral salt and 1% of a suspensory agent. Calves consumed this ration and gained weight; however, an adaptation period of about three days was necessary for the CHO-free diet.

Sixteen male Holstein calves were fed the CHO-free diet from one to three weeks of age and were then fed four different diets for the next two weeks. These diets were: (a) lactose, (b) galactose, (c) glucose, and (d) CHO-free. The carbohydrates were incorporated into the diet and fed

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at 4.5 g/Kg body weight. Calves were sacrificed at 5 weeks of age.

Lactase activities (per Mg protein x 10^{-2}) in the anterior one-third of the small intestine for the respective diets were 10.2, 7.8, 5.8, and 4.7. Values for diets A and B were greater ($P < 0.05$) than D. These data suggest that galactose is the active moiety in inducing lactase activity on diets high in lactose.

Intestinal maltase followed a trend similar to lactase with diet A significantly greater ($P < 0.05$) than D in the anterior one-third of the small intestine. There were no significant differences between any of the treatments in the lower section of the small intestines. Pancreatic amylase activity was less ($P < 0.05$) on the glucose (31.35) than on the CHO-free diet (65.18).

Fasting blood glucose was depressed on the CHO-free diet and failed to recover with the addition of any of the three carbohydrates. Removal of glucose from the blood appeared greater after calves had received carbohydrates for two weeks than after two weeks on a CHO-free ration. In this study increases in blood glucose did not parallel increases in intestinal lactase and were not satisfactory

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indicators of lactase activity. Increases in blood glucose after feeding galactose were low compared to either the glucose or lactose diets.

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By

Danny G. Britt

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Outline of Studies:

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I. INTRODUCTION

Lactose constitutes 40% of the solids in cow's milk and is the carbohydrate of preference in diets for young calves. Previous research has shown that the young calf's tolerance to lactose in liquid rations can be increased by increasing the amount of this carbohydrate in the diet. This increased tolerance appears related to stimulation of higher levels of lactase in the calf's small intestine. However, little is known regarding the mechanism of this increased lactase production.

Studies in humans have shown that dietary fructose (a hexose moiety of sucrose) stimulated increased sucrase activities. The primary objective for these studies was to investigate the possibility that a parallel situation existed in the calf with galactose being responsible for the increased lactase activities observed when lactose is increased in the diet.

Because of the possibility of all the simple sugars influencing intestinal disaccharidase activities I decided

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it would be advantageous to develop a carbohydrate-free (CHO-free) ration for calves. This type of diet for calves had not been previously reported in the literature. The formulation of and effects to calves on this CHO-free ration became major objectives of this study.

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II. LITERATURE REVIEW

Milk is important in the nutrition of mammals and survival in the early stages of life is dependent on their ability to digest and utilize the milk constituents. Cow's milk contains approximately 4.5 per cent lactose while human milk contains 6-7 per cent lactose which is only 16 per cent as sweet as sucrose (78). Lactose thus constitutes a major portion of the total solids of milk with fat contributing approximately 3.8 per cent and protein 3.4 per cent (33). This carbohydrate constituent of milk accounts for approximately 40% of the total solids and occurs either as free lactose or as lactose containing oligosaccharides (18). The equilibrium between free and bound lactose in unheated milk occurs at a concentration of eight parts free lactose to one part bound lactose (35).

Site of Lactose Digestion and Location of Lactase

It has been known since the nineteenth century that lactose, a disaccharide composed of glucose and galactose,

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requires a beta-galactosidase (lactase) for hydrolysis and absorption. The site of lactose digestion was early recognized to be the small intestine. Cajori (13) identified a lactose splitting enzyme in the duodenal and jejunal mucosa of the dog, in water extracts of the mucosa and to a lesser extent in jejunal juices and in the liver. Dahlqvist and Borgstrom (25) found lactose was digested and absorbed in the duodenum and proximal jejunum in the human; maltose in the jejunum and proximal ileum and sucrose in the distal jejunum and ileum. In contrast to this Koldovsky et al. (57) found greater beta-galactosidase activities in the ileum than in the jejunum of infant rats, but the accumulation of reducing substances in the serosal fluid was equal. He concluded that there could be either increased active transport or different beta-galactosidases in different sections of the small intestines. Siddons (93) found disaccharidase activities were located mainly in the small intestine of the young bovine and showed a non-uniform pattern of distribution along the small intestine. Lactase was highest in the proximal and middle section and maltase activity was highest in the distal section.

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There was controversy in the early twentieth century over whether lactose digestion occurred in the lumen or in the mucosal layer of the small intestine. Early work (14) indicated that sucrose and lactose were absorbed much more rapidly than would be predicted on the basis of the enzymatic activity of the juice. Starch disappeared from an intestinal loop at a rate commensurate with the amylase content of the juice. The workers concluded that either the disaccharides were being absorbed unhydrolyzed or hydrolysis occurred at the mucosal surface. Borgstrom et al. (10) in a similar calculation found that lactose was absorbed where the luminal content of lactase was very low or non-existent. Wasserman (108) studied the movement of radioactive lactose from the center of the intestine into the mucosal cells. This study indicated that lactose can penetrate directly into the mucosal cells before hydrolysis and can be hydrolyzed inside the cell.

Mucosal hydrolysis was further verified by Dahlqvist and Borgstrom (25) in humans who were fed disaccharides and intestinal contents were removed by intubation techniques. The low level of hydrolysis which occurred in the contents and the low glycosidic activities relative to the calculated amount of carbohydrate absorbed

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indicated that disaccharides are absorbed unhydrolyzed followed by subsequent intracellular hydrolysis. In an in-vitro hydrolysis of disaccharides using hamster tissue, Miller and Crane (68) calculated the relative distribution of the products of hydrolysis between the tissue and medium. These concentration relationships indicated that hydrolysis of the disaccharides, sucrose, and maltose, occurs within the epithelial cell.

In further attempts to determine the location of the disaccharidases, Miller and Crane (69) isolated epithelial brush border membrane as a morphologically distinct unit from homogenates of intestinal mucosa of hamsters. Virtually all the invertase and maltase activities were found in this fraction of the homogenate. Dahlqvist (23) reported that in humans disaccharidase activity comes from at least 6 separate enzymes. These were composed of 5 different alpha glucosidases, four of which have maltase activity, and the fifth lactase which hydrolyzes both β-glucosides.

Eichholz (36) has since isolated five fractions by density-gradient centrifugation from tris disrupted intestinal brush borders from hamsters and found lactase activity in both the major and minor membrane components.

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He has also digested isolated brush border membranes with low concentrations of papain and obtained a multienzyme particle containing disaccharidase activity which could be separated on Sephadex G-200 columns (37). Additional support for two lactase components was obtained by Swaminathan and Radhakrishnan (103, 104). Chromatography of proteins from monkey intestinal mucosa on DEAE-Sephadex gave two peaks possessing beta-galactosidase activity. Both fractions hydrolyzed lactose and cellobiose and were inhibited by tris, urea, and gluconolactone.

Effect of Age on Lactase Activity

Early studies showed that young mammals have greater lactose hydrolyzing ability than older animals. Huber et al. (50) reported lactase activity twice as high in newborn calves as in six-week-old calves. No sucrase activity has been found in calves and maltase levels were low and did not change with age (32, 50, 105). Siddons (93) later reported similar results.

Okamoto et al. (74) found efficient glucose and lactose utilization in calves when measured by blood glucose increases after oral administration of these

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carbohydrates. No apparent hydrolysis of sucrose, dextrin, or starch was detected. Response to lactose was greater at 3 and 4 weeks than at 1-2 weeks. Dollar and Porter (32) used calves to 4 weeks of age and reported similar trends. Amylolytic activity of the pancreas and disaccharidase activities of the small intestine showed excellent correlation with the blood sugar response.

Intestinal lactase activity was also shown to be highest in newborn rats and pigs. During the suckling period the activity gradually decreased to 10% of the initial value and remained constant during later life (43). In the same study no distinct decrease in lactase activity with age was observed in guinea pigs. Intestinal sucrase increases with age in rats and it is 2/3 of the adult value at 23 days (9). Walker (106) has reported constant lactase values in sheep between one and five weeks of age.

Upon examination of postmortem intestinal specimens from premature and full term human fetuses, Auricchio et al. (6) found that activity of all disaccharidases was present from 3 months of gestation. Also, lactase activity did not reach a maximum until late in gestation and was significantly higher in infants that had survived for a

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Disaccharidase Stimulation and Adaptation

Weinland (110) as early as 1899 suggested lactose might stimulate lactase synthesis and this question to date has not been completely settled. Plimmer (76) offered some of the early proof that lactose adaptation does not occur. Heilskov (45) found a decreased lactase activity with age in the intestines of calves and rabbits and could not confirm lactase adaptation. Fisher et al. (40) found no lactase adaptation after lactose feeding.

As suggested by Herzenberg and Herzenberg (47) lactase adaptation may occur in two ways; either an increased concentration of enzyme per unit of protein or an increased amount of protein with the same enzyme concentration per unit of protein. Riggs and Beaty (83) have shown that during adaptation the weight of the intestinal mucosa of rats increases about 50 per cent and, specific activity was not altered. Fischer et al. (39) reported that lactose disappeared faster from the intestine in adapted animals than non-adapted.

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DeGroot and Engel (30) found lactose had a growth retarding effect when 10, 15, or 25 per cent was included in the diet of rats. Replacing the lactose with corresponding amounts of the constituent monosaccharides (glucose and galactose) resulted in a considerable increase in growth. The decreased growth was attributed to an enzyme (hydrolyzing ability) deficiency. Cargell et al. (16) also noted a slow rate of hydrolysis of lactose in young rats fed a 68 per cent lactose diet. A slight increase in total lactase was shown while specific lactase activity decreased 30 per cent. In adult rats total and specific gut lactase increased 50 to 60 per cent after nine days on the high lactose diet. This effect could not be duplicated with glucose and galactose and no increased lactose hydrolysis could be shown in rats perfused in vivo with lactose (11). Cain et al. (15) have shown lactase stimulation upon force-feeding lactose.

No increase in lactase activity was observed when rats and guinea pigs were maintained on diets containing 25 per cent lactose for several months after weaning (43). Reddy et al. (80) found that lactose increased lactase activities in both germfree and conventional rats at 30 days of age but not at 60 days. The changes in

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Normal male rats fed a 70 per cent sucrose diet had sucrase activities similar to those of the controls. However, animals fed a carbohydrate-free, high-casein diet for 1 day or longer had significantly lower sucrase activities than controls (9). Deren et al. (31) also found a stimulation of disaccharidases when a diet containing carbohydrate was fed to fasted rats. Sucrase was increased twofold but lactase was unaffected. The sucrose diet gave the greatest response in total sucrase however the specific activity of sucrase was unaffected. Maltase, palatinase, and sucrase activities in rats increased significantly after feeding a low-protein or protein-free diet; however, lactase increases were not significant (102). It was suggested that this was an adaptation to a high carbohydrate diet. In contrast, Prosper et al. (77) found that young rats deprived of protein up to 45 days did not have a changed level of lactase, sucrase, or maltase in any segment of the intestine.

Larner and Gillespie (61) found no difference between oligo 1-6 glucosidase, maltase, and invertase

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activities in intestinal extracts prepared from germ-free or non-germ-free rats. They concluded that intestinal microorganisms are not responsible for the production of these intestinal enzymes. However, Reddy (80) found germ-free rats had higher disaccharidase levels than conventional rats. In digestibility studies, microbial breakdown products of disaccharides were not beneficial to the calf (32).

Lactase, sucrase, and maltase activities were reduced to 50% of control values in both jejunal mucosal homogenates and brush border preparations from puppies with a severe iron deficiency (48). Methotrexate gave a significant depression of lactase, maltase, and alkaline phosphatase in the intestinal mucosa of rabbits (1).

An early experiment (42) in calves fed synthetic milk demonstrated that lactose was better utilized than glucose or corn starch and lactose gave feces a better consistency. Raven and Robinson (79) did not find scouring in calves fed a diet containing 66% lactose. Huber et al. (54) found an increased specific activity of lactase and increased total lactase levels when 5% or 15% lactose was added to whole milk in the rations of male Holstein calves from 3 to 77 days of age. In contrast, Siddons (93) found

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no marked differences between the carbohydrase activities of calves fed solely on milk and those of calves given a concentrate-hay diet from 6 weeks of age.

In further work Huber et al. (53) found gains significantly greater on lactose than on starch in 8-month-old steers fed liquid diets by nipple pail. Maximum increases in blood sugars following administration of lactose averaged three times that for starch. Calves conditioned to lactose gave higher blood sugar responses to both the lactose and starch test meals than those conditioned to starch. In pre-ruminant calves glucose, galactose, and lactose were readily utilized by all calves and the utilization of glucose and galactose increased with age, whereas that of lactose remained constant (94).

