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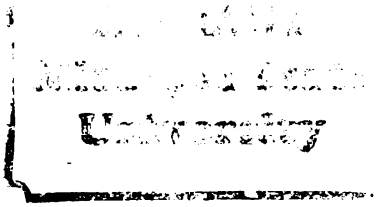

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Spray Dried Fish Solubles, Soy Protein Concentrate and Limestone in Milk Replacers for Young Calves

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SPRAY DRIED FISH SOLUBLES, SOY PROTEIN CONCENTRATE AND LIMESTONE IN MILK REPLACERS FOR YOUNG CALVES

By

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ABSTRACT

SPRAY DRIED FISH SOLUBLES, SOY PROTEIN CONCENTRATE AND LIMESTONE IN MILK REPLACERS FOR YOUNG CALVES

By

Oriel Fajardo de Campos

In a first experiment, 168 Holstein calves at Cornell, Kansas State and Michigan State Universities were used to test the partial substitution of spray-dried fish solubles (SDFS) and soy protein concentrate (SPC) for milk protein (MP) in milk replacers. Calves were placed on experiment between 3 and 8 days of age and were fed experimental diets as the only source of nutrients for 6 weeks. Replacers were reconstituted with water to 14% solids and fed at 8, 9, 10, 11, 12 and 12% body weight from 1st to 6th week. Calves were fed twice daily from open pails. Experimental diets consisted of (1) 13% crude protein (CP) as MP; (2) 19% CP as MP; (3) 19% CP, 13% MP, 6% SDFS; (4) 19% CP, 13% MP, 6% SPC; (5) 19% CP, 13% MP, 3% SDFS, 3% SPC; (6) 23% CP as MP; and, (7) 23% CP, 13% MP, 10% SDFS.

Average daily body weight gains were higher for diets containing higher levels of MP (2 and 6), intermediate for 4 containing 6% CP from SPC, and lower for the negative control (diet 1) and those containing SDFS. Fecal socres, treatment for sicknesses, and rectal temperatures were higher for diets containing SDFS. Plasma amino acids sampled

when calves were 3 and 6 weeks old showed higher total essential amino acids for 19 and 23% than 13% MP or milk substitute diets. Xylose absorption test at 6 weeks failed to reveal impaired capacity for gut absorption due to feeding lower quality protein. On diet 7, mortality rate was 30% compared to a mean of 12% for other diets, indicating that 10% protein from SDFS was excessive.

The second experiment was a digestibility trial involving diets 1 to 4 from experiment 1, with four calves per treatment. Calves were placed on experiment between 3 and 8 days of age and were fed and managed similarly to trial 1 for 2 weeks. Lowered digestibilities of dry matter and organic matter, and less retained nitrogen were associated with decreased weight gains in calves fed SDFS or SPC. Necropsy results also raised the possibility of SDFS causing allergy in young calves.

A third experiment, involving 16 male calves, was conducted to study the buffering effect of limestone in the small intestine of pre-ruminant calves fed milk replacers containing 50% of the protein from SPC. Experimental diets were: (A) 19% CP as MP; (B) same as A, but containing .8% limestone; (C) 19% CP, 9.5% MP, 9.5% SPC; and, (D) same as C, but containing .8% limestone. Calf management, feeding criteria and dilution rates were the same as in trial 1.

It was observed that SPC in milk replacers resulted in 20% lower weight gains, and lower dry matter and protein digestibilities. Apparent crude protein retention was also reduced, but intake of nutrients, feed efficiency, fecal score and rectal temperature were not different between protein sources. Limestone resulted in no significant changes

in the already mentioned parameters, although apparent nutrient digestibilities and protein retention were lowered when limestone was fed. Xylose absorption tests performed when calves were 3 and 6 weeks old showed differences due to age, but not treatment. Analysis of digesta obtained from different sections of the gut of 6 week old calves sacrificed 6 hours after feeding revealed that (a) abomasal pH was higher than previously reported for calves fed whole milk; (b) small intestinal pH was above 6 for both protein sources which may explain the ineffectiveness of adding limestone to the replacers; (c) the use of SPC resulted in a higher pH in the large intestine and feces.

DEDICATION

To my wife, Lea, and our children, Oriel Junior, Susana and Adriana.

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I would like to thank Dr. J.T. Huber for assistance and guidance throughout my graduate course. Also, I wish to express my appreciation to Dr. W.G. Bergen, Dr. J.L. Gill and Dr. H. Ritchie for serving on my Graduate Committee.

