THE EFFECT OF SOY PROTEIN ON DIGESTIBILITY OF CALF MILK REPLACERS
AND ON INTESTINAL ABSORPTIVE ABILITY AS DETERMINED
BY THE XYLOSE ABSORPTION TEST

by

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INTRODUCTION

Calf nutrition is an important aspect of the dairy industry. Since the placental transfer of antibodies does not occur in the bovine, the calf is born essentially without immunity, and for this reason calves should receive colostrum as soon as possible after birth. Even with the passive immunity derived from the absorption of the antibodies in colostrum, the calf is quite vulnerable to disease during the first several weeks of life.

The ration fed to a calf during this period is of extreme importance, and should always be of high quality, since there is no reason to compound natural immune deficiency with a nutritional problem. Milk is the best liquid diet for young calves, but routine feeding of milk to calves is expensive. Feeding milk replacers to young dairy calves has become popular when there is an economic advantage to sparing saleable milk.

Research continues in development of lower cost milk replacers which provide adequate nutrition. Since protein is ordinarily the most expensive nutrient in milk replacers, research involving the quest for more economical protein sources has received the most emphasis. Animal proteins, including those of milk origin, are generally more expensive than plant proteins, and considerable work has been done with vegetable proteins in calf milk replacers. Soybeans are high in protein and have a balanced amino acid profile. Processed soy products, however, have generally produced unsatisfactory results when fed in milk replacers to young calves.
This research was conducted to study the digestibility and the effect on intestinal absorptivity of three calf milk replacers containing different soy protein sources, two soy protein concentrates and a soy flour. In Experiment 1, digestion trials were conducted to determine protein digestibilities. In Experiment 2, a xylose absorption test was used to evaluate the absorptive ability of the intestine.
SOY MATERIALS IN CALF MILK REPLACERS - A REVIEW

Nature of the Digestive Tract of the Pre-ruminant

The delicate nature of the digestive tract of the newborn calf has been known for many years (Hayward, 1902). The young pre-ruminant uses fat, protein, and carbohydrate from milk as efficiently as the young of other species. However, some digestive abilities of the pre-ruminant to utilize nutrients from other sources are limited, develop more slowly, or do not develop compared to young non-ruminant animals (Porter, 1969). Attempts to formulate adequate substitutes for milk using more economical plant proteins have met with limited success in the young calf.

Protein Requirements of the Calf

The protein requirements of the calf depend mainly on size and daily protein deposition in tissues. The amount of nitrogen retained per unit of gain is fairly constant, approximately 32 g of nitrogen retained per kilogram of gain (Erbersdobler and Gropp, 1973).

Plant Products in Calf Milk Replacers

Fat Utilization

Many animal and vegetable fats are well digested by the young calf, although diarrhea is a common problem when vegetable fats are fed (Porter, 1969). Jacobson and Cannon (1947) reported that hydrogenated soybean oil was as well digested as butter oil, although calves fed expeller-processed soybean oil scoured often, and this scouring was not affected by vitamin supplementation.

Early Use of Non-soy Plant Protein

Hayward (1902) reported that after 10 days of age, calves could be fed a milk replacer with high plant protein and maintain growth similar to that
achieved with skim milk. Maynard and Norris (1923) found satisfactory growth when a milk replacer high in plant protein was fed to calves beginning at 4 weeks of age. No soy materials were used in these two studies, in which rations consisted mainly of wheat flour, corn meal, oat groats, linseed meal, and blood flour.

Soy Protein Utilization

Early Use of Soybeans for Calves

Norton and Eaton (1946), using a limited-milk feeding system, found satisfactory growth of calves fed dry calf starters containing up to 18% soybean meal and no animal protein. This study indicated that soy protein, fed in a dry calf starter which was digested in the rumen, could be utilized by the young calf if some milk was also provided.

Soy products have received a great deal of emphasis in experimental milk replacers since soybean proteins are one of few vegetable proteins with a complete and balanced amino acid composition (Porter, 1969). Reports of soybeans being utilized as a milk substitute for human infants suggested the use of soy flour in calf milk replacers (Shoptaw, 1936). He used soy flour as the only source of protein, and observed diarrhea, rough coats, poor health, and slower growth compared to milk-fed calves. Some reasons for the poor performance of calves fed soy materials are acceptability, rate of passage, trypsin inhibitor, and diarrhea.

Factors Affecting Calf Performance

Acceptability. Shoptaw (1936) reported that some calves did not readily consume a soy flour ration. Smith et al. (1970) reported that soy flour rations were readily accepted by calves for two or three feedings and thereafter were
rejected. In these two studies soy flour was the only protein source in the milk replacers.

Rate of Passage. The slow release of protein from the abomasum of the calf is important for digestion. It is detrimental to the calf if proteins leave the abomasum too quickly. In milk-fed calves free amino acids and other soluble nitrogen compounds are released from the abomasum first, then whey proteins, with caseins last. Many substitutes for milk protein do not clot sufficiently in the abomasum and leave too rapidly. This is most important for the very young calf which secretes predominantly rennin instead of pepsin in the stomach, since only casein is a substrate for rennin (Erbersdobler and Gropp, 1973). Shoptaw et al. (1937) found that soy flour left the calf's stomach faster than whole milk or a milk replacer containing dried skim milk, although gastric secretion did not differ among the three treatments. Colvin et al. (1969) reported that passage of dry matter and total nitrogen from the calf's abomasum was more rapid for soy flour diets than for milk. However, Smith et al. (1970), using a polyethylene glycol marker, reported a slower flow of digesta from the abomasum when soy flours were fed and a normal flow, compared to milk, when an isolated soy protein was fed. Transit time through the small intestine was faster for soy flours than for milk, and all soy products used affected nitrogen flow (Smith et al., 1970).

Trypsin Inhibitor. Raw soybeans have a heat-labile trypsin inhibitor (TI) and other factors causing a decrease in intake and growth, and an increase in secretion of pancreatic enzymes (Porter, 1969). Raw soybeans contain a TI with five fractions, some of which are more sensitive to inactivation by heat or alkali treatment than others (Obara and Watanabe, 1971). The cystine content
and the formation of disulfide bridges may play an important role in the stability of TI (Obara and Watanabe, 1971). Birk (1961) isolated a highly-active acetone-insoluble TI from ether-extracted soy flour which was completely inactivated by autoclaving at 15 lbs/in² for 20 minutes. It was unaffected by heating for 1 hour at 105 to 108 C, or by treatment with HCl or pepsin. The stability of this soybean TI to acid and pepsin suggested that it might pass through the stomach undamaged and reach the site of trypsin and chymotrypsin action, and thereby might have a large role in the growth-depressing activity of soy materials (Birk, 1961).

Gorilli and Nicholson (1971) added a purified soybean TI to milk and an all-milk milk replacer. Reduced trypsin and chymotrypsin activities were found when the TI was added at 1 mg/ml, but not at lower levels. Diarrhea was common at the 1 mg/ml level. Total nitrogen and percent non-protein nitrogen declined in the intestinal contents, but pancreatic enzyme secretions were not affected. Kakade et al. (1974) reported that heating the TI had no effect on growth and digestibility in calves. The pancreas size of calves fed soy diets in which TI had not been heated was smaller than in calves fed a diet with heated TI (Kakade et al., 1974).

Fully-cooked soy flour contains an inactive form of TI which is activated at pH 7 to 9 (Ramsey and Willard, 1975). The TI can be destroyed by heating in water, but the extent of destruction is dependent on the concentration of flour in water. Thermo-alkali processing was found to reduce greatly the TI activity of soy flour and soy protein concentrate (Coblentz et al., 1976). The presence of TI might be an explanation for the observation that newborn calves often have not performed well on milk replacers containing large quantities of soy flour. The TI could have a greater effect on a younger calf
which secretes less trypsin than an older calf (Ramsey and Willard, 1975).

**Diarrhea.** A major problem in replacing milk is furnishing adequate energy without causing diarrhea (Wallace et al., 1951). Nitsan et al. (1971) reported a lower incidence of diarrhea in calves fed soy products than in those fed an all-milk milk replacer. However, other reports have not supported this observation. Loose feces have been observed with calves fed diets containing soy products (Shoptaw, 1936; Gorrill and Nicholson, 1971; Smith and Wynn, 1971; Coblentz et al., 1976).

**Form of Soy Materials Used**

**Whole Soybeans.** Raw soybeans have caused a decrease in intake and growth in calves and an increase in pancreatic enzyme secretion (Porter, 1969). Benevenga and Ronning (1963) reported that calves grew very little when fed a ration containing 56 percent ground cooked soybeans, 28 percent whey, and 16 percent hydrogenated vegetable oil. Extensive hair loss was seen, which the addition of vitamins did not help. Calves fed raw or partly-heated soybeans as the only protein source did not grow (Nitsan et al., 1971).

**Soybean Meal.** Stein et al. (1953) reported satisfactory growth of calves fed a milk replacer containing 30 percent soybean meal which replaced blood flour, dried whey, and part of dried skim milk. Later reports have not shown similar success. Toasted soybean meal, providing 73 percent of the total protein in a milk replacer, produced poor growth, low protein digestibility (50 percent), and decreased fat and mineral absorption (Nitsan et al., 1972).

**Soy Flour.** Soy flour is a less expensive protein source than soy protein concentrate or soy protein isolate, and various soy flours have been used in
experimental work. A high-fat soy flour and a solvent-extracted soy flour, both used at 20 percent of a milk replacer, resulted in slower growth in the first month than in milk-fed calves. Calves fed the solvent-extracted soy flour grew at twice the rate of calves fed the high-fat soy flour in the first 8 weeks (Wallace et al., 1951). Stein and Knodt (1954) and Stein et al. (1954) reported that solvent-extracted soy flour could replace up to a maximum of 43 percent of the nonfat dry milk solids in a milk replacer, and could also replace dried whey, corn solubles, and blood meal, if dried brewer's yeast and whey solubles were also fed. If soy flour constituted more than 43 percent of the ration, growth and appetite declined, and poor calf appearance was observed.

Using milk replacers containing soy flour and milk products, Gorrill and Thomas (1967) found that calves lost weight when fed a ration in which 60 percent of the protein was supplied by a 50 percent protein soy flour. However, a milk replacer containing 86 percent of its protein from a 71 percent protein soy flour produced growth equal to milk. Lassiter et al. (1959) reported satisfactory growth of calves when soy flour was fed as 11 percent of a milk replacer with dried skim milk and whey, but not when soy flour constituted 20 or 28 percent of the dry milk replacer. Average daily gains from birth to 40 days were unsatisfactory for calves fed soy flour at 33.4 or 41.9 percent of the diet (Fries et al., 1958). Smith and Wynn (1971) reported that calves fed soy flour as the only protein source, even if heated, lost weight and had disturbed digesta flow, diarrhea, and high antibody titres. Growth did not differ among calves fed a ration containing 33 percent of a 52.4 percent protein soy flour or rations with the same soy product with addition of 3.5 percent lactose or 5 percent dried whey (Noller et al., 1956a).
Soy Protein Concentrate. Soy protein concentrates are products prepared from high quality, clean, dehulled soybeans by removing most of the oil and water-soluble non-protein constituents and which contain not less than 70 percent protein on a moisture-free basis. Gorrill and Nicholson (1969) reported that a milk replacer, in which Promosoy, a commercial soy protein concentrate, provided 70 percent of the total protein, had nitrogen digestibility and retention similar to an all-milk milk replacer. No significant difference in gain was found between the all-milk and soy-milk fed calves from birth to 7 weeks. Schmutz et al. (1967) fed milk and milk replacers containing milk products and 8, 10, or 16 percent Promosoy to calves. When solids were fed at 10 percent of the milk replacer, calves fed milk replacers with 8 or 16 percent Promosoy gained at a slower rate than calves fed milk or a milk replacer with 10 percent Promosoy.

In a five-week growth study, Promosoy successfully replaced 22 percent, but not 44 percent, of the crude protein from milk sources in a calf milk replacer (Morrill et al., 1969, 1971). Replacing part of milk protein with soy protein caused an increase in calf growth (Morrill et al., 1969, 1971; Schmutz et al., 1967), possibly due to supplementation of one or more limiting amino acids in milk protein (Morrill et al., 1971).

Using calves larger than 60 kg, Erbersdobler and Gropp (1973) reported good growth from milk replacers containing 34 or 68 percent of the total protein from soy protein concentrate. Growth was less than that produced from all-milk products, possibly due to lower digestibility or enzyme inhibitor residues (Erbersdobler and Gropp, 1973). Nitsan et al. (1971) reported that a soy protein concentrate should be properly heated before its inclusion in a calf milk replacer.
Soy protein concentrate satisfactorily furnished 50 to 88 percent of the protein in calf milk replacers when a concentrate with 16 percent protein was provided. When properly prepared, these materials produced growth of calves up to 85 percent of that of milk-fed animals (Nitsan et al., 1972).

Akinyele et al. (1975) reported satisfactory growth when soy protein concentrate was substituted for 30, but not 84, percent of milk protein in a calf milk replacer. Escano et al. (1973) reported no significant difference in growth between calves fed a milk replacer with all-milk protein and those fed a milk replacer in which part of the protein had been provided by soy protein concentrate.

The nitrogen digestibility of soy protein concentrate was 80 percent for three-week-old calves and 85 percent for five-week-old calves (Nitsan et al., 1972). Porter and Hill (1963) reported digestibilities of 75 percent in one- and two-week-old calves and 87 percent in four- and five-week-old calves. Gorrill and Nicholson (1969) found the protein digestibility of a milk replacer, with 70 percent of the protein provided by soy protein concentrate, to be 81.6 percent, while that of an all-milk milk replacer was 87.3 percent in calves at 3 and 6 weeks of age.

In comparison, the digestibility of milk protein by calves was greater than 90 percent (Blaxter and Wood, 1952; Whitnah, 1943). Parrish et al. (1953) reported digestibilities of milk protein of 92, 83, 86, and 93 percent in calves 1 to 2, 3 to 4, 5 to 6, and 14 to 17 days old, respectively.
Effect of Processing Methods

**Heat Treatment.** Raw soybeans have caused weight loss and health problems when fed to calves. The heating of soy products caused an increase in their nutritive value in rats (Kakade et al., 1971) and in calves (Kakade et al., 1974). Heat treatment has improved nutritional quality of soy proteins, but gains were still lower than from all-milk proteins (Thompson et al., 1974). Nitsan et al. (1971) reported that calves fed raw or partly-heated soybeans as the only protein source did not grow. Calves fed fully-heated soy proteins grew at 35 percent of the rate of calves fed all-milk proteins in the first 10 days of life (Nitsan et al., 1971). Heating improved the digestibility of the soy protein from 72 percent for raw soybeans to 79 percent for partly-heated to 88 percent for fully-heated. Soybeans were steam-heated at 120°C for 5 minutes (partly-heated) or 25 minutes (fully-heated).