Velu et al. (105) found that glucose produced greater blood sugar responses than lactose and galactose during the first 6 weeks of age in calves, while lactose and galactose gave slightly higher responses at one week than at 3 or 6 weeks. In calves varying in age from 22 to 600 days, ingestion of sucrose and maltose caused diarrhea at all ages; but diarrhea due to glucose and lactose was more frequent in the older animals (51). Rojas et al. (91) found calves utilized dietary lactose very effectively up

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to 6 weeks of age; however, when lactose was doubled there was an increase in galactose excretion equal to 8% of the ingested lactose and an unthriftness of the animal was noted. Supplementation of 3.5% lactose to a basal vegetable milk replacer ration was not beneficial (72).

Lactose Digestion in Humans

In recent reviews Simoons (95, 96) concludes that the group differences found in primary adult lactose intolerance among the world's peoples are largely genetic in origin. He hypothesized that a form of selection had occurred under which tolerance became commonplace among adults in an ethnic group after consumption of lactose-rich milk over many generations.

Intolerance of lactose has been shown to exist as a cause of milk intolerance in adults (5, 21, 22, 34, 56, 67). Subjects with lactose intolerance exhibited a flat blood glucose curve upon oral administration of lactose. They had mild diarrhea and bloating after ingestion of 1 or more glasses of milk. Twenty of twenty-one subjects with normal lactase activity had a maximum blood sugar rise of 25 mg/100 ml of blood or more after ingestion of 50 gm

of lactose while eleven of fourteen hypolactasia patients had rises less than 25 mg/100 ml of blood (67). In most of these studies lactose intolerance appeared to be a trait developed in adult life. Tolerance tests with glucose plus galactose gave normal increases (5).

Lactase deficiency has been found to cause impaired lactose digestion in these humans (26, 29). Lactase assays revealed two distinct groups which corresponded to the two groups which were separated on their ability to absorb lactose. Only 13.7% of non-absorbers drank milk but they had consumed milk early in life (22). The ability to absorb lactose can be restored with the addition of lactase to a lactose test meal (56, 71). Some cases of milk intolerance have been identified in children who responded favorably to omission of lactose from the diet; and upon mucosal assay revealed lactase deficiencies (27).

Symptoms of milk intolerance may be precipitated by intestinal lesions or exaggerated when intestinal surgery is performed (56, 67). Delayed gastric emptying may contribute to a slow rise in blood sugar after oral intake of lactose, so the limitations of the oral lactose tolerance test to diagnose lactase deficiency are apparent (80); but it is useful to screen subjects for lactase

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deficiencies (75) as is also the pH of the stool material (27). A generalized lactase deficiency is common in patients having extensive damage to the small intestinal mucosa (92). Kwashiorkor does not exert an abnormal effect on lactase. Five-and-one-half years after exposure to the disease no abnormal digestive enzyme levels were found (20). Although, malnourished infants exhibited an immediate disaccharidase deficiency which was believed partially responsible for the diarrhea observed in marasmic children (8). Diarrhea induced by drugs, ionizing radiation, etc. may also decrease disaccharidase levels (64). Obese subjects undergoing a 2 to 4 week fast did not have altered small intestine histology but decreases in sucrase, maltase, and palatinase activities were observed (59).

Lactose intolerance has been shown to be more prevalent in certain races. In a survey of 40 healthy subjects, 20 Negro and 20 white, Bayless and Rosensweig (7) reported that 19 of the 20 Negroes gave a history of milk intolerance while only 2 of the whites gave such a report. Lactose intolerance occurred in 20 of the 21 milk intolerant subjects. Deficient lactase levels were observed in 14 of the 20 Negroes and in only one of 20 whites. The occurrence of lactase deficiency in Negroes has been further

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Also, the incidence of lactose intolerance was found to be higher in the oriental race (5, 7, 17). Huang and Bayless (49) studied orientals living in the United States and found responses to milk and lactose similar to native orientals and concluded that lactase deficiency in orientals is probably genetically controlled. Alzate et al. (4) have also reported a high incidence of lactose intolerance in Colombian Chami Indians in which 14 of 24 healthy adults were shown to be lactase deficient.

Dietary Regulation of Glycolytic Enzymes and Disaccharidases in Humans

Cuatrecases et al. (22) found no increases in lactose absorption or jejunal lactase activities in response to dietary lactose fed for periods up to 5 days in seven lactose non-absorbers. Four lactose absorbers treated similarly for 45 days also showed no change. The feeding of 50 grams of lactose daily to 50 young adult Thais for a 4-week period did not alter the lactose tolerance test or levels of lactase (58). Orientals living and raised in the United States, although receiving milk products, are

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still lactase deficient (49). In a study of galactosemic patients Kogut et al. (60) found that 90% of those tested hydrolyzed lactose in spite of its exclusion from their diets since early infancy. This and previous reports suggest that intestinal lactase in humans does not appear to be dependent upon lactose intake.

However, Rosensweig and Herman (85, 87) have reported that in humans dietary sucrose or fructose caused increases in jejunal sucrase and maltase activities. The sucrase to lactase and maltase to lactase ratios also increased significantly. Fructose feeding gave results similar to sucrose, so fructose (a monosaccharide) appears to be the active moiety of the sucrose molecule in stimulating enzyme activity. Neither lactose, galactose, glucose, nor maltose had any effect on enzyme levels.

After sucrose was introduced into the diet, three to seven days were required for the increase in levels of sucrase and maltase as well as for the increases in the ratios of sucrase and maltase to lactase. When the subjects were placed on a carbohydrate-free diet three to five days were required for either a decrease or increase in enzyme concentrations coincides well with the turnover time of human intestinal epithelial cells (84, 86).

Individual sugars have been found to influence glycolytic enzymes in rat jejunum. Fructose had an adaptive effect upon fructokinase and fructose-1-phosphoaldolase while glucose exerted an adaptive effect upon hexokinase and glucokinase. The addition of calories in the form of protein to fasted rats caused a non-specific increase in the activity of all jejunal glycolytic enzymes. Changes in the jejunal glycolytic enzymes due to diet reflect those in the liver (100, 101).

Rosensweig et al. (90) have reported similar results in adaptation of human glycolytic enzymes. However in humans the time required for adaptive changes in glycolytic enzymes was one day or less (89). This differs from the 2 to 5 days required for disaccharidase adaptation, indicating that glycolytic enzymes and disaccharidases adapt by different mechanisms. In further studies of dietary regulation of enzymes Stifel et al. (98) found galactose stimulated galactose metabolizing enzymes in rat jejunum more than fructose, sucrose, or glucose.

In additional work from the same laboratory, Stifel et al. (99) have found that intestinal phosphofructokinase and pyruvate kinase are stimulated by six hormones in rats. Estradiol gave the greatest stimulation in female rats and

its effect was blocked by testosterone; while in the male rat, testosterone gave the greatest stimulation and was blocked by estradiol. In human subjects oral folic acid stimulated jejunal glycolytic enzyme activities but had no effect on disaccharidase activity (88).

Effect of Carbohydrate-Free Diets

No studies using carbohydrate-free diets in bovines were found in the literature; however, chicks on a carbohydrate-free diet, where non-protein energy came from soybean oil, exhibited growth equal to chicks fed a high-glucose diet (12). Renner (82) found that the chick's requirement for carbohydrate can be met with fat without diverting amino acids from protein to carbohydrate. In the absence of dietary carbohydrate when non-protein energy was supplied by soybean oil, diammonium citrate was less effective in promoting growth than L-glutamic acid or L-aspartic acid. Addition of carbohydrate improved the growth of chicks fed diammonium citrate (81). The feeding of carbohydrate-free diets also tended to decrease glycolytic enzymes and increase gluconeogenic enzyme activity (3), and caused a change in NAD/NADH (2). Ginsburg and

Heggeness (43) found the rate of glucose and galactose absorption in infant and adult rats fed a carbohydrate-free diet was 75% of that in animals fed high-glucose diets.

Carbohydrate Utilization in Young Calves

One to two hours after birth the whole blood content of glucose in calves averaged 62 mg per 100 ml and reducing sugars were 127 mg per 100 ml. The difference was due mainly to fructose which declined to 3 mg per ml by 24 hours. Glucose in both plasma and corpuscles increased after birth and peaked at day 2. The peak for reducing sugars in all blood fractions was at birth (111). Blood glucose averaged 95 mg per 100 ml of blood during the first week of life, declined to 60 mg per 100 ml at 5 weeks, and remained constant at this level (66, 105).

Blood sugar levels declined faster on a high roughage than on a milk diet and averaged 45 to 65 mg per 100 ml at about 8 weeks (19, 107). Blood glucose can be increased by the feeding of glucose (105). In addition to the increase in blood glucose upon feeding glucose, Siddons (94) found galactose caused a marked increase in

the concentration of total blood reducing sugars but a small rise in blood glucose. Infusion of galactose into the blood of pigs increased glucose levels (97). Davis and Brown (28) found that glucose was utilized at a rate of 8.3 grams per hour per 100 lb of body weight in two-week-old calves.

Little response in blood glucose to feeding starch has been documented in calves (41, 109). However Huber et al. (52) reported up to 79% digestibility of starch in milk replacers fed young calves. Apparent utilization increased between 10 and 24 days of age. Other workers (73) found that ground corn was not satisfactorily utilized until the calves were 25 days of age. It has been reported (51, 63) that very little response in total reducing sugars occurs when sucrose, starch, or corn carbohydrates are introduced directly into the omaso-abomosal area. Larson et al. (62, 63) reported that calves could utilize maltose at nine months and Dollar and Porter (32) reported utilization at 9 weeks. However Huber et al. (49) found sucrose and maltose caused diarrhea in calves from 22 to 600 days of age.

Grossman et al. (44) found a high carbohydrate diet increased amylase activity in rats and a high protein

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diet decreased amylase activity in the pancreas. Morrill et al. (70) found the amylase concentration and flow rates higher for calves fed all their protein from milk than from soybean sources. Hudman et al. (55) found total amylase per pig increased from 1 to 35 days.

There is evidence to suggest both adaptability and non-adaptability in lactase levels in mammals. The use of milk and milk products in the diets of children and adults in underdeveloped regions necessitates knowledge on the occurrence of adaptation if it occurs and secondly, what controls this adaptation if and when it does occur.

In this study I hope to determine whether or not lactose adaptation does occur in calves. This information will help to better understand carbohydrate digestion in the calf and what controls its digestive enzyme levels. Some extrapolation to other species may be possible; but, as already pointed out, marked species differences in the adaptive phenomenon appear to occur.

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III. MATERIALS AND METHODS

Preliminary Studies

Effect of Freezing on Disaccharidase Activity

This study was conducted to determine if changes in disaccharidase activities occurred upon freezing of intestinal tissue. Segments of small intestine from veal calves were obtained from a local slaughterhouse. Upon sacrifice the small intestine was immediately removed from the calves and segments were placed in ice and brought to the laboratory for subsequent disaccharidase analysis.

Immediately upon return to the laboratory the intestinal segments were split lengthwise and blotted dry. The mucosa was separated from the serosa by scraping with a glass slide. The mucosa was then placed in a cold metal cup of a Sorvall Omini-Mixer (Ivan Sorvall, Inc.) and homogenized in 4 parts W/V of distilled water. The homogenates were then divided into two aliquots. One portion was analyzed fresh and the other was frozen at -20C for two weeks and then analyzed for enzyme activities.

The fresh sample and frozen sample upon thawing were treated identically. The homogenates were placed in a RC 2-B Sorvall refrigerated centrifuge and spun at 4000 x g for 15 minutes to remove undisrupted cells and other debris. The supernatants were removed and the pellet discarded. The supernatant was then diluted to the proper concentration for the assay procedure as described by Dahlqvist (24). This consisted of incubating 0.2 ml of the enzyme solution with either 0.1 ml of a 0.056 molar solution of lactose or maltose in a 0.1 molar maleate solution as a preservative. The incubation was for one hour at 37°C. At this time the hydrolysis was stopped by placing the tubes in boiling water for one minute and then were cooled. Blanks were made of the substrate and enzyme solution which were immediately placed in the boiling water.

The incubation mixture was then diluted with 1.6 ml of distilled water; a 0.5 ml portion was then transferred into another test tube, mixed with 3 ml of Tris-glucose-oxidase (TGO) reagent and incubated at 37°C for 1 hour. The TGO reagent was prepared from the Glucostat glucose reagent (Worthington Biochemical Co.) in the following way: The contents of the chromogen vial were

dissolved in 1 ml of detergent solution (10 ml of Triton X-100 in 40 ml of 95% ethanol) and 2-3 ml of .5 molar Tris-HCl buffer at a pH of 7.0. The contents of the Glucostat vial were then dissolved in another 4-5 ml of the Tris buffer and the two solutions were mixed and diluted to 100 ml with Tris-HCl buffer.