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INTRODUCTION

Replacement of milk products in the young dairy calf's diet with plant and/or animal products has been attempted due to the increasing demand for milk in human diets and the consequent high price accompanying this demand. Many dairymen utilize colostrum and waste milk as substitutes for whole milk for young females, but young males raised for market beef or veal depend heavily on commercial milk replacers.

The proportion of milk consumed by a calf relative to it's mother's production is greater in areas where dairying is less developed and where the milk is less available for human consumption. In such areas, the calf may consume as much as 50% of the dam's production compared to 10% or less for more developed systems of management. Thus, the formulation of high quality milk replacers is not only a concern for developed countries, but also for underdeveloped ones.

Schugel (1974) described the history of milk replacers. The first milk replacers were developed in the fifties, and were based on skimmilk, buttermilk, whey and animal fat. At that time, problems related to fat incorporation and overheating of the skimmilk during the drying process resulted in a reduced energy content of the replacer, a high incidence of diarrhea and often poor performance of calves. Solving these problems resulted in better formulas and improved performance of calves fed milk replacers.

In 1966, the price of skimmilk increased and its inclusion in milk replacers became less economical. Casein and whey were available at moderate cost and the industry shifted to these products with little reduction in quality. Later, as a result of the world's short supply of casein, researchers have investigated non-milk proteins, mainly from the soybean and fish industries; but these protein sources have not proved entirely satisfactory.

The objective of this work was to study the partial substitution of milk protein by soybean protein concentrate and/or spray dried fish solubles. Also, the addition of limestone in milk replacers for young calves was evaluated. The parameters measured were growth, feed efficiency, scours, digestibility of nutrients, nitrogen retention, xylose absorption in the small intestine, and plasma amino acid concentration.

REVIEW OF LITERATURE

Efficient digestion and utilization of nutrients require that food pass at a suitable rate through the alimentary tract with appropriate enzymes and absorptive mechanisms that will allow the breakdown of complex molecules and absorption of the products.

Young calves are sensitive to protein quality in these liquid rations and only highly digestible proteins with a favorable profile of essential amino acids are desirable in milk replacers. In particular, the first 3 weeks of life are the most critical for high quality protein in young calves (Huber and Slade, 1967; Makdani et al., 1971c; Norman, 1971) since protein digestibility increases with age (Noller et al., 1956; Gorrill et al., 1975; Bell et al., 1979).

The sequence of digestive events in the abomasum and small intestine of the young calf fed milk has been determined in systematic studies using fistulated animals (Henschell et al., 1961a; Ash, 1964; Smith, 1964; Mylrea, 1966a). These events will be considered in the development of this review.

Protein Digestion in the Abomasum

In preruminating calves the stimulus of drinking milk causes the formation of the esophageal groove which directs liquids through the omasum into the abomasum (Titchen and Newhook, 1975). Thus, the first enzymatic action on dietary protein takes place in the abomasum.

Hydrochloric acid is produced in the abomasum and its production increases with age Porter (1969). Cholinergic reflexes stimulate 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).

Before feeding, the abomasal contents consist of a fairly clear, slightly viscous fluid containing small milk clots and has pH of 1-2. During feeding, milk clots rapidly as it enters the abomasum (Hill, 1968), and clotting is complete within a minute or so after finishing the meal. The pH values of abomasal contents increase to around 6 (Mylrea, 1966a) immediately after feeding, but as acid secretion increases pH slowly decreases and reaches pre-feeding values after about 5 hours (Porter, 1969). Only after most of the whey fluids leave the abomasum, does the pH of the abomasal fluids become sufficiently low for dissolving of the casein clot by pepsin (Mylrea, 1966a; Ternouth et al., 1974a).

There is some evidence that quality of diet affects HCl production in the abomasum. Tagari and Roy (1969) concluded that a diet containing "severely" pre-heated spray dried skimmilk powder resulted in higher pH values in the pyloric outflow compared to "mildly" pre-heated powder. This difference was probably due to a reduction in acid secretion on the heat damaged diet. Colvin et al. (1969) showed a higher pH of digesta leaving the abomasum from calves fed soybean flour than in those fed whole milk. At 40% substitution of milk protein, fish protein concentrate appeared a better stimulus than soybean flour for gastric acid secretion (Ternouth, 1971).

In the abomasum, milk protein clots through action of rennin and pepsin. The peptic chief cells appear to respond primarily to direct cholinergic neural stimulation, although gastrin is also implicated (Gregory, 1974). Soy, fish and whey proteins may inhibit rennin secretion, but pepsin does not seem to be affected by protein substrate (Garnot et al., 1977).

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). For clotting by rennin, the optimum pH is 6.5; whereas, the optimum pH for proteolytic activity is 4.0 for rennin and 2.0 for pepsin (Tagari and Roy, 1969).