**Acid or Alkali Treatment.** Colvin and Ramsey (1968) reported that the nutritive value of fully-cooked soy flour was improved by acid treatment at pH 4 for 5 hours at 37°C prior to addition to the milk replacer. Compared to calves fed untreated soy flour, calves fed fully-cooked acid-treated soy flour grew twice as rapidly (Colvin and Ramsey, 1968), or slightly faster (Sudweeks and Ramsey, 1972). Kakade et al. (1971) found that acid treatment of lecithinated fully-cooked soy flour (15 percent lecithin added) increased weight gains by 27 percent in rats. Acid treatment had no effect on raw or heated soy flour without added lecithin, leading to the conclusion that the beneficial effects of acid treatment must somehow have involved lecithin (Kakade et al., 1971). Willard and Ramsey (1972) reported that treatment of soy flour with anhydrous hydrogen chloride improved its nutritive value for the calf.
Treatment of fully-cooked soy flour with alkali (pH 10.6 for 5 hours at 37 C) was found to increase growth of calves and rats over untreated flour to the same degree as acid treatment (Colvin and Ramsey, 1969). Thermoalkali processing (pH 10.5 at 85 C) of a soy protein concentrate for 5 minutes was found to produce increased but unsatisfactory growth over the untreated product in calves when fed as 75 percent of the protein in a 20 percent protein milk replacer (Coblentz et al., 1976). Treatment for a shorter or longer time caused weight losses in calves in the first two weeks of life. Rats performed satisfactorily on the thermoalkali-processed soy products. Feeding of untreated soy flour to calves was found to yield higher plasma concentrations of certain amino acids 2 to 4 hours after eating than feeding of acid- or alkali-treated soy flour (Sudweeks and Ramsey, 1973).

**Effect of Supplementary Materials**

**Methionine.** In rats, the addition of 0, .05, or .25 percent methionine to a 10 percent protein ration in which soy flour was the only source of protein produced average gains (grams), protein efficiency ratios, biological values, and protein digestibility coefficients of 122.2, 2.69, 68, 78; 147.5, 3.06, 69, 78; and 163.5, 3.44, 80, 80, respectively, in a 28-day study (F. J. Seegraber, unpublished data). This increase in nutritional value of soy protein due to supplementary methionine has not been observed in calves. Gorrill and Nicholson (1969) reported that addition of methionine at .1 percent of milk replacer dry matter did not increase calf growth or nitrogen retention. Addition of methionine to soy flour had an adverse effect on calf growth (Thompson et al., 1974).
Vitamins. Thompson et al. (1974) found no effect on calf gain due to adding fat or the vitamins B₁₂, folic acid, or choline to soybeans. Vitamin supplementation did not affect hair loss in calves fed ground cooked soybeans (Benevenga and Ronning, 1963).

Antibiotics. Growth of calves fed soy products was found not to be affected by addition of penicillin or streptomycin (Thompson et al., 1974) or chlortetracycline (Ramsey and Witaszek, 1972).

Pepsin. The addition of pepsin to calf milk replacers containing soy flour had no effect on growth (Fries et al., 1958), or decreased growth slightly (Lassiter et al., 1959). Henschel et al. (1961b) reported that pepsin and rennin had similar activity on casein, but pepsin was more effective than rennin in the breakdown of large soy protein fragments.

Other Factors Affecting Soy Protein Utilization

pH. The pH of the abomasal contents of the newborn calf did not reach a sufficiently low level for effective peptic proteolysis until the calf was several weeks old (Henschel et al., 1961a). Similar findings have been reported for the young pig. The pH of the stomach contents of the pig was about 6 in the first few days of life and decreased thereafter, but it never went below 3.5 in the first 7 weeks of life (Kratzer and Porter, 1962). Henschel et al. (1961b) reported that the pH of a fasting calf's abomasum was 2, which increased to 5 after a feeding of milk and returned to 2 in 5 hours. Colvin et al. (1969) reported that the pH of the calf's abomasal contents decreased faster after feeding milk than soy flour diets.
Using *in vitro* tests, Kratzer and Porter (1962) found that casein, whey proteins, soybean flakes, and two isolated soy proteins were digested by pepsin with a pH optimum at 1.8 to 2.0. Raising the pH of digestion to 4 caused a greater decline in the rate of digestion of soy than of milk protein, which indicated that milk proteins might be more rapidly digested by pepsin than soy proteins.

**Age of Calf.** Noller et al. (1956a) reported a critical period in a calf's life from birth to 25 days during which soy materials were not well digested. After this period soy products were better utilized. Digestibility of whole milk varied very little with age (Noller et al., 1956b). Soybean protein digestibility increased in calves between 7 and 46 days of age (Nitsan et al., 1971, 1972).

**Effect of Soy Protein on Enzyme Activity.** The activities of the pancreatic enzymes trypsin and chymotrypsin were lower in calves fed a milk replacer with 60 percent of the protein supplied by a 50 percent protein soy flour than in calves fed milk or a milk replacer with 86 percent of the protein supplied by a 71 percent protein soy flour (Gorrill and Thomas, 1967). Gorrill et al. (1967) reported adverse effects of soy protein on the pancreatic exocrine function of the calf. Kakade et al. (1974) observed that pancreas size was smaller in calves fed soy diets with unheated TI than in calves fed soy diets with heated TI.

**Sensitivity to Soy Protein.** Smith et al. (1970) suggested the possibility of an allergic reaction to soy protein in the gut of the calf. The flow of digesta through the digestive tract was not affected the first time soy flour was fed, but was more rapid after subsequent feedings. The reactions of calves fed soy flour resembled the effects of wheat gluten in human malabsorption.
cases (Smith and Wynn, 1971). Hemagglutination techniques showed high titres of antibodies to soy protein in calves fed diets in which soy flour was the only protein source. In calves fed soy flour or soy protein concentrate added to milk, antibody titres were low for 2 weeks, then increased to high values after 6 to 10 weeks.
General Considerations of Xylose

Xylose is a five-carbon sugar with chemical formula $\text{C}_5\text{H}_{10}\text{O}_5$. This naturally-occuring aldopentose is found in wood, fruits, and many other sources of plant origin, from which it has been extracted with heat and acid treatment. The discovery of xylose is attributed to E. Koch who first isolated it from wood in 1881 (Miller and Lewis, 1932a). Prior to 1918 little xylose was used in biological experimentation due to its high cost. At this time a method of xylose isolation from corn cobs or cottonseed hulls was discovered by Hudson and Harding (1918) which produced high yields of purified xylose at low cost, thus providing a readily available supply for chemical, biological, medical, or other scientific uses.

Absorption of Xylose

Active Absorption vs. Passive Diffusion. Different sugars are absorbed from the intestine at different rates, in general hexoses faster than pentoses. Xylose has commonly been used in absorption and excretion studies with animals. The question of the mechanism of intestinal absorption of xylose has been studied by many workers with variable results. Xylose was readily absorbed from the intestine, but at a much slower rate than glucose (Miller and Lewis, 1932b). In some reports xylose was considered to be absorbed only passively from the intestine, since the energy-requiring process of active transport was thought to be limited to sugars normally utilizable in the body. This view
was presented in reports of studies using the rat in vivo (Wilbrandt and Laszt, 1933; Verzar, 1935) and the isolated intestine of the hamster in vitro (Crane and Mandelstam, 1960; Crane, 1960, 1962).

Two criteria are required for confirmation of active transport of a sugar: absorption against a concentration gradient; and inhibition of transport when energy-yielding reactions are inhibited (Wilson and Vincent, 1955). Wilson and Vincent (1955) and Crane (1960) reported that glucose and galactose were accumulated against a concentration gradient in hamster intestine, while xylose and arabinose were not. Similar results were found by Wilson and Landau (1960). Crane (1960) proposed a common structural requirement for all actively transported sugars, a hexopyranose ring with a hydroxyl group at carbon 2, which excluded xylose and other pentoses. Verzar (1935) reported that in rats glucose and galactose were absorbed at a constant rate independent of the luminal concentration of sugar, which required active cell processes, while with xylose the quantity absorbed was proportional to the concentration, indicating passive diffusion.

Active absorption of xylose was reported to occur in vitro with the intestine of the frog (Csaky and Lassen, 1964; Lassen and Csaky, 1966), the hamster (Alvarado, 1964, 1966; Bihler et al., 1969; Barnett et al., 1970), the chicken (Alvarado, 1964, 1967), and the rat (Faust et al., 1967), and in vivo in the dog (Levitt et al., 1969), the rabbit (Beyreiss et al., 1965), and the rat (Csaky and Lassen, 1964; Csaky and Ho, 1965). The concept of a mobile carrier system for xylose absorption in the intestine, shared by glucose, was proposed by Csaky and Lassen (1964), Beyreiss et al. (1965), Alvarado (1967), and Levitt et al. (1969). Levitt et al. (1969) reported that active transport of glucose
was five times that of xylose, which suggested a carrier molecule with a higher affinity for glucose than for xylose (Salomon et al., 1961). This concept was supported in a study with dogs (Annegers, 1968) in which glucose reduced the absorption rate of xylose, but the reverse did not occur. Earlier, McDougall (1935) reported that in rabbits the rate of absorption of glucose and xylose was not reduced by continued intravenous injection of the sugars even when the sugar concentration became higher in blood than in the intestine, thus suggesting active absorption of both sugars. This was confirmed for xylose by Peters and Van Slyke (1946). Bockus (1964) reported that xylose is absorbed by passive or facilitated diffusion, but not actively. Small et al. (1959) proposed that absorption of xylose may occur by both active and passive processes in the intestine, similar to reabsorption of xylose in the kidney.

**Role of Sodium Ions.** Several reports indicated that sodium ions played an integral role in active transport processes in the intestine. In the frog and the rat, substitution of sodium ions by lithium or potassium caused a decrease in xylose absorption (Csaky and Lassen, 1964). Faust (1962) reported that substitution of sodium ions by lithium in rats caused a decline in glucose absorption, but had no effect on xylose, and concluded that xylose was not actively absorbed. Later Rosensweig et al. (1965) and Faust et al. (1967) obtained the same results and concluded that some active absorption mechanisms were independent of sodium. Alvarado (1967) found that the sodium ion gradient determined the direction of net xylose movement into or out of the cell. Crane (1964) postulated that the net uphill movement of sugar into the cell was a consequence of a downhill gradient of sodium ions into the cell maintained by an outwardly directed, energy-dependent sodium pump.
Factors Inhibiting Xylose Absorption

Many factors have been found to inhibit xylose absorption. Among these were actively transported sugars which competed for the carrier mechanism (Bihler et al., 1969; Barnett et al., 1970); phlorizin (Larralde and Giraldez, 1957; Hart and Nissim, 1964; Salem et al., 1965; Alvarado, 1966); mono-iodoacetic acid (Peters and Van Slyke, 1946; Wilson and Vincent, 1955; Beyreiss et al., 1965); arbutin (Alvarado, 1964); aminopterin (Small et al., 1959); somatostatin (Wahren and Felig, 1976); neomycin sulfate (Small et al., 1966); oligomycin and 2,4-dinitrophenol (Faust et al., 1967).

Also inhibiting absorption of xylose in the small intestine were the amino acids D-histidine and L-methionine in the hamster (Duthie and Hindmarsh, 1966). Thyroxin was found to have no effect on xylose absorption in rats (Ponz, 1945), however, Broitman and Zamcheck (1963) reported increased urinary xylose in thyroxin-treated rats. Hyperthyroid human patients showed an accelerated absorption of glucose, galactose, and xylose (Peters and Van Slyke, 1946). Harland and Clark (1963) reported that rats kept at 4 C absorbed more xylose than those kept at 27 to 31 C, and that thiouracil treatment caused a decrease in xylose absorption. Beck et al. (1962) found that normal variations in gastric emptying did not influence xylose absorption 30 minutes after administration in man. Total body irradiation of rats caused a significant decrease in xylose and glucose absorption (Farrar et al., 1956). Hypophysectomized rats excreted less xylose than did normal animals (Csernay et al., 1962). Absorption of glucose and xylose was found to be greatest in rat intestine in vitro at pH 6.5 to 7.5, and absorption decreased on both sides of this range (Ponz and Larralde, 1950). Gellhorn and Moldavsky (1934) reported that changes
in pH to either side of neutral caused an increase in xylose and glucose absorption, due to alteration in gut permeability. Using rabbit intestine in vitro, Auchinachie et al. (1930) found that xylose diffused through dead segments faster than glucose, but the reverse was true in living segments at low temperatures. The enzyme alkaline phosphatase apparently was involved with intestinal absorption of glucose, but not xylose, in rats (Tuba and Dickie, 1954).

**Rate of Xylose Absorption**

Small et al. (1959) confirmed the findings of Verzar (1935) that glucose is absorbed at a constant rate while xylose is absorbed at a rate proportional to its concentration. Fullerton and Parsons (1956) reported that xylose absorption is directly proportional to the water absorption rate in the rat intestine, while glucose absorption also involves a constant. The relative rates of absorption of five monosaccharides were galactose > glucose > fructose > xylose > arabinose in the rat (Cori, 1925; Wilbrandt and Laszt, 1933; Hele, 1950) and in man (McCance and Madders, 1930). In the rat, xylose differed from other sugars in that much more was absorbed during the first hour than in subsequent hours. After the first hour, xylose absorption followed a straight line as other sugars did from the beginning (Cori, 1925). In man, McCance and Madders (1930) found that absorption of xylose proceeded rapidly and at a linear rate for 1.5 hours and then almost ceased, even when a large amount still remained in the intestine. This report was not in agreement with the findings of Verzar (1935) and Small et al. (1959) that xylose was absorbed at a rate proportional to its luminal concentration. It was also reported by McCance and Madders (1930) that xylose was absorbed in the proximal parts of the small intestine, with
little or no absorption in more distal sections. This agreed with the findings of Skala et al. (1963) that the absorption of xylose in the terminal ileum was considerably lower than in the more proximal parts of the intestine of the rat. Likewise, Hagen (1952) found that isotonic solutions of glucose and xylose were absorbed readily from the small intestine of the guinea pig, but not from the colon. Davidson and Garry (1940) reported that xylose was absorbed as fast as glucose from the caudal region of the small intestine of the cat in vitro, which differed from absorption in the rat.

Phosphorylation of Xylose

Hele (1950) reported that the absorption of sugar from the intestine is accompanied by an increased phosphorylation of sugars in the mucosa. He suggested that hexokinase participates directly in sugar absorption and functions in a phosphorylation-dephosphorylation cycle with phosphatase. Xylose was reported to be phosphorylated in vitro, but to a lesser degree than glucose, galactose, or fructose (Hele, 1950). Hele (1953a,b) found that the phosphorylation rates of different sugars in vitro (galactose > glucose > fructose > xylose) had the same relationship to each other as did their absorption rates in vivo. Earlier work by Davidson and Garry (1941) had reported that, while the absorption of galactose and glucose may have been accelerated by phosphorylation in the rat intestine in vivo, xylose was not phosphorylated and its absorption depended on simple diffusion. Sols and Crane (1954) found that xylose acted as a competitive inhibitor of glucose for brain hexokinase. In a related study, Crane and Sols (1954) reported that xylose-5-phosphate did not serve as a non-competitive inhibitor in brain hexokinase reactions. The authors postulated that specificity of substrate for brain hexokinase involves a ring
structure with hydroxyl groups at carbons 1, 3, 4, and 6 of the molecule. Turner (1950) reported that, after an oral dose of glucose, fructose, or xylose, there was for each sugar a characteristic decrease in the concentration of serum inorganic phosphate, indicating possible phosphorylation of xylose in vivo. This was confirmed for pentoses in man by Wyngaarden et al. (1957). The phosphorylation hypothesis for active transport of xylose and other sugars was refuted by Crane (1962), who found that the specificity of active transport for the hydroxyl group at carbon 2 of the sugars was not consistent with the phosphorylation capacity of kinases.