At the same time a standard glucose-benzoic acid solution, containing 100 mg of glucose and 2.7 grams of benzoic acid made up to 1000 ml with distilled water and subsequently diluted so as to contain 0, 10, 30, 50 μg glucose per ml, was incubated with the TGO reagent. After allowing the color to develop for 1 hour all tubes were read in a Coleman Junior II Spectrophotometer at 420 mμ. The amount of glucose in the enzyme containing tubes and blanks was interpolated from a standard curve calculated from the absorbance of a set of standard glucose tubes.

The disaccharidase activity of the preparation analyzed was calculated in the following manner:

$$\text{Disaccharidase activity (units/ml)} = \frac{(a-b) \cdot d}{n \cdot 540}$$

a = amount of glucose (μg) found in aliquot of the incubated sample

b = amount of glucose (μg) found in aliquot of the corresponding blank.

d = dilution factor of the enzyme solution used for mixing with the substrate

n = number of glucose molecules that are liberated per disaccharide molecule hydrolyzed

Units of activity = μ moles disaccharide hydrolyzed/ min.

Optimum pH for Bovine Lactase

To determine the optimum pH for the incubation mixture when lactase and maltase were being determined, the pH in a series of solutions of the 0.056 M lactose or maltose solutions in 0.1 M maleate buffer was adjusted as follows with 1 N NaOH: 5.0, 5.2, 5.4, 5.6, 5.8, 6.0, 6.2, 6.4, 6.6, 6.8, 7.0. Supernatant from Study I was used to determine the response to variable pH. The sample preparation and incubation procedures were similar to those described in Study I.

Location of Maximum Lactase and Maltase Activity

A third study was conducted to determine where the maximum lactase and maltase activities occurred in the small intestine of the young bovine. From this work I

hoped to develop a satisfactory sampling procedure without having to remove the entire mucosa.

The small intestine was removed immediately upon sacrifice of the calves and the mesentery was quickly stripped so an accurate measurement of the distance from the pyloric sphincter could be made. Segments of 4.0 to 5.0 cm were then removed for disaccharidase analyses at the following distances from the pyloric sphincter: 0.3, 0.45, 0.6, 0.9, 1.2, 1.5, 1.8, 2.7, 3.6, 5.4, 7.2, 16.2, and 28.5 meters.

The segments were immediately placed in ice, transported to the laboratory and prepared in a manner similar to that described in Study I; however, all the homogenates were frozen in this study. Subsequently lactase and maltase activities were determined on duplicate samples of each segment. To remove the variation of different concentration of mucosa the results were calculated on activity per mg of protein, as well as on activity per ml of homogenate.

Composition of
Carbohydrate-Free Diet

In my attempt to study the effect of lactose on the disaccharidases in the small intestine I decided to feed a ration essentially free of carbohydrate up to two weeks of age and then introduce specific carbohydrates back into the diet and measure their effect on enzyme activities. No reference to such a diet for calves was found in the literature so we formulated a ration in which 70% was a protein premix and 30% was fat.

TABLE I

Composition of Protein Premix

Ingredient	Per Cent
Casein	32.0
Promosoy	60.0
Vitamins (Appendix Table I)	1.0
Dicalcium phosphate	4.5
Trace mineral salt	1.5
Super-Col (suspension agent)	1.0

The fat source was emulsified white grease from beef. Rations were fed at 1% of body weight in water at 7% of body weight.

Two Holstein male calves which had received colostrum for 3 days after birth were placed on the ration. The calves were placed in individual stalls in a heated barn and fed twice daily at approximately 8 AM and 3 PM. At weekly intervals calves were weighed and the amount of feed was adjusted. Solids for fourteen feedings were placed in plastic bags to be mixed with warm water and fat at the time of feeding. Each mixture was allowed to stand for five minutes before feeding to allow swelling of the suspension agent. Adequate time was allowed for each calf to consume its entire ration.

The calves remained on the carbohydrate-free diets for approximately six weeks and then were returned to a normal ration. After an adjustment period of 3-5 days the calves seemed to adapt to the ration and gained weight during the last 3 weeks of the trial.

Experiment

Design

Sixteen male Holstein calves which had received colostrum for three days and whole milk for three days were placed on the basal ration low in carbohydrate for two

weeks. (Additional information about the calves, Appendix, Table II.) The calves were treated in a manner similar to that described in Study IV. After two weeks on the basal ration the calves were then randomly alloted to four groups. Each group was then fed one of four rations for an additional two-week period. These rations were: basal, glucose, galactose, and lactose. Specific carbohydrates were added at 45% of the diets with the protein mix and fat making up 40% and 15% respectively.

Rations were continuously fed at 1% of body in water at 7% of body weight. Calves were maintained in a similar environment and fed in the same manner as previously described. The calves were weighed weekly and the rations were adjusted accordingly.

At the end of the fourth week on the treatment the calves were fasted for 16 hours and sacrificed by electrical shock at the MSU Veterinary Diagnostic Laboratory. Immediately after the shock the jugular vein was severed to allow drainage of blood. The calf's body cavity was quickly opened and the pancreas was removed and placed in ice. The small intestine was also removed and the mesentery was separated from the intestine.

For the first 6 M of the small intestine, a 2.5 cm segment was removed at 0.6 M intervals. The composites of these ten segments will be referred to as the upper section. For the remainder of the length of the small intestine a 2.5 cm segment was sampled at 1.2 M intervals and composited and comprised the lower section. Plastic bags containing the intestinal segments and the entire pancreas from each calf were placed in ice for transportation to the analytical laboratory.

Upon returning to the laboratory the intestinal segments were split, blotted dry, and the mucosa removed and homogenized in a manner similar to that described in Study II. These samples were then divided into 4 aliquots and frozen at -10 C in glass test tubes for subsequent analyses of lactase and maltase. Pancreas were weighed and stored at -10 C.

Blood Glucose Studies

Analysis of fasting blood glucose and blood glucose response to the diet was performed at three different dates during the treatment period. The first analysis (I) was performed after the calves had been on the carbohydrate-free

ration for 10 days; the second immediately after the calves had received their first meal of the various treatments; and the final analysis (III) at one day prior to sacrifice of the animals when they were approximately five weeks old.

Blood Sampling

On each sampling date four blood samples (A, B, C, D) were taken at different intervals with respect to feeding. In all instances collection was performed in the morning. The calves received their normal afternoon meal at 3 PM. At 6 AM after the calves had fasted for 15 hours blood samples (A) were withdrawn. The calves were then fed their morning meal in the usual manner; however, they were closely observed during eating to insure complete consumption of the meal. At 30, 90, and 210 minutes, respectively, after consumption of the meal samples B, C, and D were taken.

Blood was sampled from the jugular vein of each calf by gently restraining the calf and inserting a needle attached to a ten ml evacuated rubber stoppered glass test tube (Vacutainer; Becton, Dickinson and Company) containing 17.5 mg of sodium fluoride and 14.0 mg of potassium oxalate.

The sodium fluoride was added to prevent glycolysis and bacterial growth and the potassium oxalate was added to prevent clotting. Approximately 7 ml of blood was withdrawn at each sampling. Unnecessary excitement in the calves was avoided.

Blood samples were immediately placed in ice and transported to the laboratory where protein-free filtrates were prepared using the whole blood, barium hydroxide, and zinc sulfate. The resulting filtrate was approximately of neutral pH and the level of blood glucose was estimated by the glucose oxidase method (Worthington Biochemicals). Each sample was analyzed in duplicate and the amount of glucose was interpolated from a standard curve.

Statistical Analysis

Data were analyzed by analysis of variance procedures using a split-plot design and the significance of differences between means within location was tested according to Duncan's New Multiple Range Test. Sample calculation Appendix Table XIV.

IV. RESULTS

Preliminary Studies

Comparison of Lactase Activity of Fresh and Frozen Tissue

Lactase activities for fresh and frozen tissues were determined at three different pH values (5.6, 5.8, 6.0) because the optimum pH and stability to freezing for calf intestinal lactase were unknown. Assays on the fresh tissue were performed as quickly as possible after collecting from the animal. However, there was some delay in transporting to the analytical laboratory. The tissue was placed in ice while being returned to the laboratory and no attempt was made to keep it alive. The calf from which samples were taken was a veal calf of unknown age but presumed to be about 8-12 weeks old. Its previous diet was unknown. Table II shows that lactase activities for fresh and frozen tissue were similar between pH values of 5.6 and 6.0. Assays on the frozen and fresh tissue were performed from the same homogenate. Thus, frozen tissue was considered acceptable for lactase determination.

TABLE II

Lactase Activity of Fresh vs. Frozen Tissue

pH	Lactase Activity/ml of Homogenate	
	Fresh Tissue	Frozen Tissue
5.6	.178	.181
5.8	.216	.191
6.0	.206	.174

Optimum pH for Lactase Assays

After finding no differences in activities in fresh and frozen tissue, I used some of the remaining supernatant from the first calf to further ascertain the optimum pH for subsequent assays. The pH of incubation mixtures was adjusted with NaOH using a pH meter with a glass electrode. The pH values were not adjusted during the incubation. The results are presented in Figure 1 and show that the optimum pH for assays of lactase and maltase under conditions of this study was between 5.6 and 6.0. A median value of 5.8 was chosen at which the remainder of our assays were determined.

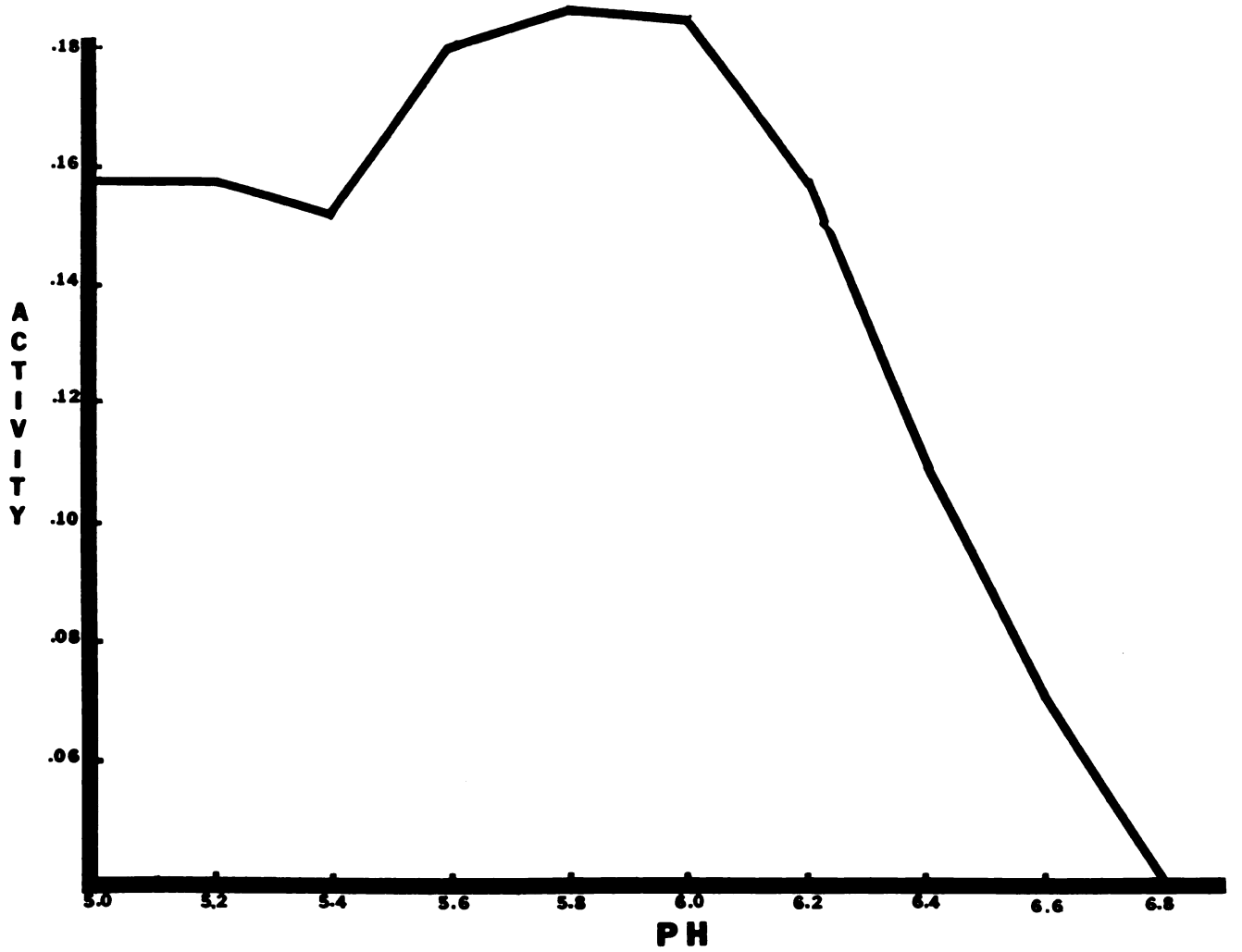

Figure 1.--Optimum pH for Assaying Lactase and Maltase Activities in the Young Bovine.

