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FACTORS AFFECTING UTILIZATION OF SOYBEAN PROTEINS INCLUDED IN MILK REPLACERS FOR YOUNG CALVES

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# FACTORS AFFECTING UTILIZATION OF SOYBEAN PROTEINS INCLUDED IN MILK REPLACERS FOR YOUNG CALVES

By

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ABSTRACT

FACTORS AFFECTING UTILIZATION OF SOYBEAN PROTEINS INCLUDED IN MILK REPLACERS FOR YOUNG CALVES

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First, twenty-four calves (8/treatment) were fed milk replacer containing 19% crude protein (CP) from a) 100% milk protein (MP); b) 66% modified soybean protein + 34% MP (MS); c) 66% heated soybean flour + 34% MP (HS).

MP resulted in better gain and feed efficiency. Organic matter and CP digestibility for MP, MS and HS were: 90.8%, 87.2%, and 85.3%; and 82.6%, 72.1% and 64.1%, respectively.

A xylose absorption test was performed by feeding .5 g D-xylose/kg body weight (BW) and a xylose disappearance test was performed by intravenous injection of .25 g D-xylose/kg BW. Calves fed MP had 16% greater xylose absorption than those fed MS or HS, with a peak of 39.2 mg xylose/100 ml plasma at 150 min after feeding xylose. Calves fed soybean proteins showed greater capacity of clearance of xylose from the blood.

Two calves/treatment were sacrificed and the small intestine contents and structural integrity of villi were studied. Gastro-intestinal tract contents were rapidly neutralized at the duodenum. Greater acidity from the small to the large intestine contents were

observed in calves fed soybean than MP, acidity of content of calves fed HS was greater than MS. CP of content of the distal part of small intestine was higher for calves fed soybean than MP. Wide morphological variation in size and shape of villi were observed, with greater differences within animals than between treatments.

Superior performance of calves fed MP was related to a greater digestibility of nutrients and absorptive capacity of digested nutrients. No reason was evident for higher blood xylose clearance of calves fed soybean.

Second, sixteen calves were fed milk replacer (23% CP) from a) 100% MP; b) 66% soybean protein concentrate + 34% MP (S). Eight calves were sensitized to soybean and eight were not sensitized. Afterwards each calf received one of the diets during 10 days followed by the other for an additional 10 days.

Feeding S resulted in lower gain and feed efficiency. Higher rectal temperatures, increased diarrhea and villi atrophy were observed in calves fed S and suggested an allergic reaction to soybean protein which were associated with the poor performance observed.

DEDICATION

In memory of my Mother (October 22, 1915 - July 8, 1983)

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# INTRODUCTION

Whole milk is generally considered the best feed for young calves, however approximately 70% of dairy calves in the U.S. are raised on milk replacers for economic reasons (Schugel, 1973). Milk replacers are generally more expensive than colostrum or disposable milk, but cheaper than saleable milk (Appleman and Otterby, 1973). Properly formulated milk replacers, fed at recommended rates, have been used for many years as satisfactory liquid diets for calves after 3-5 days of age. Calves fed milk replacers normally do not show as much bloom as calves fed whole milk or colostrum, but usually make acceptable weight gains (Jones, 1975). Replacement of milk products in calf milk replacers with plant and/or animal products has been extensively utilized because of the increasing demand for milk in human diets and the consequent high milk prices accompanying this demand.

Soybean products are the most common non-milk source of protein in calf milk replacers. Soybean flour is a finely ground material resulting after screening the ground, dehulled, oil-extracted meal. Soybean protein concentrate is prepared from dehulled ground beans which have been fat-extracted and further extracted with ethanol and water to remove water-soluble non-protein constituents. It must have not less than 70% crude protein (Church, 1984). Soybean protein concentrate and especially manufactured soybean flour are satisfactory

protein sources for milk replacers, provided they undergo sufficient treatment to destroy the trypsin inhibitor factor.

The objective of the present study was to further elucidate factors limiting the nutrient utilization of soybean products in milk replacers for young calves and relate such factors to calf performance.

# REVIEW OF LITERATURE

The digestive system of the young calf undergoes a rapid transformation during the first few months after birth. The calf is born a nonruminant and becomes a functioning ruminant by 6 weeks. Economic reasons have lead to the development of milk replacers with the objective of reducing the amount of whole milk used by the calf. On the other hand it must be remembered that digestion of nutrients is a complex process in that the food must pass through the alimentary tract at a suitable rate to permit action of digestive enzymes in order for normal absorption to occur.

The milk is the natural food for the calf, but studies to replace the milk nutrients have not always been successful. Reviews by several authors (Huber, 1969; Porter, 1969; Radostits and Bell, 1970; Roy, 1974) have shown that young calves are very sensitive to protein quality during the first 3 weeks of life, but protein digestibility increases with age allowing the use of some proteins of lower quality than generally found in milk and milk products.

The objective of this review was to summarize various aspects of protein digestion by young calf, especially that of alternative sources such as soybean protein which has been used in milk replacers.

## Protein Digestion in Preruminant Calves

Milk and milk-type liquids fed to young calves are shunted directly into the abomasum through the esophageal groove. The rumen is effectively bypassed when the animals consume their liquid diet in a state of "juvenile excitement" (butting movement with heads, tail wagging, etc.), but when the animals drink to quench thirst or are forceably drenched, the liquids normally enter the rumen (Ørskov et al., 1970). The nipple is more effective than open pail feeding in stimulating reflex closure of the esophageal groove (Wise and Anderson, 1939; Wadleigh and Mowat, 1978). Whey has been shown to be a poorer stimulator of esophageal groove function than milk protein, soy flour or fish protein concentrate (Guilhermet et al., 1973) and there is some suggestion that fish protein concentrate may pass into the rumen and cause bloat (Makdani et al., 1971b).

Due to the esophageal groove, the first enzymatic action on dietary protein occurs in the abomasum. Pepsinogen, prorennin, and HCl are the main digestive secretion of the abomasum (Huber, 1969). Pepsinogen is changed to active enzyme in one of two ways. As HCl mixes with the precursors, the pH falls below 5.0 and activation is initiated. In addition, small amounts of pepsin act autocatalytically. The result is that several peptides are cleaved from the pepsinogen moleculas to produce the enzyme with pH optima varying between 1.8 and 3.5 (Sanford, 1982). Cholinergic reflexes stimulate abomasal hydrochloric acid secretion directly by acting on parietal cells, or indirectly by facilitating gastrin secretion and potentiating its effect on parietal cells (Debas et al., 1974). The peptic chief

cells appear to respond primarily to direct cholinergic neural stimulation, although gastrin was also implicated (Gregory, 1974).

Coagulating activity of the abomasal mucosa exists in the ruminant fetus (Kirton et al., 1971). Most of this action is due to rennin for which the general proteolytic activity is lower than of pepsin (Thivend et al., 1980). Gastric secretions evolve with the age of the calf and the quantity of milk ingested. In the preruminant calf fitted with a gastric pouch, the daily quantities of gastric juice collected increased up to the fifth or seventh week, then decreased until week 32. Rennin activity decreases according to age, while pepsin tends to increase slightly (Thivend et al., 1980; Henschel et al., 1961a,b). Abomasal protease production markedly increases immediately after feeding but then decreases to below prefeeding levels (Ash, 1964).

Gastric secretions measured as the quantity of acid passing out of the abomasum of the preruminant calf, doubled between days 7 and 24 and tripled between days 24 and 63, but amounts of $Na^+$, $K^+$ and $Cl^-$ did not increase significantly (Roy and Stobo, 1975). In young preruminant calves fitted with a gastric pouch however, large cyclic variations were noted between the second and thirty-second week for acid secretion as well as for the output of $Na^+$, $K^+$ and $Cl^-$ without showing large changes with age (Thivend et al., 1980). The origin and the processing of proteins greatly affect gastric secretion. Acid secretion decreased when milk protein was replaced by soybean protein, and increased with fish protein (Roy and Stobo, 1975; Williams et al., 1976). Digesta from calves given diets with "mildly"

preheated spray-dried skim-milk powder (MHM) was more acid 1 to 3 h after feeding than digesta from calves given diet with "severely" preheated spray-dried skim-milk powder (SHM). There was also a tendency for a greater output of acid by the pouches when calves were given diet MHM (Williams et al., 1976). Tagari and Roy (1969) concluded from a study of pyloric outflow, that MHM probably stimulated greater acid secretion than SHM. From measurements of $Cl^-$ minus $Na^+$ in the duodenal outflow, Ternouth et al. (1974), concluded that there was about 11% greater acid secretion with MHM. Soy flour was a less effective stimulant to acid secretion than fish protein concentrate or MHM (Williams et al., 1976). This cannot be explained by any great difference in the ratio undigested:digested protein in the duodenal fluid (Termouth et al., 1975) but it may result from differences in the products of protein degradation (Williams et al., 1976). Hill (1968), however, pointed out that there is no direct evidence available on dietary factors which stimulate gastrin production and release in ruminants.

At weaning, rennin activity in the calf and the lamb decreased while the pepsin activity remained nearly constant (Thivend et al., 1980), or increased slightly (Guilloteau et al., 1983). This change was partially reversible. When animals were given a liquid feed containing casein, the rennin activity increased again, however, without reaching the preweaning level and pepsin activity was not affected (Garnot et al., 1977). The amount of rennin secreted decreased with the casein content of the milk (Garnot et al., 1977). After weaning pepsin activity is predominant, but rennin is still

present (Thivend et al., 1980). Hydrochloric acid secretion is probably very low at birth but increases rapidly (Thivend et al., 1980). Abomasal parietal cell development in the lamb is great during the first 3 days after birth (Roy and Stobo, 1975). The pH of abomasal content is 5-8 before the first drinking but drops to 3.0 by 2 days (Thivend et al., 1980). Protease and acid output are related to diet. Acid production was increased by presence of milk in the abomasum (Ash, 1964).

Development of a firm abomasal clot by milk or milk replacer has a useful physiological function (Frantzen et al., 1971; Roy and Stobo, 1975; Tagari and Roy, 1969; Thivend et al., 1979) by improving protein and fat digestion through prolonging retention time in the abomasum. Clots help digestion by releasing the nutrients more slowly into the small intestine where digestive enzymes can act more effectively. A firm curd prevents excessive quantities of undigested protein from reaching the small intestine (Roy and Stobo, 1975; Thivend et al., 1979) and is particularly important in calves less than 3-4 weeks of age, because their digestive system has not fully developed (Thivend et al., 1979).

Attempts to substitute vegetable proteins for milk proteins in liquid diets have usually resulted in poorer calf performance (Radostits and Bell, 1970). This has been attributed, among other factors, to the lack of a normal coagulum in the abomasum (Roy et al., 1977). A strong curd formation may also influence fat digestion since fat globules are enmeshed in the abomasal clot (Hill et al., 1970; Ternouth et al., 1980). A longer retention time in the abomasum would

allow greater opportunity for fat hydrolysis by pregastric esterase (Radostits and Bell, 1970; Ternouth et al., 1980). Lister and Emmons (1976) suggest that coagulation appears to be a requirement for young calves less than 3 weeks of age fed a diet based on skim milk but not for older calves.

Other workers have suggested that curd formation in the abomasum is not important for normal growth and health of calves (Owen and Brown, 1958; Netke et al., 1962). Owen and Brown (1958) reported that calf growth was not adversely affected when they added citrate to whole milk to prevent clotting. Netke et al. (1962) were unable to show any effect of low or high milk pH, which was assumed to reduce curd, on calf health and weight gains.

Jenkins and Emmons (1982) suggest that abomasal chymosin-casein curd formation may provide a beneficial physiological function in calves fed whole milk and that coagulation may not be useful for milk replacers containing a mixture of skim-milk powder and soluble, poor quality protein when it promotes a different absorption pattern for essential amino acids.

Factors which may increase curd formation are: a lower pH of the skim-milk (over a range of 5.6-6.6), higher concentration of skim-milk solids (over a range of 5-20%), higher concentration of rennin, lower temperatures of heat treatment of skim-milk prior to spray drying, and higher temperature of coagulation, 37 vs. 30°C (Emmons and Lister, 1976). Defatted rapessed flour (4% wt/wt of reconstituted low-heat skim-milk) has little effect on curd firmness whereas whey powder (4%) and fish protein concentrate (2%) decreased

gel strength of the reconstituted skim-milk by approximately 30%. Soybean meal (4%) and soy protein isolate (2%) markedly reduced curd firmness; addition of $CaCl_2$ (0.1-0.4%) restored coagulability and curd firmness. However, addition of $CaCl_2$ does not restore gel firmness in reconstituted, severely heated skim-milk powder as occurs with a low-temperature product. Addition of fat to skim-milk containing 15 or 20% total solids, followed by homogenization, has little effect on curd firmness at pH 6.1. Fat addition, however, decreases curd firmness of skim-milk containing 10% total solids and nearly prevents coagulation in skim-milk containing 5% total solids (Emmons et al., 1976). In vitro substitution of skim-milk powder in calf milk replacer with acid-precipitated casein and whey results in extended coagulation time with rennin (pH 6.1, 39°C) and weaker curd strength (Jenkins, 1982). Replacement of casein by soya flour or fish-protein concentrate impairs curd formation in the abomasum, and in comparison with milk protein, both cause a reduction in rennin and pepsin secretion (Williams et al., 1976).

Abomasal contents have a pH of 1 to 2 before feeding and consist of a fairly clear, slightly viscous fluid containing small milk clots. During feeding the milk rapidly clots as it enters the abomasum (Hill, 1968). The acidity of abomasal contents decreases (pH 6.0) immediately after feeding (Mylrea, 1966a); but as acid secretion increases, the pH slowly decreases and reaches pre-feeding values in about 5 hours (Porter, 1969). By this time most of the whey fluids have left the abomasum and the pH of abomasal contents is

low enough for dissolving of the casein clot by action of pepsin (Mylrea, 1966a; Ternouth et al., 1974).

In the abomasum, milk protein clots through action of rennin and pepsin which separates milk into a casein clot and whey. The whey appears in the duodenum within 5 minutes after feeding, but the casein clot is slowly degraded and its products are subsequently discharged into the duodenum (Mylrea, 1966a). Pepsin has only one-third the milk-clotting activity of rennin at near neutral pH but over 20 times the proteolytic activity at a lower pH (Raymond et al., 1973). The optimum pH for clotting by rennin is 6.5, whereas the optimum pH for proteolytic activity is 4.0 for rennin and 2.0 for pepsin (Tagari and Roy, 1969). There is little difference between results with pepsin and rennin for the "in vitro" digestion of raw milk (Henschel et al., 1961b). Both enzymes have similar amino acid sequences (Pedersen and Foltmann, 1973), but differ drastically in their activity on ribonuclease (RN) as substract. Pepsin inactivates RN and rennin does not (Bang-Jensen et al., 1964).

The movement of digesta from the abomasum of a calf fed whole milk is a slow, well-controlled process (Ash, 1964; Mylrea, 1966a). The rate of flow of digesta is more rapid immediately after feeding, reaches a peak during the first hour and decreases progressively with time. However a considerable flow continues for many hours (Ash, 1964). With increasing time, the digesta contains a greater proportion of endogenous origin, but certain dietary constituents such as nitrogeneous components and fat continue to be found for many hours (Mylrea, 1966b; Toullec and Mathieu, 1973). Ternouth et al. (1974)

suggested that the major factor regulating abomasal emptying is the tension of the abomasal wall and that the enteric inhibition of abomasal emptying is relatively unimportant for 12 hours postprandially. Smith and Sissons (1975) found that duodenal outflow of calves receiving milk protein or soybean products for one or two occasions (unsensitized calves) was different from calves receiving certain soybean products, for a number of these feedings (sensitized calves). For unsensitized calves, the rates of flow of total digesta from the abomasum after giving synthetic milk prepared from different protein sources were similar to those after cow's milk was given. Total digesta flow was greater in the first few hours after a feed consisting of a mineral solution was given than after cow's milk was given. For sensitized calves, rates of flow of total digesta from the abomasum were greatly affected by the nature of the protein source used in the diet. Soybean flour (heated or unheated) generally caused inhibition of flow for some hours after feeding; soybean protein isolate (isoelectric) had a similar but lesser effect; whereas soybean concentrate (prepared by alcohol extraction of a soybean flour) and milk protein had little or no effect.

In contrast to Smith and Sissons (1975) increased outflow of undigested protein during the first hour after feeding was observed in calves fed "severely" heat-treated skim-milk powder (Tagari and Roy, 1969), or when milk protein was replaced by soybean flour (Colvin et al., 1969), fish protein concentrate (Ternouth et al., 1975) or whey powder (Gorrill and Nicholson, 1972a).

In spite of the conclusion of Guilloteau et al. (1979) that suppressing transit of digesta into the abomasum (by infusion into the duodenum) and accelerating the rate of passage have an adverse effect on nitrogen and liquid digestibilities, the data previously mentioned and the observation by Christison and Bell (1976) that a more digestible pea protein left the abomasum faster than a poorly digested pea product suggests that the rate of passage of crude protein through the abomasum was not a major factor in determining the crude protein digestibility. Smith and Sissons (1975) suggest that an abomasal inhibition of outflow is a sign of more general disorders such as an induced change in the duodenum perhaps resulting from a gastrointestinal allergy.

After being partially digested by gastric enzymes in the abomasum, dietary protein goes to small intestine where hydrolysis continues by action of the trypsin, chymotrypsin and carboxypeptidase present in the pancreatic juice, and by proteases present in intestinal mucosa cells (Huber, 1969; Porter, 1969; Radostits and Bell, 1970).

Pancreatic flow increases with age, but in very young calves it increases during feeding and decreases 1-2 hours after feeding. By four months of age the flow becomes more constant, approaching the pattern observed in mature sheep (McCormick and Stewart, 1967). Huber et al. (1961b) found that the levels of pancreatic proteases were low in newborn calves but increased three-fold by 2 weeks of age with little change to 6 weeks. There doesn't appear to be a marked increase in the activity of these enzymes with age but total amounts available for digestion increase markedly because of higher pancreatic flow rates

(Gorrill and Thomas, 1965; Gorrill et al., 1967). In contrast, maximum pancreatic enzyme activity occurred only in adult cattle (Clary et al., 1969; Track and Bokermann, 1973). Pelletier and Dunnigan (1983) showed that glucocorticoid administration to young calves has beneficial effect upon the development of the pancreas, but could have a detrimental effect on growth of the animal.

The proteolytic enzymes in the pancreatic juice are released as inactive precursors. Trypsinogen changes spontaneously to the active form in solution, but this change may be accelerated in two ways. First, enterokinase, secreted into the intestinal contents, resists proteolytic digestion and catalyses the conversion of trypsinogen to trypsin. Second, activated trypsin converts more precursors into the active form. Trypsin hydrolyses peptide bonds within the protein molecule (endopolypeptidase), particularly those in which the carboxyl groups are provided by an amino acid with a positively-charged side chain such as lysine or arginine. Chymotrypsinogen is activated to chymotrypsin by trypsin. Chymotrypsin acts preferentially on peptide bonds in which the carboxyl groups are provided by tyrosine or phenylalanine. Carboxypeptidase is also changed from its zymogen by action of trypsin that cleaves animo acids with the free carboxyl groups from the end of peptide chains (Sanford, 1982).

After proteolytic enzymes from gastric and pancreatic juices as well as some derived from sloughed epithelial cells have exerted their effects on dietary proteins, a mixture of free amino acids and small peptides remains. Two distinct groups of enzymes are thought

to be involved in the subsequent peptide hydrolysis; one is associated with the brush border membrane, while the other is located in the cytoplasm of the intestine epithelial cells (Sanford, 1982).