**Xylose Penetration into Cells**

The examination of xylose penetration into cells of various tissues in the body has been conducted by several authors. Cori and Goltz (1925) reported that the epithelial cells of the intestine were selectively permeable for different sugars, while those of the peritoneal cavity were equally permeable for all sugars. The same authors found that muscle cells of mice were less permeable to sugars than liver cells. Both hexoses and pentoses reached equilibrium at the same rate. After oral administration in the rat, xylose content of blood, liver, and kidney, but not muscle, increased (Miller and Lewis, 1932b).

**Role of Insulin.** The effect of insulin on the process of xylose penetration into intracellular space has been documented. Goldstein et al. (1953) found that, under the influence of insulin, glucose, galactose, and xylose entered the intracellular compartment more readily. They suggested that only sugars having the same configuration as glucose at carbons 1, 2, and 3 were aided by
insulin to enter certain cells. DeVananzi (1958) reported that insulin, glucose, or insulin plus glucose accelerated removal of xylose from the blood of normal dogs. A three-fold increase in xylose disappearance from blood occurred in man after intravenous injection of insulin. This effect was not due to increased renal excretion and suggested that insulin affected the volume of distribution of xylose in body fluids (Segal et al., 1957). Insulin accelerated the disappearance of xylose in nondiabetics, but it had no effect on blood xylose in insulin-treated diabetics, possibly due to the presence of insulin antibodies (Field and Johnson, 1960). Using rat tissue in vitro, Park et al. (1957) found no xylose penetration into muscle in the absence of insulin, but large amounts with insulin. They proposed that the muscle membrane was a site of insulin action, distinct from phosphorylation, and involved combination of a sugar with a constituent of the cell membrane. Likewise, after intravenous xylose injection in cats, no xylose was found in lung, intestine, skin, spleen, or muscle tissue without exogenous insulin. With insulin, however, xylose penetration into muscle, brain, and heart tissue increased (Sacks and Bakshy, 1957). Insulin acted to facilitate the transfer across the cell membrane of glucose and those sugars with the same steric configuration as glucose at carbon atoms 1, 2, and 3, which included xylose. The transport of a sugar into the cell appeared to precede any phosphorylation reaction (Sacks and Bakshy, 1957).

Sloan et al. (1976) found that insulin might have stimulated sugar uptake by cells of rat soleus muscle at some site prior to the cell membrane. Korbl et al. (1977) reported that insulin, anaerobic conditions, 2,4-dinitrophenol, and salicylate all stimulated xylose uptake by rat soleus muscle. Randle and Smith (1958) substantiated these findings and also reported a similar effect
due to cyanide and arsenite in the isolated rat diaphragm. Stimulation of xylose transport by insulin in rat diaphragm muscle was reported by Kipnis and Cori (1957) and Carlin and Hechter (1961). The penetration of xylose into cells occurred against a concentration gradient (Eichhorn and Hechter, 1961). Dailey et al. (1965) reported a linear relationship between insulin concentration and xylose uptake by rat diaphragm muscle. Norman and Hiestand (1959) found that triiodothyronine and cortisol had no effect on xylose distribution in vitro, but in vivo both hormones increased xylose penetration into rat muscle with or without exogenous insulin. Using rat tissue in vitro, Menozzi et al. (1961) reported that the transfer of xylose into the cell was accelerated by insulin in epidydimal tissue, but not in mesenteric tissue. Menozzi and Gatto (1961) found that thyroxin slowed xylose transport in vitro. Insulin decreased intracellular concentration of sodium, while thyroxin increased it (Menozzi and Gatto, 1961). Pozza et al. (1958) found that xylose injection into normal dogs did not stimulate insulin secretion. The conclusion was drawn that insulin secretion was probably stimulated by sugars which were both utilizable and insulin-sensitive. There was no apparent relationship between chemical structure and the ability to cause insulin release (Pozza et al., 1958). The ability of sugars to enter cells apparently was not connected with their utility since xylose cannot be utilized freely (Peters and Van Slyke, 1946).

Metabolism of Xylose

The metabolism of xylose in the body has been studied in many species. Lynch et al. (1969) reported that in man 60 percent of absorbed xylose was metabolized, while the remaining 40 percent was excreted. After oral xylose
administration, an increase in blood glucose was seen in the rat (Blatherwick et al., 1936), the rabbit (Blanco, 1928) and man (Wyngaarden, et al. 1957). Harding et al. (1933) did not find an increase in blood glucose after xylose administration in man, nor did Siddons et al. (1969) in the calf. (Blatherwick et al., 1936) reported no change in glycogen formation or lactic acid production in rabbit liver or muscle following a feeding of xylose. Similarly, no increase in liver or muscle glycogen was observed in the rat after administration of D-xylose (Miller and Lewis, 1932a,b), or L-xylose (Larson et al., 1940). The latter authors found that L-xylose was absorbed at less than one-tenth the rate of D-xylose in the rat, which supported the active transport theory of D-xylose absorption, since both isomers did not simply diffuse at the same rate. Miller and Lewis (1932a) concluded that xylose was not metabolized in the rat. No lactic acid production from xylose was found to occur in the rat jejunum (Wilson, 1956). Similar results were reported by Haarmann and Stratmann (1932). The increase in xylose excretion in thyroxin-fed rats may have reflected an altered metabolism of xylose in these animals (Broitman et al., 1961b).

Marble and Strieck (1932) suggested that xylose may have been metabolized after they observed a rise in respiratory quotient in all cases following xylose administration in dogs and human patients. Nottdurft (1937) reported similar observations in the guinea pig, but not in the rat, and concluded that the rat cannot utilize xylose aerobically or anaerobically. Thomas and Gradinescu (1931) found an acceleration of respiration in young dogs and cats after an oral dose of xylose. The metabolism of labeled xylose to CO₂ in the
chick was reported by Wagh (1967). Haarmann and Stratmann (1932) reported the breakdown of xylose in the tissues. Wainio (1947) reported the oxidation of xylose in mammalian liver slices. Xylose-\(^{14}\)C was partly metabolized to \(^{14}\)CO\(_2\) in man (Wyngaarden et al., 1957) at a rate of 16 percent of an intravenous dose in 6 hours (Segal and Foley, 1959). Only a small portion of the \(^{14}\)C-activity in urine was unmetabolized xylose (Segal and Foley, 1959). However, Wyngaarden et al. (1957) reported that no significant labeling of urinary products other than that of unchanged xylose was detected in man. Stjernholm and Noble (1961) found with \(^{14}\)C labeling that xylose was slowly converted to glycogen in the rabbit via the transketolase-transaldolase sequence. Using a similar technique in the mouse, Hiatt (1957) reported that one percent of xylose carbon was found in glycogen, a pattern consistent with the conversion of xylose to hexose via the pentose phosphate pathway. McCormick and Touster (1957) reported that xylose-\(^{14}\)C was oxidized to \(^{14}\)CO\(_2\) by the rat and the guinea pig by way of xylitol, which was converted to xylulose and other intermediates of the pentose phosphate shunt. Earlier Larson et al. (1939) reported that xylulose was transformed to glucose by the dog. Pitkaenen and Svinhufvud (1965) found that xylose administration in man resulted in excretion of 8 to 18 percent of the dose as the metabolic product, D-threitol.

**Microbial Metabolism of Xylose.** The microbial fermentation of xylose in the intestinal tract has been studied. Heneghan (1963) found that the absence of microflora in rats and mice produced a two-fold increase in xylose absorption. Monocontamination with pure cultures lowered xylose absorption due to bacterial metabolism of xylose. In some human patients with low xylose excretion tests,
Goldstein et al. (1970) found that coliforms isolated from the upper small intestine could metabolize xylose. This microbial activity could cause multi-gram amounts of an oral xylose dose to disappear, resulting in an erroneously low estimation of xylose absorption. Kreula and Rauramaa (1974) introduced xylose via fistula into the rumen of a mature cow and found it to be extensively metabolized, with eight percent recovered in feces and one percent in urine in 5 days.

**Excretion of Xylose**

**Clearance from Blood.** The clearances or rates of removal from the blood of many substances, including xylose, have been studied to investigate the function of glomerular filtration. According to Hemingway (1935) the clearance rate becomes a measure of glomerular filtration if the substance studied is eliminated only through the glomeruli. Inulin, creatinine, and urea were commonly used as references in evaluating the clearances of xylose and other sugars. The order of clearances was found to be inulin = creatinine > sucrose > xylose > urea in normal animals. These results, in all or in part, were found following intravenous injection in the frog (Forster, 1938), the rabbit (Cope, 1933b; Kaplan and Smith, 1935), the dog (Richards et al., 1934; Hemingway, 1935; Pitts, 1936), the sheep (Shannon, 1937), and in man (Keith et al., 1934). Xylose clearance was normally greater than that of glucose in the rabbit, but the two were equal when phlorizin was used as an inhibitor of reabsorption in the kidney (Cope, 1933a). Xylose removal from the blood of rabbits was drastically increased with phlorizin diabetes and decreased with tartrate nephritis (Corley, 1926a, b). Similar results were reported by Fishberg and Dolin (1930), but these differences were not seen when galactose was used instead of xylose.
(Corley, 1927). In man no significant rise in xylose clearance was seen following phlorizination (Chasis et al., 1933), which indicated tubular reabsorption of xylose did not occur, or was not an important process.

**Glomerular Filtration and Reabsorption.** Shannon and Smith (1935) reported that xylose was normally reabsorbed in man by the renal tubules from glomerular filtrate, which was in part an active process. The 10 percent of xylose reabsorption which was not removed by phlorizin may have been due to passive diffusion of xylose from tubular urine back into the blood. Small et al. (1959) found that the rat kidney displayed limited active reabsorption of xylose, with 78 percent being passively excreted. White and Monaghan (1933) also reported tubular reabsorption of xylose. Wilbrandt (1954) assumed active reabsorption of a substance in the kidney if the ratio of the concentration of the substance in urine to the concentration in plasma fell below 1.0, and found this did occur with xylose. He also found that saturation of the transport mechanism by high concentrations of glucose blocked the reabsorption of xylose completely, a situation analogous to intestinal absorption. Similar findings were reported by Shannon (1938) in studies with the dog. Hamburger (1922) observed that xylose was partially retained by the frog kidney *in vitro*.

**Xylose as an Indicator of Renal Function.** Jolliffe et al. (1932a) set forth the desirable properties of a substance used to evaluate glomerular filtration under physiological conditions. These criteria were:

1. It must be determinable in plasma and urine with quantitative accuracy.

2. It must be non-toxic and exert no local stimulating or depressing effect on the kidney.
3. It must be completely filterable from plasma.

4. It must not be secreted or reabsorbed by the renal tubules.

In the same study, the authors reported that xylose was excreted, but not reabsorbed, by all animals studied and possessed all four desirable properties. Xylose caused no local effects on renal function (Jolliffe et al., 1932b). Fish have been used to test the efficacy of xylose as an indicator of glomerular function. Jolliffe (1930) reported that fish with aglomerular kidneys did not excrete xylose. Xylose was found to be completely filterable by fish with glomerular kidneys (Jolliffe, 1930; Clarke and Smith, 1932).

**Xylose Absorption and Excretion Tests in Man**

**Xylose Excretion Tests.** Apparently the first published use of an oral xylose test in man was that reported by Ebstein (1892). He fed a diabetic 25 g of xylose and observed that he was still excreting nonfermentable reducing substance (NRS) in urine 9 days later. He attributed this to some disturbance of sugar tolerance in diabetics, possibly related to kidney damage. Fishberg and Friedfeld (1932) developed an oral xylose test for the diagnosis of impaired renal function in human subjects. Normal fasted men excreted 25 percent of a 50 g oral dose in 24 hours, while several patients with kidney malfunction excreted as low as two percent. Wuest and Solmsen (1946) reported that 46 percent of an oral xylose dose was excreted by a normal man in 24 hours. Pre-administration NRS in blood was 28 ± 5 mg/100ml, and rose to 80 mg/100ml within 3 hours after xylose administration, then decreased to 45 mg/100ml in the next 2 hours. Most of the xylose was removed from blood within 5 hours, which suggested a shortening of the urine collection period.
from 24 to a more manageable 5 hours (Fishberg and Friedfeld, 1932). The test was revised for children, using a dose of 1 g of xylose per three pounds of body weight to a maximum of 50 g (Fishberg and Friedfeld, 1933). The normal kidney concentrated xylose to 2.5 percent of urine in 5 hours. The functionally deficient kidney concentrated less xylose and xylose was retained for a longer period in the blood. In this way kidney function was estimated by xylose in the blood of infants when urine collection was difficult (Fishberg and Friedfeld, 1933). Mathematical models of xylose excretion from the normal kidney were presented by Fishberg and Dolin (1930), Fishberg and Friedfeld (1932, 1933), Domínguez and Pomerene (1934), and Domínguez et al. (1937). A xylose excretion constant for normal men was calculated which was independent of moderate exercise and moderate changes in diuresis (Domínguez and Pomerene, 1934).

**Xylose Absorption Tests.** The use of the oral xylose test as an indicator of intestinal absorption was suggested by Helmer and Fouts (1937). The authors stated that xylose was not metabolized, passed unchanged through the liver, was eliminated by the kidney, was not reabsorbed by renal tubules, and could be accurately recovered from urine and blood. Since no difference in xylose clearance was found in normal individuals when the dose was administered orally or intravenously, the authors felt that changes in absorption from the digestive tract might influence the excretion of orally administered xylose. Patients were fed 25 g of xylose. Urine and blood samples were taken hourly for 5 hours. Xylose in blood disappeared at a rate proportional to the actual momentary concentration of NRS in blood, and this was independent of urine flow. Following an oral dose, any marked deviation in xylose excretion from
that observed after administration of an IV dose was concluded to be due to changes in intestinal absorption. Patients with low xylose excretion and low xylose absorption curves were considered to have poor absorption even when kidney function was low. Impaired kidney function caused low xylose excretion in some cases of pernicious anemia, but no constant abnormality in absorption was noticed in these patients (Helmer and Fouts, 1937). In a similar kind of investigation, Gamble et al. (1969) reported that the presence of elevated serum xylose with low urinary excretion indicated that renal impairment rather than intestinal malabsorption was the problem in rheumatoid arthritis.