Localization of Activity in Small Intestines

The relation of lactase and maltase activities in the small intestine to distance from the abomasum is presented in Figures 2 and 3, respectively, for two veal calves. The activities of both enzymes closely parallel one another for both calves. Both lactase and maltase increased during the first 3 meters, remained at a high level for approximately the first one-third of the small intestine and then decreased to approximately zero in the latter two-thirds.

The data show that it would be impossible to remove a random section of small intestine and be assured of maximum disaccharidase activity or of a representative sample. Thus, a sampling scheme was devised by taking intestinal segments 2.5 cm in length at 0.6 M intervals from the first 6 M and at 1.2 M intervals for the remaining length of the small intestine. Mucosa was scraped from the segments and composited separately for the anterior one-third and the posterior two-thirds of the small intestines.

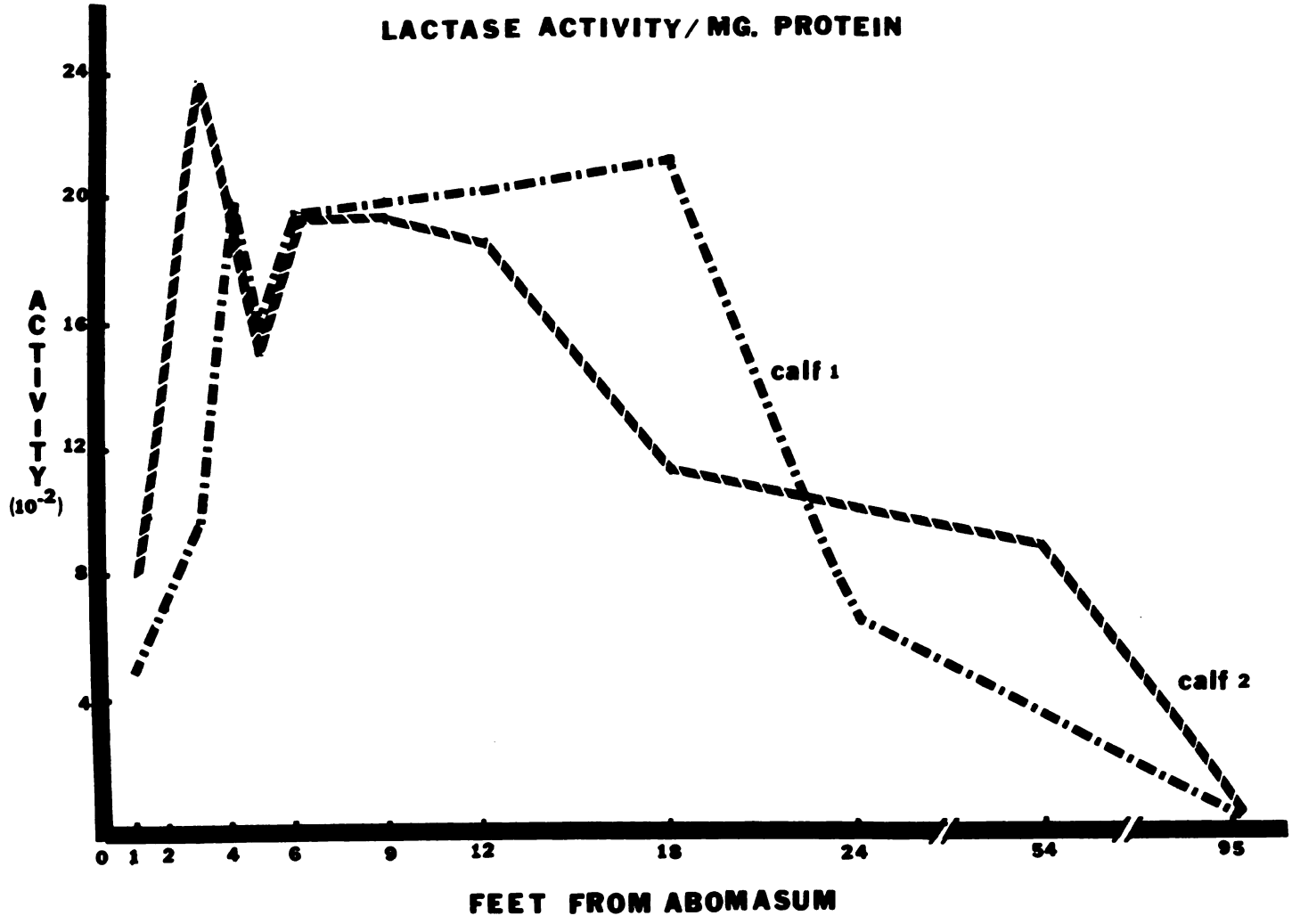

Figure 2.--Relation of Distance from the Abomasum to Lactase Activity in the Bovine Small Intestine.

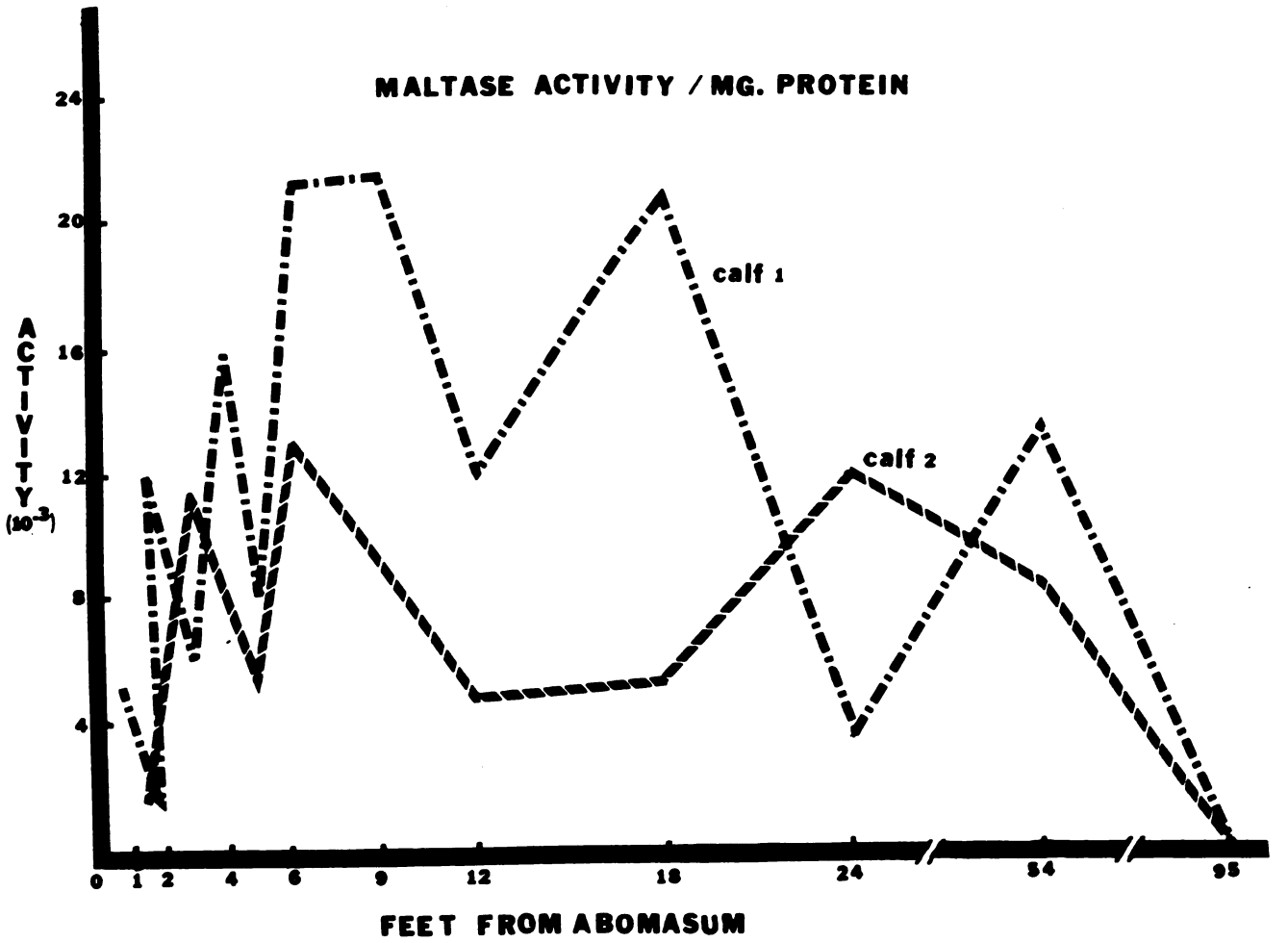

Figure 3.--Relation of Distance from the Abomasum to Maltase Activity in the Bovine Small Intestine.

Development of a
Carbohydrate-Free Diet

In an effort to formulate a carbohydrate-free diet a thorough search of the literature revealed no reference to a carbohydrate-free diet for baby calves. However, carbohydrate-free diets have been used in chickens (81, 82) and rats (43). At this stage in the young bovine's development I assumed that it was more closely related to a monogastric than a ruminant. Data from chicks grown on a carbohydrate-free diet suggest that the calf could survive on such a ration. To make a ration which could be fed as a milk replacer, a protein premix (Table I) was formulated which could be stored and then mixed with fat and water at feeding time. The ratio of protein to fat was similar to that in normal milk replacers (2:1).

Content of vitamins in this diet (Appendix Table I) was approximately twice that of the current recommendations for synthetic milk replacers (Huber and Thomas).[1] This was done because no information is available on vitamin requirements of calves on carbohydrate-free diets. The same

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[1]J. T. Huber and J. W. Thomas. Unpublished review. Vitamin requirements in young ruminants. Dept. of Dairy (D149), MSU, East Lansing.

procedure is used in our laboratory when sources of protein, which may have unknown affects on vitamin requirements in the young bovine, are being studied for growth effects.

Two male Holstein calves approximately one week of age from the University herd which had previously received colostrum were placed on the ration. The calves readily consumed the diet in quantities similar to those of normal milk replacers. During the first few feedings the solid portion (especially the minerals) had a tendency to settle out. However, this problem was alleviated by the addition of a suspensory agent (Super-Col) at 1% of the dry mix. This ration was fed for approximately six weeks. A roughened hair coat was noted after one week. However, this disappeared before the end of the 6-week feeding period. During the first few days of treatment feces were slightly fluid and exhibited a white, sticky appearance. Calves became adjusted to the ration after about one week as indicated by a cessation of weight loss. However, the color and consistency of feces did not change throughout the experiment.

The general appearance of the animals also improved after one week on the ration, and by the end of the

six-week period they were gaining weight. During the entire six-week period the calves appeared healthy. This study indicates that during the first six weeks of life the young bovine does not have an absolute requirement for dietary carbohydrates.

Experiment

Adjustment to Ration

Eighteen male Holstein calves approximately 3 days old which had received colostrum were obtained from Green Meadows Farm at Elsie, Michigan. These calves were transported 25 miles in mid-November at below freezing temperatures. For three days they received whole milk and were simultaneously treated with antibiotics to help prevent colds or pneumonia.

During the three-day adjustment period, one of the calves contracted pneumonia and died. Sixteen of the remaining seventeen calves were placed on the CHO-free ration on day four and seemed to do well. However, during the second and third weeks, three of the calves contracted pneumonia and died. These calves were replaced by calves from the MSU dairy herd.

As in the preliminary study, a slight looseness of the feces with a white, sticky consistency was noted when the calves were initially placed on the carbohydrate-free rations. This looseness disappeared in 2-3 days but color did not change.

After two weeks on the carbohydrate-free diet calves were randomly allotted to four treatment groups for the remainder of the trial. These were lactose, galactose, glucose, and control (the carbohydrate-free ration). When the carbohydrates were included in the rations a slight fecal looseness was again noted which subsided in approximately three days. During the final two weeks on the rations two more calves died. Death occurred suddenly and with no increase in body temperatures, but a slight fecal looseness was noted. Post-mortem examination revealed some lung congestion, but no overt signs of pneumonia were observed before death. None of the remaining calves showed any greater tendency for illness than did calves in the barn on normal rations.