Large increases of trypsin and chymotrypsin activities occur in digesta of calves shortly after feeding (Gorrill et al., 1967; McCormick and Stewart, 1967; Gorrill and Nicholson, 1971; Ternouth et al., 1975). However, this large output of enzymes from the pancreas does not coincide with the main outflow of total nitrogen and lipids from the abomasum which occur 5 to 10 hours after feeding (Ternouth et al., 1975). The increase in pancreatic flow (but not enzyme concentration) 6 to 12 hours after feeding was due to stimulation by secretin (Ternouth et al., 1976). Gastrin has also been shown to stimulate both flow and enzymatic activities of pancreatic fluid (Henriksen and Worning, 1969).

Pancreatic exocrine secretion is controlled by a neural and hormonal mechanism (Thomas, 1967; Harper, 1972). The quantity of enzymes secreted by the pancreas decreases when these enzymes are prevented from entering the duodenum (Taylor, 1962). The presence of trypsin into the intestine may have a feedback regulatory mechanism on pancreatic secretion through modulation of the release of secretin (Davicco et al., 1979). McCormick and Stewart (1967) suggested that the increase in pancreatic flow at feeding time may be a cephalic effect, but it doesn't appear to be the gastric induced phase.

Diet may affect pancreatic secretion. When the calf is given solid feed which is digested in the rumen, daily pancreatic secretion (11.3-13.5 ml/kg liveweight) was lower than when it was fed milk

(19.7 ml/kg liveweight) (Ternouth et al., 1977). However, trypsin activity, per unit pancreatic weight is greater in the ruminant than the preruminant (Shingoethe et al., 1970).

Calves fed milk replacer containing soybean flour with a high content of trypsin inhibitor showed a reduction of trypsin and chymotrypsin activities of the pancreas and intestinal contents, as well as lower *in vitro* protein digestion incubation (Gorrill and Thomas, 1967). Calves fed an all-milk diet secreted a significantly greater quantity of pancreatic juice and higher concentrations of trypsin and chymotrypsin than those receiving a high soybean-protein diet (Gorrill et al., 1967; Gorrill and Nicholson, 1971). Roy (1969) suggested that the diarrhea in calves fed soybean protein with a high level of trypsin inhibitor could be due to protein and peptide accumulation in the large intestine and subsequent fermentation by microorganisms. Accumulation of undigested protein in the lower intestine of animals fed soybean trypsin inhibitor was observed in calves (Gorrill and Nicholson, 1971), chicks (Coates et al., 1970) and rats (Carroll et al., 1952).

## Carbohydrate Digestion in Preruminant Calves

Whereas monosaccharides can be absorbed and passed into the blood stream intact, di- and poly-saccharides require hydrolysis to monosaccharides before effective utilization can take place. Siddons et al. (1969) found that glucose, galactose and xylose were absorbed from the intestines of preruminant calves of up to 106 days of age, and each caused large increases in concentrations of blood reducing

sugars. Responses to glucose and galactose were greater with 50-day-old than with younger calves, and at this age the maximum increases due to glucose were greater than galactose, although increases were similar with younger calves. When glucose was offered the entire increase was in blood glucose, but galactose caused only a small increase in blood glucose, and feed did not affect glucose in blood. Fructose caused no increases in blood reducing sugars of 10-day-old calves, but small increases were found in 30- and 50-day-old animals. Fructose feeding also resulted in severe scouring in young calves.

Lactose caused marked increases in the levels of both blood reducing sugars and blood glucose when fed to fasted calves and there was little difference in the response of calves of different ages up to 50 days (Dollar and Porter, 1957; Huber et al., 1961). Maltose caused only small increases in reducing sugar levels, though the response increased slightly with age (Porter, 1969). Sucrose and starch caused no increase in the level of blood reducing sugar of preruminant calves (Porter, 1969; Siddons et al., 1969).

At birth the calf has large quantities of intestinal lactase as demonstrated by Dollar and Porter (1957) and Huber et al. (1961b). With advancing age, lactase levels gradually decline (Huber et al., 1961b), which may be a reflection of the animal's decreasing dependence on milk or of a greater proportion of milk entering the rumen which is subsequently digested by rumen microflora (Radostits and Bell, 1970). The intestinal lactase levels increased significantly when calves were fed whole milk with increasing amounts of added lactose (Huber et al., 1964). Adaptation to lactose was also strongly indicated by efficient

gains and high blood sugar responses in yearling steers after being changed from a hay-grain to a suckled liquid diet high in lactose (Huber et al., 1967). These data suggest that postruminal utilization of lactose is highly regenerative in the bovine.

Lactase is a beta-D-galactosidase found in the brush border of the intestinal mucosa which hydrolyzes lactose into D-glucose and D-galactose at an optimum pH range of 5.5 to 6.0 (Radostits and Bell, 1970). Highest lactase is found in the duodenal part of the small intestine, and activity decreased distally from the duodenum (Huber et al., 1961b). No lactase was found in the colon or in pancreatic secretions (Heilskov, 1951a,b). A relative deficiency of lactase would result in excessive quantities of lactose in the small intestine which, in turn might result in fermentative diarrhea (Huber et al., 1964; Weijers and van de Kamer, 1965).

At birth the calf is unable to utilize maltose, but maltase activity was observed by seven weeks of age (Dollar and Porter, 1957). Very young calves show little evidence of maltase activity and can utilize maltose only if given supplementary maltase (Dollar and Porter, 1957). However, maltose utilization, as shown by increase in reducing sugar activity after feeding maltose, increased markedly between 8 and 50 days of age (Huber et al., 1961b). It has also been demonstrated that young calves are unable to utilize dietary sucrose when fed in milk or milk replacers (Okamoto et al., 1959; Huber et al., 1961a,b; Siddons et al., 1969; Coombe and Siddons, 1973).

Ruminants secrete no salivary amylase (Kay, 1966). Pancreatic amylase is low at birth (Huber et al., 1961a) and compared to other

species, is still poorly developed at nine months of age (Larsen et al., 1956), but large increases were reported by Ternouth et al. (1971) from 24 to 63 days in Ayrshire calves with a slower rate of increase in Friesians.

Digestion trials have indicated a measurable disappearance of starch fed to young calves (Shaw et al., 1918; Maynard and Norris, 1923) however only a low increase in blood reducing sugar occurred after feeding starch (Huber et al., 1961b). Liang et al. (1967) showed that milk-fed calves absorbed and utilized volatile fatty acid placed into the cecum or colon suggesting that preruminant calves may be able to benefit from some of the undigested dietary starch as an energy source. Growth studies have corroborated digestibility data to indicate poor utilization of starch (Flipse et al., 1950; Dollar and Porter, 1957; Huber et al., 1967; Huber et al., 1968).

A small increase in starch digestibility with age has been reported (Shaw et al., 1918; Noller et al., 1956; Huber et al., 1968). However there was no increase in ability of yearling steers to digest starch after being adapted to a liquid ration high in starch (Huber et al., 1967), nor was any adaptation to maize starch shown when included in liquid ration for 10- to 14-week-old calves (Raven and Robison, 1965).

At 6 weeks of age, about 50% of dietary starch was digested and absorbed from the small intestine (Coombe and Smith, 1972). Extensive studies in France have shown that inclusion of 75 g starch or dextrins/kg in liquid diets from 13 days of age markedly depressed growth. Raw starch or poorly soluble dextrins caused constipation

whereas hydrothermically treated starches or soluble dextrins caused diarrhea (Mathieu and Thivend, 1968; Mathieu et al., 1970).

Sucrase is absent in the intestinal mucose of ruminants (Dollar and Porter, 1957; Huber et al., 1961a). Consequently, low digestibilities of sucrose (Huber et al., 1961a; Henschel et al., 1963; Morrill et al., 1965), severe diarrhea upon ingestion of sizeable quantities of sucrose (Netke et al., 1960), and no increment in blood reducing sugars after feeding sucrose solution have been reported (Dollar et al., 1957; Dollar et al., 1959; Huber et al., 1961a,b). On the other hand, an active fermentation of sucrose by microorganisms in the lower digestive tract has been reported (Henschel et al., 1963; Morrill et al., 1965).

Undergraded or partially degraded carbohydrates which reach the large intestine and cecum are substrates for microbial fermentation resulting in organic acid production (Norris et al., 1925). Organic acid concentration of about 10 meq/100 ml have been reported in the lower digestive tract of calves on milk plus high-lactose or hay-grain rations (Huber and Moore, 1964). This concentration is as high as those reported in the rumen. Calves which received only milk exhibited concentrations about 50% as high as those on high lactose or on hay plus grain diets (Huber and Moore, 1964). Liang et al. (1967) showed efficient absorption of acetate, propionate, and butyrate from the small and large intestines of calves with 52 to 87% of the $^{14}C$ appearing in the expired $CO_2$ during the first day.

## Soybean Proteins for Milk Replacers

The soybean is one of the few vegetable materials that contains protein having a reasonably well-balanced amino acid composition (Porter, 1969). However, raw soybeans contain a heat-labile trypsin inhibitor and other factors causing a depression in food intake, poor growth, increased secretion of pancreatic enzyme and hypertrophy of the pancreas in young rats and chicks (Michelsen and Yang, 1966; Sambeth et al., 1967).

Four main soybean products have been used in milk replacers for calves: soybean meal, soybean flour, soybean protein concentrate and soybean protein isolate. They average 45, 60, 70 and 90% crude protein and 45, 35, 25 and 5% carbohydrate, respectively. A summary of calf performance when calves received the different soybean protein sources in milk replacers can be seen in Table 1.

The use of soybean products to feed young calves has resulted in poorer performance than milk (Williams and Knodt, 1951; Kakade et al., 1974; Thompson et al., 1974). Reduced body weight gain and decreased dry matter, crude protein, fat, and ash digestibility have been linked to increases in the soybean products in milk replacers for calves (Nitsan et al., 1971, 1972; Pejic and Kay, 1979). Negative results have been related to the presence of soybean trypsin inhibitor (SBTI) (Gorrill et al., 1967; Gorrill and Thomas, 1967), residual carbohydrates such as neutral arabinogalactans, acidic polyssacharides and arabinans (Kellor, 1974), and the antigenic globulins glycinin and beta-conglycinin (Sissons and Smith, 1976; Kilshaw and Sissons,

TABLE 1.--Summary of dairy calves performance on different soybean protein sources in milk replacer.

| Authors | Products | Level of protein replacement (%) | Age (days) | ADG[a] (kg) | DMD[b] (%) | PD[c] (%) |
|---|---|---|---|---|---|---|
| Gorrill and Thomas (1967) | SPC[d]-50% | 50 | 4-42 | -.11 | -- | -- |
| | SPC[d]-71% | 86 | 4-42 | .33 | -- | -- |
| Gorrill and Nicholson (1969) | SPC[d] | 0(milk) | 0-49 | .53 | 90.6 | 87.3 |
| | | 70 | 0-49 | .55 | 88.8 | 81.6 |
| Gorrill et al. (1971) | SPC[d] | 22 | 2-35 | .51 | -- | -- |
| Morrill et al. (1971) | SPC[d] | 0(milk) | 7-35 | .46 | -- | -- |
| | | 22 | 7-35 | .51 | -- | -- |
| | | 43 | 7-35 | .37 | -- | -- |
| Nitsan et al. (1971) | SPC[d] | 0(milk) | 10-56 | .40 | 95.5 | 97.0 |
| | | 83 | 10-56 | .31 | 92.8 | 89.8 |
| Gorrill and Nicholson (1972a) | SPC[d] | 0(milk) | 14-49 | .38 | 90.0 | 85.0 |
| | | 70 | 14-49 | .35 | 85.0 | 77.0 |
| Nitsan et al. (1972) | SPC[d], SBM[e] | 0(milk) | 8-50 | .41 | -- | -- |
| | | 65(SPC) | 8-50 | .33 | -- | 79.4 |
| | | 73(SBM) | 8-50 | .25 | -- | 52.0 |
| Kakade et al. (1976) | HS[f] | 70 | 14 | -- | 84.0 | 55.0 |
| Roy et al. (1977) | SF[g] | 0(milk) | 0-21 | 1.11 | 95.0 | 94.0 |
| | | 36 | 0-21 | .85 | 89.0 | 84.0 |
| | | 70 | 0-21 | .67 | 80.0 | 66.0 |

TABLE 1.--Continued.

| Authors | Products | Level of protein replacement (%) | Age (days) | ADG[a] (kg) | DMD[b] (%) | PD[c] (%) |
|---|---|---|---|---|---|---|
| Barr et al. (1978) | MSP[h] | 0(milk) | 3-28 | .50 | -- | -- |
| | | 50 | 3-28 | .46 | -- | -- |
| | | 72 | 3-28 | .45 | -- | -- |
| Bring and Barr (1979) | MSP[h] | 0(milk) | 3-42 | .67 | -- | -- |
| | | 70 | 3-42 | .64 | -- | -- |
| Jenkins (1981) | SPC[d]-SPI[i] | 0(milk) | 3-31 | .44 | 93.6 | 90.8 |
| | | 51 | 3-31 | .20 | 91.4 | 85.8 |
| Campos et al. (1982a) | SPC[d] | 0 | 5-47 | .61 | -- | -- |
| | | 33 | 5-47 | .59 | -- | -- |
| Campos et al. (1982b) | SPC[d] | 0 | 4-46 | .40 | -- | -- |
| | | 33 | 4-46 | .02 | -- | -- |
| Huber and Campos (1982) | SPC[d] | 0 | 4-46 | .42 | -- | -- |
| | | 33 | 4-46 | .35 | -- | -- |
| Campos and Huber (1983) | SPC[d] | 0 | 4-46 | .26 | 87.6 | 85.2 |
| | | 50 | 4-46 | .22 | 85.4 | 76.8 |
| Akinyele and Harshbarger (1983) | SPC[d]-SF[j] | 0(milk) | 5-21 | .30 | 92.0 | 90.1 |
| | | 100(SPC) | 5-21 | -.07 | 70.0 | 56.6 |
| | | 100(SF) | 5-21 | -.11 | 71.0 | 61.3 |

TABLE 1.--Continued.

[a]Average daily gain

[b]Dry matter digestibility

[c]Protein digestibility

[d]Soybean protein concentrate

[e]Soybean meal

[f]Heated soybean

[g]Soybean flour (thermo-alkali treated)

[h]Modified soybean protein

[i]Soybean protein isolated

[j]Soybean flour

1979a; Kilshaw and Slade, 1980), as well as to unknown factor(s) (Gertler and Nitsan, 1970; Nitsan et al., 1971).

Gorrill and Thomas (1967) found poor growth, but no hypertrophy of the pancreas in calves given a methionine-supplemented diet in which soybean flour containing trypsin inhibitor supplied 60% of the protein. The pancreatic juice and intestinal contents of calves contained less trypsin and chymotrypsin activities than those of calves given milk or a soybean flour having a very low trypsin inhibitor. The deleterious effects of the presence of SBTI were confirmed by Colvin and Ramsey (1968) although their major finding was that the nutritive value of trypsin inhibitor-free soybean flour was markedly improved by treatment with acid at pH 4 for 5 h at 37°C. Calves given the acid-treated soybean flour grew at nearly twice the rate of those receiving the untreated material. Kakade et al. (1976) did not find differences in weight or enzymatic activities of pancreas in calves due to SBTI. They concluded that SBTI plays a minor role, if any, in calf nutrition. The reduction in pancreatic secretions in calves fed SBTI contrasts to studies with chicks and rats in which extracts from raw soybeans induced pancreatic hypertrophy and hypersecretion of digestive enzymes (Haines and Lyman, 1961; Garlich and Nescheim, 1966; Michelsen and Yang, 1966). Response of swine pancreas to raw soybeans is similar to that observed in calves (Hooks et al., 1965). Diets containing soybean flour have also been reported to reduce gastric acid secretion (Williams et al., 1976) and retention time of protein in the abomasum (Colvin et al., 1969; Smith et al., 1970) with a resultant decrease in proteolysis. Attempts to improve digestibility of isolated soybean

protein used in milk replacers by pepsin and pancreatin supplementation had a detrimental effect in calf performance (Jenkins, 1981). Supplementation with these enzymes, separately or together, also reduced apparent digestibility of dry matter, and nitrogen, but had no effect on feed intakes (Jenkins, 1981). In vitro studies have shown that both pepsin and the pancreatic proteases have much better hydrolytic activity on milk protein than on soybean protein (Jenkins et al., 1980; Jenkins, 1981).

Willard and Ramsey (1972) suggested that the further removal of the small amounts of trypsin inhibitor in heated soybean flour by acid or alkali treatment improves nutritional value, but this finding was not supported by Sissons and Smith (1976).

An inactive form of trypsin inhibitor in fully-cooked soy flour that is converted to an active form in the pH range of 7 to 9 was reported by Ramsey and Willard (1975). This inhibitor can be destroyed by heating the soy flour in water, but the extent of destruction appears dependent on the concentration of flour in the water. The presence of trypsin inhibitor in fully-cooked soy flour is offered as a possible explanation for the observation that newborn calves often do not perform well on milk replacers containing large quantities of this product.

Acid or alkali treatment of toasted soybean flour (Colvin and Ramsey, 1968, 1969; Colvin et al., 1969) or extraction with alcohol (Nitsan et al., 1972; Smith and Sissons, 1975) has improved its quality. Thermo-alkali treatment with lower temperatures inactivates SBTI more effectively than thermo-acid treatment (Wallace et al., 1971).

The presence of complex carbohydrates in soybean products, mainly in soybean meal and soybean flour which have 45 and 35% carbohydrates, may explain some of the poor results reported when these products are fed to young calves. Roy et al. (1977) concluded that feeding of soybean flour may have detrimental effects due to the presence of trypsin inhibitor, haemagglutimins, a large content of oligosaccharides (which probably cannot be utilized by the calf) and phytin.

Roy et al. (1977) also observed a lower Ca absorption in calves fed milk replacers containing soybean flour than all milk. This was attributed to the phytin in soybean flour, which accounts for 70% of the total phosphorous, and is not only unavailable for simple-stomached animals but interferes with the absorption of calcium, iron and zinc (Taylor, 1965; O'Dell, 1969; Thompson and Erdman, 1984). Disappearance from the gut of considerable carbohydrate from soy protein sources (Nitsan et al., 1971, 1972; Kakade et al., 1976) is presumably due to intestinal fermentation. Feeding of high chlorotetracycline to depress intestinal fermentation did not improve growth in calves fed untreated soybean flour (Colvin and Ramsey, 1969).

Enzymatic pre-digestion of soybean flour did not stimulate growth, even though the carbohydrate fraction underwent extensive degradation (Colvin and Ramsey, 1968). Removal of the water-soluble carbohydrates from soybean flour did not improve growth and addition of the water-soluble carbohydrates to a replacer containing soybean flour did not depress growth (Sudweeks and Ramsey, 1972).

Smith and Wynn (1971) reported large amounts of hemaglutinates in calf blood, when milk protein was completely replaced by soybean flour or when successive feedings of soybean flour was interpersed with feedings of normal milk diets. Smith and Sissons (1975) found that soybean flour and isolated soybean protein caused inhibition of the digesta flow from the abomasum for a few hours after feeding. They suggested this inhibition was a sign of more general disorders caused by a factor entering the duodenum which induced a change in the way the calf responded, probably as the result of a gastrointestinal allergy. Calves given soybean flour showed high titres of serum antibodies to an antigen from soybean flour.