**Normal and Abnormal Results of Xylose Tests.** Since the report of Helmer and Fouts (1937), the oral xylose test has been used extensively in human medicine as a clinical tool for the diagnosis of intestinal malabsorption. In normal individuals urinary xylose excretion and blood xylose concentration are remarkably constant following an oral dose of xylose. Normal individuals excreted 4.68 g in 5 hours, or 19% of the oral dose of 25 g (Helmer and Fouts, 1937). Fourman (1948) reported that normal persons excreted about 6 g (24%) of xylose in 5 hours after an oral dose of 25 g, whereas patients with malabsorption eliminated as little as 2 g in that time. Brien et al. (1952) found that 6.14 g was excreted in 5 hours. A decrease in plasma inorganic phosphate was seen, indicating active absorption. The 19 g not excreted may have been metabolized, since a sugar capable of phosphorylation is usually capable of metabolism in the body. Blood xylose reached a maximum value in 1 hour and approached fasting levels in 5 hours (Brien et al., 1952). Xylose excretion in normal persons was 6.5 g in 5 hours according to Benson et al. (1957), while untreated patients with tropical sprue excreted about 1 g.
Blood xylose rose to about 40 mg/100ml in 1 to 2 hours in normal adult subjects. Similar blood values were reported in children by Lanzkowsky et al. (1963). Butterworth et al. (1959) reported average xylose excretion of 5.7 g in normal individuals, and less than 3.0 g in cases of malabsorption. An average xylose excretion of 7.2 g was found by Fowler and Cooke (1960). Christiansen et al. (1959) reported a five-hour urinary xylose excretion of 6.5 g after a 25 g dose, with a plasma xylose maximum of 35 mg/100ml in 1 to 2 hours. Santini et al. (1961), using a 5 g dose, reported average five-hour excretion of 1.8 g in normal individuals, which was a somewhat higher percentage (36%) than the range of 19 to 29% reported for 25 g doses (Wiseman, 1964). Levinson and MacFate (1969) reported that normal persons excreted 5 g or more of an ingested 25 g dose, while 2.5 to 5 g indicated secondary malabsorption and less than 2.5 g was a sign of severe malabsorption. Lynch et al. (1969) found that 4.3 to 7.5 g were excreted in normal cases, with less than 3 g indicating malabsorption. They also reported that 60 to 70 percent of a 25 g oral dose was absorbed in the first 5 hours. Bockus (1964) found that 4.5 g was normally excreted, 3 to 4.5 g was borderline, and less than 3 g was abnormal.

Normal blood xylose was at least 20 mg/100ml within 2 hours after a 25 g dose (Bockus, 1964). Normal blood values with low urine xylose indicated poor renal excretion, while low blood and low urine values indicated intestinal disease (Helmer and Fouts, 1937; Bockus, 1964). A close correlation was found between the plasma xylose absorption curve and urinary xylose excretion when renal function was normal (Benson et al. 1957; Meeuwisse and Dano, 1965). For this reason, after an oral xylose dose the simpler method of a five-hour
urine collection has been commonly used instead of blood sampling to test for intestinal absorptive ability in human subjects. Xylose was reported to be stable in urine (Levinson and MacFate, 1969).

**Criteria for an Absorption Test.** Althausen (1939) proposed a set of criteria for a substance to be used in tests of intestinal absorption. The desired properties of a suitable substance were:

1. It should be susceptible to phosphorylation, since this process is involved in the absorption of some important food elements.

2. It should be water soluble.

3. The outcome of the test should not depend on concentration of the substance because dilution by gastric and intestinal secretions is variable.

4. The test should be independent of the rate of gastric evacuation which cannot be controlled and varies with many factors.

5. It should be absorbed as such without preliminary hydrolysis since this introduces a new enzyme system.

6. It should be capable of quantitative determination and should not occur in the medium used for determination of the test.

7. If it is utilized in the body or excreted during the test, it should be possible to determine its rate of utilization or excretion to rule out the possibility that abnormalities of these functions might interfere with the test.

As mentioned previously, the phosphorylation requirement for active absorption of sugars has been questioned (Crane, 1962). However, the other criteria are all met by the xylose test.

Fordtran et al. (1962) described the ideal sugar for absorption tests as one which is substantially but incompletely absorbed in the small intestine; is not metabolized in the body; and is quantitatively excreted in the urine. The authors added that the ideal sugar had not been found.
Human Malabsorption and the Xylose Test

Celiac Disease and Sprue. In humans the oral xylose tolerance test has been used successfully in the diagnosis and follow-up treatment of many intestinal diseases, the most prominent of which are the malabsorption syndromes of celiac disease and sprue. Celiac disease is the most common cause of malabsorption in children (Hubble and Littlejohn, 1963). The cause of this disease appears to be a sensitivity to gluten (Kendall et al., 1972). That patients with celiac disease consistently exhibited low xylose excretion was shown by: Fowler and Cooke (1960); Clark (1962); Hubble and Littlejohn (1963); McCrae (1963); Ingomar et al. (1964); and Doran et al. (1966). The improvement in absorptive ability of the intestine in celiac patients placed on a gluten-free diet has been documented by Zareba and Laskowski (1967), Kendall et al. (1972), and Harms (1973) by use of the xylose test. Hubble and Littlejohn (1963) concluded that the xylose test is an excellent test for malabsorption in childhood.

Primary malabsorption syndrome is a genetically-transmitted complex metabolic disorder affecting enzymatic pathways involved in intestinal absorption of many nutrients. Celiac disease in childhood and sprue in later life are different phases of the same syndrome. The xylose absorption test is routinely used in screening suspected cases of sprue. Low xylose excretion values were reported in cases of sprue by: Benson et al. (1957); Adlersberg (1959); Butterworth et al. (1959); Sessions et al. (1962); Santiago-Borrero et al. (1971). The usefulness of the xylose test for malabsorption syndromes has also been confirmed by Florkiewicz et al. (1965); Rafes et al. (1969); Hara et al. (1970); and Mary et al. (1975).
Normal xylose excretion after intravenous injection in patients with sprue confirmed that malabsorption accounted for low xylose excretion following an oral dose (Butterworth et al., 1959). Low blood and urine levels in sprue patients were found by Gardner and Santiago (1956) and Finlay and Wightman (1958). Remission of sprue, whether spontaneous or induced by cortisone or a gluten-free diet, was associated with a normal or greatly improved xylose test (Finlay and Wightman, 1958). Urinary excretion was a more reproducible indicator of malabsorption than blood xylose levels with sprue (Gardner and Santiago, 1956), while blood levels were a better means of discriminating between normal and pathological values with celiac disease (Ingomar et al., 1964).

Malnutrition. Conditions of malnutrition have also been evaluated by the xylose absorption test. Patients with kwashiorkor and marasmus showed a high incidence of abnormally low xylose excretion (Amin et al., 1969; Sawhney and Kaul, 1971). The degree of xylose malabsorption bore a linear relationship to the severity of malnutrition. Gurson and Sauer (1969) reported low xylose excretion in subjects with marasmus. IV tests showed normal renal function, so low excretion was due to poor intestinal absorption. Duque et al. (1975) studied adults with severe protein malnutrition. Xylose malabsorption correlated with electron microscopic studies, indicating microvillar abnormalities in the jejunum. Xylose absorption has been of great value as a screening test for malabsorption in many countries. Using the xylose test, widespread sub-clinical malabsorption due to malnutrition has been shown in Liberian children (Rhodes et al., 1971) and in adults in Puerto Rico (Angel et al., 1963), Nigeria (Falaiye, 1969), and India (Baker et al., 1971).
Other Diseases. Other health problems which have been associated with xylose malabsorption include: rotavirus and adenovirus gastroenteritis (Mavromichalis et al., 1977); myxedema (Broitman et al., 1961, 1964; Risek et al., 1962); steatorrhea (Chanarin and Bennett, 1962; Shiner et al., 1962; Zareba and Laskowski, 1966); Addison's disease (Thaddea, 1943); giardiasis (Velez and Orrego, 1963; Wright et al., 1977); liver cirrhosis (Arimasa, 1964); mental retardation (Chapman et al., 1966); systemic bacterial infections (Cook, 1972); atrial and ventricular septal defects (Markiewicz et al., 1976); coarctation of the aorta (Markiewicz et al., 1975); strongyloidosis (Velez and Orrego, 1963); lymphosarcoma (Velez et al., 1963); compression of celiac artery (Garvin et al., 1977); and chronic colitis (Vartio, 1963). The xylose test was especially useful in cases of celiac disease, idiopathic sprue, myxedema, steatorrhea, and small intestinal derangements, but less so with regional enteritis, pancreatic disease, and post-gastrectomy (Beck et al., 1962; Zamcheck, 1962; Finlay et al., 1964).

No relationships between xylose absorption and the following diseases were found: eczema (Fry et al., 1965); pellagra and ancylostomiasis (Abdalla et al., 1963). No consistent effect on xylose absorption was found in patients after gastrectomy (Beno et al., 1968). Thyrotoxic patients tended to show an increase in xylose absorption (Broitman et al., 1961a, 1964), as did patients with acute and chronic hepatitis (Arimasa, 1964). Reduced luminal resistance in the intestine correlated with xylose absorption (Ritchie and Salem, 1965).

Carlsson and Dehlin (1972) reported that chronic alcoholics had significantly greater xylose absorption than normal subjects, and their pancreatic exocrine function did not differ from normal. Small et al. (1960) and Broitman et al.
(1961b) found that alcohol caused decreased xylose absorption in rats. Zamcheck (1962) reported increased urine xylose with acute alcoholism. Small et al. (1960) found decreased xylose absorption in chronic alcoholics after an acute drinking bout.

Sensitivity to cow-milk albumin induced a malabsorptive state in susceptible children which was accompanied by reduced xylose absorption. Subsequent treatment with diets free of bovine milk were shown to improve the situation as xylose test results increased (Buehrdel et al., 1975).

Other Factors Affecting Xylose Tests

Geographical Area. Average five-hour urinary xylose excretion in normal Japanese men was 8.07 g after a 25 g dose (Arimasa, 1964). This value was higher than average values in Europe and North America and may have reflected the effect of different diets over many years, especially with regard to carbohydrate content. The five-hour excretion after an IV dose did not differ from xylose excretion in other reports. Keusch et al., (1970) reported that Americans upon arrival in Thailand had significantly higher xylose excretion values than Thai natives, but after 8 months in Thailand, both groups were equal. The two-hour blood xylose level was lower in Americans than Thais and did not change during 8 months. The authors urged caution in interpreting intermediate values (3 to 5 g) of xylose excretion in the tropics. Than-batu et al. (1971) suggested that the behavior of the kidneys in the tropics may normally differ from that in temperate zones. The discrepancy in xylose test results may have been due to variation in xylose metabolism and/or renal handling in the tropics.
**Age Effect.** The effect of age on the values obtained in the xylose absorption test has been studied with variable results. An increase in xylose absorption from infancy through older childhood was reported by: Møllerberg and Soederhjeim (1965); Allen and Vest (1966); Zareba et al. (1966); Bunke (1970); Harms (1973); and Heimann et al. (1977). Lanzkowsky et al. (1963) reported lower xylose recovery in children than in adults. Deckert et al. (1967) found that xylose excretion steadily decreased from infancy through older childhood and to adulthood. Jones (1951) and Small et al. (1960) found that xylose absorption was greater in newborn rats than in adult animals. Guth (1968) and Fowler and Cooke (1960) found no difference in xylose excretion in man between the ages of 30 to 69 and 20 to 62 years, respectively. Thereafter, both reports indicated progressively decreasing urine xylose with age. Lynch et al. (1969) and Kendall (1970) reported that elderly persons exhibited higher blood xylose and lower xylose excretion, which was due to a deterioration in glomerular filtration rather than a reduction in intestinal absorption. Finding similar results, Rosin et al. (1970) concluded that the xylose excretion test was not a reliable screening procedure for malabsorption in elderly patients due to poor renal function. Mehta (1970) found that the age and sex of patients did not influence the xylose absorption rate.

**Effect of Dose.** Several levels of dosage have been used in the xylose absorption test in man, the most common being the 25 g dose which was first used by Ebstein (1892) and later by Helmer and Fouts (1937). This dose has been given regardless of body size to human adults with satisfactory results. In some human patients, the 25 g dose caused unpleasant side effects, such
as diarrhea and nausea (Santini et al., 1961; Clark and Harland, 1967). The use of a 5 g dose was suggested by Santini et al. (1961), who found that this dose gave results comparable to the 25 g dose, but without the side effects. In normal subjects, 1.8 g of a 5 g dose was excreted in 5 hours in one study (Clark and Harland, 1967), and 1.75 g in another (Castro et al., 1964). These values corresponded to excretions of 35 to 36 percent of the administered dose, compared to 23 percent of the 25 g dose (Clark and Harland, 1967). Comparative studies showed xylose excretion at the two dosage levels to be closely correlated in normal individuals (Joske and Haagensen, 1964; Kryszewski, 1968). Xylose absorption was more rapid and more complete with the 5 g test, but the 5 g test was a less sensitive indicator of malabsorption than the 25 g test, which was considered to be more suitable for routine use (Joske and Haagensen, 1964). Rinaldo and Gluckmann (1964), using doses of 5, 25, and 50 g, and Mehta et al. (1971), using doses of 5, 10, 15, 20, and 25 g, concluded that the 25 g dose was the most practical and effective for diagnostic differentiation between normal subjects and those with sprue. Mehta et al. (1971) found many false negative tests with doses less than 25 g.

In the rat it has been shown that a larger percentage of xylose was excreted at lower concentrations than at higher (Clark and Harland, 1967). An increased dose of xylose corresponded to an increased rate of xylose absorption in chicks (Wagh and Waibel, 1967). Corley (1928) reported that xylose levels of 2 or 3 g per kg of body weight produced similar blood concentration and renal excretion, but smaller doses produced lower values. Bolton et al. (1976) reported no increase in maximum plasma xylose concentration or in urinary xylose output in the horse at dosages in excess of .5 g/kg, whereas doses of .12 and .25 g/kg caused lower blood and urine xylose values.
Advantages of the Xylose Test

Klassen and Lanzkowsky (1964) described the oral xylose test as simple, reproducible, inexpensive and valuable in the investigation of malabsorption. Digestion of xylose is unnecessary and its absorption is independent of bile, intestinal, or pancreatic secretions. Since xylose is absorbed in the duodenum and jejunum, its primary use is for demonstration of malabsorption due to lesions of the cranial small intestine. Factors affecting general body metabolism and nutrition influence the absorption, metabolism, and excretion of xylose (Zamcheck, 1962). Clinical abnormalities in the test resulted only from abnormal intestinal absorption and defective glomerular filtration (Finlay et al., 1964). The rate of false positives in patients without malabsorption was less than 2 percent (Beck et al., 1962).