The calves lost body-weight during the four weeks on the experiment (Appendix Table II). However, these weight losses occurred after the first and second ration changes; thus, at the end of the experiment, the calves

were gaining weight. The average weight losses on the four treatments were not significantly different but the calves on the carbohydrate-free ration tended to lose less weight than the calves receiving glucose, lactose, or galactose treatments (2.8 vs. 4.7, 3.0 and 3.5 Kg respectively).

After four weeks on the experiment the calves were sacrificed and digestive tracts were inspected for any lesions or obvious signs of malfunction. No lesions were found; however, several of the calves showed some lung congestion. A small amount of shavings (approximately 100 CC/calf), which had been used for bedding, was found in the rumen which had been non-functional as indicated by little or no papillary development.

Amount of Intestinal Mucosa

The percent of mucosa in the upper and lower sections of the small intestine is shown in Table III. These values were calculated by taking the total weight of the intestinal segments, removing the mucosa with a glass slide, and dividing the total intestinal weight into the mucosal weight. No significant differences in the mucosal

percentage were noticed between any of the groups for either the upper or lower sections or for total mucosal weight. Values for individual calves are given in the Appendix Table III.

TABLE III

Mucosal Weight and Percent Mucosa of the Upper and Lower Sections of the Small Intestine

Treatment*	Upper Section of Small Intestine	
	% Mucosa	Mucosa Wt (gm)
Galactose	57.00 ± 0.84	8.80 ± 1.85
Glucose	59.87 ± 4.08	7.55 ± 1.25
Lactose	54.49 ± 7.68	7.13 ± 1.18
CHO-Free	57.24 ± 3.62	7.52 ± 0.29
Treatment*	Lower Section of Small Intestine	
Galactose	56.60 ± 2.82	9.06 ± 1.62
Glucose	53.15 ± 10.31	9.24 ± 2.97
Lactose	54.51 ± 4.35	9.41 ± 1.86
CHO-Free	56.96 ± 1.62	10.73 ± 1.13

*Each treatment is the mean of four calves.

The composite weight of small intestinal segments and the total length of the small intestines are given in Table IV. There were no significant differences in any of these parameters. Individual calf values are presented in Appendix Table IV.

TABLE IV

Small Intestine Weight and Length as Affected by Diet

Treatment*	Total Intestinal Segment Wt (gm) Upper Section	Total Intestinal Segment Wt (gm) Lower Section	Total Intestinal Length (m)
Galactose	15.47 ± 3.40	16.40 ± 2.37	18.90
Glucose	12.65 ± 2.21	17.27 ± 3.39	20.12
Lactose	13.10 ± 1.56	17.22 ± 2.71	19.82
CHO-Free	13.19 ± 1.33	18.83 ± 1.76	19.97

*Each treatment is the mean of four calves.

Pancreatic Weights

Total pancreatic weights and the pancreatic weight expressed on body weight showed no significant differences between treatments (Table V). However, highest values were noted from calves receiving lactose. Individual values are shown in Appendix Table V.

TABLE V

Pancreatic Wt as Affected by Diet

Treatment*	Pancreas Wt (gm)	Pancreas Wt (gm/100kg BW)
Galactose	22.78 ± 4.01	11.83 ± 1.62
Glucose	21.19 ± 5.65	11.26 ± 1.69
Lactose	27.49 ± 2.71	13.91 ± 1.21
CHO-Free	23.69 ± 2.85	12.95 ± 2.34

*Each treatment is the mean of four calves.

Lactase Activity

Disaccharidase assays were performed in duplicate as quickly as possible after the samples were stored (2-4 weeks). Table VI summarizes lactase activities for the upper section of the small intestines. The individual calf values are in the Appendix, Table VI. Lactose treatment resulted in significantly greater ($P < 0.05$) lactase activity (per mg tissue protein) than either glucose or the CHO-free ration and galactose was intermediate. The same ranking (Lactose > galactose > glucose > CHO-free) was seen for total lactase and for the lactase:maltase ratios but differences between treatments for these parameters were not significant.

TABLE VI

Intestinal Lactase Activity[1] (Upper Section) as Affected by Diet

Treatment[2]	Lactase Activity	Lactase/ Maltase	Lactase Activity/ mg Protein (10-2)
Glucose	0.998 ± 0.279	8.188 ± 1.737	5.818[b] ± 1.716
Lactose	1.646 ± 0.308	9.230 ± 2.284	10.200[a] ± 0.593
Galactose	1.622 ± 0.597	8.371 ± 1.260	7.582[ab] ± 1.890
CHO-free	0.958 ± 0.100	7.633 ± 0.338	4.740[b] ± 0.571

[1]Lactase Activity = μmoles disaccharide hydrolyzed/min.

[2]Each treatment is the mean of four calves

[ab]Means not having the same superscript are significantly different ($P < 0.05$).

The lower section of the small intestine had significantly lower ($P < 0.005$) lactase activities than the upper section in all three expressions of activity (Table VII). In all expressions of activity the CHO-free group was lowest. When the activity was placed on a tissue protein basis, galactose gave the greater values followed by lactose, glucose, and CHO-free; but per gram of tissue, glucose and lactose were reversed. However, none of the comparisons approached statistical significance.

Appendix Table VII gives the individual values for lactase activity in the lower section.

TABLE VII

Intestinal Lactase Activity[1] (Lower Section) as Affected by Diet

Treatment[2]	Lactase Activity	Lactase/ Maltase	Lactase Activity/ mg Protein (10^{-2})
Glucose	.324 ± .063	2.218 ± 0.294	1.785 ± 0.321
Lactose	.304 ± .051	4.349 ± 1.993	1.805 ± 0.540
Galactose	.422 ± .203	3.336 ± 1.551	2.186 ± 0.820
CHO-Free	.232 ± .040	2.022 ± 0.316	1.152 ± 0.140

[1]Lactase activity = μmoles disaccharide Hydrolyzed/min

[2]Each treatment is the mean of four calves.

Maltase Activity

Maltase activity in both the upper and lower sections of the small intestine are given in Table VIII. When expressed on a per gram of tissue basis, a dietary trend similar to that observed for lactase activity was shown in the upper section (lactose > galactose > CHO-free > glucose). No differences due to treatment were noted in the lower section. When placed on a tissue protein basis the

lactose-fed calves were significantly ($P < .05$) higher in maltase activity than those fed the CHO-free diet. Maltase did not decline in activity from the upper to lower sections as was true for lactase. In fact, some groups were slightly higher in the lower than upper section. The individual calf data are presented in Appendix Table VIII.

TABLE VIII

Intestinal Maltase Activity[1] as Influenced by Dietary Carbohydrate Source

Treatment[2]	Maltase Activity		Maltase Activity/ mg Protein (10^{-2})	
	Upper	Lower	Upper	Lower
Glucose	.115 ± .014	.143 ± .014	.684[ab] ± .125	.766 ± .053
Lactose	.238 ± .100	.124 ± .023	1.243[a] ± .278	.761 ± .132
Galactose	.185 ± .056	.124 ± .010	.882[ab] ± .179	.723 ± .092
CHO-Free	.125 ± .000	.115 ± .010	.606[b] ± .039	.590 ± .036

[1]Maltase activity = μmoles disaccharide hydrolyzed/min.

[2]Each treatment is the mean of four calves.

[ab]Means not sharing the same superscript are significantly different ($P > 0.05$).

Pancreatic Amylase Activity

Pancreatic amylase, expressed as activity per gram of tissue, was significantly greater ($P < 0.05$) for the

CHO-Free group than the glucose group and was followed in activity by the lactose and galactose groups. On a tissue protein basis the same ranking is noted with activities for the CHO-Free being greater than the glucose fed calves; however, there were no significant differences among treatments. Individual calf values for pancreatic amylase activities are given in Appendix Table IX.

TABLE IX

Pancreatic Amylase Activity[1] as Affected by Diet

Treatment[2]	Activity/gm Tissue	Activity/mg Protein
Glucose	$31.35^{a} \pm 12.50$	.235 ± .087
Lactose	$42.08^{ab} \pm 3.54$	.351 ± .020
Galactose	$34.73^{ab} \pm 9.31$	.256 ± .063
CHO-Free	$65.18^{b} \pm 12.91$	.459 ± .087

[1]Activity = mg maltose released in 10 minutes.

[2]Each treatment is the mean of four calves.

[ab]Means not sharing the same superscript are significantly different ($P < 0.05$).

Blood Glucose Studies

Blood glucose responses to the different rations are presented as absolute values (Table X) and as changes from the fasting level (Table XI). Figures 4 and 5 give changes in blood glucose in graphic form. Values for individual calves can be seen in Appendix Tables X, XI, XII, and XIII.

In Table X the values on "A" bleeding date should be approximately equal for all calves because they all had received the CHO-Free ration for 2 weeks. Fasting blood glucose levels at this bleeding reached a very low level approximating those of an adult ruminant. Values increased slowly after consumption of the CHO-Free ration and were still increasing at 210 minutes after feeding. If values for "A" bleeding date are averaged across treatments the blood glucose expressed as mg glucose/100 ml of whole blood at 0, 30, 90, and 210 minutes are 47.5, 47.9, 52.7, and 61.0 and if expressed as a change from fasting level the values are 0.4, 5.2, and 13.5, respectively.

These results show that the calves which received the CHO-free ration attained blood glucose levels similar to the mature ruminant earlier in life than if they had been receiving a normal ration. The inclusion of

TABLE X

Blood Glucose Responses to the Various Experimental Rations (Mg Glucose/100 ml Whole Blood)

Treatment		Time After Feeding (Minutes)			
		0	30	90	210
Glucose	A	37.6	39.5	43.2	67.9
	B	36.8	85.5	116.8	128.8
	C	39.2	78.3	118.3	88.4
Lactose	A	63.8	54.8	58.5	59.1
	B	47.5	77.9	108.9	103.0
	C	45.3	70.0	100.1	66.7
Galactose	A	41.5	48.3	49.8	52.0
	B	44.6	59.1	87.2	93.0
	C	46.0	60.0	62.1	64.8
CHO-Free	A	47.2	48.8	59.2	65.0
	B	46.7	56.8	46.1	52.7
	C	43.5	41.2	40.3	42.0

A = After two weeks on a CHO-Free ration.

B = First meal after switching to a carbohydrate ration.

C = After two weeks on a carbohydrate ration.

TABLE XI

Blood Glucose Changes from Fasting Level at Three Time Periods when Fed Four Experimental Rations (Changes in mg Glucose/100 ml Whole Blood)

Treatment		Time After Feeding		
		30 Min.	90 Min.	210 Min.
	A	1.9	5.6	30.3
Glucose	B	48.7	80.0	92.0
	C	39.1	79.1	49.2
	A	9.0	5.3	4.7
Lactose	B	30.4	61.4	55.5
	C	24.7	54.8	21.4
	A	6.8	8.3	10.5
Galactose	B	14.5	42.6	48.4
	C	14.0	16.1	18.8
	A	1.6	12.0	17.8
CHO-Free	B	10.1	.6	6.0
	C	2.3	3.2	1.5

A = After two weeks on a CHO-Free ration.

B = First meal after switching to a carbohydrate ration.

C = After two weeks on a carbohydrate ration.