Preruminant calves fed heated soybean flour produced high titres of serum IgG and IgE antibodies specific for soybean protein glycinin and beta-conglycinin (Kilshaw and Sissons, 1979a,b). Barratt et al. (1978) reported a complement-fixing IgG1 preciptin as the predominant antibody in calves fed alcohol-extracted soybean protein. The soybean antigen was shown resistent to proteolysis and, to a lesser degree, to the microbial action of rumen fluid. No evidence of tolerance was seen and previously sensitized calves responded with marked increases in antibody levels. Biopsy studies showed morphological disturbances to the villi and lamina propria of the intestine. Benzyl isothiocyanate has also been identified as a prominent allergen in soybeans. Calves fed whole milk to which 90 mg benzyl isothiocyanate was added showed poor growth rates and persistent diarrhea, identical to those fed milk replacer containing soybean flour (Gardner et al., 1982).

Gradual deterioration in villus integrity was reported by Seegraber and Morrill (1982a,b) in calves fed soybean proteins. Abnormalities included an absence of villi, while those remaining were short, blunted and convoluted. Deterioration of villi tended to be reversed with a return toward normal size and shape when the diet was changed to milk. These data were supported by Kilshaw and Slade (1982) who reported partial atrophy of villi and crypt enlongation in calves given heated soybean flour. The first exposure to soybean protein caused a slight shortening of villi, but after successive feedings, animals developed marked mucosal abnormalities and severe diarrhea. MacDonald and Ferguson (1976) suggested that a local cell-mediated immunologic reaction may be the cause of villus atrophy, crypt hyperplasia, and malabsorption of food. Kilshaw and Slade (1980) showed that successive feeding of heated soybean flour to preruminant calves caused progressively larger increases in intestinal permeability and rising titers of serum antibodies (IgG, IgA and IgM) to soybean proteins. This response was observed in about 50% of the calves tested.

An essential function of the gastrointestinal mucosa is exclusion from the circulation of bacteria, toxins and food antigens. An effective barrier is formed by the interaction of immunologically specific and non-specific defense mechanisms (Walker, 1976). It is generally believed that in normal circumstances only trace quantities of macromolecules pass unchanged from the gut into the blood. A break in the mucosal barrier might therefore have pathogenic consequences (Walker and Isselbacher, 1974). Inflammatory reactions in the

intestinal mucosa can increase the absorption of undegraded protein (Bloch et al., 1979). This is suggested to occur in food allergies (Paganelli et al., 1979) and might explain some of the observed responses of calves to soybeans.

Methionine has been shown to be the most limiting amino acid in soybean protein for rats, chicks and pigs (Almiquist et al., 1942; Hays et al., 1959; Berry et al., 1962). Lysine was lower in the alkali-treated than untreated soybean concentrate (Gorrill, 1970), and lysine, cysteine and serine were reduced when isolated soybean protein was mildly treated with .02N NaOH (DeGroot and Slump, 1969). Gorrill and Nicholson (1969) reported no increase in growth or nitrogen retention in young calves fed milk replacers containing 70% of the dietary protein from soybean protein concentrate which was supplemented with methionine (0.1% of dry matter) but Porter and Hill (1964) showed improved calf performance when methionine was supplemented to isolated soybean protein, thereby increasing its content from 1.5 to 2.3 g/100 g of protein.

The maximum level of milk protein substitution by soybean protein varies with the type of soybean protein source used. Gorrill and Thomas (1967) reported that 86% replacement of milk protein by soybean protein concentrate resulted in weight gains similar to that of calves fed whole milk. Gorrill and Nicholson (1969) concluded that the soybean protein concentrate could supply up to 70% of the protein in milk replacer for rearing dairy calves. In contrast, Morrill et al. (1971) stated that soybean protein concentrate could successfully replace 22%, but not 44% of total protein in milk replacers.

Nitsan et al. (1971) and Nitsan et al. (1972) concluded that milk replacers containing soybean protein concentrate up to 88% of the total protein can be used if a calf starter containing cereal grains and soybean meal of 16% crude protein is also supplied. Similar results were reported by Guilhoteau et al. (1977). Roy et al. (1977) reported that up to 36% milk protein could be replaced by protein from a thermo alkali-treated soybean flour without markedly affecting calf performance.

## Other Protein Sources for Milk Replacers

Milk is the natural source of protein for the newborn calf. Proteins of raw milk and of spray-dried milk are of high biological value and are generally well utilized. Casein is quite satisfactory as the sole source of protein in synthetic diets for 1-2 week old calves has resulted in values for percentage nitrogen retention and apparent nitrogen digestibility of 35 and 86, respectively; compared values for raw milk of 49 and 91 (Porter, 1969). Problems arise in heat-treatment of milk if the time-temperature relationship is such that the whey proteins, including the Ig, are denatured. Such milk powders have a slow rate of clotting; and instead of forming a firm curd, a flocculent precipitate is produced (Tagari and Roy, 1969). Gastric acid production and proteolysis are reduced causing passage of undigested casein into the duodenum. Pancreatic secretion and protease outflow are also reduced (Ternouth and Roy, 1973). A lower over-all digestibility of protein, fat and ash is observed. Such diets may predispose calves to enteric infection if they have

received only marginal passive immunity or are exposed to an adverse microflora (Roy, 1974).

Fish products have been used to replace part of the milk protein in milk replacers for calves. Marked variability in nutritional value was obtained from the different fish species and from the various processing procedures (Miller, 1956; Morrison and McLaughlan, 1961; Morrison et al., 1962; Makdani et al., 1971a; Jenkins et al., 1982; Campos et al., 1982; Huber and Campos, 1982).

Much of the early work using fish meal (Rupel and Wilson, 1962; Genskow et al., 1968; Wendlandt et al., 1968; Gorrill et al., 1975) or fish flour (Harshbarger and Gelwicks, 1965; Huber and Slade, 1967; Williams and Rust, 1968) was unsuccessful because of the low quality and isolubility of the material.

Dried fish solubles are made of stickwater, an aqueous extract from cooked fish usually obtained from fish meal plants where fish are cooked and separated by pressure into an aqueous extract and a fish pulp (Lassen, 1965). Inclusion of even moderate amounts of spray-dried fish solubles in milk replacers produced poor growth, low feed efficiency, and high calf mortalities (Campos et al., 1982a,b).

When fish protein concentrate replaced up to 35% of the protein in calf milk replacer, growth was satisfactory, but depressed gains resulted from feeding more fish (Huber and Slade, 1967; Genskow et al., 1968; Makdani et al., 1971a). Proposed causes for the negative results with fish protein concentrate were low protein digestibility and poor nitrogen retention (Matre, 1970; Sleiman and Huber, 1971); poor quality of essential amino acids (Sleiman and Huber, 1971; Roy et al., 1977);

vitamin E deficiency (Genskow et al., 1968; Michel et al., 1972); toxic compounds associated with the solvent (Munro and Morrisson, 1967; Makdani et al., 1971a); aplastic anemia (Picken et al., 1952; Schultze et al., 1959); and high ash content of the fish protein concentrate (Gillespie, 1971; Makdani et al., 1971a). Gorrill et al. (1975) concluded that isopropanol-extracted fish protein concentrate could replace up to 62% of the total nitrogen in milk replacers without having a detrimental effect on calf performance.

Dehulled rapeseed meal was used to supply 30% of the protein in milk replacers with no significant decrease in calf performance (Gorrill et al., 1976); while dehulled, low-erucic-acid, low-glucosinolate, full-fat rapeseed replaced up to 50% of the protein in milk replacers without significantly affecting lamb performance (Seoane et al., 1976). Gorrill et al. (1974) concluded that rapeseed protein concentrate, but not rapeseed flour, could supply at least 25% of the total nitrogen in lamb milk replacers.

Meat meals were shown to be a poor source of protein in milk replacers, and resulted in low nitrogen retention and decreased nutrient utilization. Meat meals contain mainly collagen, which is approximately 13% hydroxy-proline and largely unavailable to the calf (Raven, 1972; Polzin et al., 1976). On the other hand, blood meal and blood flour were of comparable value in milk replacers, resulting in slightly lower growth than all-milk protein (Brumbaugh and Knodt, (1952)

Fababean was substituted for up to 25% of the milk protein in milk replacers (Wittenberg and Ingalls, 1979); alfalfa for up to 50%

(Alpan et al., 1979) and crab meal for up to 20% (Patton et al., 1975). These sources all gave acceptable calf performance.

## Summary of the Literature Review

The literature reviewed has shown that soybean protein may replace most of milk protein in milk replacers for baby calves. However, the use of soybean products has resulted in poorer performance than milk. Reduced body weight gain and decreased dry matter, crude protein, fat, and ash digestibility have been linked to increased incorporation of soybean products. Negative results have been related to the presence of soybean trypsin inhibitor, residual carbohydrates such as neutral arabino galactans, acidic polyssacharides and arabinans, soybean antigenic globulins (glycinin and beta-conglycinin), the low levels of methionine and lysine, as well as to unknown factor(s).

The objective of Experiment 1 was to test digestibility and absorption of the "modified soybean protein" and related these to calf performance. In Experiment 2 was detect any alterations of small intestinal mucosa caused by incorporation of large amounts of soybean protein in milk replacers were studied.

# MATERIAL AND METHODS

## Experiment 1

This experiment was conducted from August 31 to November 9, 1982 with the objective to compare digestibility and absorption of a "modified soybean protein" and heated soybean flour with milk protein as protein sources in milk replacers for baby calves.

During a 4 week period twenty-four male Holstein calves (3-4 days of age) were purchased from a commercial dairy farm and transported to the Michigan State University Dairy Cattle Center (MSUDCC). The day calves arrived they were identified by an ear tag, weighed, navels were desinfected with iodine solution and calves were started on the experimental ration. Also, each calf received an injectable solution containing 1,000,000 IU of vitamin A, 150,000 IU of vitamin $D_3$, 136 IU of vitamin E and 2 mg of selenium. Based on its body weights each group of 6 calves was split into 2 blocks, heavy and light, and calves in each block were randomly allotted to one of the 3 experimental diets. Except for the digestibility and xylose test periods, calves were kept indoors in tie stalls bedded with straw.

From 4 to 52 days of age each calf was fed its designated milk replacer as the only source of nutrients at 8, 9, 10, 11, 12, 12, 12 and 12% of body weight from 1 to 8 weeks, respectively. Solids content after mixing with warm water (approximately 37°C) was 14%. Replacers

were prepared just before feeding and fed twice daily (12 hr intervals) from open pails. Fresh, clean water was available at all times.

The experimental diets all contained 19% crude protein (%DM) from varying sources. Treatments were:

Treatment MP: 100% milk protein;

Treatment MS: 66% modified soybean protein + 34% milk protein;

Treatment HS: 66% heated soybean flour + 34% milk protein.

Modified soybean protein was obtained by uniformly mixing defatted soybean flour with a solution of 33.7 parts of 95% ethanol and 6.3 parts of water and placing the mixture in a closed reactor where it was heated at 121°C for 30 minutes under a pressure of 1.055 $kg/cm^2$. Afterwards, the flour was sprayed into a drying chamber where the alcohol and most of the water were evaporated. The material was then recovered as spray-dried, modified soybean protein.

Ingredient composition of milk replacers is shown in Table 2. As milk replacers were being fed, a sample was taken from each sack and composited for laboratory analysis. Milk replacers were analyzed for dry matter (forced air oven at 70°C), nitrogen (macro-Kjeldahl), ether extract and ash as described by A.O.A.C. (1980). Chemical composition of milk replacers is shown in Table 3.

On days 8 and 29, calves were moved to metabolism cages for collection of feces and urine to determine digestibility of nutrients and nitrogen retention. Collection periods lasted from 3 to 5 days depending on calf's capacity to tolerate the collection conditions. Urine was collected in pans containing 10 ml of a mixture 50% concentrated sulfuric acid and 50% water which were placed under metabolism

TABLE 2.--Ingredient composition of milk replacers used in Experiment 1 (% of spray dried material)[a].

| Ingredient | Treatment | | |
|---|---|---|---|
| | MP | MS | HS |
| Nonhydroscopic edible whey | 65.58 | 55.38 | 55.74 |
| Fat - Milk concentrate[b] | 23.03 | 22.22 | 22.45 |
| Casein[c] | 10.14 | -- | -- |
| Modified soybean protein[c] | -- | 21.15 | -- |
| Heated soybean flour[c] | -- | -- | 20.56 |
| Pre-mix (vit.-min.)[d] | 1.25 | 1.25 | 1.25 |

[a]All milk replacers contained 250 g/ton neomycin base and 100 g/ton oxitetracycline.

[b]40% fat as homogenized white grease which was spray dried with 60% whey and contained 7% protein.

[c]Casein - 90% protein; modified soybean protein - 50% protein; heated soybean four - 50% protein.

[d]44,000 U.S.P. units vit. A/kg; 11,000 U.S.P. units vit. D3/kg; 44 U.S.P. units vit. E/kg; 6.6 mg thiamine/kg; 6.6 mg riboflavin/kg; 2.6 mg niacin/kg; 13.0 mg d- pantothenic acid/kg; 0.11 mg biotin/kg; 110 mg ascorbic acid/kg; 6.6 mg pyridoxine hydrochloride/kg; 0.55 mg folic acid/kg; 0.07 mg vit. $B_{12}$; 2,650 mg choline chloride/kg; iron 100.0 ppm; copper 10.0 ppm; cobalt 0.1 ppm; zinc 40.0 ppm; manganese 40.0 ppm; iodine 0.25 ppm; and selenium 0.1 ppm.

TABLE 3.--Chemical composition of milk replacers used in Experiment 1.

| Measurement | Treatment | | |
|---|---|---|---|
| | MP | MS | HS |
| | (%) | | |
| Dry matter (DM) | 96.92 | 96.66 | 96.79 |
| | (% of DM) | | |
| Crude protein | 20.22 | 19.81 | 19.25 |
| Ether extract | 8.67 | 8.88 | 8.49 |
| N-free extract | 63.43 | 63.58 | 64.53 |
| Ash | 7.68 | 7.73 | 7.73 |

cages. Feces were collected with light harnesses. A composite sample of feces for each collection period was made by saving 10% of feces excreted daily and mixed with that of other days from the same calf. A composite sample of urine for each collection period was prepared by saving 1% of the urine excreted daily. Fecal samples were kept at -20°C until analyzed for dry matter (forced air oven at 70°C), nitrogen (macro-Kjeldahl), ether extract and ash (as described by A.O.A.C. 1980). Urine samples were kept at -20°C until analyzed for nitrogen (macro-Kjeldahl) as described by A.O.A.C. (1980).

Food intake was recorded daily. Calves were weighed on the first and last day of experiment and at weekly intervals. Fecal consistency was rated each day for each calf on a scale from 1 to 4 (Larson et al., 1977) with 1 being normal and 4 quite fluid. Rectal

temperatures were measured daily just before the morning feeding, except during collection periods.

A jugular blood sample was taken at 5 hr after feeding to determine plasma urea nitrogen on 22 and 44 days of the feeding trial. Plasma was separated by centrifugation at 8,000 g for 20 min and kept at -20°C until analyzed for urea as described by Fawcet and Scott (1960).

A xylose disappearance test was performed on day 41 of the trial to evaluate the rate of xylose clearance from the blood of each calf. Calves were fasted for 24 hr before administering, by intravenous injection, .25 g xylose/kg body weight in 20% water solution.

A xylose absorption test was performed on day 50 of the trial. Calves were fasted for 24 hr before administering, via nipple bottle, .5 g xylose/kg body weight in a 10% water solution. For both xylose tests, jugular blood was sampled just before and 30, 60, 90, 120, 150, 180, 240 and 300 minutes after administration of xylose. Urine also was collected during the blood sampling period. Plasma and urine were kept at -20°C until analyzed for xylose by the orcinol/ferric chloride, spectrophotometric method as described by Seegraber and Morrill (1979).

Two calves randomly chosen from each treatment were sacrificed by electrocution on the last day of the experiment. After removal of the entire digestive tract, weights were recorded for rumen, abomasal, small intestine (divided in three equal length sections), cecal and large intestinal contents. Samples of contents were kept at -20°C until analyzed for dry matter, crude protein and ash as described by

A.O.A.C. (1980). The pH was also measured for all digestive tract contents at the time of collection.

Two equally spaced 2.5 cm x 2.5 cm tissue samples were taken from each of the three sections of small intestine just after the animal was sacrificed. These samples were taken to the scanning electron microscopy laboratory and placed in a solution of isotonic sodium chloride with a pH of 6.0. They were then fixed for 2 hours in 4% glutaraldehyde buffered at pH 7.2 with 0.1 M sodium cacodylate buffer. Samples were then rinsed twice for 15 minutes in the buffer alone, dehydrated in a graded ethanol series, and dried at the critical point in a Balyers Critical Point Drier using $CO_2$ as the transitional gas. Tissue samples were mounted on stubs, coated with gold, and examined in a Jeol JSM-35C scanning electron microscope using beam-accelerating voltages of 15 KV at magnifications ranging from 48 to 10,000 X. Selected fields were photographed using a Polaroid camera. The villi morphology and integrity shown in the micrographs were evaluated by 5 independent evaluators and classified in a 1 to 5 scale with 1 being for normal and 5 for quite abnormal or absent villi.

Treatment for illness were recorded daily. Life-Guard (Norden Laboratories, Inc., Lincoln, NE) was used as an electrolyte and for body fluid replacement in dehydrated calves. Administration of LS/50 (17.6 mg/kg body weight spectinomycin + 11.0 mg/kg body weight lincomycin obtained from The Upjohn Company, Kalamazoo, MI) was used for calves exhibiting body temperatures in excess of 39.5°C for more than 24 hr.

Data were analyzed as a completely randomized block or as a split-plot design. Either the Bonferroni t test or designed orthogonal contrasts were used to compare means, as indicated in the footnotes of tables. Analysis of variance for all variables was as described by Gill (1978a,b,c), and these analyses are shown in Table A.1.

Experiment 2

This experiment was conducted from April 2 to June 18, 1984 with the objective to detect alterations in small intestinal villi which might be caused by incorporation of large amounts of soybean protein into milk replacers for baby calves.

Sixteen male Holstein calves were purchased from the same commercial dairy farm as those obtained in Experiment 1 and transported to the MSUDCC. Procedures for receiving these calves were the same as described for Experiment 1.

Upon arrival at the MSUDCC, calves were fed colostrum obtained from their dams for 1 feeding and herd colostrum for the subsequent 3 feedings. The colostrum was followed by whole milk for 2 more days. From 5 to 46 days of age each calf was fed its designated milk replacer as the only source of nutrients at 8, 9, 10, 11, 12, 12 and 12% body weight from 1 to 7 weeks, respectively. Solids content of all milk replacers after mixing with warm water (approximately 37°C) was 14%. The milk replacers were prepared just before feeding and fed twice daily (at 12 hr intervals) from an open pail. Fresh, clean water was available at all times.

Initial treatment of these calves differed from those in Experiment 1 in that they were removed immediately after birth from their dams in an attempt to avoid any viral infections which might occur.

The experimental milk replacers contained 23% crude protein and protein sources were:

Treatment M: 100% milk protein;

Treatment S: 66% soybean protein concentrate + 34% milk protein.

Ingredient composition of milk replacers is shown in Table 4. At opening a sample was taken from each sack of milk replacer and used for compositing prior to laboratory analysis. Replacers were analyzed for dry matter (forced air oven at 70°C), nitrogen (macro-Kjeldahl), ether extract and ash as described by A.O.A.C. (1980). Chemical composition of milk replacers is shown in Table 5.

One-half of the calves were sensitized to soybean protein by feeding milk replacer S during a 21-day period while the others received milk replacer M and were not sensitized. After the initial 21-day period, the calves within each group were randomly assigned to one of the milk replacers for 10 days which was followed by the other milk replacer for 10 more days. The outline of the experiment is as follows:

TABLE 4.--Ingredient composition of milk replacers used in Experiment 2 (% of spray dried material).

| Ingredients | Treatment | |
|---|---|---|
| | M | S |
| Nonhydroscopic edible whey | 58.82 | 52.12 |
| Fat - Milk concentrate[a] | 23.00 | 23.20 |
| Casein[b] | 17.40 | -- |
| Soybean protein concentrate[c] | -- | 23.90 |
| Vit. Mineral Premix[d] | 0.78 | 0.78 |

[a]40% fat as homogenized white grease, which was spray dried with 60% whey and contained 7% protein.