Limitations of the Xylose Test

Several limitations of the xylose test have been noted. The rate and extent of absorption is dependent on the area available for absorption. If only a small area of the intestine is damaged, xylose may be completely absorbed in the five-hour test, especially if the dose is only 5 g (Sammons, 1965). Luecking and Gruettner (1971) compared intestinal biopsies to the xylose test in celiac disease, and found inconsistency in the effectiveness of the xylose test in demonstrating cases of villous atrophy. The jejunal biopsy was also favored by Krawitt and Beeken (1975), who found a 30 percent error in cases of normal and abnormal absorption with the xylose test. They concluded that the xylose test yielded little guidance for diagnosis or therapy and was superfluous when a biopsy could be obtained. The widely held concept that xylose is a foreign sugar in the body was contradicted by Weicker and
Grasslin (1966), who reported that xylose can always be found in the prosthetic groups of mucopolysaccharides and glycoproteins obtained from the urine and serum of normal patients and those with tumors.

Comparison with Other Absorption Tests.

Xylose has compared favorably with other commonly used absorption tests. Bockus (1964) and Petrovic and Nikolic (1969) found the glucose tolerance test to be less sensitive than the xylose test in demonstrating malabsorption. Although the glucose test may indicate severe intestinal malabsorption, the xylose test is preferable since it is a specific indicator whose plasma concentration is less altered by insulin levels, degree of excitement, length of fasting, and diet (Bolton et al., 1976). The xylose test was more reproducible than either the fructose or glucose test (Turner, 1950; Brien et al., 1952). Similar results were reported when the xylose test was compared to the fat absorption test (Christiansen et al., 1959) or the vitamin A absorption test (Wolfish et al., 1955; Christiansen et al., 1959).

Fordtran et al. (1962) proposed an absorption test using 3-methylglucose, a synthetic sugar actively absorbed in the intestine, not metabolized in the body, and almost quantitatively excreted in the urine, with 90 percent being recovered in normal subjects. This sugar, however, was found to be much less sensitive in diagnosing malabsorption than xylose, probably because xylose was incompletely absorbed in the normal intestine. Any disease of the small intestine, especially in the upper jejunum where xylose is preferentially absorbed, will decrease xylose absorption. Glucose and 3-methylglucose, however, can also be absorbed in the ileum, and intestinal diseases must be relatively more extensive before absorption of these hexoses is decreased (Fordtran et al., 1962).
Modification of the Xylose Test

Recent suggestions for modification of the xylose test have been made. Lyster et al. (1972) advocated the use of radioactively-labeled $^{14}$C-xylose for absorption tests, with the $^{14}$C-activity of blood and urine samples being used for xylose analysis. Frolkis and Bushkova (1977) used the oral xylose test in conjunction with meat or glucose feeding and found that meat caused a much greater increase in xylose absorption in the control group than in those with malabsorption. As reported by previous workers, glucose decreased xylose absorption. The authors recommended xylose testing after meat and glucose loading for functional investigation of the small intestine. Chandrasekaran et al. (1976) recommended a combined lactose-xylose tolerance test to evaluate absorptive function in infancy.

Xylose Malabsorption and Histological Changes.

Several malabsorption syndromes, characterized by villous atrophy, edema, and necrosis in the intestinal wall, have been found to correlate with the xylose absorption test in man (Adlersberg, 1959), horse (Bolton et al., 1976) and miniature swine (Bicks et al., 1967). The condition in miniature swine was produced experimentally by 2,4-dinitrochlorobenzene, which may have produced a condition similar to gluten sensitivity in humans afflicted with sprue (Bicks et al., 1967).

Xylose Absorption Tests in Animals

Animal tissues, both in vivo and in vitro, have been used extensively as models for xylose absorption, metabolism, and excretion, as this review has demonstrated. However, the xylose test as an aid to the diagnosis of conditions of malabsorption in livestock or companion animals has received
very little emphasis, in contrast to human malabsorption, for which the xylose test has been widely used. Xylose tests have been reported for the horse (Bolton et al., 1976; Roberts, 1974), the pony (Breukink, 1974), and the dog (Hill et al., 1970).

Hill et al. (1970) administered xylose to dogs at .5 g/kg of body weight, obtaining a normal peak blood xylose concentration of 45 mg/100 ml in 60 to 90 minutes. Urine was not collected. An abnormal xylose curve was found with mucosal damage of small intestine, but not with pancreatic insufficiency, which supports the findings of Beck et al. (1962) in humans.

The oral glucose tolerance test was used in the horse to assess sugar transport through the small intestine and to evaluate pancreatic endocrine function (Roberts and Hill, 1973). Loeb et al. (1972) fed corn starch as a source of glucose to horses by stomach tube. The glucose test has also been used for ponies (Breukink, 1974).

A xylose dose of .5 g/kg was concluded to be most appropriate for the horse. At this dose a peak plasma xylose concentration of approximately 20 mg/100 ml was reached 1 hour after administration (Bolton et al., 1976). Roberts (1974) reported that a dose of 2 g/kg was required to produce a maximum plasma xylose level of 30 mg/100 ml after 2 hours. He concluded that the xylose test in the horse might be useful, but was too expensive for routine use, being twenty-five times the cost of the glucose test.

Effects of Xylose in the Body

Xylose affected the appetite center of sheep in a way similar to glucose. Intravenously injected xylose, considered to be non-utilizable, elicited a feeding response related to the osmolarity of the solution injected, as did an injection of glucose (Seoane and Baile, 1973).
The feeding of large amounts of dietary xylose to young pigs produced poor appetite, poor growth, low nitrogen retention, high urine sugar, cataracts in eye lenses, decreased voluntary activity, and rough hair coats (Wise et al., 1954). Glucose-fed controls were normal in all respects. Similar experiments with rats (Darby and Day, 1939, 1940; Sterling and Day, 1951; Booth et al., 1953) confirmed the cataractogenic ability of xylose. Feeding galactose or maintaining high levels of blood glucose has also caused cataracts, as in human diabetics. The calf lens in vitro has been used as a model for studying the process of cataract formation caused by xylose (Van Heyningen, 1958).

**Xylose Test and Protein Deficiency**

The effect of insufficient dietary protein on xylose absorption has been shown in human cases of kwashiorkor (Amin et al., 1969; Sawhney and Kaul, 1971), and in the rat (Small et al., 1960). In the latter study, a protein-deficient diet resulted in decreased xylose absorption in the youngest animals. Apparently no work has been done with the xylose absorption test as an indicator of malabsorption in young calves fed diets containing experimental protein sources.

**Use of Xylose in Calves**

Xylose was found to be the only free sugar at measurable amounts in the abomasum of two- and six-month-old calves (Barhydt and Dye, 1957). Xylose was largely fermented in the rumen, with a small amount escaping rumen degradation and being absorbed in the small intestine. When 100 g of xylose were introduced into the abomasum by fistula, blood samples showed a peak xylose value of 20 mg/100ml over 6 hours. Siddons et al. (1969) infused sugars into the duodenum of pre-ruminant calves and found xylose to be absorbed almost as readily as glucose during a two-hour absorption period. No increase in blood glucose after xylose absorption was observed.
A report of research using the xylose absorption test in pre-ruminant calves is presented in Experiment 2 of this thesis.
EXPERIMENT 1.

PROTEIN DIGESTIBILITIES OF THREE CALF MILK REPLACERS
CONTAINING SOY PROTEIN SOURCES

SUMMARY

Three calves, beginning at 2 to 3 days of age, were used in each of two digestion trials. Four milk replacers were formulated which contained one-third of the protein from casein (CS) or one of three soy products: Promocaf (PC), an experimental soy protein concentrate (SPC), or an experimental soy flour (SF). Rations were isonitrogenous and isocaloric. A 3 x 3 Latin square design was used during the three-week trials. In trial 1, average protein digestibilities and standard errors were 76.9 ± 6.7, 63.9 ± 5.1, and 40.1 ± 15.6% for CS, SF, and SPC, respectively. In trial 2, average protein digestibilities and standard errors were 37.9 ± 2.0, 42.0 ± 4.4, and 41.7 ± 18.1% for PC, SF, and SPC, respectively.

Increases in protein digestibility with age had been expected with calves in the first 3 weeks of life, but digestibilities of soy rations were lower in some periods of the trials than in previous periods. Impaired digestive and/or absorptive ability due to soy protein is postulated and tests to evaluate malabsorption are suggested.

INTRODUCTION

The search for more economical sources of protein in calf milk replacers has included investigations of soy products. Soybeans are high in protein and soy proteins have a more nutritionally balanced amino acid composition than do
most other vegetable proteins (Porter, 1969).

Nitrogen digestibilities of soy products have generally been lower than those of milk proteins in the young calf (Noller et al., 1956b; Porter and Hill, 1963; Gorrill and Nicholson, 1969; Porter, 1969; Nitsan et al., 1972). Although the nutritional value of soy proteins has been improved by treatment with heat (Nitsan et al., 1971; Thompson et al., 1974), acid (Colvin and Ramsey, 1968, 1969), or alkali (Colvin and Ramsey, 1969; Coblentz et al., 1976), soy materials, when used to provide all or a high proportion of the protein in milk replacers, have generally produced slower growth of calves than milk products in the first few weeks of life (Wallace et al., 1951; Stein et al., 1954; Fries et al., 1958; Lassiter et al., 1959; Morrill et al., 1971; Nitsan et al., 1971, 1972; Thompson et al., 1974; Coblentz et al., 1976).

The processing of experimental soy materials used in this study suggested that they might produce improved results in calf rations. The protein digestibilities of milk replacers containing one of three soy products or casein are compared in calves in the first 3 weeks of life.

MATERIALS AND METHODS

Soy Products. Three soy products were used as protein sources in calf milk replacers: Promocaf (PC), a commercial soy protein concentrate; an experimental soy protein concentrate (SPC); and an experimental soy flour (SF).

Details of the processing methods of SF and SPC (Far-Mar-Co, Inc.) were not available. General considerations in the preparation of soy flours and soy protein concentrates follow.

Soy flours are prepared by grinding dehulled, solvent-extracted, unheated soybean flakes to pass a fine screen. These products contain approximately
40 to 50 percent protein on a dry basis.

Soy protein concentrates are prepared from clean, dehulled soybeans by removing most of the oil and water-soluble non-protein constituents. These materials contain not less than 70 percent protein on a moisture-free basis.

Promocaf (Central Soya Co.) is prepared from defatted soybean flakes which are extracted by a water-alcohol solvent procedure which immobilizes the protein fraction and removes water-soluble carbohydrates, minerals, and other minor constituents. The extracted product is freed of solvent, dried, and ground to desired particle size. It is free of trypsin inhibitors and other antigens common to soybeans.

**Milk Replacers.** Nutrient compositions of main ingredients of the milk replacers are presented in table 1. Casein (CS) was used as a control. Throughout this paper, each milk replacer will be referred to by the abbreviation of the protein source it contains, i.e., PC, SPC, SF, or CS. Milk replacers were formulated so that one-third of the total protein was provided by one of these products. Most of the remaining two-thirds of the protein was provided by dried skim milk and dried whey. Ingredients and the nutrient contents of the rations are presented in tables 2 and 3. Milk replacers were isonitrogenous, at 20 percent protein, and isocaloric, with calculated net energy of 2.2 Mcal/kg. Each contained 12 percent fat. Rations were formulated to furnish published nutrient requirements of the calf (N.R.C., 1971). A vitamin/trace mineral premix (table 4) and an antibiotic premix were added.

**Digestion Trials.** Three two- to three-day-old calves were used in each of two trials. In trial 1, SPC, SF, and CS were compared; in trial 2, PC, SPC,
and SF were used. In a 3 x 3 Latin square design, calves were fed milk replacers for one-week periods and reassigned weekly during the three-week experiment. Calves were housed in metabolism stalls. Milk replacers were fed at eight percent of body weight daily in two equal feedings with dry matter constituting 13 percent of the rations. No dry feed or water was provided. During each one-week period a four-day preliminary adaptation time and a three-day collection span were used.

Calves were fitted with harnesses to which plastic bags were attached for fecal collection, similar to the procedure described by Noller et al. (1956b). Bags were checked four times daily and changed as needed. Feces were frozen immediately after collection. Following the three-day collection period, feces from each animal were combined, weighed, and composite samples were taken. Protein content of samples was determined by the Kjeldahl procedure (\(\%N \times 6.25\)). Average daily fecal protein excretion was calculated and protein digestibilities were determined.

Statistical Analysis. Protein digestibilities were analyzed by analysis of variance for a Latin square design (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

The protein digestibilities from trials 1 and 2 are presented in table 5. No differences in protein digestibility due to rations, periods, or animals were found in either trial (\(P>.05\)).

As expected, in trial 1 the digestibility of protein in the CS milk replacer was higher than those of SF or SPC. The average value for CS, 76.9, was lower
than reported digestibility coefficients of milk proteins in calves. Average milk protein digestibilities in calves were 93.8 percent from 5 to 45 days of age (Blaxter and Wood, 1952) and 87.3 percent at 3 and 6 weeks (Gorrill and Nicholson, 1969). Parrish et al. (1953) reported digestibilities of colostrum and milk protein to be 92, 83, 86, and 93 percent at 1 to 2, 3 to 4, 5 to 8, and 14 to 17 days of age, respectively. Noller et al. (1956b) reported the digestibility of milk protein to be 90.1, which varied very little with age of the calf from birth to 25 days.

The digestibility of soy protein in the calf increased with age from 1 to 3 weeks (Nitsan et al., 1972), and from 7 to 46 days of age (Nitsan et al., 1971).

The expected increase in soy protein digestibility with age did not occur in this study. The protein digestibility of SF in both trials increased from week 1 to week 2 but then decreased to an intermediate level in period 3. In trial 2, the protein digestibility of PC decreased slightly in the second week and continued to decline in week 3. The digestibility of protein in SPC increased markedly with age in trial 1, but exhibited a decline between week 1 and week 3 in trial 2. The extraordinarily low values for SPC in period 1 of trial 1 and period 2 of trial 2 seemed to indicate a high degree of variability in the digestibility of SPC proteins.

In trial 2 the average protein digestibility of PC was 37.9. This value is considerably lower than the 81.6 for three- and six-week-old calves fed a milk replacer in which 70 percent of the protein was supplied by Promocaf and the remainder by milk products (Gorrill and Nicholson, 1969).

Dietary protein is very important in the rapidly growing young calf for cellular structure and enzyme formation. This is especially true in the cells
of the digestive tract, which are among the most active in the body. It seems reasonable that a dietary protein insufficiency may rapidly affect intestinal epithelial cells and secretion of digestive enzymes.

Since the remainder of the protein in the milk replacers, other than that provided by soy materials, was supplied by milk products, which are more readily digested, the soy protein may have been responsible for the low apparent digestibility of protein in the PC, SF, and SPC rations.