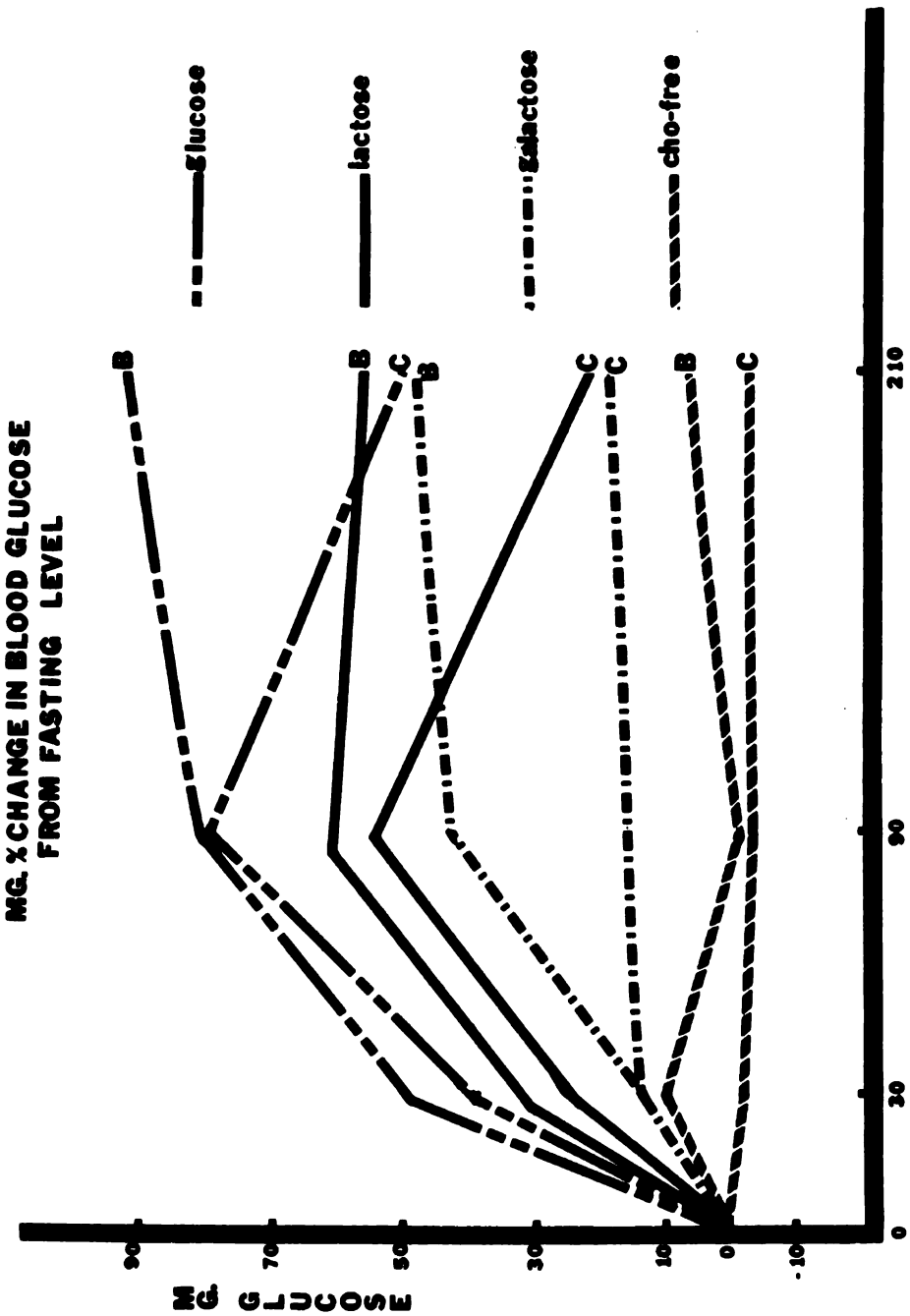

Figure 4.--Changes in Blood Glucose Levels after a 15 Hour Fast when Fed the Four Experimental Rations.
(B = First feeding of a carbohydrate ration after two weeks on a carbohydrate-free ration; C = After two weeks on a carbohydrate containing ration. Mg glucose equals mg glucose/100 ml whole blood and 0, 30, 90, and 210 equals time in minutes of blood sampling after consuming diet following a 15 hour fast.)

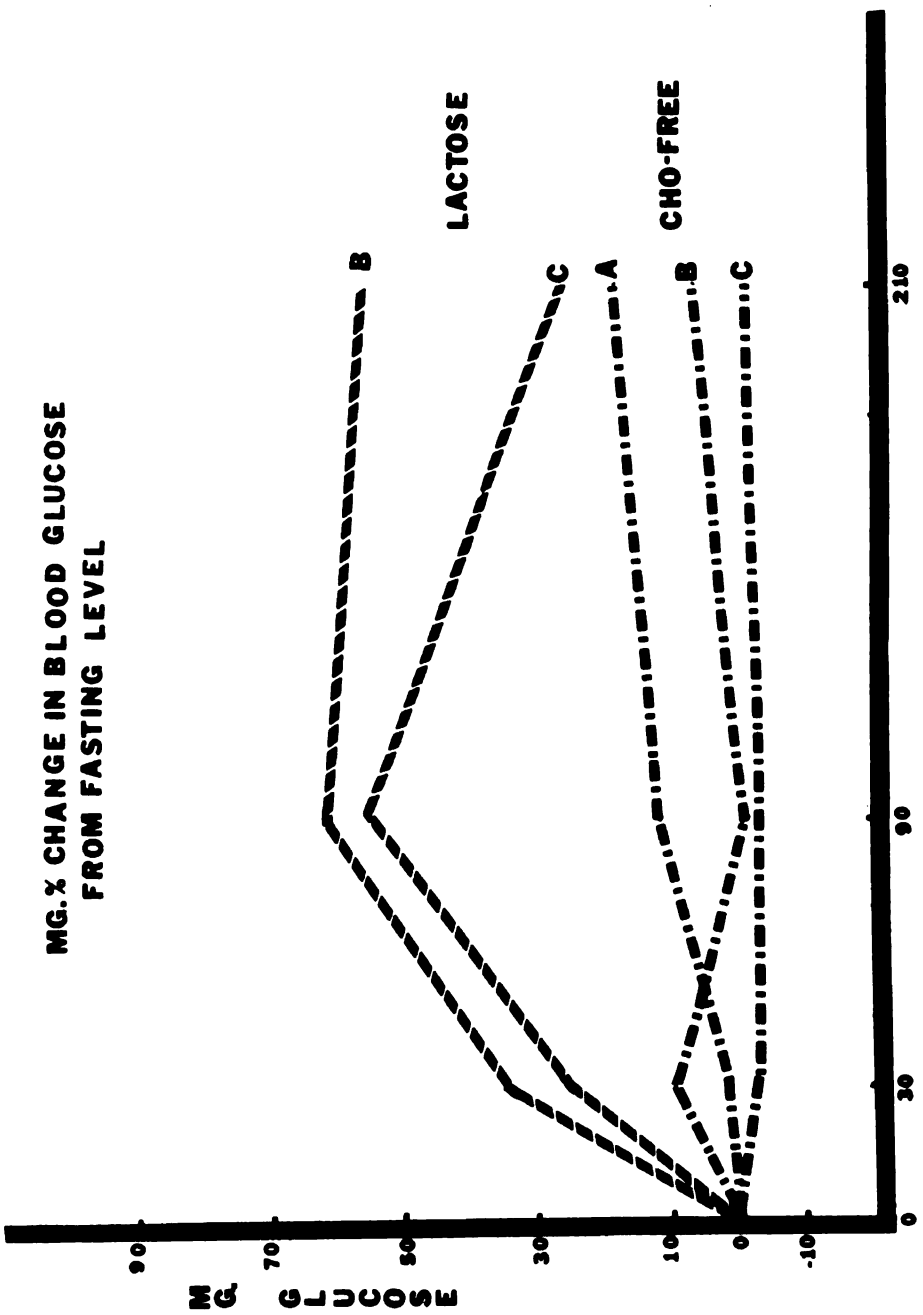

Figure 5.--Changes in Blood Glucose Levels after a 15 Hour Fast when Fed either a Lactose or a CHO-Free Diet.

(A = Two weeks on a carbohydrate-free ration; B = First feeding of a carbohydrate containing ration after two weeks on a carbohydrate-free ration; C = After two weeks on a carbohydrate containing ration. Mg glucose = mg glucose/100 ml whole blood and 0, 30, 90, and 210 = time in minutes of blood sampling after consuming diet following a 15 hour fast.)

carbohydrates in the calves' diets did not increase this low fasting level of blood glucose which resulted from feeding the CHO-free ration during early life. Blood glucose had not returned to fasting levels by 210 minutes after feeding. Calves which had received glucose or lactose for 2 weeks peaked at about 90 minutes and started to decline 210 minutes after feeding. Calves on CHO-free and galactose rations were still increasing at the 210-minute bleeding.

The glucose ration resulted in the greatest increases in blood glucose after feeding and the CHO-free ration the least, with lactose and galactose intermediate (Table XI and Figure 4) but, increases for lactose were much higher than for galactose. After calves had received carbohydrate for two weeks ("C" bleeding date) they reached their peak in blood glucose at a similar time as they did on their first day of carbohydrate feeding ("B" bleeding date). Level of blood glucose decreased at a faster rate after attaining maximum concentration at age "C" than "D." This may indicate a faster removal from the blood of adapted animals.

Figure 5 compares the lactose and CHO-free rations at the three post-feeding times and at three ages.

Maximum increases in blood glucose due to lactose feeding did not increase with time on the ration; nor did calves on the CHO-free ration seem to increase in their ability to synthesize glucose from other organic substances as the time on the ration increases.

V. DISCUSSION

Storage of Samples

Results of Studies I through III are discussed in the first few paragraphs. The calves used in the preliminary studies were veal calves obtained from a local slaughterhouse. The previous diets of these calves were unknown. Since I was not interested in absolute enzyme levels, comparative values from these calves were adequate.

Because of limited access to the diagnostic laboratory, the lengthy incubation periods specified in the analyses of the disaccharidases and the amount of time required for sample preparation, it was desirable to prepare the homogenates and freeze them for subsequent analysis. The first endeavor of this project was to determine the stability of bovine lactase to freezing. Freezing the mucosal cells in an isotonic solution might increase the disaccharidase activity by rupturing the cell walls and releasing the enzyme. This idea was based on the work of Eichholz (36, 37) in which disaccharidase activity was

found to be localized in subcellular particles. However, upon freezing no significant differences in disaccharidase activity were noted. I concluded that I was getting release of the enzyme protein with our homogenization procedure and freezing did not increase this release. In addition it appeared that freezing was not destroying the enzyme protein. This is in agreement with a report by Siddons (93) who found that carbohydrase activities in the supernatant of a mucosal homogenate remained constant when stored at -20° for at least 4 weeks.

The optimum pH for bovine lactase found agreed with the values obtained by Young et al. (111) and Siddons (93).

Sample Collection and Location of Lactase and Maltase Activity

In efforts to develop an optimum sampling procedure for representative disaccharidase activity I found a non-uniform pattern of distribution of both lactase and maltase throughout the length of the small intestine in the young bovine. This pattern of lactase activity in the small intestine agreed with that reported by Siddons (93); except that the location of maximum lactase activity in the intestine was somewhat different. Siddons (93) found maximum

activity in the middle section while mine was in the proximal 1/3. This may be due to different sampling schemes; my scheme divided the proximal 1/3 on a length basis while Siddons (93) defined the proximal section as approximately the upper 10% with respect to length. Siddons' calves were also much younger, being approximately two weeks of age while the calves in this study were 12 weeks old. Huber et al. (50) have shown that the decrease of lactase with age of calves up to 6 weeks was due primarily to a reduced activity in the middle 1/3 of the small intestine. In general the pattern for lactase observed in this study is not in disagreement with that shown by Huber et al. (50) or Siddons (93) under slightly different conditions.

A possible explanation for the retention of higher lactase in the anterior than the posterior portion of the small intestine with advancing age is that smaller amounts of lactose reaches the small intestine as age increases because of decreased dietary intake and rumen development. As less lactose enters the small intestine a larger proportion would be digested and absorbed in the anterior portion, resulting in a decreased stimulation in the posterior area.

Apparent conflict with published data was in the location of the maltase activity. Siddons (93) has reported

an increase in maltase activity in the lower section of the small intestine while we found some decrease. This might have been due to differences in diet and age between the two studies.

Calf Response to a CHO-Free Diet

A CHO-free diet was developed which was fed to two calves from one to six weeks of age. The calves survived on the diet but it was not suitable for production purposes. How this ration affects a calf after six weeks of age is not known. The ration was readily consumed by the calves and they were gaining weight and seemed to be in good health at the end of the six-week period. Up to six weeks of age the calf appears not to have an essential requirement for dietary carbohydrates if survival is used as the criterion. However, if growth or maintenance of a normal level of blood glucose is used, carbohydrates appear essential.

The reason for the poor growth response in the baby calves may have been due to a deficiency of some nutrient. Renner (81, 82) has found baby chicks can survive and grow on a CHO-free diet. The chicks apparently synthesized the

needed carbohydrate from fat without diverting amino acids from protein. The low blood glucose in calves on the CHO-free diet indicates that gluconeogenesis from fat and protein is not supplying the animals with sufficient glucose for optimum growth.

The vitamin allowances were based on a review by Huber and Thomas[1] on needs for synthetic milk diets. Approximately twice the level of vitamins recommended by Huber and Thomas were added which was thought to meet any increased requirements on such an unusual ration as was fed. In addition, it was necessary to add a suspension agent (Super-Col) because of settling of the ration when mixed with water just prior to feeding. The suspension agent had little nutritive value and only a slight ration dilution effect (1% of dry mixture).

When the calves were initially placed on the CHO-free diet an adjustment period of approximately three days was required. During this period a roughness of the hair coat and slight scouring was noted. Also, when carbohydrates were introduced back into the diet slight scouring

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[1]J. T. Huber and J. W. Thomas, Unpublished review. Vitamin requirements in young ruminants. Dept. of Dairy (D-149), MSU, East Lansing.

was again noted. A possible explanation is that the calf adapted to the CHO-free diet by increasing its proteolytic, gluconeogenic and lipid-metabolizing enzymes, with a corresponding decrease in its carbohydrate-metabolizing enzymes. When carbohydrates were reintroduced, a re-adaptation was needed.