[b]90% protein.

[c]Procon 2000 - 67% protein.

[d]44,000 U.S.P. units vit. A/kg; 11,000 U.S.P. units vit. D3/kg; 44 U.S.P. units vit. E/kg; 6.6 mg thiamine/kg; 6.6 mg riboflavin/kg; 2.6 mg niacin/kg; 13.0 mg d- pantothenic acid/kg; 0.11 mg biotin/kg; 110 mg ascorbic acid/kg; 6.6 pyridoxine hydrochloride/kg; 0.55 mg folic acid/kg; 0.07 mg vit. $B_{12}$; 2,650 mg choline chloride/kg; iron 100.0 ppm; copper 10.0 ppm; cobalt 0.1 ppm; zinc 40.0 ppm; manganese 40.0 ppm; iodine 0.25 ppm; and selenium 0.1 ppm.

TABLE 5.--Chemical composition of milk replacers used in Experiment 2.

| Measurement | Treatment | |
|---|---|---|
| | M | S |
| | (%) | |
| Dry matter (DM) | 96.83 | 97.30 |
| | (% of DM) | |
| Crude protein | 23.00 | 23.06 |
| Ether extract | 7.21 | 7.53 |
| N-free extract | 62.90 | 62.33 |
| Ash | 6.89 | 7.08 |

| PO | P1 | P2 | Calves |
|---|---|---|---|
| Non Sensitized | M | S | 4 |
| M | S | M | 4 |
| Sensitized | M | S | 4 |
| S | S | M | 4 |

PO - Preliminary sensitization period (21 days)

P1 and P2 - Treatment periods (10 days each)

M - Milk replacer with 23% crude protein; 100% as milk protein

S - Milk replacer with 23% crude protein; 66% as soybean protein concentrate + 34% as milk protein

During the second week each animal was surgically fitted with a plastic, Y-shaped, duodenal cannula 1 cm in diameter positioned about 100 cm posterior to the pyloric sphincter in order to permit

biopsy of intestinal mucosa. Initial anesthesia was induced by halothane gas using a face mask, after which an endotracheal tube was introduced and anesthesia was maintained with halothane gas. Calves were placed in left lateral recumbency and the skin of the right flank was prepared for aseptic surgery. A 10 cm incission was made 7 cm caudal to the 13th rib. The descending duodenum was isolated and a purse-string suture was placed into the antimesenteric border. A 2 cm incision was then made in the intestine such that it could be closed by tightening the purse-string suture. The cannula was then inserted into the incision and the suture was drawn tight. A second purse-string suture was placed over the first so as to invert the cut edges of the intestine. A small perforation was made in the greater omentum and the cannula was passed through the hole. A stab incision was then made through the body wall and skin just caudle to the 13th rib and the cannula was further passed through to the outer surface of the body. A plastic flange was then placed over the cannula to hold it tightly against the abdomen. The incisions in the muscular layers of the abdomen and the skin were then closed with sutures.

Intestinal mucosa samples were taken by biopsy during the surgery to fit the cannula and on the last day of each period. Biopsy samples were taken at the site of surgery and 40 to 50 cm caudal to the cannula. Tissues were fixed immediately in a 10% phosphate buffered formalin solution. Villi were stained with 1% new methylene blue solution and twenty villi were measured from each sample by use of a dissecting microscope. Measurements of villi were made the same day or the day following biopsy.

Feed intake of calves was recorded daily and body weights on the first and last days of the experiment, at weekly intervals and on the first day of each experimental period. Fecal consistency was rated daily similar to the method described for Experiment 1. Rectal temperatures were recorded daily just before the morning feeding.

Treatments for illness were recorded daily. Life-Guard (Norden Laboratories, Inc., Lincoln, NE) was used as an electrolyte and body fluid replacement in dehydrated calves. All calves were given 1 gm ampicillim (Omnipen-N, Wyeth Laboratories Inc., Philadelphia, PA) immediately following surgery and twice a day during the next 3 days. Ampicillim (1 gm per dose) also was given twice daily for 3 days to all calves exhibiting rectal temperatures higher than 39.7°C for more than 12 hr. Five hundred mg kanamycin (Kantrim, Veterinary Products Bristol Laboratories, Syracuse, NY) were added to medication of calves when the rectal temperatures did not decrease and/or when calves showed inappetence. Calves which refused to eat were force-fed. Data from the surgery and PO period were analyzed as a completely randomized design and those from P1 and P2 periods were analyzed as split-plots, with treatments as sub-plots in a crossover Latin square design. The F-test was used to compare means. Analysis of variance for all variables were as described by Gill (1978a,b,c), and are shown in Tables A.2 and A.3.

## RESULTS AND DISCUSSION

### Experiment 1

Initial body weights were not different between treatments, but final weights (Table 6) were higher ($P<.05$) for calves fed milk protein (MP) than modified soybean protein (MS) or heated soybean flour (HS). Similar results were observed for body weight gain, gains as a percent on initial body weight (Table 6) and average daily gains (Table 7).

A significant ($P<.05$) interaction of treatment x age was observed for body weight, total gain and gain as a percent of initial body weight suggesting that differences between treatment in weight gains become greater with increased age (Figure 1, 2 and 3).

Although soybean products have replaced up to 60 or 70% of the milk protein in milk replacers for young calves with generally satisfactory growth, body weight gains were usually lower than those on only milk protein (Morrill et al., 1971; Nitsan et al., 1972; Pejic and Kay, 1979; Huber and Campos, 1982; Campos et al., 1982; Beynen and van Gils, 1983). De Gregorio et al. (1972) reported no difference in calf weight gains when milk replacer protein sources were either modified soybean protein (66% milk protein replacement) or all milk, but the authors reported higher ($P<.05$) calf weight gain for calves fed all milk or modified soybean protein than heated soybean flour.

TABLE 6.--Effect of feeding milk protein (MP), modified soybean protein (MS), or heated soybean flour (HS) on calves body weight (BW), total weight gain (TG) and gain as a percent of initial body weight (% BW) of calves at different ages Experiment 1.

| Age (days) | BW (kg)[k] | | | TG (kg)[k] | | | % BW[k] | | |
|---|---|---|---|---|---|---|---|---|---|
| | MP | MS | HS | MP | MS | HS | MP | MS | HS |
| 0 | 43.26 | 42.35 | 41.13 | -- | -- | -- | -- | -- | -- |
| 8 | 42.98 | 42.48 | 40.76 | -0.29 | 0.13 | 0.54 | -0.64 | 0.21 | -1.05 |
| 15 | 42.24 | 39.98 | 39.00 | -1.03 | -2.38 | -1.95 | -2.43 | -5.99 | -5.16 |
| 22 | 43.61 | 41.09 | 40.05 | 0.35 | -1.26 | -1.31 | 0.86 | -3.24 | -2.58 |
| 29 | 46.74 | 43.55 | 42.20 | 3.48 | 1.20 | 1.41 | 8.07[i] | 2.70[j] | 2.69[j] |
| 36 | 49.43 | 45.25 | 43.61 | 6.16[g] | 3.48[h] | 2.95[h] | 14.15[g] | 6.74[h] | 6.29[h] |
| 43 | 52.01 | 48.28 | 46.26 | 8.75[g] | 5.93[h] | 5.38[h] | 20.28[g] | 14.09[h] | 12.75[h] |
| 50 | 56.83[i] | 51.45[j] | 49.26[j] | 13.56[c] | 9.10[d] | 8.49[d] | 31.36[c] | 21.37[d] | 20.08[d] |
| 52 | 59.11[c] | 54.14[d] | 50.99[d] | 15.85[c] | 11.79[d] | 10.38[d] | 36.51[c] | 27.35[d] | 24.16[d] |
| Means | 48.47[e] | 45.39[f] | 43.70[f] | 5.85[a] | 3.50[b] | 3.10[b] | 13.52[a] | 7.91[b] | 7.15[b] |
| ± SEM | ±2.01 | ±2.01 | ±2.01 | ±0.78 | ±0.78 | ±0.78 | ±1.74 | ±1.74 | ±1.74 |

[a,b]Means in the same row with different superscripts are different (P<.005).

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

[e,f]Means in the same row with different superscripts are different (P<.10).

[g,h]Means in the same row with different superscripts are different (P<.15).

[i,j]Means in the same row with different superscripts are different (P<.20).

[k]Orthogonal Contrast (MP vs MS + HS; MS vs HS).

TABLE 7.--Effect of feeding milk protein (MP), modified soybean protein (MS), or heated soybean flour (HS), on average daily gain (ADG), feed intake (FI), and feed efficiency (G/I) of calves as measured for each week of the experiment Experiment 1.

| Period (ages-days) | ADG (g)[n] | | | FI (kg)[o] | | | G/I[o] | | |
|---|---|---|---|---|---|---|---|---|---|
| | MP | MS | HS | MP | MS | HS | MP | MS | HS |
| 8-14 | -106 | -357 | -252 | 3.24 | 2.86 | 3.22 | -0.259[a] | -0.912[c] | -0.574[b] |
| 15-21 | 197 | 159 | 150 | 4.01 | 3.56 | 3.68 | 0.357 | 0.317 | 0.289 |
| 22-28 | 447 | 352 | 307 | 4.54[l] | 3.91[m] | 4.06[m] | 0.689[l] | 0.616[m] | 0.540[m] |
| 29-35 | 384[h] | 243[h,i] | 202[i] | 5.29[l] | 4.81[m] | 4.68[m] | 0.509[j] | 0.364[k] | 0.339[k] |
| 36-42 | 370 | 432 | 379 | 5.07[l] | 4.60[m] | 4.41[m] | 0.514[m] | 0.677[l] | 0.598[m] |
| 43-49 | 687[h] | 453[i] | 429[i] | 5.55[l] | 5.12[m] | 4.92[m] | 0.869[d] | 0.602[e] | 0.615[e] |
| Means | 330[a] | 214[b] | 202[b] | 4.62[f] | 4.15[g] | 4.16[g] | 0.446[a] | 0.277[b] | 0.301[b] |
| ± SEM | ±21 | ±21 | ±21 | ±0.17 | ±0.17 | ±0.17 | ±0.032 | ±0.032 | ±0.032 |

[a,b,c]Means in the same row with different superscripts are different (P<.005).

[d,e]Means in the same row with different superscripts are different (P<.01).

[f,g]Means in the same row with different superscripts are different (P<.025).

[h,i]Means in the same row with different superscripts are different (P<.05).

[j,k]Means in the same row with different superscripts are different (P<.10).

[l,m]Means in the same row with different superscripts are different (P<.15).

[n]Bonferroni t test.

[o]Orthogonal Contrast (MP vs MS + HS; MS vs HS).

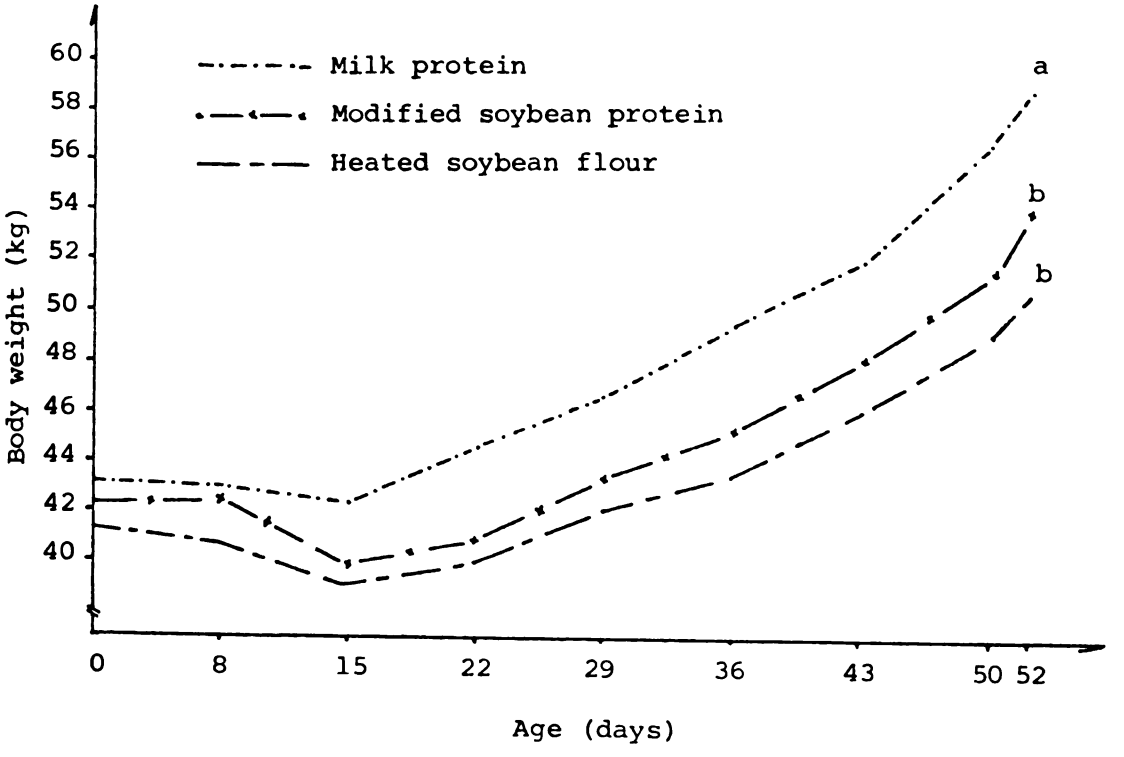


Figure 1.--Effect of feeding milk protein, modified soybean protein, and heated soybean flour on body weight of calves at different ages. For each age, points with different letters are different ($P<.05$) Experiment 1.

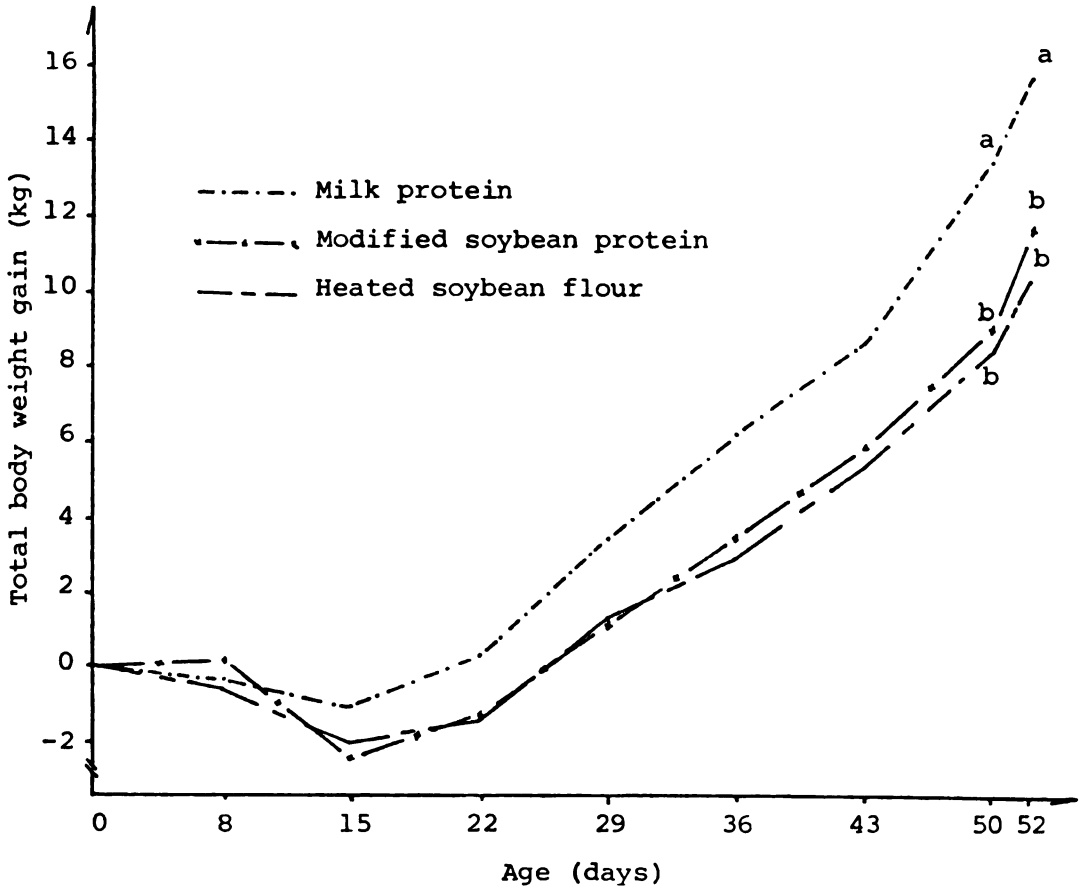


Figure 2.--Effect of milk feeding protein, modified soybean protein and heated soybean flour on calves total body weight of calves at different ages. For each age, points with different letters are different ($P<.05$) Experiment 1.

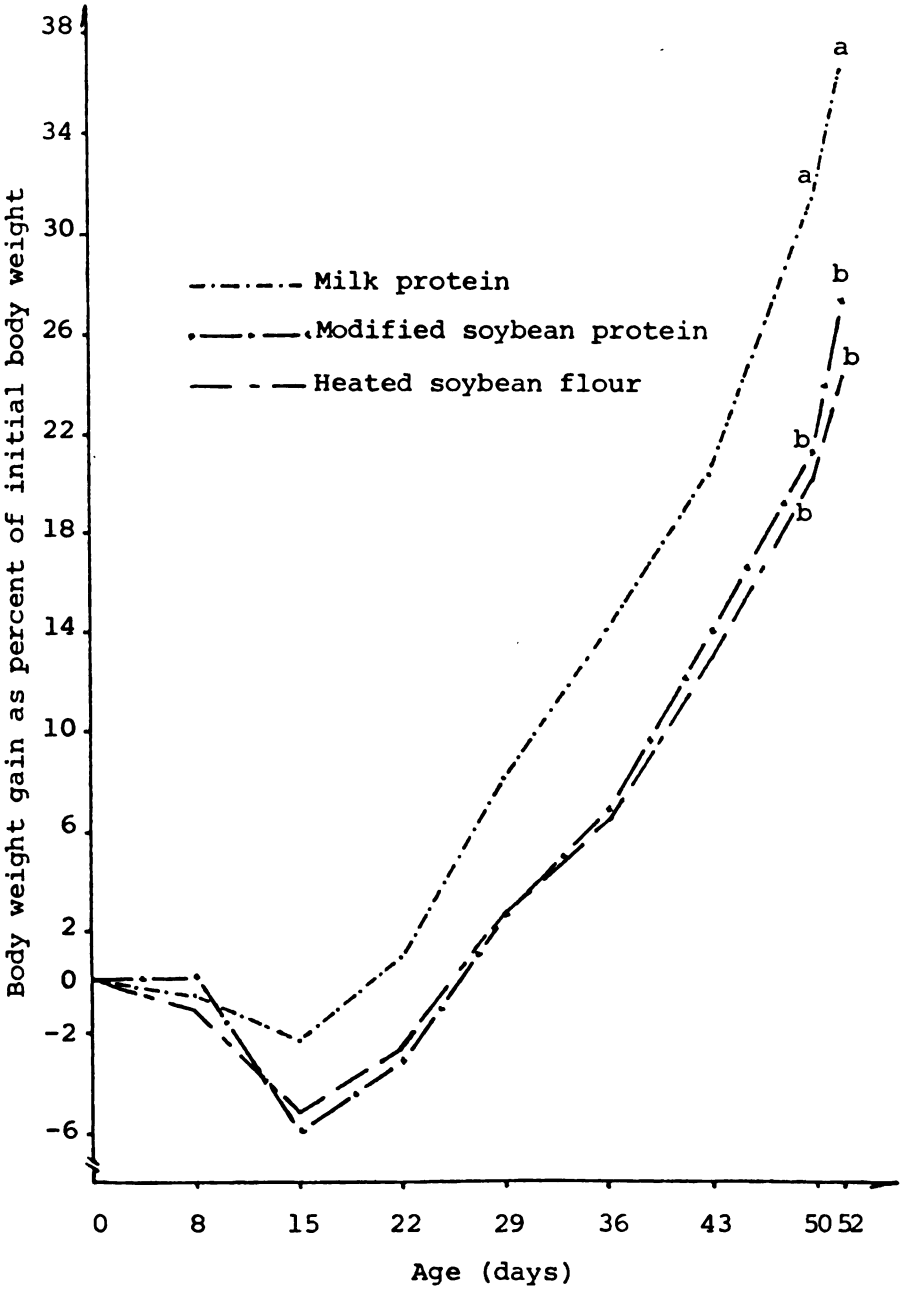


Figure 3.--Effect of feeding calves milk protein, modified soybean protein, and heated soybean flour on body weight gain as percent of initial body weight. For each age, points with different letters are different ($P<.05$) Experiment 1.