The decrease in protein digestibility in the first few weeks of life, a time when it would be expected to increase, suggests that soy proteins may have had a detrimental effect on the ability of the mucosal cells of the intestinal wall to absorb the products of protein digestion, or soy proteins may have caused a decrease in secretion and/or activity of proteolytic enzymes, or both. Since the antitrypsin activity of the soy products used had been destroyed by processing, the inhibition of proteolytic enzyme action was not the likely cause of reduced protein digestibility. Soy protein has been shown to have adverse effects on the pancreatic exocrine function of the calf (Gorrill et al., 1967; Gorrill and Thomas, 1967).

If soy protein did have an adverse effect on the ability of the intestinal epithelium to absorb protein, the absorption of other nutrients may also have been affected. The use of oral tests for malabsorption in calves fed soy protein during the first few weeks of life may give further insight into changes in intestinal capacity for absorption of carbohydrates, such as glucose or xylose, and fat.
### TABLE 1. NUTRIENT CONTENT OF MAIN INGREDIENTS IN MILK REPLACERS *

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Protein (%)</th>
<th>Fat (%)</th>
<th>Calcium (%)</th>
<th>Phosphorus (%)</th>
<th>Calculated net energy (Mcal/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy flour (SF) (^a)</td>
<td>46.0</td>
<td>.5</td>
<td>.22</td>
<td>.64</td>
<td>1.76</td>
</tr>
<tr>
<td>Soy protein concentrate (SPC) (^b)</td>
<td>70.0</td>
<td>.3</td>
<td>.22</td>
<td>.64</td>
<td>1.91</td>
</tr>
<tr>
<td>Promocaf (PC) (^c)</td>
<td>70.0</td>
<td>.3</td>
<td>.22</td>
<td>.64</td>
<td>1.91</td>
</tr>
<tr>
<td>Casein (CS) (^d)</td>
<td>90.0</td>
<td>---</td>
<td>.10</td>
<td>.10</td>
<td>1.98</td>
</tr>
<tr>
<td>Dried skim milk (DSM) (^e)</td>
<td>34.7</td>
<td>1.2</td>
<td>1.34</td>
<td>1.10</td>
<td>1.94</td>
</tr>
<tr>
<td>Fat product (^f)</td>
<td>7.0</td>
<td>60.0</td>
<td>.40</td>
<td>.30</td>
<td>3.52</td>
</tr>
<tr>
<td>Dried whey (^g)</td>
<td>12.2</td>
<td>.8</td>
<td>.98</td>
<td>.81</td>
<td>1.94</td>
</tr>
</tbody>
</table>

\(^a\) Treated soy flour, Far-Mar-Co, Inc., Hutchinson, KS.

\(^b\) Ultra-Pro soy protein concentrate, Far-Mar-Co, Inc., Hutchinson, KS.

\(^c\) Commercial soy protein concentrate, Central Soya Company, Decatur, IN.

\(^d\) Ultra supreme sodium caseinate, Erie Casein Company, Erie, IL.

\(^e\) Milk Specialties Company, Dundee, IL.

\(^f\) Ho-Milc fat product (contains milk protein and animal fat), Merrick Foods, Inc., Union Center, WI.

\(^g\) Milk Specialties Company, Dundee, IL.

\(^*\) Dry matter basis
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>SF</th>
<th>SPC</th>
<th>PC</th>
<th>CS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SF</td>
<td>14.48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPC</td>
<td></td>
<td>10.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC</td>
<td></td>
<td></td>
<td>10.08</td>
<td></td>
</tr>
<tr>
<td>CS</td>
<td></td>
<td></td>
<td></td>
<td>7.40</td>
</tr>
<tr>
<td>DSM</td>
<td>17.38</td>
<td>16.12</td>
<td>16.12</td>
<td>13.68</td>
</tr>
<tr>
<td>Fat product</td>
<td>18.88</td>
<td>18.78</td>
<td>18.78</td>
<td>18.92</td>
</tr>
<tr>
<td>Dried whey</td>
<td>49.26</td>
<td>55.02</td>
<td>55.02</td>
<td>60.00</td>
</tr>
<tr>
<td>Vitamin/trace mineral premixa</td>
<td>.5</td>
<td>.5</td>
<td>.5</td>
<td>.5</td>
</tr>
<tr>
<td>Antibiotic premixb</td>
<td>.1</td>
<td>.1</td>
<td>.1</td>
<td>.1</td>
</tr>
<tr>
<td>Total</td>
<td>100.60</td>
<td>100.60</td>
<td>100.60</td>
<td>100.60</td>
</tr>
</tbody>
</table>

aNational Vitamin Products Company, Minneapolis, MN (composition in table 4).

bTM-50D (Terramycin added at .005% of ration), Pfizer Company, Terre Haute, IN.
TABLE 3. CALCULATED NUTRIENT COMPOSITION OF MILK REPLACERS

<table>
<thead>
<tr>
<th>Milk replacer</th>
<th>Ingredient</th>
<th>Quantity per 100 kg of dry milk replacer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein (kg)</td>
<td>Fat (kg)</td>
</tr>
<tr>
<td>SF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SF</td>
<td>14.48</td>
<td>6.66</td>
</tr>
<tr>
<td>DSM</td>
<td>17.38</td>
<td>6.03</td>
</tr>
<tr>
<td>Fat product</td>
<td>18.88</td>
<td>1.32</td>
</tr>
<tr>
<td>Dried whey</td>
<td>49.26</td>
<td>6.01</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100.00</strong></td>
<td><strong>20.02</strong></td>
</tr>
<tr>
<td>SPC, PC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPC or PC</td>
<td>10.09</td>
<td>7.06</td>
</tr>
<tr>
<td>DSM</td>
<td>16.13</td>
<td>5.60</td>
</tr>
<tr>
<td>Fat product</td>
<td>18.78</td>
<td>1.31</td>
</tr>
<tr>
<td>Dried whey</td>
<td>55.00</td>
<td>6.71</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100.00</strong></td>
<td><strong>20.68</strong></td>
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<tr>
<td>CS</td>
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<td></td>
</tr>
<tr>
<td>CS</td>
<td>7.40</td>
<td>6.66</td>
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<tr>
<td>DSM</td>
<td>13.68</td>
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<tr>
<td>Fat product</td>
<td>18.92</td>
<td>1.32</td>
</tr>
<tr>
<td>Dried whey</td>
<td>60.00</td>
<td>7.32</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100.00</strong></td>
<td><strong>20.05</strong></td>
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</table>
TABLE 4. NUTRIENTS PROVIDED BY VITAMIN/TRACE MINERAL PREMIX<sup>a,b</sup>

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity/kg of dry milk replacer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vitamins</strong></td>
<td></td>
</tr>
<tr>
<td>Vitamin A</td>
<td>15,000 I.U.</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>5,000 I.U.</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>300 I.U.</td>
</tr>
<tr>
<td>Niacin</td>
<td>2.6 mg</td>
</tr>
<tr>
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</tr>
<tr>
<td>Riboflavin</td>
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<td>Pyridoxine</td>
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<tr>
<td>Thiamine</td>
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<tr>
<td>Folic Acid</td>
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<tr>
<td>Vitamin B&lt;sub&gt;12&lt;/sub&gt;</td>
<td>.07 mg</td>
</tr>
<tr>
<td><strong>Minerals</strong></td>
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<tr>
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<tr>
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<tr>
<td>Magnesium</td>
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<sup>a</sup>National Vitamin Products Company, Minneapolis, MN.

<sup>b</sup>Added to dry milk replacer at .5 percent.
### TABLE 5. DIGESTIBILITY OF PROTEIN IN TRIALS 1 and 2

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<thead>
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<th>Milk Replacer</th>
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<th>SF</th>
<th>SPC</th>
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<td>40.1</td>
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<td><strong>Standard Error</strong></td>
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<table>
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<td><strong>Standard Error</strong></td>
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EXPERIMENT 2.

EFFECT OF SOY PROTEIN ON INTESTINAL ABSORPTIVE ABILITY OF CALVES
AS DETERMINED BY THE XYLOSE ABSORPTION TEST

SUMMARY

A xylose absorption test was used to determine intestinal absorptive ability in young calves. Five calves per group were fed milk (control) or one of three milk replacers with one-third of the total protein provided by a soy product. Soy materials used were: Promocaf, a commercial soy protein concentrate (PC); an experimental soy protein concentrate (SPC); and an experimental soy flour (SF).

After a 24-hour fast, calves were fed xylose at .5 g/kg body weight as a 10% aqueous solution. Urine was collected for 5 hours. Jugular blood was sampled at 0, .5, 1, 1.5, 2, 2.5, 3, 4, and 5 hours after xylose administration. Tests were run weekly on each calf from 1 through 5 weeks of age.

Urinary xylose excretion, as a percent of xylose fed, was higher in the control group than in the groups fed PC, SPC, or SF at week two (P<.05), week three (P<.005), week four (P<.005), and week five (P<.001). Mean five-hour urinary xylose excretion values and standard errors over the entire experiment were 12.4 ± 1.0, 4.2 ± .6, 4.2 ± .7, and 4.3 ± .5 percent of xylose administered for calves fed milk, SF, SPC, and PC, respectively.

Mean peak increases in plasma xylose concentration and standard errors were 55.7 ± 2.6, 44.4 ± 2.5, 42.8 ± 2.4, and 45.3 ± 1.8 mg/100ml for calves fed milk, SF, SPC, and PC, respectively. Peak values for control calves were higher than
those for calves fed SF, SPC, or PC at weeks four (P<.05) and five (P<.01). No significant differences in time required to reach peak value were observed.

Neither plasma xylose concentration nor urinary xylose excretion differed among the SF, SPC, and PC treatment groups. Soy protein may have caused impaired absorption of xylose in calves fed SF, SPC, or PC.

The xylose absorption test appears to be an effective method of investigating intestinal absorptive ability in the young calf. Urinary xylose may be a more accurate indicator of malabsorption than plasma xylose.

INTRODUCTION

The protein digestibilities of milk replacers containing soy products tended to decrease in the second or third week of Experiment 1. Although some increases were seen, these were not as great as might have been expected in calves during the first few weeks of life. These findings suggested that the intestinal absorptive ability of calves may have declined as the experiment proceeded. Since the remainder of the protein in the milk replacers, other than that provided by soy materials, was from milk sources, it was thought that soy protein may have caused a deterioration in intestinal integrity and that this condition may have become progressively worse with continued feeding of soy products in calf milk replacers.

D(+)−xylose absorption and excretion tests have been used extensively to assess intestinal absorptive capability and renal functions in man (Helmer and Fouts, 1937; Fourman, 1948; Benson et al., 1957; Christiansen et al., 1959; Fowler and Cooke, 1960; Santini et al., 1961; Meeuwisse and Dano, 1965). Similar tests using xylose have been reported for the dog (Hill et al., 1970), the horse (Roberts, 1974; Bolton et al., 1976), and the pony (Breukink, 1974).
Apparently no work has been reported on use of the oral xylose test in preruminant calves. In this study a xylose absorption test was used to evaluate intestinal absorptive ability of calves fed milk replacers containing soy proteins.

MATERIALS AND METHODS

Twenty Holstein bull calves were assigned randomly at 1 or 2 days of age to one of four rations with five calves per group. Treatments consisted of either SF, SFC, or FC milk replacer, or milk. Milk replacers were identical to those used in Experiment 1 (tables 1,2,3,4). Calves were fed liquid rations once daily at 8% of body weight to a maximum of 3.64 kg with dry matter constituting 13% of the milk replacers on an as-fed basis. No dry feed was provided. Water was available ad lib. Calves were housed in elevated metal stalls. Calf weights were recorded weekly and ration levels adjusted accordingly.

A xylose absorption test was conducted weekly on each calf from 1 through 5 weeks of age. Calves were fasted for 24 hours prior to testing and were placed in metabolism crates during the testing procedure. Xylose was administered via nipple pail at a rate of .5 grams per kilogram of body weight as a 10% aqueous solution. No water was allowed during the test. Jugular blood was sampled just prior to and at .5, 1, 1.5, 2, 2.5, 3, 4, and 5 hours after ingestion of xylose. Heparin was used as an anticoagulant. Urine was collected quantitatively during the five-hour test period. The collection of urine was suggested by the close relationship between the blood xylose concentration curve and five-hour urinary xylose excretion in normal human subjects and those with malabsorption (Meeuwisse and Dano, 1965), and in normal horses (Bolton et al.,
1976). It was of interest to observe whether this similarity also occurred in the calf.

Plasma was separated by centrifugation and stored in frozen state until chemical analysis. Urine was refrigerated until analysis for xylose content. Xylose determinations in blood and urine were made by the orcinol/ferric chloride spectrophotometric method described by Frânek (1970), with the modifications for plasma reported by Bolton et al. (1976). The details of this procedure are included in the Appendix.

Statistical analyses. To determine the feed effect, at each time of blood sampling (0, .5, 1, 1.5, 2, 2.5, 3, 4, and 5 hours after xylose administration), plasma xylose values of calves on the four different rations were analyzed as a one-way analysis of variance within each age group (weeks 1 through 5). Conversely, at each sampling time, plasma xylose values of calves of different ages were analyzed as one-way analyses of variance within each ration treatment in order to determine the age effect. In addition to these analyses, a two-way analysis of variance was done at each sampling time to assess the effects of ration, age, and age x ration interaction. Urine xylose data, as a percent of xylose administered, were treated similarly to the plasma data, with one-way analyses of rations alone and ages alone, and a two-way analysis of rations, ages, and ration x age interaction. Similar analyses were done for peak plasma xylose concentration. In all analyses, significant treatment effects were followed by the least significant difference test (Snedecor and Cochran, 1967).
RESULTS

Plasma xylose concentration

Pre-administration xylose levels. Blood samples taken just prior to xylose administration showed small but variable levels of plasma xylose as determined by the test. These values, presented in figure 1, were deducted from the post-administration xylose measurements as had been done with the horse (Bolton et al., 1976) and man (Klassen and Lanzkowsky, 1964). Calves fed SF, SPC, and PC all had higher (P<.005) pre-administration plasma xylose concentrations than did milk-fed calves over the experiment as a whole. On a weekly basis, the differences were significant in the third, fourth, and fifth weeks. Pre-administration plasma xylose concentrations (mg/100ml), expressed as means with standard errors, and averaged over 5 weeks, were 7.4 ± .7, 12.2 ± .6, 10.9 ± 1.1, and 12.8 ± .8 for calves fed milk, SF, SPC, and PC, respectively. There was no significant effect due to age alone, nor due to ration x age interaction.

Xylose absorption curves. The xylose absorption curves for five milk-fed calves are presented in figure 2. Peak plasma xylose levels were attained at 2 or 2.5 hours after xylose administration in all 5 weeks of the experiment for milk-fed calves. Xylose absorption tended to increase with age in milk-fed control animals, since the 2 and 2.5 hour levels were higher (P<.05) at the fourth and fifth weeks than at the first and second weeks. The absorption curve for the milk-fed control group is characterized in all 5 weeks by a rapid rise in blood xylose during the first hour, followed by a much less rapid increase and gradual decline in the first and second weeks. At 3 weeks, the rapid rise continued to 1.5 hours. The four- and five-week old calves exhibited the steep
rise in plasma xylose concentration up to 2 hours after administration, whereafter a slight increase or decrease occurred to 3 hours, followed by a gradual decline.