This hypothesis is further substantiated by Solimano et al. (102) who found that there was a rise in intestinal disaccharidases and a fall in peptidase levels when rats were placed on a high carbohydrate, low protein diet. Weight changes also support this hypothesis in that the calves initially lost weight when placed on the CHO-free diet but gained weight after adjustment to the ration. When carbohydrates were re-introduced into the ration calves initially lost weight but at the time of sacrifice they were gaining weight.

The calves remained on both the CHO-free and carbohydrate diets for two weeks. In the work of Rosensweig et al. (86) with humans, the turnover time for sucrase and maltase was 2-5 days. Assuming a similar turnover time in calves, a two-week period should have been sufficient for adaptation to dietary disaccharides to occur.

The increased pancreatic amylase in pancreas of calves on the CHO-free diet was not expected. In apparent conflict with our findings, Grossman et al. (44) noted that a high-carbohydrate diet increased amylase activities in pancreatic juice but were decreased on a high protein diet. However, a possible explanation is that the CHO-diet stimulated release of pancreatic amylase which resulted in lower activities in the pancreatic tissue than on a CHO-free diet.

Lactase and Maltase Adaptation

Because of possible variables influencing intestinal length such as inheritance, degree of stretch during measurement and others, no differences due to treatment were expected or found.

If adaptation to lactose or carbohydrates was to have occurred by increasing the amount of mucosa in the small intestine as demonstrated by Herzenberg and Herzenberg (47) in rats, an increase in the percentage of mucosa should have been noted. However, no significant treatment differences or trends were noted for this parameter. Thus, adaptation to lactose appeared to be due to an increase in

the specific activity of the lactase and not to increased mucosal weight. However, our methods of sampling and scraping of the mucosa may have prevented detection of small differences which could have been significant.

These findings are also in contrast to reports by Fisher (38) and Huber et al. (54). Fisher found lactose feeding significantly increased the cleaned small intestine weight of rats. She also found that the total β-D-galactosidase activity of the small intestinal mucosa was increased by lactose feeding but the specific activity was not altered. Huber et al. (54) showed both an increase in specific lactase activity and a higher per cent of intestinal weight as mucosa as lactose levels in calf diets were increased from 0 to 75% of the total solids.

The disagreement with the studies of Huber et al. (54) concerning per cent of mucosa might be explained by differences in weighting and measuring techniques or amount of lactose fed. In the Virginia study (54) up to 75% of the total solids consumed was lactose compared to 45% in our study.

Using lactase to maltase ratios to express lactase activity appeared unsatisfactory. This means of expression was based on the report of Rosensweig et al. (85) who found

lactase was constant in intestinal biopsy samples from human subjects; so basing induced changes in sucrase and maltase upon their ratio to lactase appeared to increase precision of the estimate. In young calves lactase normally decreases with age (50, 93) but if adaptation occurred, this pattern might be reversed on certain rations. In previous work (49, 50, 62, 63) maltase was not greatly affected by age or diet, and appeared to be a satisfactory divisor. However, treatments caused significant changes in both maltase and lactase activities, resulting in no significant differences among treatments when expressed by the ratio method.

In the upper section of the small intestine a significant increase in specific activity of lactase was found on the lactose ration compared to the glucose and CHO-free rations. Lactose feeding also increased specific lactase activity in rat studies by Herzenberg and Herzenberg (47) and in calf studies by Huber et al. (54). These studies (47, 54) as well as the present research are in disagreement with Siddons (93) who found no difference between the lactase levels of calves fed solely on milk and those receiving hay and grain. Partial explanation of this discrepancy might be the levels of lactose fed. In the study

by Huber et al. (54) where lactose significantly increased specific gut lactase, 75% of the ration dry matter was lactose. In previous work, Huber et al. (50) found 3% lactose had no affect on lactase activities when compared to a 3% addition of sucrose and starch. The difference between the rations in this work approximates the difference in the levels fed by Huber et al. (54). Moreover, Huber et al. (54) showed a linear relationship between lactose in the diet and intestinal lactase activity, but the difference between the hay-grain ration and the milk ration was not significant even though activities on the latter tended to be higher.

Another difference between our work and that of Siddons (93) may be the basal level of lactase induced by the CHO-free diet. Blair et al. (9) found that a CHO-free diet markedly decreased sucrase activities in rats. Sucrase is thought to be an inducible enzyme in this species.

A possible explanation for not obtaining a significant increase in lactase in the lower tract may be that the dietary lactose was completely hydrolyzed in the upper tract and insufficient lactose reached the lower tract to stimulate lactase production. As previously discussed the degree of rumen function is important in determining the

amount of lactose that reaches the lower tract. Siddons' calves (93) may have had somewhat functional rumens while ours did not. In order to stimulate lactase production it is postulated that lactose must reach the small intestine.

The higher maltase on the lactose and galactose diets than the CHO-free diets contrasts with previous work (49, 50, 62, 63) in which little change was observed in specific maltase activity until at least nine weeks of age. This dietary effect might be explained by the extremely low level of maltase activity on the CHO-free diet.

For both expressions of maltase activity, the lower tract showed no increase which supports the previous suggestion that complete digestion and absorption of the carbohydrates occurred in this trial before they reached the lower tract. There was little difference in maltase activity between the upper and lower sections. Except for the glucose diet, the upper section had a slightly greater activity than the lower section. This disagrees with that reported by Siddon (93) who reported that the greatest maltase activity is in the lower section of the small intestine.

In all cases the galactose diet gave lower responses than the lactose in lactase activities but higher responses than either the glucose or CHO-free diets. This indicates that galactose is probably the active moiety in stimulating increased lactase on high-lactose diets. The reason that the galactose diet did not give a greater lactase response than lactose, even though it was in approximately twice the molecular concentration, may have been due to its location in the lumen of the gut. Lactose is cleaved at the brush border of the mucosal cell and is ready for rapid movement into the cell where stimulation of enzyme production probably occurs (85). When fed as a monosaccharide galactose is absorbed at a slower rate than lactose, as indicated by the blood sugar data. Moreover, absorption is probably distributed farther down the digestive tract with a net result of lower concentrations entering the mucosal cells of the upper small intestine where stimulation evidently occurs (Table VII).

This work parallels that of Rosensweig et al. (85) in which fructose was found to be the active unit in stimulating increased sucrase activity. Broitman et al. (11) found that glucose and galactose fed together had no effect on gut lactase activity in rats which again may have been

due to the site and rapidity of monosaccharide absorption in the small intestine.

Blood Glucose Studies

The calves on the CHO-free ration declined extremely fast to a low of 40-60% mg glucose in their blood. These levels approach the values which have been reported for a mature ruminant (19, 66, 105, 107). The calves on the CHO-free ration showed some capability to synthesize glucose as suggested by the gradual increase in blood glucose after feeding. However, once the low fasting level of blood glucose had been reached, it was not altered by the inclusion of carbohydrates in the diet. It appears that the change in glucose levels similar to those found in the mature ruminant is irreversible under conditions of this experiment.

Our work suggests that the digestive and gluconeogenic enzymes are under different control systems. Apparently the young calf on a CHO-free diet cannot sufficiently increase its gluconeogenic production to maintain blood glucose at the high levels normally found and once levels have decreased to those similar to a mature ruminant, some

control in glucose metabolism will not permit their increase even after carbohydrates are restored to the diet.

As could be expected, when glucose was included in the ration it gave greater increases in blood glucose than galactose or lactose; however, response to galactose was less than to lactose. Siddons et al. (94) reported that upon infusion of galactose into the blood of calves there was an increase in blood reducing sugars comparable to glucose infusion, but only a small amount of the increase in reducing sugars was due to glucose when galactose was infused. In addition, when galactose is released from the disaccharide at the brush border it may be in a much more advantageous position to be absorbed than when free in the lumen. Siddons et al. (94) found glucose was preferentially absorbed from a glucose-galactose mixture in calves. In addition, Cargell et al. (16) reported that glucose was absorbed as rapidly as it was formed in the white rat while galactose was absorbed at a somewhat slower rate.

Ginsburg and Heggeness (43) have shown that rats receiving a CHO-free diet absorbed glucose and galactose only 75% as fast as animals fed high-glucose diets. If increases in blood glucose are used as the criterion to indicate ability to absorb glucose, calves in our study

exhibited less capacity to absorb glucose after two weeks on carbohydrate rations; which appears in direct contradiction to the findings of Ginsburg and Heggeness (43). However, an alternate and more plausible explanation is that while on the CHO-free diet the calves decreased in their ability to remove and utilize glucose from the blood; thus, lower maximum increases of blood glucose were seen 2 weeks after introduction of carbohydrate ("C" date), after calves had regained some ability to utilize glucose, than immediately afterwards ("B" date). Apparently the turnover time of the transport mechanism is great enough so that two weeks on the CHO-free ration was insufficient to deplete it, or it is not dependent on carbohydrate for stimulation. On the other hand, glycolytic enzymes require carbohydrates for their induction and a two-week period appears sufficient to deplete these enzymes.

In the present study, little difference was seen in blood glucose response to lactose feeding after two weeks on a lactose ration compared to two weeks on a CHO-free ration. Fisher and Sutton (39) found the rate of intestinal absorption of lactose as measured by blood glucose response was greater after previous feeding of a diet containing 25% lactose for six weeks than on a milk

ration. Dollar and Porter (32) also found excellent correlation of lactase activity with blood sugar responses; and Huber et al. (53) suggested blood glucose responses were related to lactase activity as did Deren et al. (31).

This difference could be explained by not having an increased lactase level; however, a significant increase in lactase due to lactose feeding has already been shown. Another explanation might be that I was not challenging the calves with enough lactose to utilize the extra hydrolyzing ability. A third explanation already discussed is that even though there might have been increased hydrolysis of lactose, an increased removal of glucose from the blood masked an increase in blood glucose levels.

VI. SUMMARY

The effect of different carbohydrates on carbohydrase activities in the digestive tract and on blood glucose of young bovines was studied with the use of a CHO-free diet.

A CHO-free diet was formulated and consisted of 30% fat and 70% protein premix which contained 92% protein, 1% vitamins, 4.5% dicalcium phosphate, 1.5% trace mineral salt, and 1% suspensory agent. The fat and protein premix was mixed with warm water twice daily at the time of feeding. The liquid diet contained 14% solids and was fed at 7% of body weight per day. This ration was consumed by the calves and they gained weight; however, an adaptation period of three days was necessary to adjust to the CHO-free diet.

To measure the effects of carbohydrates on carbohydrase activities in the young calf, 16 male Holstein calves were fed the CHO-free diet from one to three weeks of age and were then divided into four treatment groups

and were fed different diets for the next two weeks. These diets were lactose, galactose, glucose, and CHO-free. The carbohydrates were incorporated into the diet and fed at 4.5 g/Kg of body weight. Calves were sacrificed at five weeks of age.

Lactose significantly ($P < 0.05$) increased lactase activity (on a protein basis) in the anterior one-third of the small intestines compared to the glucose and CHO-free diet. Galactose gave an intermediate value suggesting it may be the active moiety responsible for the lactase stimulation on high lactose diets.

Intestinal maltase was greater ($P < 0.05$) on the lactose ration than on the CHO-free in the anterior one-third of the small intestine. There were no significant differences between any of the treatments in the lower section of the small intestines. Pancreatic amylase activity was less ($P < 0.05$) on the glucose than on the CHO-free diet.

Fasting blood glucose was depressed on the CHO-free diet and failed to recover with the addition of any of the three carbohydrates. Removal of glucose from the blood appeared greater after calves had received carbohydrates for two weeks than after two weeks on a CHO-free ration.

In this study increases in blood glucose did not parallel increases in small intestinal lactase and were not satisfactory indicators of intestinal lactase.

These data lead one to conclude that the stimulation of lactase is due to the galactose moiety in the lactose molecule and that lactose has a non-specific stimulatory affect on disaccharidases. Also that carbohydrates stimulate the release of pancreatic amylase, thus resulting in lower amylase concentrations in the pancreatic tissue.

This study has raised several questions for further experimentation on the carbohydrate-digesting apparatus of a young bovine. Further refining of the CHO-free diet could be done to definitely prove the essentiality of carbohydrates in the diet of the young calf. Additional work is also needed on the effect of a CHO-free diet on the glycolytic, gluconeogenic, and lipid-metabolizing enzymes of the calf. Hormones may play an essential role in control of these enzymes and might help to explain why some calves grow better initially than others on a normal ration. The ability of galactose to substitute for glucose should be further investigated. Moreover little is known on the turnover time of disaccharidases in the small intestine of the young bovine.