Calves on all treatments lost weight during the 2 first weeks, but the negative gains were greater ($P<.05$) for calves fed MS than MP, and HS was intermediate (Table 7 and Figure 4). This body weight loss may be related to a poor ability of baby calf's digestive system to digest and absorb nutrients (Huber, 1969; Porter, 1969; Radostits and Bell, 1970; Radostits, 1975; Thivend et al., 1980), to the normal decline in body water content (Houpt, 1977), or to stress when calves were kept in metabolic cages. Average daily gains were also diminished during the 5th week when calves again were kept in metabolic cages for collection of feces and urine (Table 7).

Treatment means for feed intake were higher ($P<.025$) for MP than MS or HS (Table 7 and Figure 5) which might be expected because calves were fed as a percent of body weight.

Feed efficiency (body weight gain/feed intake) was higher ($P<.005$) for MP than MS or HS (Table 7) and the interaction of treatment x age was significant ($P<.005$) again showing a widening of differences between MP and soybean sources as age increased (Figure 6). These results all suggest inferior utilization of soybean than milk protein and agree with previously published data (Gorrill and Nicholson, 1969; Morrill et al., 1971; Roy et al., 1977).

Only a few of the calves were able to tolerate for the entire 5 days the metabolism stalls for total collection of feces and urine during the 2nd week of the trial. Thus, it was not possible to draw firm conclusions from these data, but trends showing better nutrient digestibility for MP than for the soybean protein sources were evident (Table 8).

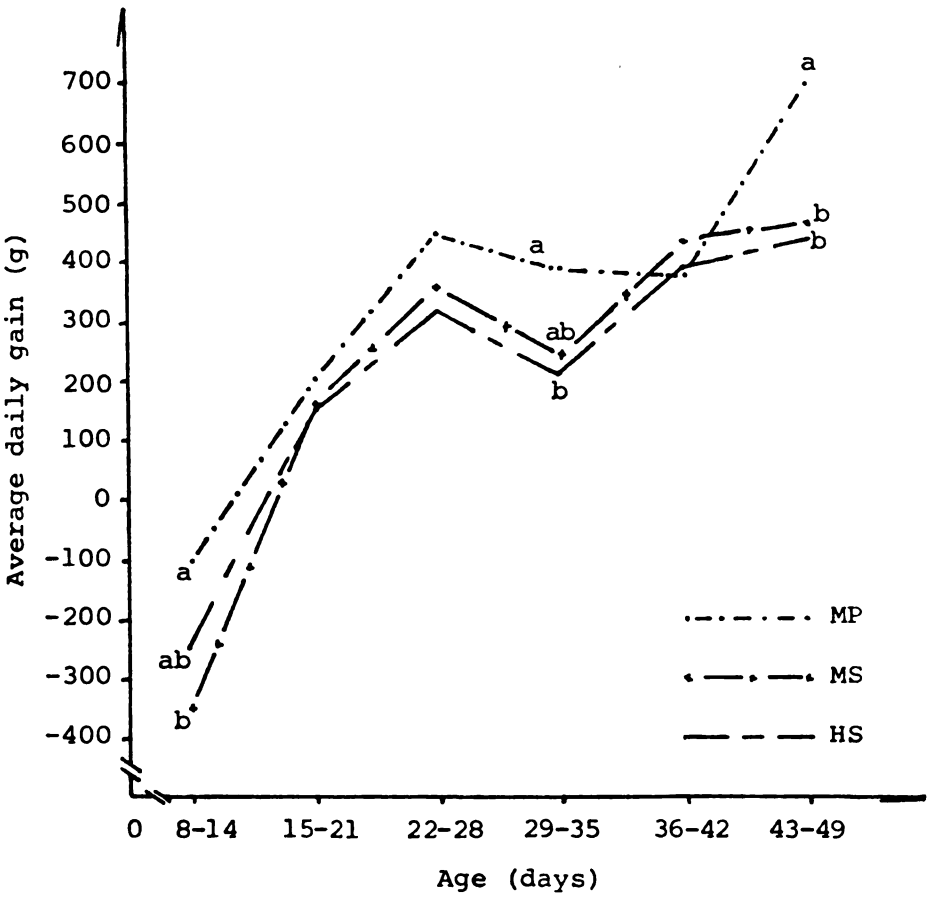


Figure 4.--Effect of feeding milk protein (MP), modified soybean protein (MS), and heated soybean flour (HS) on average daily gain of calves at different ages. For each age, points with different letters are different ($P<.05$) Experiment 1.

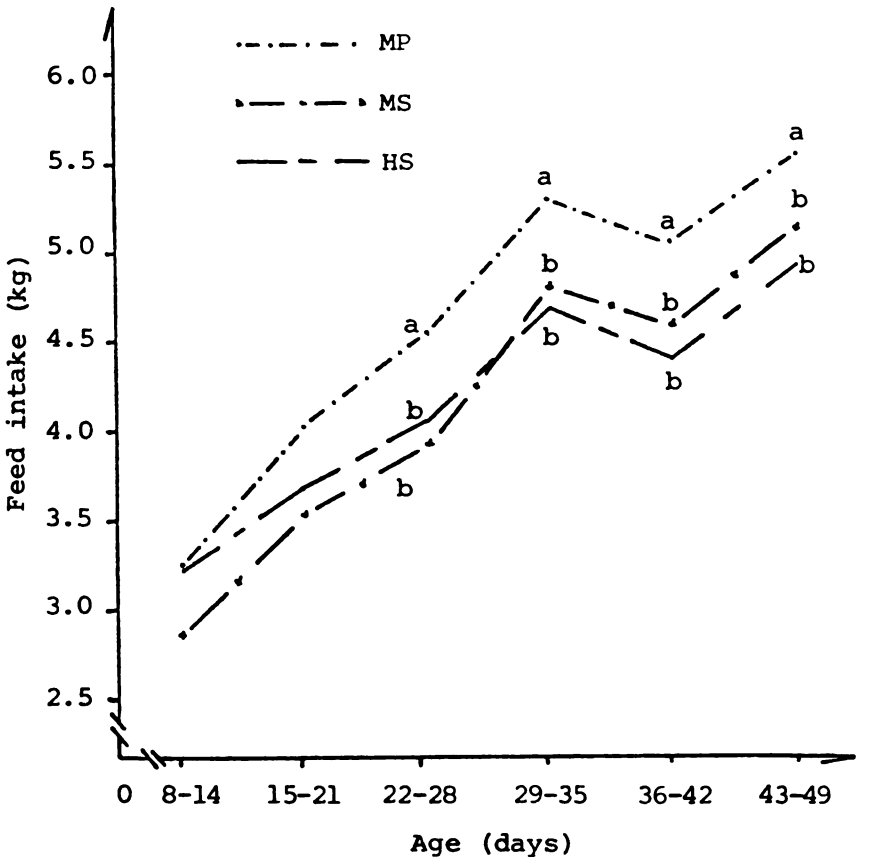


Figure 5.--Effect of feeding milk protein (MP), modified soybean protein (MS), and heated soybean flour (HS) on feed intake of calves at different ages. For each age, points with different letters are different ($P<.15$) Experiment 1.

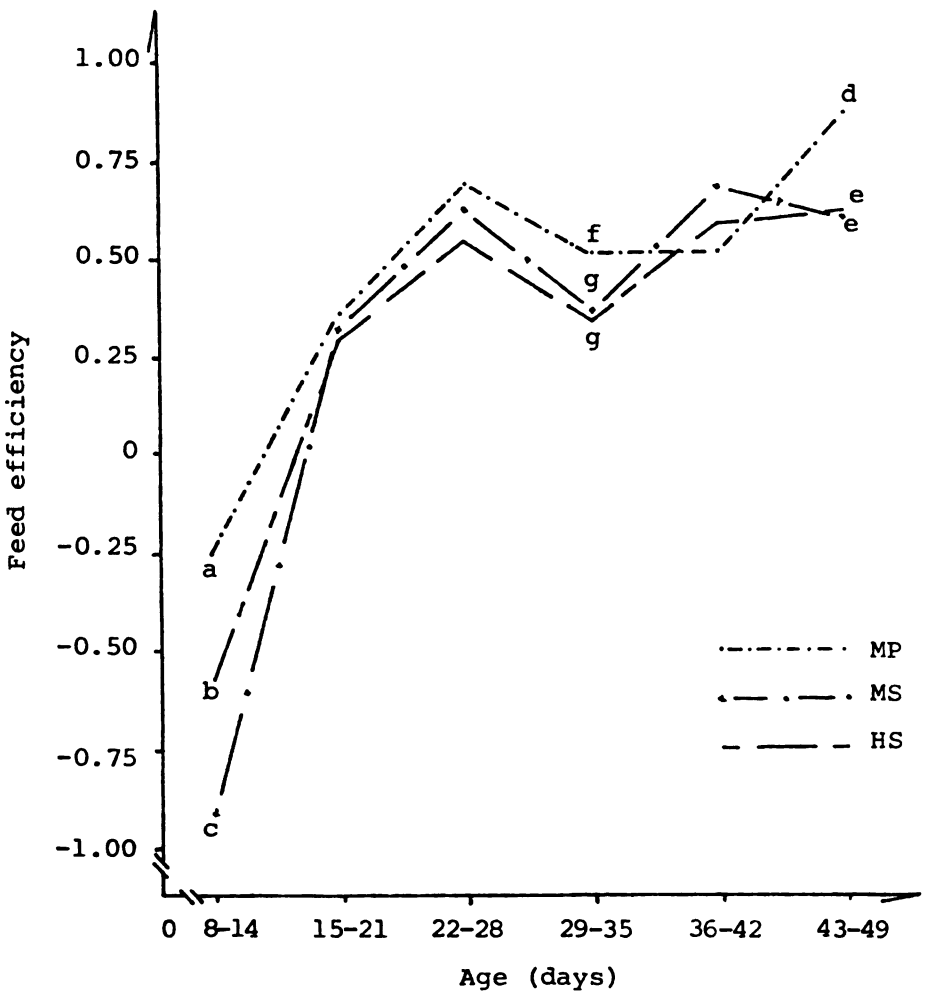


Figure 6.--Effect of feeding milk protein (MP), modified soybean protein (MS), and heated soybean flour (HS) on feed efficiency (body weight gain/feed intake) of calves. For each age, points with different letters are different: a,b,c ($P<.005$); d,e ($P<.01$) and f,g ($P<.10$) Experiment 1.

TABLE 8.--Dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), nitrogen-free extract (NFE) and ash digestibilities and nitrogen (N) retention in two-week old calves fed milk protein (MP), modified soybean protein (MS) or heated soybean flour (HS) Experiment 1.

| Variable | Treatment (means ± SEM)[c] | | |
|---|---|---|---|
| | MP | MS | HS |
| No. of calves | 4 | 3 | 5 |
| | Digestibility (%) | | |
| DM | 82.32 ± 3.46 | 73.11 ± 3.99 | 77.78 ± 3.09 |
| OM | 83.41 ± 3.50 | 74.08 ± 4.04 | 78.36 ± 3.13 |
| CP | 64.13 ± 8.26 | 43.16 ± 9.53 | 49.50 ± 7.38 |
| EE | 72.31 ± 9.81 | 60.59 ± 11.33 | 74.85 ± 8.78 |
| NFE | 91.19[a] ± 1.55 | 85.31[b] ± 1.79 | 87.43[a,b] ± 1.38 |
| Ash | 69.22 ± 3.63 | 61.44 ± 4.17 | 70.91 ± 3.24 |
| | Retention (g/day) | | |
| N | 14.13 ± 6.27 | -7.47 ± 7.24 | 4.72 ± 5.61 |

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

[c]Bonferroni t test.

The digestibility trial at 5 weeks showed that MP resulted in greater utilization ($P<.05$) of dry matter (DM), organic matter (OM), crude protein (CP), and ether extract (EE) than HS while MS was intermediate, particularly for digestibility of CP and EE. No statistical differences between treatment were observed for digestibility of nitrogen-free extract (NFE) or for nitrogen (N) retention (Table 9).

The grestest reduction in digestibility occurred for CP which was 22% lower for HS and 13% lower for MS than for MP. There have been numerous reports showing that soybean proteins are poorly digested by the young calf during the first few weeks of life (Noller et al., 1956; Porter, 1969; Morrill et al., 1971; Roy et al., 1977). The poor digestibility of soybean proteins in young calves has been attributed to a number of causes, including lack of a normal coagulum in the abomasum (Roy and Stobo, 1975; Jenkins, 1981), a reduction of pancreatic secretion of trypsin and chymotrypsin (Gorrill and Thomas, 1967; Ternouth and Roy, 1973), the presence in soybean products of trypsin inhibitors (Colvin and Ramsey, 1968; Gorrill and Nicholson, 1971), and materials provoking a gastrointestinal allergy (Smith and Sissons, 1975; Sissons and Smith, 1976). Digestibility of soybean protein has been improved by acid treatment (Colvin and Ramsey, 1968), alkali treatment (Gorrill and Nicholson, 1972b) and alcohol treatment (Sissons et al., 1971; De Gregorio et al., 1982). Heating also has been reported to improve utilization of soybean products (Nitsan et al., 1971), but inclusion of pepsin and/or pancreatin in milk

TABLE 9.--Dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), nitrogen-free extract (NFE) and ash digestibilities and nitrogen (N) retention in five-week old calves fed milk protein (MP), modified soybean protein (MS) or heated soybean flour (HS) Experiment 1.

| Variable | Treatment (means ± SEM)[d] | | |
|---|---|---|---|
| | MP | MS | HS |
| No. of calves | 8 | 6 | 6 |
| | Digestibility (%) | | |
| DM | 89.56 ± 1.03[a] | 86.20 ± 1.19[a,b] | 84.38 ± 1.19[b] |
| OM | 90.72 ± 0.99[a] | 87.16 ± 1.14[b] | 85.30 ± 1.14[b] |
| CP | 82.53 ± 1.85[a] | 72.07 ± 2.14[b] | 64.09 ± 2.14[c] |
| EE | 94.06 ± 0.70[a] | 92.19 ± 0.81[a,b] | 91.38 ± 0.81[b] |
| NFE | 92.87 ± 1.07 | 91.15 ± 1.23 | 90.83 ± 1.23 |
| Ash | 75.71 ± 1.81 | 74.56 ± 2.09 | 73.36 ± 2.09 |
| | Retention (g/day) | | |
| N | 58.08 ± 6.79 | 39.46 ± 7.84 | 29.47 ± 7.84 |

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

[d]Bonferroni t test.

replacers containing soybean protein did not improve protein digestibility or calf performance (Jenkins, 1981).

Nitrogen retention tended to be 49% lower for HS and 32% lower for MS than for MP. This reduction of N retention may be explained by the lower digestibility of soybean than milk protein. Treatment differences observed for growth and feed efficiencies (Table 6 and 7) also may be explained by differences in digestibility.

Plasma urea nitrogen (PUN) concentrations showed a tendency to be lower ($P<.10$) for MP than for MS or HS on day 23, and lower ($P<.15$) for HS than MP or MS on day 44. Moreover, PUN was higher on day 44 than on day 23 for MP ($P<.10$) but the reverse was true for HS ($P<.15$). No difference between days was observed to MS (Table 10).

The PUN concentrations of this experiment, ranged from 11.4 to 14.0 mg/100 ml, were higher than 5.4 to 6.3 mg/100 ml reported by Campos (1982), or 8.3 to 11.3 mg/100 ml observed by Foldager (1976); but were similar to 11.5 mg/100 ml reported by Williams and Smith (1973); and were lower than 21.2 to 24.6 mg/100 ml reported by Williams and Smith (1974a,b) or 24.0 to 32.0 mg/100 ml reported by Nitsan et al. (1972).

The lower PUN of calves on MP than MS or HS on day 23 was probably a reflection of the greater uptake of amino acids from blood to support the more rapid gains. The lower digestibility of soybean protein and a possible imbalance of amino acid at the tissue level due to its low methionine content are factors which may also have contributed to the higher PUN values (Williams and Smith, 1974a; Eggum, 1970; Roy, 1980). However, on day 44 the growth advantage of MP over

TABLE 10.--Effect of feeding milk protein (MP), modified soybean protein (MS) or heated soybean (HS) on plasma urea nitrogen (PUN) concentration of calves at different ages Experiment 1.

| | PUN (mg/100 ml)[E] | | | |
|---|---|---|---|---|
| Age | MP | MS | HS | SEM |
| 23rd day | 11.39[a,B] | 13.55[b] | 13.28[b,C] | ±1.18 |
| 44th day | 13.97[c,A] | 13.17[d] | 11.45[e,D] | ±1.18 |

[a,b]Means in the same row with different superscripts are different (P<.10).

[c,d,e]Means in the same row with different superscripts are different (P<.15).

[A,B]Means in the same column with different superscripts are different (P<.10).

[C,D]Means in the same column with different superscripts are different (P<.10).

[E]Orthogonal Contrast (MP vs MS + HS; MS vs HS).

HS was even higher, but PUN was lower for calves fed HS than MP. This may be related to the lower CP digestibility of HS than MP coupled with increased gains on HS on day 44 compared to day 23.

Absorption and excretion tests of xylose have been used extensively to measure intestinal absorptive capability and renal function in men (Benson et al., 1957; Butterworth et al., 1959; Hill et al., 1981), dogs (Hill et al., 197), horses (Roberts, 1974; Bolton et al., 1976), and calves (Seegraber and Morrill, 1979; Campos and Huber, 1983).

The D(+)xylose absorption test (O-XYL) showed a higher (P<.005) plasma O-XYL concentration for calves fed MP than soybean protein

(Table 11) and treatment differences were greatest at 120 to 180 minutes after oral xylose (Figure 7).

The plasma O-XYL concentrations of this experiment were higher than those reported by Bolton et al. (1976) for horses, similar to those of Benson et al. (1957) and Hill et al. (1981) for human beings and Seegraber and Morrill (1979) for calves, but lower than those of Campos and Huber (1983) for calves.

The curves of plasma O-XYL concentration plotted against time (Figure 7) showed that peak concentrations occurred 150 minutes after the oral dose for calves fed MP, but the curve for calves fed soybean protein were abnormal with no distinguishable peaks (Hill et al., 1970; Bolton et al., 1976; Seegraber and Morrill, 1979). The curve shown for calves fed MP indicates superior absorptive capacity of the intestine compared to calves fed soybean protein (Seegraber and Morrill, 1979) and corroborates the superior weight gains and nutrient digestibilities which were observed for the MP treatment (Table 6 to 9).