Calves fed SF, SPC, and PC milk replacers (figures 3, 4, 5) exhibited xylose absorption curves with a lower (P<.05) increase in plasma xylose during the first hour than milk-fed calves. There were no significant differences due to age within the milk-replacer groups, with the exception of the 30-minute xylose levels from the calves fed SF, in which values for weeks two and three were higher than that of week one, and the week-two value was greater than that of week four (P<.05). Unlike in the control group, xylose absorption did not tend to increase with age in calves fed SF, SPC, or PC. Over the entire 5 weeks, average peak plasma xylose levels (mg/100ml) attained, and the times at which peak value occurred, were 39.1 ± 2.4 at 2 hours, 36.2 ± 2.3 at 3 hours, and 42.0 ± 2.0 at 2 hours for calves fed SF, SPC, and PC, respectively. These values were all lower (P<.05) than that for milk-fed calves, 50.9 ± 2.7 mg/100ml at 2.5 hours, but did not differ among themselves. Gradual declines followed these peak values. In general, xylose absorption for the milk replacer groups tended to be relatively low in week one, followed by an improvement in weeks two and three, with a subsequent decline in weeks four and five. Differences in xylose absorption curves by weeks were not significant for calves fed milk replacers.

Average peak plasma xylose concentrations (mg/100ml) over the five-week period (figure 6), without regard for time of peak, were 55.7, 44.4, 42.8, and 45.3 for calves fed milk, SF, SPC, and PC, respectively. The milk-fed control group average was higher than the others (P<.01). There were no significant
differences among the three milk replacer-fed groups. Average post-administration times required to reach peak plasma xylose concentration were 2.6, 2.3, 2.8, and 2.4 hours for milk, SF, SPC, and PC, respectively. No differences were significant among these values (figure 7) due to ration or age.

For one- and two-week-old calves there were no significant differences in xylose absorption curves among the four rations (figures 8,9). At 3 weeks (figure 10), calves fed milk or SF had a higher (P<.05) plasma xylose concentration 1.5 hours after xylose administration than did calves fed SPC. Differences between the milk-fed group and the three milk replacer-fed groups were most apparent at 4 and 5 weeks of age (figures 11,12). Milk-fed calves showed a marked increase in ability to absorb xylose in the last 2 weeks of the experiment, when peak plasma xylose levels reached an average of 62 mg/100ml at 2.5 hours in week four, and 60 mg/100ml at 2 hours in week five. Although xylose absorption by the milk replacer-fed calves decreased somewhat from week three in weeks four and five, the differences were not significant, and peak xylose concentrations tended to remain near 30 to 40 mg/100ml. At 4 weeks of age, calves fed milk had higher (P<.05) plasma xylose levels than the groups fed SF, SPC, or PC at 2, 2.5, and 3 hours after xylose administration (figure 11). At 5 weeks, differences between control and milk replacer-fed animals were evident at 2 hours (P<.005), 2.5 hours (P<.005), 3 hours (P<.001) and 4 hours (P<.05). Both milk- and PC-fed groups had greater (P<.05) plasma xylose levels than did the SF- and SPC-fed groups at 5 hours after administration in the fifth week.
One-half hour after xylose administration. Figure 13 shows average plasma xylose concentrations at .5 hour post-administration for all tests conducted. There were no significant differences due to rations, but there were due to weeks. Two- and three-week-old calves had higher (P<.05) plasma xylose levels than did one-week-old calves. Within the SF group, two-week-old calves had higher (P<.05) xylose levels than did one- and four-week-old calves, and three-week values were greater (P<.05) than one-week levels.

One hour after xylose administration. Plasma xylose levels at 1 hour are presented in figure 14. The milk group was higher (P<.05) than the milk replacer groups overall. No significant differences were found due to rations at any one age, nor due to ages alone.

One and one-half hours after xylose administration. Plasma xylose levels at this time of sampling are in figure 15. Overall, the milk fed group was higher than the SPC or PC groups (P<.05) and the SF group was also higher than the SPC group (P<.05). At 3 weeks of age, calves fed milk or SF had higher blood xylose than those fed SPC (P<.05). No differences due to age alone were observed.

Two hours after xylose administration. Greater differences among treatment groups began to be evident after 2 hours had elapsed (figure 16). Overall the milk-fed group was higher than the SF, SPC, and PC treatments (P<.05). Control calves had higher plasma xylose concentrations than the animals fed milk replacers at week four (P<.05) and week five (P<.005). Within the control group higher xylose levels were seen at weeks four and five than at weeks one and two (P<.05). There were no significant differences within any of the other treatment groups, and there were none due to age alone.
Two and one-half hours after xylose administration. The results at this time (figure 17) are very similar to those at 2 hours. Overall, calves on milk had higher levels of plasma xylose than those on SF, SPC, or PC (P<.005), and there were no significant differences among the groups fed milk replacers. As at the previous sampling time, the control group was higher than the others at week four (P<.05) and week five (P<.005), but not during the first 3 weeks of the experiment. Calves fed milk had greater values at weeks four and five than at weeks one and two (P<.05), but no similar differences occurred with the SF, SPC, or PC groups. There were no differences due to age alone.

Three hours after xylose administration. As with blood samples taken at 2 and 2.5 hours, plasma xylose at 3 hours (figure 18) was greater (P<.005) in the milk-fed control calves overall than in those fed milk replacers with no significant differences among the SF, SPC, and PC groups. Control animals exhibited higher plasma xylose at weeks four (P<.05) and five (P<.005) than the other groups, as with blood samples taken at 2 and 2.5 hours. At 3 hours the values within the control group at weeks four and five were not significantly different from earlier weeks. No significant differences were observed within the SF, SPC, or PC groups, or due to age alone.

Four hours after xylose administration. At this time (figure 19), the control group was still higher than the others overall (P<.05). There were no significant differences among rations in weeks one through four. At week five, the milk-fed calves had higher (P<.05) plasma xylose than did those fed SF, SPC, or PC. There were no significant differences due to age alone, or due to age within any of the groups.
Five hours after xylose administration. The five-hour plasma xylose data are presented in figure 20. No overall differences due to rations were observed. At week five, the milk and PC groups were greater than the SF and SPC groups (P<.05). There were no differences within ration treatments or among ages.

**Urinary xylose excretion**

Cumulative xylose excretion was determined from the five-hour urine collection and data are presented as a percent of xylose fed (figure 21). Overall, the milk-fed calves excreted a higher (P<.001) percent of the oral xylose dose in 5 hours than did calves fed SF, SPC, or PC. Mean percentages of xylose excreted (average of five calves) with standard errors were 12.4 ± 1.0, 4.2 ± 0.6, 4.2 ± 0.7, and 4.3 ± 0.5% for calves fed milk, SF, SPC, and PC, respectively. No significant differences were observed among the milk replacer groups. The differences between the control group and the other groups became more evident as the weeks progressed. At 1 week, the difference was not significant. The disparity in xylose excretion between calves fed milk and calves fed milk replacers was seen at week two (P<.05), week three (P<.005), week four (P<.005), and week five (P<.001). There were no significant differences in xylose excretion within any of the four groups from week to week. No differences were observed due to age alone.

No significant differences in plasma xylose concentration or urinary xylose excretion due to age x ration interaction were determined.

**Discussion**

Xylose absorption and excretion tests have been effectively used as indicators of malabsorption in the dog (Hill et al., 1970), the horse (Bolton et al., 1976), and in man (Fourman, 1948; Benson et al., 1957; Butterworth et al., 1959;
Christiansen et al., 1959; Santini et al., 1961). Xylose tests have been used in man to evaluate intestinal absorptive capacity of patients during the course of treatment for malabsorption syndromes, such as celiac disease and idiopathic sprue with special gluten-free diets (Zareba and Laskowski, 1967; Kendall et al., 1972; Harms, 1973). Apparently no work has been reported using the xylose absorption test to compare the effects of different rations on intestinal absorptive ability in animals.

With respect to xylose absorption, as indicated by plasma xylose concentration and urinary xylose excretion, the four treatment groups easily fell into two categories, with milk-fed control animals on the one hand, and those fed SF, SPC, or PC milk replacer on the other. The differences between the control group and the other groups became larger and statistically more significant as the experiment proceeded from week one to week five.

If the xylose absorption curves (figure 2) from the milk-fed calves are assumed to be normal, results from weeks four and five, especially the latter, appear to correspond closely with results from similar xylose tests conducted on other species. A comparison of xylose absorption tests in normal subjects of different species is presented in table 6. Xylose absorption curves with normal adult dogs, given xylose at .5 g/kg of body weight, exhibited a rapid initial increase in plasma xylose concentration to a maximum increase of 59.8 mg/100ml at 1 hour after administration (Hill et al., 1970). Xylose tests in the horse (Bolton et al., 1976), after a dose of .5 g/kg, showed a rapid initial increase in plasma xylose after administration, but at 1 hour a mean peak level of 20.6 mg/100ml was attained which was only about one-third that of the dog. Another report using the horse showed a peak plasma xylose increase of only 8.15 mg/100ml at 1 hour after an oral dose of .5 g/kg (Roberts, 1974). Using
human subjects, Benson et al. (1957) reported a peak increase in plasma xylose of approximately 40 mg/100ml at 1 to 2 hours after oral administration of 25 g of xylose. Results with milk-fed, five-week-old calves in this study show a mean maximum plasma xylose increase of 60.1 mg/100ml at 2 hours after oral administration of a .5 g/kg dose.

Five-hour urinary xylose excretion has been shown to correlate with plasma xylose concentration as an indicator of malabsorption in cases where renal function is normal in man (Benson et al., 1957; Meeuwisse and Dano, 1965). Urine collection is simpler, costs less, and is less distressing to patients than the numerous venipunctures required in blood sampling to establish a xylose absorption curve. For these reasons, urine collection without blood sampling is the most common method of xylose absorption testing currently used in man. Urine was collected in this experiment in an attempt to ascertain whether the close relationship between plasma xylose concentration and urinary xylose excretion seen in man in cases of normal absorption and malabsorption would also be observed in calves.

Five-hour urinary xylose excretion in milk-fed calves averaged 12.4% of xylose fed over the five-week experiment, with a range from 10.6% at week one to 14.3% at week five. These values were lower than those found in normal human cases: 26% (Benson et al., 1957), 27% (Christiansen et al., 1959), 29% (Fowler and Cooke, 1960), and 22% (Fourman, 1948) with 25 g doses, and 36% (Santini et al., 1961) with a 5 g dose.

Calves fed SF, SPC, or PC milk replacer excreted less urinary xylose than milk-fed calves in every week of the experiment. There were no significant differences within any of the ration treatments by weeks. This indicated that
adverse effects took place very shortly after the initiation of feeding these milk replacers, and, therefore, very early in life. Since it was unlikely that soy protein in the milk replacer would adversely affect kidney function, it was thought that malabsorption was caused by changes in the intestinal mucosa. This hypothesis could be investigated by intravenous injection of xylose and subsequent urine collection to determine the rate of xylose clearance from blood. In this way glomerular filtration would be an important factor in xylose elimination, whereas intestinal absorption would have no influence. This procedure has been used in man (Helmer and Fouts, 1937; Butterworth et al., 1959; Gurson and Saner, 1969) to determine whether low xylose excretion is due to renal or intestinal malfunction.

Since xylose is not normally found in the animal body, its presence in fasting pre-administration plasma samples at low levels in this study called for an explanation. The orcinol-ferric chloride method, which was used in the xylose determinations on blood and urine in this experiment, was also tried on glucose. Glucose standards alone and added to blood samples in concentrations identical to those used for xylose standards were evaluated by the analytical procedure. It was found that glucose resulted in an absorbance which was 10% of that of an identical concentration of xylose. With this association between glucose and the method of xylose determination established, the pre-administration levels of "xylose" were attributed to blood glucose. Since average overall pre-testing plasma xylose levels were 7.4, 10.9, 12.2, and 12.8 mg/100ml for calves fed milk, SPC, SF, and PC, respectively, multiplication by ten produced blood glucose levels of 74, 109, 122, and 128 mg/100ml, which are within a reasonable range for young calves
(Kennedy et al., 1939). The observation that calves fed SF, SPC, and PC had significantly higher pre-administration levels (figure 1) than milk-fed calves in the last 3 weeks of the experiment was unexpected and puzzling.

A definite increase in ability to absorb xylose was seen in milk-fed calves during the five-week experiment (figure 2). However, with calves fed SF (figure 3), SPC (figure 4), or PC (figure 5), no similar increase was seen. It seems that normally the ability of the calf's intestine to absorb carbohydrate increases with age in the first 5 weeks of life. The inclusion of soy protein as one-third of the total protein in calf milk replacers caused not a decrease in absorptive ability, but a failure of intestinal absorptive capacity to improve in the early weeks of life. Through the duration of the experiment, the disparity between the control and other groups increased with time. For one- (figure 8) and two-week-old calves (figure 9), there was no significant difference in xylose absorption among the four rations. Significant differences began to appear at week three (figure 10), and were progressively more obvious in weeks four (figure 11) and five (figure 12).

In another method of presentation (figures 13-20), plasma xylose levels were examined at each post-administration time of sampling. Significant differences among rations did not occur at .5 hour (figure 3), but began to be seen at 1 hour (figure 14), when the control group began to exhibit higher plasma xylose levels than the other groups. This trend continued at 1.5 hours (figure 15). At 2, 2.5, and 3 hours after administration (figures 16, 17, 18, respectively), near the peak of the xylose absorption curve, xylose levels for milk-fed calves were significantly higher than for the other
treatments at the fourth and fifth weeks. The xylose levels at 4 hours (figure 19) again showed an overall significant difference between the control and soy-fed groups, but no difference was evident at 5 hours (figure 20), with the exception of week five. These figures show that xylose absorption normally increased from 1 week of age to 5, but this did not occur when calves were fed SF, SPC, or PC milk replacer.

Abnormal xylose absorption curves are characterized by a low peak, delayed peak, or the so-called "flat" curve (Hill et al., 1970; Bolton et al., 1976), which has no distinguishable peak and no marked decline. According to these criteria, the curves representing SF, SPC, and PC in weeks four (figure 11) and five (figure 12) were considered to be abnormal due to low peaks or flat curves. All the curves for milk, SF, SPC, and PC during weeks one (figure 8) and two (figure 9) were flat curves and, therefore, not of the normal pattern, which indicated that a certain advancement in intestinal absorptivity was required before the typical normal curve was seen with milk-fed calves (weeks four, figure 11, and five, figures 2 and 12). Delayed peak values were not a factor in determining abnormal xylose absorption curves in this study since there were no significant differences among the four treatments in time required to reach peak plasma xylose concentration following oral administration (figure 7).