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APPENDIX

APPENDIX

TABLE I

Composition of Vitamin Premix[1]

Vitamin A	3.164 (gm)
Vitamin D	5.498
Vitamin E	14.677
Thiamine	1.592
Riboflavin	1.100
Niacin	6.364
Pyridoxine	1.600
Biotin	.464
Pantothenate	4.772
Choline chloride	636.300
Vitamin B_{12}	14.680

[1]This was made up to 1.6 Kg with Promosoy.

APPENDIX

TABLE II

Descriptive Data for Male Holstein Calves Employed in Experiment I

Ration	Calf Number	Origin[A]	Weight (kg)	
			Begin	End
CHO-free	520	G	43	39
"	487	G	38	34
"	486	G	39	38
"	491	G	44	42
			$\bar{X}$ 41.0	38.3
Glucose	517	G	43	36
"	523	G	42	35
"	943	B	54	50
"	482	G	35	34
			$\bar{X}$ 43.5	38.8
Lactose	518	G	46	39
"	522	G	43	39
"	904	B	46	42
"	490	G	41	39
			$\bar{X}$ 44.0	39.8
Galactose	851	B	42	40
"	519	G	40	34
"	521	G	45	43
"	484	G	46	42
			$\bar{X}$ 43.3	39.8

[A]G refers to calves obtained from Green Meadows Farm, Elsie, Michigan and B refers to calves obtained from the dairy herd at Michigan State University.

APPENDIX

TABLE III

Mucosa Weight of the Small Intestines as Affected by Diet

Treatment	Calf #	Upper Section		Lower Section	
		Mucosa Wt (gm)	% Mucosa	Mucosa Wt (gm)	% Mucosa
Galactose	851	8.80	56.45	9.83	56.04
	519	7.05	58.26	7.00	53.03
	521	8.00	56.74	8.65	59.75
	484	11.39	56.56	10.78	57.59
	$\bar{X}$	8.80	57.00	9.06	56.60
Glucose	517	7.20	65.45	7.10	55.25
	523	7.40	59.68	6.65	38.22
	943	9.30	58.68	13.04	64.74
	482	6.32	55.68	10.17	57.39
	$\bar{X}$	7.55	59.87	9.24	53.15
Lactose	518	7.58	64.07	7.30	51.41
	522	7.50	55.15	8.95	57.01
	904	8.07	53.41	11.80	59.33
	490	5.39	45.33	9.60	50.29
	$\bar{X}$	7.13	54.49	9.41	54.51
CHO-free	520	7.00	61.40	9.63	57.05
	487	7.58	52.90	9.90	55.62
	486	8.26	58.62	11.50	55.96
	491	7.25	56.03	11.90	59.23
	$\bar{X}$	7.52	57.24	10.73	56.96

APPENDIX

TABLE IV

Small Intestine Weight and Length as Affected by Diet

Treatment	Calf No.	Total SI Length (m)	Upper Section	Lower Section
			Total SI Segment Wt (gm)	Total SI Segment Wt (gm)
Galactose	851	19.5	15.59	17.54
	519	19.5	12.10	13.20
	521	18.3	14.10	16.15
	484	18.3	20.12	18.72
	$\bar{X}$	18.90	15.47	16.40
Glucose	517	18.3	11.00	12.85
	523	19.5	12.40	17.40
	943	23.2	15.85	21.12
	482	19.5	11.35	17.72
	$\bar{X}$	20.12	12.65	17.27
Lactose	518	18.3	11.83	14.20
	522	19.5	13.60	15.70
	904	22.0	15.11	19.89
	490	19.5	11.89	19.09
	$\bar{X}$	19.82	13.10	17.22
CHO-free	520	22.0	11.40	16.88
	487	18.3	14.33	17,80
	486	20.7	14.09	20.55
	491	18.9	12.94	20.09
	$\bar{X}$	19.97	13.19	18.83

APPENDIX

TABLE V

Individual Pancreas Weight as Affected by Diet

Treatment	Calf No.	Pancreas Weight (gm)	Pancreas Weight/ 45 kg B.W.
Galactose	851	22.43	25.20
	519	19.18	25.57
	521	21.05	22.39
	484	28.46	30.93
	$\bar{X}$	22.78	26.02
Glucose	517	23.55	29.81
	523	16.87	21.91
	943	28.08	25.30
	482	16.28	22.00
	$\bar{X}$	21.19	24.76
Lactose	518	30.78	31.73
	522	28.60	33.26
	904	24.81	26.97
	490	25.78	30.44
	$\bar{X}$	27.49	30.60
CHO-free	520	26.18	30.80
	487	25.00	33.33
	486	23.97	28.54
	491	19.63	21.34
	$\bar{X}$	23.69	28.50

APPENDIX

TABLE VI

Lactase Activity (Upper Section) as Affected by Diet

Treatment	Calf No.	Lactase Activity/ gm Tissue	Lactase Activity/ mg protein (X10-2)	Lac/Mal
Galactose	851	.643	3.135	5.314
	519	.933	5.788	7.585
	521	1.604	10.246	11.217
	484	3.307	11.157	9.368
	$\bar{X}$	1.622	7.582	8.371
Glucose	517	.282	1.990	3.710
	523	1.642	10.102	11.217
	943	.991	6.641	7.341
	582	1.076	4.540	10.056
	$\bar{X}$	.998	5.818	8.188
Lactose	518	1.157	8.532	15.026
	522	1.818	10.644	10.509
	904	1.165	10.312	6.773
	490	2.443	11.310	4.61
	$\bar{X}$	1.646	10.200	9.230
CHO-free	520	.765	4.432	7.083
	487	1.240	6.420	8.532
	486	.925	3.828	7.773
	491	.900	4.281	7.143
	$\bar{X}$	.958	4.740	7.633

APPENDIX

TABLE VII

Intestinal Lactase Activity (Lower Section) as Affected by Diet

Treatment	Calf No.	Lactase Activity/ gm Tissue	Lactase Activity/ mg Protein (X10-2)	Lac/Mal
Galactose	851	.137	.849	1.070
	519	.153	.921	1.645
	521	.392	2.685	2.762
	484	1.007	4.289	7.867
	$\bar{X}$	.422	2.186	3.336
Glucose	517	.170	1.130	1.560
	523	.278	1.427	2.242
	943	.464	2.580	2.994
	582	.382	2.002	2.076
	$\bar{X}$	.324	1.785	2.218
Lactose	518	.372	2.670	10.140
	522	.412	2.701	2.747
	904	.223	.479	3.431
	490	.210	1.371	1.077
	$\bar{X}$	.304	1.805	4.349
CHO-free	520	.153	.921	1.645
	487	.180	.900	1.385
	486	.327	1.411	2.404
	491	.268	1.377	2.653
	$\bar{X}$	.232	1.156	2.022

APPENDIX

TABLE VIII

Intestinal Maltase Activity (in Upper and Lower Sections) as Affected by Diet

Treatment	Calf No.	Upper Section		Lower Section	
		Maltase Activity	Maltase/ Protein (10^{-3})	Maltase Activity	Maltase/ Protein (10^{-3})
Galactose	851	.121	.470	.128	.650
	519	.123	.805	.093	.553
	521	.143	.914	.146	.981
	484	.353	1.338	.128	.706
	$\bar{X}$	.185	.882	.124	.723
Glucose	517	.076	.550	.109	.729
	523	.141	.865	.126	.631
	943	.135	.921	.155	.853
	582	.107	.398	.184	.852
	$\bar{X}$	.115	.684	.143	.766
Lactose	518	.077	.602	.086	.647
	522	.173	1.011	.150	.982
	904	.172	1.494	.065	.442
	490	.531	1.863	.195	.974
	$\bar{X}$	.238	1.243	.124	.761
CHO-free	520	.108	.623	.092	.596
	487	.145	.658	.130	.493
	486	.119	.491	.136	.675
	491	.126	.653	.101	.596
	$\bar{X}$	.125	.606	.115	.590

APPENDIX

TABLE IX

Pancreatic Amylase Activity as Affected by Diet

Treatment	Calf No.	Total Amylase	Amylase/mg Protein
Galactose	851	36.90	.262
	519	7.80	.075
	521	48.60	.336
	484	45.60	.351
	$\bar{X}$	34.73	.256
Glucose	517	16.20	.127
	523	21.90	.167
	943	68.70	.494
	582	18.60	.152
	$\bar{X}$	31.35	.235
Lactose	518	51.90	.351
	522	36.90	.307
	904	36.90	.405
	490	42.60	.341
	$\bar{X}$	42.08	.351
CHO-free	520	62.10	.419
	487	33.00	.250
	486	69.90	.498
	491	95.70	.668
	$\bar{X}$	65.18	.459

APPENDIX

TABLE X

Blood Glucose Response of CHO-Free Calves to Diet

Calf No.	Period	Time After Feeding (Min.)			
		0	30	90	210
		Mg Glucose/100 ml of Whole Blood			
486	A	46.8	48.4	73.6	68.9
	B	55.4	44.4	48.3	54.4
	C	45.6	45.1	78.0	78.0
487	A	32.1	40.5	60.0	65.2
	B	52.2	62.0	44.5	43.9
	C	39.7	44.1	70.1	89.2
491	A	52.7	54.0	61.2	60.8
	B	38.5	39.6	50.6	73.2
	C	35.9	36.4	38.0	32.8
520	A	57.2	52.4	42.0	--
	B	40.6	81.1	40.9	39.4
	C	52.7	39.3	45.1	38.2

APPENDIX

TABLE XI

Blood Glucose Response of Glucose Fed Calves to Diet

Calf No.	Period	Time After Feeding (Min.)			
		0	30	90	210
		Mg Glucose/100 ml of Whole Blood			
482	A	51.5	52.6	64.2	83.1
	B	54.9	129.0	149.3	170.7
	C	38.2	66.6	138.7	144.6
943	A	39.0	36.9	33.3	90.5
	B	37.2	74.9	106.3	89.4
	C	38.7	81.6	117.2	96.5
517	A	24.8	28.4	28.8	40.0
	B	21.2	49.6	98.2	131.5
	C	40.5	83.2	106.2	57.2
523	A	35.2	40.0	46.4	58.0
	B	33.0	88.3	113.3	123.6
	C	39.3	81.9	111.2	60.4

APPENDIX

TABLE XII

Blood Glucose Response of Lactose fed Calves to Diet

Calf No.	Period	Time After Feeding (Min.)			
		0	30	90	210
		Mg Glucose/100 ml of Whole Blood			
904	A	61.7	63.5	63.9	61.2
	B	51.7	71.0	89.1	98.5
	C		71.8	127.9	40.0
490	A	49.5	49.5	56.7	58.1
	B	40.7	72.1	120.0	79.8
	C	32.2	55.1	60.8	59.8
518	A	88.8	48.0	47.2	56.4
	B	47.0	81.5	131.5	141.0
	C	53.5	78.9	125.0	94.9
522	A	55.2	58.3	66.0	60.8
	B	50.4	86.8	95.1	92.5
	C	50.2	74.1	86.5	71.9

APPENDIX

TABLE XIII

Blood Glucose Response of Galactose Fed Calves to Diet

Calf No.	Period	Time After Feeding (Min.)			
		0	30	90	210
		Mg Glucose/100 ml of Whole Blood			
484	A	29.5	31.6	53.1	50.0
	B	83.4	70.0	54.9	55.4
	C	58.8	45.6	66.2	82.3
851	A	40.1	41.4	50.4	58.1
	B	27.0	71.5	103.4	83.1
	C	23.9	42.6	31.7	40.0
519	A	47.2	64.0	38.0	45.2
	B	31.5	58.0	90.2	122.4
	C	53.9	92.8	82.8	68.6
521	A	49.2	56.0	57.6	54.6
	B	36.4	36.8	100.4	111.0
	C	47.2	58.9	67.7	68.1

APPENDIX

TABLE XIV

Sample Calculation (Lactase Activity on a Protein Basis)

Source	DF	SS	MS	F	Level of Significance
Treatment	3	69.084	23.028	3.65	.05
Error (Ans./Trt.)	12	75.629	6.302		
Location	1	193.136	193.136	56.41	.005
Trt. X Location	3	12.528	4.176	1.22	n.s.
Error (Ans./Trt. X Loc.)	12	41.083	3.424		
Total	31	391.460			

Duncan's Test

Lactase Activity/Mg Protein

		2	3	4	
α =	.01	5.789	6.035	6.193	
α =	.05	4.130	4.322	4.439	
	10.200				
	7.582	2.618			
	5.818	1.764	4.382		Significant Differences
	4.740	1.078	2.842	5.460	

Lactase Activity

		2	3	4
α =	.01	1.603	1.671	1.715
α	.05	1.143	1.196	1.229
	1.646			
	1.622	.024		
	.998	.624	.648	
	.958	.040	.664	.688