Cumulative O-XYL excretion determined from the 5 hr of urine collection taken during O-XYL absorption tests, when expressed as a percent of xylose, agrees with Seegraber and Morrill (1979) for calves fed milk protein, but are about twice as high for calves fed soybean protein (Table 12). The differences between treatments were not statistically significant, but the tendency of a high XYL excretion for calves fed soybean protein was associated with greater xylose disappearance from the blood of soybean-fed calves (Table 12 to 14 and Figure 8 and 9).

TABLE 11.--Effect of previous feeding of milk protein (MP), modified soybean protein (MS), or heated soybean flour (HS) on average plasma xylose (XYL) concentration (mg XYL/ 100 ml plasma) after oral dose of .5 g XYL/kg of body weight was administered to 50-day old calves Experiment 1.

| Treatment | Minutes after XYL oral dose[g] | | | | | | | | Treatment means ± SEM |
|---|---|---|---|---|---|---|---|---|---|
| | 30 | 60 | 90 | 120 | 150 | 180 | 240 | 300 | |
| MP | 13.68 | 28.50 | 34.68 | 37.54[e] | 39.30[c] | 38.05[e] | 32.70 | 27.69 | 31.52[a] ± 1.26 |
| MS | 10.24 | 25.33 | 29.49 | 31.64[f] | 28.85[d] | 32.74[f] | 29.68 | 24.30 | 26.53[b] ± 1.26 |
| HS | 13.67 | 26.84 | 30.86 | 31.07[f] | 30.64[d] | 29.10[f] | 27.19 | 23.02 | 26.55[b] ± 1.26 |

[a,b]Means in the same column with different superscripts are different (P<.005).

[c,d]Means in the same column with different superscripts are different (P<.05).

[e,f]Means in the same column with different superscripts are different (P<.10).

[g]Orthogonal Contrast (MP vs MS + HS; MS vs HS).

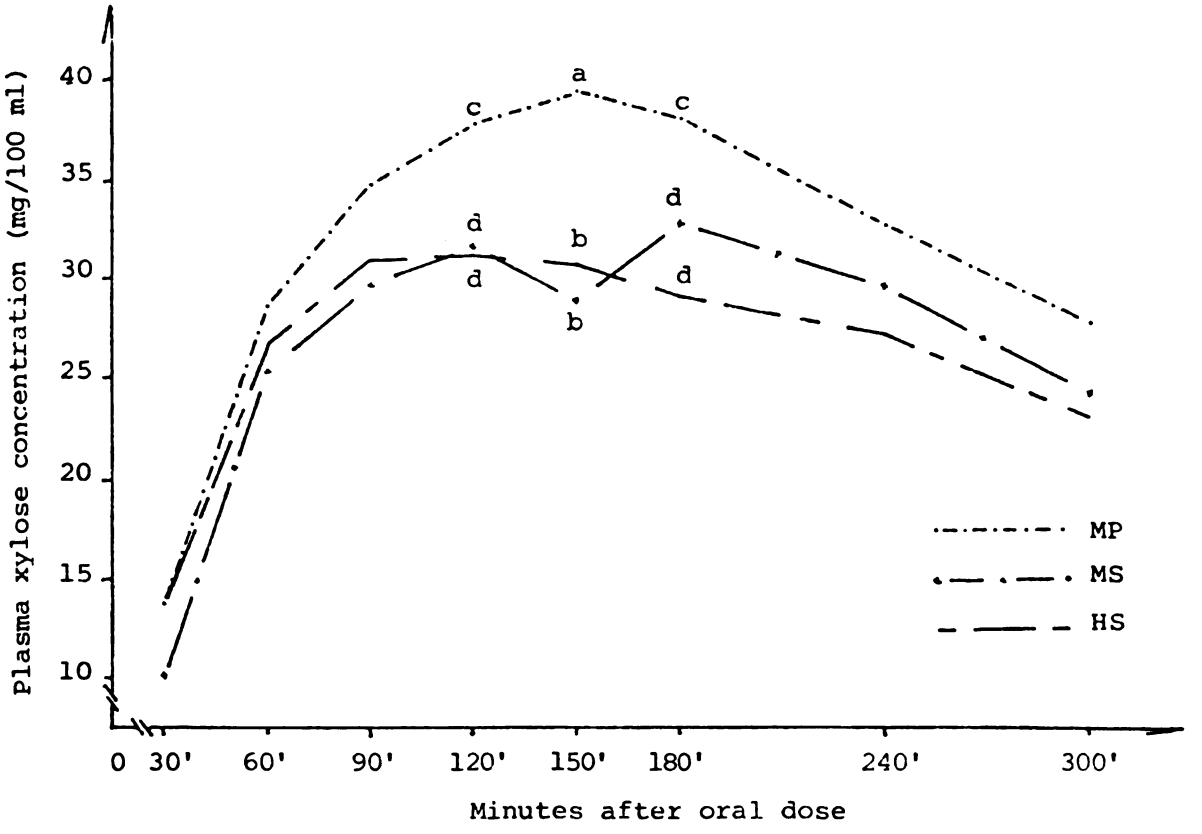


Figure 7.--Effect of feeding milk protein (MP), modified soybean protein (MS) and heated soybean flour (HS) on calf plasma xylose concentrations at different times after oral ingestion of .5 g/kg body weight. For each time points with different letters are different: a,b ($P<.05$) and c,d ($P<.10$) Experiment 1.

TABLE 12.--Percentage of oral or intravenous xylose (XYL) administered which was excreted into the urine by calves fed milk protein (MP), modified soybean protein (MS) or heated soybean flour (HS) during a 5 hr period after XYL administration Experiment 1.

| | Treatment (means ± SEM)[a,b] | | |
|---|---|---|---|
| Variable | MP | MS | HS |
| No. of calves | 7 | 8 | 7 |
| Oral XYL (%) | 12.74±1.99 | 9.47±1.86 | 10.34±1.99 |
| No. of calves | 7 | 7 | 6 |
| IV-XYL (%) | 21.86±3.24 | 20.90±3.24 | 28.02±3.50 |

[a]Means in the same row are not significantly different (P>.20).

[b]Turkey's test.

TABLE 13.--Effect of previous feeding of milk protein (MP), modified soybean protein (MS), or heated soybean flour (HS) on plasma xylose (XYL) concentration (mg XYL/ 100 ml plasma) after an intravenous dose of .25 g XYL/kg of body weight was administered to 41 day old calves Experiment 1.

| Treatment | Minutes after XYL IV dose[a] | | | | | | | | Treatment means |
|---|---|---|---|---|---|---|---|---|---|
| | 30 | 60 | 90 | 120 | 150 | 180 | 240 | 300 | |
| MP | 48.73 | 40.23 | 38.93 | 33.30 | 35.34 | 25.24 | 20.51 | 18.15 | 32.55 |
| MS | 49.08 | 42.48 | 34.66 | 31.26 | 24.60 | 23.95 | 18.73 | 15.40 | 30.02 |
| HS | 49.88 | 41.02 | 33.61 | 30.34 | 25.90 | 22.68 | 16.97 | 13.76 | 29.27 |

[a]The variance was heterogeneous. The data were analyzed transformed in a log scale (see Table 14).

TABLE 14.--Average log of plasma xylose (XYL) concentrations (mg XYL/100 ml plasma) after an intravenous dose of .25 g XYL/kg of body weight was administered to 41 day old calves fed milk protein (MP), modified soybean protein (MS), or heated soybean flour (HS) Experiment 1.

| Treatment | Minutes after XYL IV dose[i] | | | | | | | | Treatment means ± SEM |
|---|---|---|---|---|---|---|---|---|---|
| | 30 | 60 | 90 | 120 | 150 | 180 | 240 | 300 | |
| MP | 1.687 | 1.592 | 1.583[g] | 1.520 | 1.525[a] | 1.398 | 1.309[e] | 1.251[c] | 1.483[a] ± 0.018 |
| MS | 1.686 | 1.626 | 1.537[h] | 1.492 | 1.372[b] | 1.373 | 1.260[f] | 1.177[d] | 1.440[b] ± 0.018 |
| HS | 1.687 | 1.612 | 1.524[h] | 1.478 | 1.410[b] | 1.352 | 1.227[f] | 1.117[d] | 1.426[b] ± 0.018 |

[a,b]Means in the same column with different superscripts are different ($P<.025$).

[c,d]Means in the same column with different superscripts are different ($P<.05$).

[e,f]Means in the same column with different superscripts are different ($P<.15$).

[g,h]Means in the same column with different superscripts are different ($P<.20$).

[i]Orthogonal Contrast (MP vs MS + HS; MS vs HS).

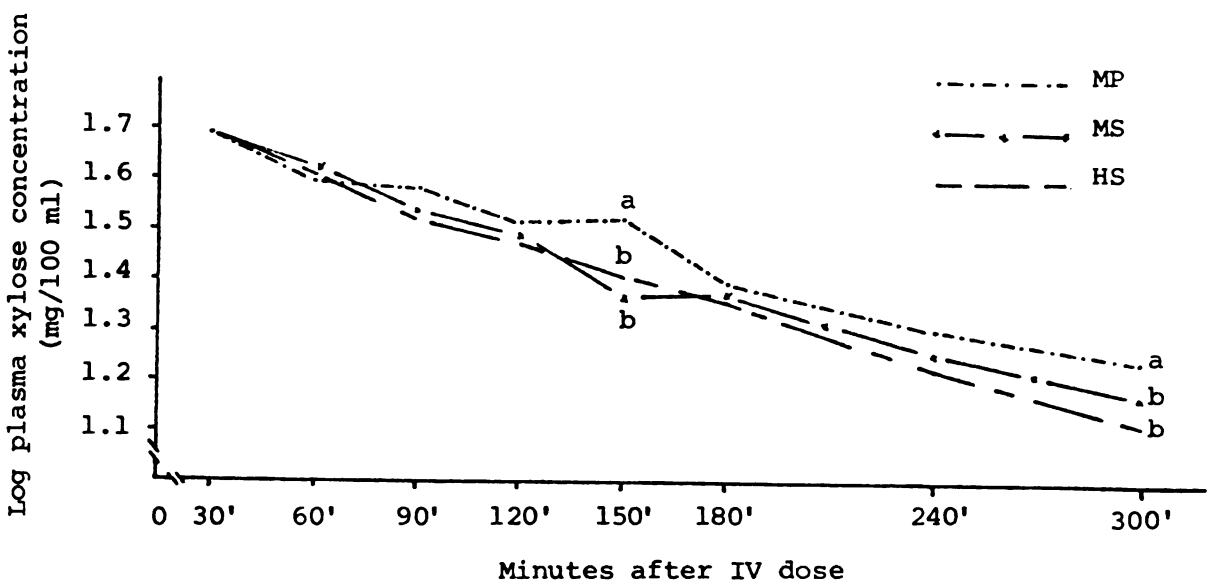


Figure 8.--Effect of feeding milk protein (MP), modified soybean protein (MS), and heated soybean flour (HS) on calf plasma xylose concentration at different times after IV dose. For each time, points with different letters are different ($P<.05$) Experiment 1.

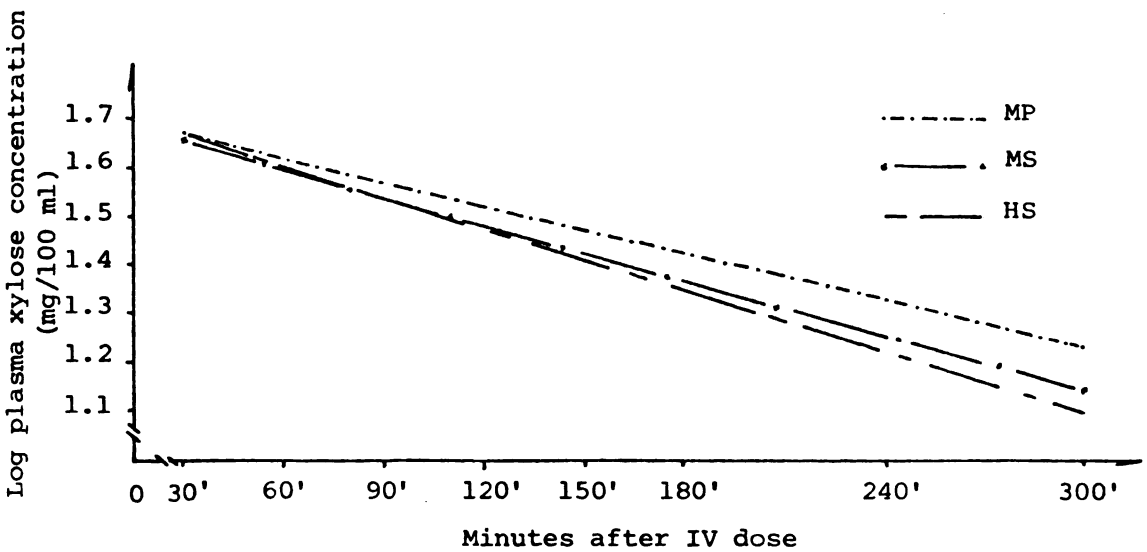


Figure 9.--Effect of feeding milk protein (MP), modified soybean protein (MS), and heated soybean flour (HS) on calf plasma xylose concentration at different times after IV dose. Data presented as linear regression of log plasma xylose concentration on time Experiment 1.

Plasma xylose concentrations after calves received the intravenous dose of xylose (IV-XYL) are in Table 13. These data showed heterogeneous variance but homogeneity was obtained by transformation to the log scale. Transformed data are shown in Table 14 and Figure 8. The plasma IV-XYL concentration was higher ($P<.025$) for calves fed MP than MS or HS (Table 14). Linear regression of the plasma IV-XYL concentration on time (Figure 8) resulted in the following equations:

For treatment MP:

$\hat{Y} = 1.7197006 - 0.0016175x$; with correlation = -0.8534941

For treatment MS:

$\hat{Y} = 1.7190983 - 0.0019063x$; with correlation = -0.8813361

For treatment HS:

$\hat{Y} = 1.7312601 - 0.0020887x$; with correlation = -0.913337

Origins of the equations were not significantly different but slopes were. The slope for HS was higher ($P<.01$) than for MP and the slope for MS tended to be higher ($P<.20$) than for MP. Slopes for HS and MS were not significantly different.

When the variation between treatment trends were analyzed by the partitioned interaction method, plasma IV-XYL clearance was higher ($P<.005$) for HS than MS or MP and was higher ($P<.005$) for MS than MP.

These data suggest greater capacity for clearance of xylose from the blood of calves fed soybean compared to milk protein with fastest clearance on the HS diet. Xylose is not normally a constituent of the blood, although small quantities may be found in urine (White and Hess, 1956). Xylose clearance from the blood occurs mainly through renal excretion but some metabolism also occurs. Enzymes have been

demonstrated from several bacterial sources by which xylose can be utilized as an energy source via the pentose phosphate pathway (Hochster and Watson, 1953). Wyngaarden et al. (1957) showed that after an intravenous infusion of D-xylose-1-$C^{14}$ into man, 13.5% of the radioactive substance was recovered as carbon dioxide in exhaled air, confirming the observations of Brien et al. (1952) that blood glucose rises and serum inorganic phosphate falls after the administration of xylose to human subjects. Hiatt (1957) found incorporation of about 1% of the administered xylose in the form of glycogen in mouse liver, while Butterworth (1959) suggested that the liver plays a role in the removal of xylose from the blood of dogs. Segal et al. (1957) found an abrupt lowering of the blood levels of xylose when the plasma insulin was increased from three to six times the fasting value and concluded that insulin affects distribution of xylose in body fluids of man.

The increased IV-XYL clearance from the blood of calves fed soybean protein compared to milk protein (Figure 9) might be explained by an increase in xylose metabolism and/or an increase of renal excretion of xylose. The tendency for a higher percentage of IV-XYL to be found in urine of calves fed HS diets (Table 12) suggests an increase in renal excretion. The reason for such an effect is not clear.

Data of contents from different sections of the gastro-intestinal tract of 2 calves per treatment slaughtered 5 hr after administration of a test meal are shown in Table 15. The rumen-reticulum-omasum contents had a pH ranging from 5.85 to 6.40 with slight tendency to be higher

TABLE 15.--Effect of feeding milk protein (MP), modified soybean protein (MS) or heated soybean flour (HS) to calves on pH, wet weight, dry matter, organic matter and crude protein of contents from different sections of the gastrointestinal tract, also on length of small intestine and body weight[h] Experiment 1.

| | Treatments | | | |
|---|---|---|---|---|
| | MP | MS | HS | SEM |
| No. of calves | 2 | 2 | 2 | |
| Contents from | | pH | | |
| R.R.O[i] | 6.40[e] | 5.95[f] | 5.85[f] | ± 0.25 |
| Abomasum | 2.95 | 3.70 | 2.80 | ± 0.25 |
| S.I.-1st 1/3[j] | 6.18 | 5.96 | 5.80 | ± 0.25 |
| S.I.-2nd 1/3[k] | 6.93[e] | 6.38[f] | 5.80[g] | ± 0.25 |
| S.I.-3rd 1/3[l] | 7.13[c] | 6.18[d] | 5.35[e] | ± 0.25 |
| L.I.Cecum[m] | 5.23[e] | 5.35[e] | 4.75[f] | ± 0.25 |
| | | Wet weight (g) | | |
| R.R.O[i] | 4,786[c] | 5,999[d] | 5,103[e] | ± 252 |
| Abomasum | 475 | 891 | 650 | ± 252 |
| S.I.-1st 1/3[j] | 498 | 385 | 395 | ± 252 |
| S.I.-2nd 1/3[k] | 359 | 643 | 476 | ± 252 |
| S.I.-3rd 1/3[l] | 259[g] | 993[e] | 523[f] | ± 252 |
| L.I.Cecum[m] | 841 | 966 | 844 | ± 252 |

TABLE 15.--Continued.

| | Treatments | | | |
|---|---|---|---|---|
| | MP | MS | HS | SEM |
| | Dry matter - DM (%) | | | |
| R.R.O[i] | 11.92 | 11.65 | 11.10 | ± 1.08 |
| Abomasum | 8.70 | 9.51 | 9.69 | ± 1.08 |
| S.I.-1st 1/3[j] | 9.56 | 10.24 | 7.90 | ± 1.08 |
| S.I.-2nd 1/3[k] | 9.16 | 6.67 | 7.34 | ± 1.08 |
| S.I.-3rd 1/3[l] | 8.55 | 6.94 | 7.40 | ± 1.08 |
| L.I.Cecum[m] | 12.16 | 10.30 | 10.02 | ± 1.08 |
| | Organic matter (%) on DM basis | | | |
| R.R.O[i] | 90.25 | 90.53 | 90.34 | ± 0.66 |
| Abomasum | 89.81 | 90.67 | 91.73 | ± 0.66 |
| S.I.-1st 1/3[j] | 89.85 | 90.09 | 89.14 | ± 0.66 |
| S.I.-2nd 1/3[k] | 88.00 | 88.01 | 88.24 | ± 0.66 |
| S.I.-3rd 1/3[l] | 84.24[a] | 87.35[b] | 88.98[b] | ± 0.66 |
| L.I.Cecum[m] | 86.08[e] | 85.88[f] | 86.37[g] | ± 0.66 |
| | Crude protein (%) on DM basis | | | |
| R.R.O[i] | 11.36 | 10.92 | 11.11 | ± 3.68 |
| Abomasum | 18.75 | 20.49 | 20.08 | ± 3.68 |
| S.I.-1st 1/3[j] | 47.48[e] | 38.74[f] | 44.99[g] | ± 3.68 |
| S.I.-2nd 1/3[k] | 45.41 | 47.92 | 42.35 | ± 3.68 |
| S.I.-3rd 1/3[l] | 31.54 | 33.21 | 28.54 | ± 3.68 |
| L.I.Cecum[m] | 18.61[c] | 25.26[d] | 26.63[d] | ± 3.68 |

TABLE 15.--Continued.

| | Treatments | | | |
|---|---|---|---|---|
| | MP | MS | HS | SEM |
| | Other measurements | | | |
| Length of S.I.[n] (cm) | 1,250 | 1,384 | 1,225 | ± 61 |
| Body weight (kg) | 65.75 | 69.40 | 55.30 | ±24.40 |

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

[c,d,e]Means in the same row with different superscripts are different (P<.10).