Dietary proteins are necessary for formation of digestive enzymes and are used as structural units of intestinal epithelial cells, which are among the most rapidly dividing cells in the body. It seems likely that these cells could be among those first affected by a lack of available dietary protein in a rapidly growing animal like the very young calf. A reduction in intestinal
integrity may result, causing poor digestion and absorption of not only protein, but all nutrients, including carbohydrates. The responsibility for subnormal xylose absorption after several weeks in calves fed SF, SPC, and PC has been placed upon the soy protein in these milk replacers because other proteins in the rations were derived from milk sources, dried skim milk and whey (table 3). Since xylose is a carbohydrate, and is actively transported, at least in part, by a process similar to glucose absorption in the intestine, (Csaky and Ho, 1965; Levitt et al., 1969), it seems reasonable that xylose absorption would be an appropriate indicator of malabsorption in the proximal small intestine.

Benson et al. (1957) stated that, in man, blood xylose values after oral doses were more variable and more inclined to overlapping between groups than urinary xylose excretion, although general tendencies were similar. In this study with calves, significant differences in peak plasma xylose concentration were observed between control and soy-fed groups at weeks four and five (figures 6,11,12,16,17,18), while significant differences in xylose excretion were evident at weeks two, three, four, and five (figure 21). These results suggest the possibility that five-hour urinary xylose excretion may be a more sensitive method of evaluating xylose absorption in the calf than is plasma xylose concentration, although the general tendencies are similar. Perhaps a five-hour urine collection and pre-administration and two-hour blood samples would be the most informative compromise, as was suggested for use in man (Benson et al., 1957).

Weight gains and fecal scores in this study were consistent with results from xylose absorption tests for calves fed milk replacers and milk.
Consistency of feces was recorded twice daily on a scale of 0 (normal) to 3 (fluid). Mean fecal scores with standard errors were $0.21 \pm 0.03$, $1.66 \pm 0.08$, $1.53 \pm 0.05$, and $1.46 \pm 0.10$ for calves fed milk, SF, SPC, and PC, respectively. Scores for the control group were lower ($P<0.001$) than those for soy-fed groups, but there were no significant differences among the three milk replacer groups. The three milk replacers containing soy protein caused serious scouring problems in young calves, which was likely a result of poor intestinal digestive and absorptive ability, as indicated by the digestion trial in Experiment 1 and the xylose absorption test in this report. Average weight gains with standard errors during the five-week experiment were $2.72 \pm 0.20$, $-0.02 \pm 0.28$, $-2.00 \pm 0.50$, and $-1.26 \pm 0.61$ kg for calves fed milk, SF, SPC, and PC, respectively. Calves in the control group gained more in five weeks than calves fed SPC or PC ($P<0.01$), and more than calves fed SF ($P<0.05$). There were no significant differences among the soy-fed groups.

Protein digestibility studies of SF, SPC, and PC in Experiment 1 suggested a reduced intestinal absorptive ability, which was demonstrated by xylose absorption tests in this report. Milk replacers in which SF, SPC, or PC provided one-third of the total protein, when fed as the only ration to calves in the first 5 weeks of life, produced unsatisfactory results. With respect to the parameters measured in Experiments 1 and 2, no significant differences were observed among groups of calves fed SF, SPC, or PC. Calves in all three groups were well below those fed milk or all-milk products in protein digestibility, ability to absorb xylose, and weight gain, and had higher fecal scores than milk-fed calves.
Histological examinations of the intestinal wall of calves following this experiment would have been an interesting endeavor, but, unfortunately, could not be done. The abnormal xylose absorption after 4 and 5 weeks in calves fed SF, SPC, and PC suggests the possibility of lesions in the mucosal epithelium, such as villous atrophy, which may correlate with malabsorption.

Results from this study indicate that the xylose absorption test may be an accurate method of evaluating intestinal malabsorption in the pre-ruminant calf.
<table>
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<th>Reference</th>
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<th>Number of subjects</th>
<th>Oral dose</th>
<th>0</th>
<th>.5</th>
<th>1</th>
<th>1.5</th>
<th>2</th>
<th>2.5</th>
<th>3</th>
<th>3.5</th>
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<td>30.4</td>
<td>40.5</td>
<td>41.0</td>
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<td>17.5</td>
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<tr>
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<td>59.8</td>
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Figure 1. Mean pre-administration plasma xylose concentrations of five calves fed milk, SF, SPC or PC from 1 through 5 weeks of age. Points with unlike letters differ at level of significance indicated.
Figure 2. Mean plasma xylose concentrations in five milk-fed calves from 1 through 5 weeks of age. Points with unlike letters differ at level of significance indicated.
Figure 3. Mean plasma xylose concentrations in five calves fed SF from 1 through 5 weeks of age. Points with unlike letters differ at level of significance indicated.
Figure 4. Mean plasma xylose concentrations in five calves fed SPC from 1 through 5 weeks of age.
Figure 5. Mean plasma xylose concentrations in five calves fed FC from 1 through 5 weeks of age.
Figure 6. Mean peak plasma xylose concentrations in five calves fed milk, SF, SPC, or PC from 1 through 5 weeks of age. Points with unlike letters differ at level of significance indicated.
Figure 7. Mean times after xylose administration required to reach peak plasma xylose concentrations in five calves fed milk, SF, SPC, or PC from 1 through 5 weeks of age.
Figure 8. Mean plasma xylose concentrations in five one-week-old calves fed milk, SF, SPC, or PC.
Figure 9. Mean plasma xylose concentrations in five two-week-old calves fed milk, SF, SPC, or PC.
Figure 10. Mean plasma xylose concentrations in five three-week-old calves fed milk, SF, SPC, or PC. Points with unlike letters differ at level of significance indicated.
Figure 11. Mean plasma xylose concentrations in five four-week-old calves fed milk, SF, SPC, or PC. Points with unlike letters differ at level of significance indicated.
Figure 12. Mean plasma xylose concentrations in five five-week-old calves fed milk, SF, SPC, or PC. Points with unlike letters differ at level of significance indicated.
Figure 13. Mean plasma xylose concentrations at .5 hour after xylose administration in five calves fed milk, SF, SPC, or PC from 1 through 5 weeks of age. Points with unlike letters differ at level of significance indicated.
Figure 14. Mean plasma xylose concentrations at 1 hour after xylose administration in five calves fed milk, SF, SPC, or PC from 1 through 5 weeks of age.
Figure 15. Mean plasma xylose concentrations at 1.5 hours after xylose administration in five calves fed milk, SF, SPC, or PC from 1 through 5 weeks of age. Points with unlike letters differ at level of significance indicated.
Figure 16. Mean plasma xylose concentrations at 2 hours after xylose administration in five calves fed milk, SF, SPC, or PC from 1 through 5 weeks of age. Points with unlike letters differ at level of significance indicated.
Figure 17. Mean plasma xylose concentrations at 2.5 hours after xylose administration in five calves fed milk, SF, SPC, or PC from 1 through 5 weeks of age. Points with unlike letters differ at level of significance indicated.
Figure 18. Mean plasma xylose concentrations at 3 hours after xylose administration in five calves fed milk, SF, SPC, or PC from 1 through 5 weeks of age. Points with unlike letters differ at level of significance indicated.
Figure 19. Mean plasma xylose concentrations at 4 hours after xylose administration in five calves fed milk, SF, SPC, or PC from 1 through 5 weeks of age. Points with unlike letters differ at level of significance indicated.
Figure 20. Mean plasma xylose concentrations at 5 hours after xylose administration in five calves fed milk, SF, SPC, or PC from 1 through 5 weeks of age. Points with unlike letters differ at level of significance indicated.
Figure 21. Mean five-hour urinary xylose excretion values, as a percent of xylose fed, in five calves fed milk, SF, SPC, or PC from 1 through 5 weeks of age. Points with unlike letters differ at level of significance indicated.
APPENDIX

XYLOSE ANALYSIS\(^{1/}\)

**Plasma xylose determination.** To a 2 ml sample of plasma was added 2 ml of .6M perchloric acid to precipitate protein. Samples were shaken and allowed to stand for 5 minutes, then centrifuged at 700 \(x\) g for 5 minutes. Supernatant was filtered through Whatman \#5 paper. To .5 ml of protein-free filtrate was added 1.5 ml water in a glass test tube. After addition of 2 ml of orcinol reagent, tubes were placed in boiling water bath. After one hour, tubes were removed, cooled, and diluted to 25 ml with water. Samples were read against blank on spectrophotometer set at 630 nm. Dilution factor = 100.

**Urine xylose determination.** Several ml of urine were filtered through Whatman \#5 paper, and .2 ml of filtrate was diluted to 100 ml with water. To a 2 ml aliquot of sample solution 2 ml of orcinol reagent was added. Tubes were heated, cooled, diluted, and read as with plasma. Dilution factor = 6250.

**Preparation of reagents**

- **Perchloric acid.** A volume of 57.04 ml of HC\(_{10}\)\(_{4}\) was diluted to 1000 ml with water to yield a .6M solution.

- **Benzoate diluant.** Quantities of .6 g of benzoic acid and 1.2 g of sodium benzoate were combined and diluted to 1000 ml with water.

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\(^{1/}\)Orcinol-ferric chloride method described by Frankel (1970) and modified for plasma by Bolton et al. (1976).
**Orcinol reagent.** A ferric chloride-hydrochloric acid solution stable at room temperature was made by diluting 2.5 g of FeCl₃ to 1000 ml with 36% HCl. Immediately before addition to sample, 1 g of orcinol (5-methyl resorcinol) monohydrate was added per 100 ml of HCl·FeCl₃ solution. This solution is stable for only about 1 hour, so it was made just before use.

**Preparation of standards**

A stock xylose standard (500 mg/100ml) was prepared by dilution of .5 g of reagent grade D(+)–xylose to 100 ml with benzoate diluant. This was refrigerated.

A 50 mg/100ml standard was made by dilution of 1 ml of stock standard to 10 ml with benzoate diluant. This solution was refrigerated.

A working standard (5 mg/100ml) was obtained by diluting 1 ml of 50 mg/100ml solution to 10 ml with water. This was prepared daily.

Volumes of 0, .25, .5, 1.0, and 2.0 ml of working standard were placed into test tubes and each diluted to 2 ml with water. After addition of 2 ml of orcinol reagent, the tubes were heated, cooled, and diluted, as were the samples. After dilution to 25 ml, the standards corresponded to 0, .05, .1, .2, and .4 mg of xylose per 100 ml of cuvette solution.

**Calculations.** The standard solution containing no xylose was used as the blank, and for this tube the spectrophotometer was set at zero absorbance at a wavelength of 630 nm. After the absorbance (A₆₃₀) of each standard and sample was read, a standard curve was established, using values of A₆₃₀ along the y-axis and the known concentrations (mg/100ml) of xylose in the standards along with the x-axis. The linear least squares method was used to calculate the slope (b) of the standard curve. Since the curve must pass through the
origin, the following formula was used: \[ b = \frac{\sum x_i y_i}{\sum x_i^2} \]

Once the value of \( b \) was determined from the standards, the concentration of xylose (mg/100ml) for each sample was calculated algebraically by dividing the appropriate reading of \( A_{630} \) by \( b \). For plasma, the cuvette xylose concentration was multiplied by 100 to obtain the actual mg of xylose per 100 ml of sample. With urine, multiplication by the dilution factor of 6250 provided a value for mg of xylose per 100 ml of urine. This figure was multiplied by ml urine \( \div 100 \) to give the actual quantity of xylose excreted in urine during the five-hour collection period. This value in grams is expressed in the data as a percentage of xylose fed to the calf.
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Cope, C. L. 1933b. The excretion of non-metabolized sugars by the mammalian kidney. J. Physiol. 80:238.


Hemingway, A. 1935. The elimination of xylose, creatinine and urea by the perfused mammalian kidney. J. Physiol. 84:458.


THE EFFECT OF SOY PROTEIN ON DIGESTIBILITY OF CALF MILK REPLACERS AND ON INTESTINAL ABSORPTIVE ABILITY AS DETERMINED BY THE XYLOSE ABSORPTION TEST

by

FRANK JOSEPH SEEGRABER, JR.

B. A., University of Massachusetts, 1970

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Animal Nutrition

Department of Animal Sciences and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1978
Soy products are a potential source of more economical proteins for calf milk replacers. Two experiments were conducted to study the effect of soy protein on digestibility and absorptive ability in calves. Three calf milk replacers were formulated to contain one-third of total protein from one of three processed soy products: Promocaf (PC), a commercial soy protein concentrate; an experimental soy protein concentrate (SPC); or an experimental soy flour (SF). The remainder of the protein was provided by milk products. Milk replacers were isonitrogenous and isocaloric and were the only rations fed to calves in Experiments 1 and 2.

In Experiment 1, two trials were conducted to determine protein digestibilities of PC, SPC, and SF in three calves from several days to 3 weeks of age. A casein (CS) ration was used as a control. Average protein digestibilities and standard errors in trial 1 were 76.9 ± 6.7, 63.9 ± 5.1, and 40.1 ± 15.6% for CS, SF, and SPC, respectively. In trial 2, protein digestibilities and standard errors were 37.9 ± 2.0, 42.0 ± 4.4, and 41.7 ± 18.1% for PC, SF, and SPC, respectively. Digestibilities of soy proteins declined from earlier to later periods of the trials, whereas an increase had been expected with calves at this age. Lower digestibilities suggested that the digestive and/or absorptive ability of the intestine may have been impaired by feeding soy proteins to young calves.

Xylose absorption tests were used in Experiment 2 to evaluate intestinal absorptive ability in calves fed PC, SPC, or SF from several days to 5 weeks of age, with five calves per group. Calves fed whole milk were used as controls. An oral xylose test was conducted weekly on each calf after a 24-hour fast. Xylose was fed at .5 g/kg body weight as a 10% aqueous solution. Jugular blood
was sampled at 0, .5, 1, 1.5, 2, 2.5, 3, 4, and 5 hours after xylose administration. Urine was collected for 5 hours. Mean peak plasma xylose concentrations and standard errors were 55.7 ± 2.6, 44.4 ± 2.5, 42.8 ± 2.4, and 45.3 ± 1.8 mg/100ml, and mean urinary xylose excretion values, as a percent of xylose fed, and standard errors were 12.4 ± 1.0, 4.2 ± .6, 4.2 ± .7, and 4.3 ± .5% for calves fed milk, SF, SPC, and PC, respectively. Plasma (P<.01) and urine (P<.001) xylose values were greater for the control group than for the soy-fed groups. No significant differences in plasma of urine xylose were found among groups fed SF, SPC, or PC. Differences between the milk-fed group and the soy-fed groups became greater as the experiment proceeded from the first to the fifth week. This observation indicated that soy proteins may have had an adverse effect on intestinal absorption.

Pre-administration plasma xylose levels were greater (P<.005) in calves fed soy products than in those fed milk. Calf growth and fecal consistency scores paralleled the results of the xylose absorption test. Urinary xylose excretion may be a more accurate indicator of malabsorption in the calf than plasma xylose concentration. The use of histological studies to confirm the results of the xylose test is suggested.