[e,f,g]Means in the same row with different superscripts are different (P<.15).

[h]Bonferroni t test.

[i]Rumen-reticulo-omasum.

[j]First one-third of small intestine (close to the pylorus).

[k]Second one-third of small intestine.

[l]Third one-third of small intestine (close to the cecum).

[m]Large intestine and cecum.

[n]Small intestine.

($P<.15$) in calves fed MP than soybean protein. Similar ruminal pH values were observed by Campos and Huber (1983) for calves fed milk but they were slightly lower on soybean protein.

The abomasum contents had a pH ranging from 2.80 to 3.70. These values are higher than the 1.0 to 2.0 reported by Mylrea (1966a) and Porter (1969) for calves fed whole milk 5 hr after a meal, but are similar to data reported by Campos and Huber (1983). No significant differences between treatments were observed.

A rapid increase of pH to about 6.0 was observed in the first section of the small intestine. The limited capacity of the small intestine of adult ruminants to neutralize acid digesta from the abomasum (Smith, 1962; Phillipson and Storry, 1965; Topps et al., 1968) in combination with large amounts of digesta passing through the pylorus results in an acidic condition (pH of 2.0 or 3.0) in the upper jejunun of adult ruminants. On the other hand, preruminant calves have been shown to have a high capacity to neutralize the acid digesta from the abomasum (Huber, 1958; Huber and Moore, 1964; Campos and Huber, 1983).

Small intestinal pH increased from the proximal for the distal portion for calves fed MP or MS but decreased for calves fed HS. The pH of small intestinal contents was higher ($P<.10$) for calves fed MP than soybean proteins, and was higher ($P<.10$) for calves fed MS than HS. Fermentation of undegraded or partially degraded carbohydrates from soybean protein diets (mainly from HS), explains the increased acidity of small intestine (Radostits and Bell, 1970), large intestine, and cecal contents (Huber, 1969; Radostits and Bell, 1970).

Crude protein values of large intestine and cecum contents were higher ($P<.10$) for calves fed soybean than milk proteins. These values reflect the lower digestibility of soybean protein (Table 9) and agree with data reported by Campos and Huber (1983). Length of the small intestine and body weight were not different (Table 15).

D-xylose absorption reflects structural integrity of the small intestine (Hill et al., 1981). In this experiment examination of intestinal segments from each section was accomplished through use of a scanning electron microscope equipped with a Polaroid camera. Pictures were evaluated on a 1 to 5 scale, when 1 represented normal morphology of villi (Figure 10) and 5 extremely abnormal (Figure 11). Normal villi were similar to those described by Mebus et al. (1975; 1977) for gnobiotic calf and abnormal villi were similar to those described by Seegraber and Morrill (1982) for a calf fed soybean protein concentrate or by Mebus et al. (1977) for calves infected by virus of human infantile gastro-enteritis. In this study no differences between treatments were noted in villi morphology (Table 16); however significant differences ($P<.01$) were observed for segments taken from different positions of the small intestine, with morphology of distal segments more normal than the proximal. Morphology of the first intestinal segment collected just proximal to the pylorus, was less normal than all others for treatment MP and HS; whereas, the first and second were different from third, fourth and sixth segments on treatment MS. These results do not agree with those of Seegraber and Morrill (1982) who associated impaired absorptive ability of the intestine of calves fed the soybean portein diets with morphological


Figure 10.--Normal villi from a scanning electron micrographs of small intestinal mucosa of a calf. Most villi have a fairly uniform appearance and surfaces show transverse furrows with Goblet cell openings Experiment 1.

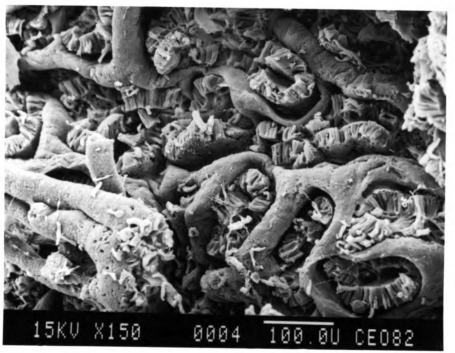


Figure 11.--An abnormal villi from scanning electron micrographs of small intestinal mucosa of a calf Experiment 1.

TABLE 16.--Effect of feeding calves milk protein (MP), modified soybean protein (MS) or heated soybean flour (HS) on villi morphology[a] Experiment 1.

| Treatment | Position of segment in small intestine[d] | | | | | | Treatment means ± SEM |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | |
| MP | 4.7[b] | 2.9[c] | 2.5[c] | 2.4[c] | 2.3[c] | 2.6[c] | 2.90±0.27 |
| MS | 3.7[b] | 3.8[b] | 2.0[c] | 2.2[c] | 2.9[b,c] | 1.9[c] | 2.75±0.27 |
| HS | 4.4[b] | 1.9[c] | 2.9[c] | 1.7[c] | 2.4[c] | 2.1[c] | 2.57±0.27 |

[a]Evaluated by 5 evaluators on a 1 (normal) to 5 scale from segments taken at 6 different positions (from proximal to caudal) in the small intestine.

[b,c]Means in the same row with different superscript are different ($P<.01$).

[d]Turkey's test.

changes in intestinal structure. In this experiment calves fed soybean protein showed lower weight gains, feed efficiencies, nutrient digestibilities and xylose absorption than calves fed milk protein, but greater differences in villi morphology were observed within animals than between treatments.

Fecal scores were no different (P>.25) between treatments (Table 17) and contrast with previous reports where soybean and other non-milk proteins resulted in more diarrhea (Gorrill and Thomas, 1967; Seegraber and Morrill, 1979; Campos, 1982). Viral infections in many of the calves at the beginning of the experiment could have minimized differences between treatment. There was no difference (P>.25) between treatments in rectal temperatures, which were all within the normal range (Table 17).

In summary, lowered digestibilities of dry matter, organic matter, crude protein and ether extract were associated with inferior body weight gains, poorer feed efficiencies and lower xylose absorption

TABLE 17.--Effect of feeding calves milk protein (MP), modified soybean protein (MS) and heated soybean protein (HS) on calves fecal scores and rectal temperatures Experiment 1.

| Variable | Treatment[a,b] | | | |
|---|---|---|---|---|
| | MP | MS | HS | SEM |
| Fecal scores (1-4 scale) | 2.965 | 2.961 | 3.016 | ±0.096 |
| Rectal temperature (°C) | 38.872 | 38.900 | 38.879 | ±0.062 |

[a]Means in the same row are not significantly different (P>.25).

[b]Orthogonal Contrast (MP vs MS + HS; MS vs HS).

for calves fed milk replacers containing 66% of the protein from soybean sources (as modified soybean protein or heated soybean flour) than 100% from milk. For these parameters, modified soybean protein tended to be superior to heated soybean flour. No differences were observed between any treatments for villi morphology, fecal scores or rectal temperatures. Greater xylose clearance from the blood during a 5 hr period was observed for calves fed soybean proteins than milk protein, suggesting that soybean diets increased xylose metabolism and excretion compared to milk. Gastrointestinal tract contents showed a tendency to be more acid in the medium and distal portions of small intestine and in the large intestine and cecum of calves fed the soybean protein sources. More crude protein was also shown in large intestine and cecum of calves fed the soybean protein, suggesting increased fermentation of undergraded or partially degraded nutrients on these diets.

Experiment 2

Initial body weights were similar for calves assigned to 100% milk protein (Treatment M) or 66% of their protein as soybean protein concentrate (Treatment S). Body weights for the last day of the 21-day sensitization period were higher ($P<.05$) for calves fed M than S. Similar treatment differences were observed for body weight gains and average daily gains (Table 18).

However, treatment had no significant effect on body weights during the two following 10-day periods (experimental periods) when the calves were shifted from one treatment to the other, but calves

TABLE 18.--Effect of feeding milk protein (M) or soybean protein concentrate (S) during the 21-day sensitization period on calves body weights, body weight gains, average daily gains, feed intakes, feed efficiencies (gain/intake), rectal temperatures, fecal scores and villi length Experiment 1.

| Variable | Treatment[g] M | Treatment[g] S | SEM |
|---|---|---|---|
| Initial body weight (kg) | 42.02 | 42.30 | ±1.17 |
| Final body weight (kg) | 46.16[c] | 41.56[d] | ±1.41 |
| Body weight gain (kg) | 4.14[a] | -0.74[b] | ±0.55 |
| Average daily gain (g) | 197[a] | -35[b] | ±26 |
| Feed intake (kg) | 11.21 | 11.12 | ±0.33 |
| Gain/intake | 0.37[a] | -0.07[b] | ±0.05 |
| Rectal temperatures (°C) | 38.76[c] | 38.95[d] | ±0.06 |
| Fecal score (1-4 scale) | 2.42[e] | 2.86[f] | ±0.18 |
| Surgery villi length (μm) | 504[e]±47.55 | 387[f]±41.18 | -- |
| Final villi length (μm) | 696 | 616 | ±67.08 |

[a,b]Means in the same row with different superscripts are different ($P<.001$).

[c,d]Means in the same row with different superscripts are different ($P<.05$).

[e,f]Means in the same row with different superscripts are different ($P<.10$).

[g]F test.

which received no soybean protein during the sensitization period had higher ($P<.005$) body weights than those sensitized with soybean protein. Body weights were also higher ($P<.05$) at the end of period 2 than 1 (Table 19).

Previous sensitization to soybean protein had no significant effect on average daily gains (ADG) during experimental periods but treatments and periods did affect gains (Table 19). Calves fed M had higher ($P<.025$) ADG than S and ADG was higher ($P<.10$) during period 2 than 1. Due to a significant ($P<.05$) sensitization x period interaction, a comparison between periods was made for non-sensitized and sensitized calves (Table 20). No differences were observed between periods for sensitized calves but a higher ($P<.01$) ADG was observed for non-sensitized calves during period 2 than 1 suggesting a lowered capacity of sensitized calves to gain weight.

Feed intake was not significantly affected by treatment during any of the periods (Table 18 and 19). However feed intake was higher ($P<.005$) for non-sensitized than previously sensitized calves during experimental periods, and was higher ($P<.025$) during period 2 than 1 (Table 19). Feed intake differences were due to variation in body weight because calves were fed according to body weight.

Feed efficiency (G/I) was higher for calves fed M than S during both sensitization ($P<.001$) and experimental ($P<.005$) periods (Table 18 and 19). The effect of sensitization and periods on feed efficiency was not significant during experimental periods, but sensitization x period and sensitization x treatment interactions were (Table 19). Due to interaction, comparisons of efficiencies between periods and

TABLE 19.--Effect of sensitization (NS = non-sensitized; SS = sensitized), treatment (M = milk protein; S = soybean protein concentrate) and experimental period ($P_1$ = period 1; $P_2$ = period 2) on body weights (BW), average daily gains (ADG), feed intakes (FI), feed efficiencies (G/I), rectal temperatures (RT), fecal scores (FS) and villi length (VL) of calves[k] Experiment 2.

| | Sensitization | | | Treatment | | | Period | | |
|---|---|---|---|---|---|---|---|---|---|
| Variable | NS | SS | SEM | M | S | SEM | $P_1$ | $P_2$ | SEM |
| BW (kg) | 52.82[a] | 48.51[b] | ± 0.74 | 51.31 | 50.02 | ± 1.55 | 47.86[e] | 53.47[f] | ± 1.55 |
| ADG (g)[i] | 502 | 459 | ±28 | 592[c] | 369[d] | ±57 | 400[g] | 561[h] | ±57 |
| FI (kg) | 7.91[a] | 7.19[b] | ± 0.13 | 7.49 | 7.60 | ± 0.23 | 7.03[c] | 8.06[d] | ± 0.23 |
| G/I[i,j] | 0.62 | 0.64 | ± 0.04 | 0.78[c] | 0.48[d] | ± 0.07 | 0.58 | 0.69 | ± 0.07 |
| RT (°C) | 38.85[g] | 38.97[h] | ± 0.04 | 38.86 | 38.99 | ± 0.06 | 38.85 | 38.96 | ± 0.06 |
| FS (1-4 scale) | 2.61 | 2.93 | ± 0.12 | 2.44[g] | 2.93[h] | ± 0.19 | 2.89 | 2.48 | ± 0.19 |
| VL (μm)[j] | 611 | 576 | ±24 | 634 | 553 | ±36 | 611 | 576 | ±36 |

[a,b]Means in the same row and under the same subtitle with different superscripts are different ($P<.005$).

[c,d]Means in the same row and under the same subtitle with different superscripts are different ($P<.025$).

[e,f]Means in the same row and under the same subtitle with different superscripts are different ($P<.05$).

[g,h]Means in the same row and under the same subtitle with different superscripts are different ($P<.10$).

[i]Significant sensitization x period interaction ($P<.05$).

[j]Significant sensitization x treatment interaction ($P<.25$).

[k]F test.

TABLE 20.--Interaction of sensitization x period on average daily gain (ADG) and feed efficiencies (G/I) of calves[e] Experiment 2.

| | Non-sensitized | | Sensitized | | |
|---|---|---|---|---|---|
| Variable | Period 1 | Period 2 | Period 1 | Period 2 | SEM |
| ADG (g) | 329[a] | 675[b] | 472 | 447 | ±81 |
| G/I | 0.45[c] | 0.80[d] | 0.70 | 0.58 | ± 0.10 |

[a,b]Means in the same row and under the same subtitle with different superscripts are different (P<.01).

[c,d]Means in the same row and under the same subtitle with different superscripts are different (P<.025).

[e]Orthogonal contrast.

between treatments were made for sensitized and non-sensitized calves (Table 20 and 21). Efficiency was higher (P<.025) for non-sensitized calves during period 2 than 1 but no significant difference between periods was observed for sensitized calves; however efficiency was higher (P<.05) for sensitized calves fed M than S, again suggesting that sensitized calves were not able to gain weight with increased age as rapidly as non-sensitized.

These growth measurements all show milk protein superior to soybean protein concentrate (SPC). Moreover negative responses to SPC were greater in calves previously sensitized to soybean protein (by feeding SPC) than non-sensitized calves which were initially fed milk protein.

The low digestibility and poor growth of calves on soybean protein-based milk replacer has been attributed by some authors to the

TABLE 21.--Interaction of sensitization x treatment (M = milk protein; S = soybean protein concentrate) on feed efficiencies (G/I) and villi length (VL) of calves[e] Experiment 2.

| | Non-sensitized | | Sensitized | | |
|---|---|---|---|---|---|
| | M | S | M | S | SEM |
| G/I | 0.71 | 0.54 | 0.86[a] | 0.42[b] | ± 0.10 |
| VL (μm) | 616 | 607 | 652[c] | 500[d] | ±51 |

[a,b]Means in the same row and under the same subtitle with different superscripts are different (P<.005).

[c,d]Means in the same row and under the same subtitle with different superscripts are different (P<.05).

[e]Orthogonal Contrast.

presence of soybean trypsin inhibitor (Gorrill et al., 1967; Gorrill and Thomas, 1967); to the failure of the milk replacers to form large, firm clots of coagulated protein in the calf abomasum resulting in a diminished opportunity for action of pepsin (Jenkins, 1981); to the lower hydrolitic activity of pepsin and pancreatic proteases on soybean proteins than on milk protein (Jenkins et al., 1980). Others have attributed the problem to a gastrointestinal allergy to soybean proteins (Smith and Sissons, 1975; Sissons and Smith, 1976; Kilshaw and Sissons, 1979a,b) detected by appearance in serum of antibodies specific for soybean proteins (Barratt et al., 1978; Kilshaw and Sissons, 1979a). Villus atrophy and crypt enlogation have also resulted from feeding soybean proteins (Kilshaw and Slade, 1982).

Rectal temperatures were higher ($P<.05$) for calves fed S than M during the sensitization period (Table 18) and sensitized calves tended ($P<.10$) to have higher rectal temperature than non-sensitized calves during the experimental periods (Table 19). Treatment and periods had no significant effect on temperatures during the experimental periods. In spite of the rotavirus infection dectected in some calves during the experiment and the medication used when elevated temperature (>39.7°C) lasted for more than 12 hr, these data suggest an allergic reaction of calves to soybean proteins.

Fecal scores tended to be higher ($P<.10$) for calves fed S than M during the sensitization and experimental periods (Table 18 and 19). Failure of milk to coagulate (Tagari and Roy, 1969; Paruelle et al., 1972) followed by faster passage of partially digested material through the lower gut (Colvin et al., 1969; Ternouth et al., 1975), might explain the more liquid feces observed in calves fed SPC (Seegraber and Morrill, 1979; Campos, 1982).

Villi length measures from biopsy samples taken at surgery tended to be higher ($P<.10$) for calves fed M than S, but no significant differences were observed in biopsies taken on the last day of sensitization period (Table 18). No treatments, sensitization or periods effect on villi length were observed during experimental periods. However, the sensitization x treatment interaction was significant (Table 19). Due to this interaction, the effect of treatment on villi length were compared for non-sensitized and sensitized animals. These data show that treatment did not affect villi length of non-sensitized

calves, however sensitized calves fed M had longer villi ($P<.05$) than those fed S (Table 21).

Villus atrophy associated with crypt hyperplasia and an increased cell renewal rate has been associated with soybean protein intolerance and coeliac disease in man (Kosnai et al., 1980; Perkkio et al., 1981) as well as with the feeding of heated soybean flour to calves (Kilshaw and Slade, 1982). Morphological disturbances to the villi and lamina propria of the intestine described by Barratt et al. (1978) were caused by feeding toasted soybean flour to calves. A cell mediated immune reaction was suggested by MacDonald and Ferguson (1976) as the cause of villus atrophy, crypt hyperplasia and malaborption in food allergy. Buckley (1982) stated that when ingested food antigens encounter the IgE-coated mast cell, degranulation occurs, leading to increased goblet cell activity, increased mucus secretion, edema of mucosal epithelial cell villi, increased protein loss from the gut, and increased absorption of foreign antigens. Kilshaw and Sissons (1979a,b) found an increase in IgG and IgE antibodies specific to the soybean storage globulins glycinin and beta-conglycinin in preruminant calves fed heated soybean flour. The soybean antigens are resistent to proteolysis; and, to a lesser degree, to the microbial action of rumen fluid (Barratt et al., 1978).

Some calves appear to be more susceptible to soybean antigens than others. Susceptible calves become sensitized after having been fed heated soybean protein and the reactions become progressively worse. Sensitivity may be retained for several weeks without reexposure to soybean antigens (Thomas and Parrott, 1974; Kilshaw and Slade, 1980).

Changes in intestinal mucosa were observed 24 hr after feeding heated soybean flour to sensitized calves, but normal morphology was again observed after 10 days after returning to milk feeding (Kilshaw and Slade, 1982).

In conclusion the data of this experiment suggest that calves fed milk replacer with 66% of its protein replaced by soybean protein concentrate (SPC) had performance inferior to calves fed all milk protein. The higher rectal temperatures, more liquid feces and villi atrophy suggested an allergic reaction to SPC which certainly was related to the poor performance. Villi length data suggest that calves were more susceptible to the villi atrophy caused by soybean protein at 2 weeks than older ages, and effects of SPC were more pronounced in calves which had been previously sensitized.

# CONCLUSIONS

Replacement of 66% of the milk protein (MP) in milk replacers with modified soybean protein (MS) or heated soybean flour (HS) resulted in decreased calf performance as measured by body weight gains and feed efficiencies. The poorer performance was related to a decreased digestibility of crude protein (22% and 13% for HS and MS, respectively). Also, calves fed soybean protein had 16% lower xylose absorption. The reason for the increased clearance of xylose from the blood of calves fed MS and HS than MP is not known. From the upper small intestine to the large intestine gastrointestinal tract contents showed greater acidity for calves fed MS than milk protein (MP) and pH was lower for calves fed HS than MS, confirming poorer utilization of nutrients for HS than MS and of MS than MP. Crude protein content from the distal portion of small intestine was also higher for calves fed soybean proteins. Rectal temperatures, fecal scores and villi morphology (evaluated through scanning microscopy at 52 days of age) were not affected by protein source. Subjective evaluation of scans showed wide morphological variation in size and shape of villi, with greater differences within animals than between treatments.

Replacement of 66% of the milk protein in a milk replacer with soybean protein concentrate (SPC) resulted in lower body weight gains and feed efficiencies in baby calves. Higher rectal temperatures, more diarrhea and greater atrophy of villi were observed in calves

fed SPC. Also effects of SPC were more evident in calves which were previously sensitized to soybean proteins by feeding SPC 21 days before the experimental periods.

These data show that milk replacers containing 66% of protein from soybean sources resulted in lower performance, decreased nutrient digestibility, less xylose absorption and higher xylose clearance from blood in young calves. Higher rectal temperatures, greater incidence of diarrhea and villi atrophy, which may suggest an allergic reaction of calves to soybean protein, was more evident in the younger calves, used in Experiment 2.

Further experimentation might test the ability of intestinal mucosa of young calves to absorb undigested peptides as well as the reasons for increased xylose clearance from the blood observed in calves fed soybean proteins compared to milk protein.

# APPENDICES

TABLE A1.--Analysis of variance for variables in Experiment 1.

| Source of variation | d.f. | Mean square | F ratio | Significance level[a] |
|---|---|---|---|---|
| Body weight (kg) | | | | |
| Treatment (T) | 2 | 421.01 | 1.45 | NS |
| Block (B) | 7 | 927.54 | 3.19 | ** |
| T x B (error a) | 14 | 290.29 | | |
| Period (P) | 8 | 631.70 | 293.81 | ***** |
| T x P | 16 | 10.83 | 5.03 | ***** |
| B x P | 56 | 4.11 | 1.91 | **** |
| Error b | 112 | 2.15 | | |
| Total body weight gain (kg) | | | | |
| Treatment (T) | 2 | 141.92 | 3.64 | * |
| Block (B) | 7 | 65.56 | 1.68 | NS |
| T x B (error a) | 14 | 39.00 | | |
| Period (P) | 7 | 688.19 | 345.82 | ***** |
| T x P | 14 | 8.36 | 4.20 | *** |
| B x P | 49 | 3.66 | 1.84 | *** |
| Error b | 98 | 1.99 | | |
| Percent of gain on initial body weight (%) | | | | |
| Treatment (T) | 2 | 775.49 | 3.99 | ** |
| Block (B) | 7 | 410.91 | 2.12 | NS |
| T x B (error a) | 14 | 194.13 | | |
| Age (A) | 7 | 3,759.10 | 492.03 | ***** |
| T x A | 14 | 41.40 | 5.42 | ***** |
| B x A | 49 | 17.99 | 2.36 | ***** |
| Error b | 98 | 7.64 | | |

TABLE A1.--Continued.

| Source of variation | d.f. | Mean square | F ratio | Significance level[a] |
|---|---|---|---|---|
| Average daily gain (g) | | | | |
| Treatment (T) | 2 | 238,641 | 11.63 | **** |
| Block (B) | 7 | 45,527 | 2.22 | * |
| T x B (error a) | 14 | 20,513 | | |
| Age (A) | 5 | 1,702,935 | 63.42 | ***** |
| T x A | 10 | 35,983 | 1.34 | NS |
| B x A | 35 | 81,440 | 3.03 | ***** |
| Error b | 70 | 26,853 | | |
| Feed intake (g) | | | | |
| Treatment (T) | 2 | $3.4544500 \times 10^6$ | 2.63 | NS |
| Block (B) | 7 | $5.7209714 \times 10^6$ | 4.36 | *** |
| T x B (error a) | 14 | $1.3120000 \times 10^6$ | | |
| Age (A) | 5 | $1.4834780 \times 10^7$ | 0.74 | NS |
| T x A | 10 | $1.5923000 \times 10^5$ | 0.01 | NS |
| B x A | 35 | $1.6639429 \times 10^5$ | 0.01 | NS |
| Error b | 70 | $2.0000360 \times 10^7$ | | |
| Body weight gain/Feed intake | | | | |
| Treatment (T) | 2 | 0.402 | 8.04 | **** |
| Block (B) | 7 | 0.149 | 2.98 | * |
| T x B (error a) | 14 | 0.050 | | |
| Period (P) | 5 | 5.380 | 116.95 | ***** |
| T x P | 10 | 0.161 | 3.40 | **** |
| B x P | 35 | 0.264 | 5.73 | ***** |
| Error b | 70 | 0.046 | | |
| Dry matter digestibility - 2nd week (%) | | | | |
| Treatment | 2 | 73.26 | 1.53 | NS |
| Error | 9 | 47.88 | | |

TABLE A1.--Continued.

| Source of variation | d.f. | Mean square | F ratio | Significance level[a] |
|---|---|---|---|---|
| Organic matter digestibility - 2nd week (%) | | | | |
| Treatment | 2 | 76.18 | 1.55 | NS |
| Error | 9 | 49.08 | | |
| Crude protein digestibility -2nd week (%) | | | | |
| Treatment | 2 | 423.53 | 1.55 | NS |
| Error | 9 | 272.65 | | |
| Ether extract digestibility - 2nd week (%) | | | | |
| Treatment | 2 | 201.02 | 0.52 | NS |
| Error | 9 | 385.07 | | |
| Nitrogen-free extract digestibility - 2nd week (%) | | | | |
| Treatment | 2 | 31.95 | 3.32 | * |
| Error | 9 | 9.62 | | |
| Ash retention - 2nd week (%) | | | | |
| Treatment | 2 | 88.76 | 1.69 | NS |
| Error | 9 | 52.56 | | |
| Nitrogen retention - 2nd week (%) | | | | |
| Treatment | 2 | 1,053.53 | 1.86 | NS |
| Error | 9 | 564.21 | | |
| Dry matter digestibility - 5th week (%) | | | | |
| Treatment | 2 | 48.88 | 5.73 | ** |
| Error | 17 | 8.53 | | |
| Organic matter digestibility - 5th week (%) | | | | |
| Treatment | 2 | 53.48 | 6.86 | *** |
| Error | 17 | 7.79 | | |
| Crude protein digestibility -5th week (%) | | | | |
| Treatment | 2 | 596.87 | 21.75 | ***** |
| Error | 17 | 27.44 | | |

TABLE A1.--Continued.

| Source of variation | d.f. | Mean square | F ratio | Significance level[a] |
|---|---|---|---|---|
| Ether extract digestibility -5th week (%) | | | | |
| Treatment | 2 | 13.48 | 3.39 | ** |
| Error | 17 | 3.97 | | |
| Nitrogen-free extract digestibility - 5th week (%) | | | | |
| Treatment | 2 | 8.61 | 0.94 | NS |
| Error | 17 | 9.08 | | |
| Ash retention - 5th week (%) | | | | |
| Treatment | 2 | 9.48 | 0.36 | NS |
| Error | 17 | 26.11 | | |
| Nitrogen retention - 5th week (%) | | | | |
| Treatment | 2 | 856.84 | 16.80 | ***** |
| Error | 17 | 50.99 | | |
| Plasma urea nitrogen (mg/100 ml) | | | | |
| Treatment (T) | 2 | 4.12 | 0.36 | NS |
| Block (B) | 7 | 7.71 | 0.67 | NS |
| T x B (error a) | 14 | 11.47 | | |
| Age (A) | 1 | 0.17 | 0.02 | NS |
| T x A | 2 | 20.28 | 1.82 | NS |
| B x A | 7 | 10.32 | 0.93 | NS |
| Error b | 14 | 11.12 | | |
| Plasma xylose concentration - oral dose (mg XYL/100 ml) | | | | |
| Treatment (T) | 2 | 528.17 | 5.22 | ** |
| Block (B) | 7 | 136.72 | 1.35 | NS |
| T x B (error a) | 14 | 101.18 | | |
| Time (M) | 7 | 1,192.82 | 24.45 | ***** |
| T x M | 14 | 29.01 | 0.59 | NS |
| B x M | 49 | 55.71 | 1.14 | NS |
| Error b | 98 | 48.78 | | |

TABLE A1.--Continued.

| Source of variation | d.f. | Mean square | F ratio | Significance level[a] |
|---|---|---|---|---|
| Plasma xylose concentration - IV dose (log mg XYL/100 ml) | | | | |
| Treatment (T) | 2 | 0.0568 | 2.90 | * |
| Block (B) | 7 | 0.0239 | 1.22 | NS |
| T x B (error a) | 14 | 0.0196 | | |
| Time (M) | 7 | 0.7043 | 140.86 | ***** |
| T x M | 14 | 0.0088 | 1.76 | * |
| B x M | 49 | 0.0056 | 1.12 | NS |
| Error b | 98 | 0.0050 | | |
| Urine xylose concentration - oral dose (mg XYL/100 ml) | | | | |
| Treatment | 2 | 20.93 | 0.76 | NS |
| Error | 19 | 27.69 | | |
| Urine xylose concentration - IV dose (mg XYL/100 ml) | | | | |
| Treatment | 2 | 94.03 | 1.28 | NS |
| Error | 17 | 73.34 | | |
| Gastrointestinal tract content - pH | | | | |
| Treatment (T) | 2 | 1.7504 | 8.95 | * |
| Animal/T (error a) | 3 | 0.1956 | | |
| Sample (B) | 5 | 8.9920 | 72.28 | ***** |
| T x B | 10 | 0.2735 | 2.20 | * |
| Error b | 15 | 0.1244 | | |
| Gastrointestinal tract content - wet matter (g) | | | | |
| Treatment (T) | 2 | 616,709 | 4.23 | NS |
| Animal/T (error a) | 3 | 145,901 | | |
| Sample (B) | 5 | 22,066,631 | 173.21 | ***** |
| T x B | 10 | 116,919 | 0.92 | NS |
| Error b | 15 | 127,399 | | |

TABLE A1.--Continued.

| Source of variation | d.f. | Mean square | F ratio | Significance level[a] |
|---|---|---|---|---|
| Gastrointestinal tract content - dry matter (%) | | | | |
| Treatment (T) | 2 | 3.86 | 0.50 | NS |
| Animal/T (error a) | 3 | 7.68 | | |
| Sample (B) | 5 | 15.21 | 6.57 | **** |
| T x B | 10 | 1.46 | 0.63 | NS |
| Error b | 15 | 2.31 | | |
| Gastrointestinal tract content - organic matter (%) | | | | |
| Treatment (T) | 2 | 6.04 | 2.05 | NS |
| Animal/T (error a) | 3 | 2.95 | | |
| Sample (B) | 5 | 18.42 | 21.15 | ***** |
| T x B | 10 | 2.30 | 2.65 | ** |
| Error b | 15 | 0.87 | | |
| Gastrointestinal tract content - crude protein (%) | | | | |
| Treatment (T) | 2 | 1.09 | 0.05 | NS |
| Animal/T (error a) | 3 | 22.93 | | |
| Sample (B) | 5 | 1,103.34 | 40.83 | ***** |
| T x B | 10 | 20.94 | 0.78 | NS |
| Error b | 15 | 27.02 | | |
| Small intestine length (cm) | | | | |
| Treatment | 2 | 14,584 | 1.98 | NS |
| Error | 3 | 7,341 | | |
| Slaughtered calves body weight (kg) | | | | |
| Treatment | 2 | 214 | 0.18 | NS |
| Error | 5 | 1,190 | | |

TABLE A1.--Continued.

| Source of variation | d.f. | Mean square | F ratio | Significance level[a] |
|---|---|---|---|---|
| Villi evaluation (1-5 scale) | | | | |
| Treatment (T) | 2 | 1.6722 | 0.40 | NS |
| Evaluators (B) | 4 | 5.1472 | 1.22 | NS |
| T x B | 8 | 0.2347 | 0.06 | NS |
| Animal/T (A/T) | 3 | 4.2277 | | |
| B x A/T | 12 | 0.5333 | 0.86 | NS |
| Sample (S) | 5 | 18.9922 | 30.69 | ***** |
| T x S | 10 | 3.1322 | 5.06 | ***** |
| Error b | 135 | 0.6188 | | |
| Fecal scores (1-4 scale) | | | | |
| Treatment (T) | 2 | 0.0601 | 0.10 | NS |
| Block (B) | 7 | 0.2779 | 0.46 | NS |
| T x B (error a) | 14 | 0.5953 | | |
| Age (A) | 7 | 5.6209 | 28.24 | ***** |
| T x A | 14 | 0.2267 | 1.13 | NS |
| B x A | 49 | 0.3114 | 1.56 | ** |
| Errob b | 98 | 0.1990 | | |
| Retal temperature (°C) | | | | |
| Treatment (T) | 2 | 0.0100 | 0.05 | NS |
| Block (B) | 7 | 0.2529 | 1.38 | NS |
| T x B | 14 | 0.1836 | | |
| Age (A) | 5 | 0.6520 | 10.79 | ***** |
| T x A | 10 | 0.0310 | 0.51 | NS |
| B x A | 35 | 0.1086 | 1.79 | ** |
| Error b | 70 | 0.0604 | | |

TABLE A1.--Continued.

[a]NS = nonsignificant (P>.10).

* = P<.10.

** = P<.05.

*** = P<.01.

**** = P<.005.

***** = P<.001.

TABLE A2.--Analysis of variance for variables in Experiment 2 (21-day period).

| Source of variation | d.f. | Mean square | F ratio | Significance level[a] |
|---|---|---|---|---|
| Initial body weight (kg) | | | | |
| Treatment | 1 | 0.3 | 0.03 | NS |
| Error | 14 | 10.9 | | |
| Final body weight (kg) | | | | |
| Treatment | 1 | 84.5 | 5.31 | ** |
| Error | 14 | 15.9 | | |
| Body weight gain (kg) | | | | |
| Treatment | 1 | 95.2 | 41.39 | *** |
| Error | 14 | 2.3 | | |
| Average daily gain (g) | | | | |
| Treatment | 1 | 215,992 | 39.88 | *** |
| Error | 14 | 5,415 | | |
| Feed intake (kg) | | | | |
| Treatment | 1 | 0.0331 | 0.04 | NS |
| Error | 14 | 0.8726 | | |
| Body weight gain/Feed intake | | | | |
| Treatment | 1 | 0.7700 | 39.07 | *** |
| Error | 14 | 0.0197 | | |
| Rectal temperature (°C) | | | | |
| Treatment | 1 | 0.1406 | 5.02 | ** |
| Error | 14 | 0.0280 | | |
| Fecal score (1-4 scale) | | | | |
| Treatment | 1 | 0.7832 | 3.14 | * |
| Error | 14 | 0.2498 | | |

TABLE A2.--Continued.

| Source of variation | d.f. | Mean square | F ratio | Significance level[a] |
|---|---|---|---|---|
| Surgery villi length (μm) | | | | |
| Treatment | 1 | 46,600 | 3.43 | * |
| Error | 12 | 13,567 | | |
| Final villi length (μm) | | | | |
| Treatment | 1 | 25,360 | 0.70 | NS |
| Error | 14 | 35,995 | | |

[a]NS = nonsignificant (P>.10).

* = P<.10.

** = P<.05.

*** = P<.001.

TABLE A3.--Analysis of variance for variables in Experiment 2 (10-day period).

| Source of variation | d.f. | Mean square | F ratio | Significance level[a] |
|---|---|---|---|---|
| Body weight (kg) | | | | |
| Sensitization (S) | 1 | 148.1 | 12.55 | **** |
| Animal/S (error a) | 14 | 11.8 | | |
| Treatment (T) | 1 | 13.4 | 0.35 | NS |
| Period | 1 | 251.5 | 6.56 | ** |
| S x T | 1 | 2.5 | 0.07 | NS |
| Residual error | 13 | 38.3 | | |
| Average daily gain (g) | | | | |
| Sensitization (S) | 1 | 14,323 | 1.16 | NS |
| Animal/S (error a) | 14 | 12,347 | | |
| Treatment (T) | 1 | 395,828 | 7.61 | *** |
| Period (P) | 1 | 205,922 | 3.96 | * |
| S x T | 1 | 68,172 | 1.31 | NS |
| S x P | 1 | 275,096 | 5.29 | ** |
| Residual error | 12 | 52,019 | | |
| Feed intake (kg) | | | | |
| Sensitization (S) | 1 | 4.18 | 16.07 | **** |
| Animal/S (error a) | 14 | 0.26 | | |
| Treatment (T) | 1 | 0.08 | 0.10 | NS |
| Period (P) | 1 | 8.54 | 9.81 | *** |
| S x T | 1 | 0.04 | 0.05 | NS |
| S x P | 1 | 0.05 | 0.06 | NS |
| Residual error | 12 | 0.87 | | |

TABLE A3.--Continued.

| Source of variation | d.f. | Mean square | F ratio | Significance level[a] |
|---|---|---|---|---|
| Body weight gain/Feed intake | | | | |
| Sensitization (S) | 1 | 0.0031 | 0.14 | NS |
| Animal/S (error a) | 14 | 0.0219 | | |
| Treatment (T) | 1 | 0.7275 | 8.68 | *** |
| Period (P) | 1 | 0.1082 | 1.29 | NS |
| S x T | 1 | 0.1508 | 1.80 | NS |
| S x P | 1 | 0.4596 | 5.48 | ** |
| Residual error | 12 | 0.0838 | | |
| Rectal temperatures (°C) | | | | |
| Sensitization (S) | 1 | 0.120 | 4.32 | * |
| Animal/S (error a) | 14 | 0.027 | | |
| Treatment (T) | 1 | 0.060 | 1.01 | NS |
| Period (P) | 1 | 0.088 | 1.49 | NS |
| S x T | 1 | 0.046 | 0.78 | NS |
| Residual error | 13 | 0.059 | | |
| Fecal score (1-4 scale) | | | | |
| Sensitization (S) | 1 | 0.2112 | 0.92 | NS |
| Animal/S (error a) | 14 | 0.2306 | | |
| Treatment (T) | 1 | 1.9012 | 3.27 | * |
| Period (P) | 1 | 1.3612 | 2.34 | NS |
| S x T | 1 | 0.6050 | 1.04 | NS |
| Residual error | 13 | 0.5821 | | |

TABLE A3.--Continued.

| Source of variation | d.f. | Mean square | F ratio | Significance level[a] |
|---|---|---|---|---|
| Villi length (μm) | | | | |
| Sensitization (S) | 1 | 9,940 | 1.04 | NS |
| Animal/S (error a) | 14 | 9,566 | | |
| Treatment (T) | 1 | 51.681 | 2.44 | * |
| Period (P) | 1 | 10,011 | 0.47 | NS |
| S x T | 1 | 40,186 | 1.90 | * |
| Residual error | 13 | 21.203 | | |

[a]NS = nonsignificant (P>.10).

* = P<.10.

** = P<.05.

*** = P<.025.

**** = P<.005.

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