Both enzymes have similar amino acid sequences (Pedersen and Foltmann, 1973), but differ drastically in their activity on ribonuclease as substrate. Pepsin inactivates it and rennin does not (Bang-Jensen et al., 1964). It is uncertain whether efficiency of protein utilization is affected by the nature of the enzymes secreted, though little difference was found between results with pepsin and rennin for the "in vitro" digestion of raw milk (Henschell et al., 1961b).

Berridge et al. (1943) postulated that the changeover from rennin to pepsin secretion is initiated when animals first eat roughage. More recent results indicate that the pattern of protease secretion in the abomasum varied considerably among individual calves, but that, in addition to rennin, some pepsin activity was in most animals sampled during the first 2 weeks of life (Henschell et al., 1961a). Henschell (1973) observed a general increase in total protease activity in the

abomasum with age. At weaning, rennin activity in the calf decreases while pepsin remains nearly constant (Thivend et al., 1980). This change is partially reversible. When the animal is given a liquid feed containing casein, pepsin activity is not affected but rennin increases; however, it does not reach preruminant levels (Garnot et al., 1977).

Development of a firm curd in the stomach of the preruminant calf has two beneficial functions. It helps digestion by releasing the nutrients slowly into the small intestine where digestive enzymes more effectively hydrolyze nutrients. Secondly, a firm curd prevents excessive quantities of undigested protein from reaching the small intestine where proliferation of pathogenic organisms in the upper gut might occur, leading to illness or even death of the calf.

Tagari and Roy (1969) found that replacers containing both low- or high-temperature treated skimmilk powders coagulate; but the former produces a firm, elastic curd, and the latter a floculent, loose curd. A solid clot is retained in the abomasum, whereas a fragile clot breaks down and passes rapidly into the small intestine. Little clotting was obtained by Colvin et al. (1969) and Emmons et al. (1976) working with soy products. Low digestibilities for non-milk proteins in young calves have been attributed to the lack of coagulation of dietary protein in the abomasum (Gorrill and Nicholson, 1969a; Paruelle et al., 1972).

Frantzen et al. (1971) demonstrated depressed dry matter and protein digestibilities by 2-week-old calves fed milk replacers in which citrate or prior treatment by acid prevented coagulation in the abomasum. On

the other hand, Owen and Brown (1958) indicated that citrate addition in whole milk to reduce curd formation did not adversely affect calf growth or health. More recently, Toullec et al. (1971) observed no effects on performance in calves given a non-clotting diet.

Several factors may affect curd formation. Emmons and Lister (1976) concluded that curd formation was increased by a lower pH of the skimmilk over a range of 5.6 to 6.6, by higher concentration of rennin, by lower temperatures of heat treatment of skimmilk prior to spray drying, and by higher temperatures of coagulation (37 vs 30°C).

Jenkins et al. (1981) studied several factors affecting "in vitro" clot formation by rennet of milk replacers. They observed that "lower pressure dispersion" of lipid into skimmilk powders produced markedly higher values for curd firmness than did "homogenization" with lipid at all solid and lipid levels tested. Rennet coagulation of skimmilk treated by the "low-pressure dispersion method" promoted firmer curds than that treated by the "homogenization method" when the skimmilk was partially (20-40%) replaced by mixtures of fish protein-whey or soybean protein-whey.

Formation of a strong curd may also influence fat digestion since fat globules are enmeshed in the abomasal clot (Hill et al., 1970; Ternough et al., 1980). A longer retention time in the abomasum would allow greater opportunity for fat hydrolysis by pregastric esterase (Radostits and Bell, 1970; Ternough et al., 1980).

After coagulation of the casein, the whey in milk moves into the small intestine within 5 minutes after feeding (Radostits and Bell, 1970).

Greatest flow of whey from the abomasum occurs shortly after feeding but overall rate is one-half the volume every 2 hours (Porter, 1969). The greater the quantity of milk fed, the longer the time required for all the whey to leave the abomasum (Ternouth et al., 1974a).

As digestion in the abomasum proceeds, the clot breaks down 3-4 hours after feeding and chyme passing into the duodenum changes from a whey-like fluid containing predominantly carbohydrate and soluble nitrogenous compounds to a thick, white opaque material containing protein and fat (Mylrea, 1966b; Toullec and Mathieu, 1973). Between 82 and 87% of the digesta fluid passes from abomasum in the first 6 hours after feeding, but less than 50% of the total fat passes (Mathieu et al., 1968; Ternouth et al., 1974a).

Feeding causes a considerable increase in the rate of outflow of abomasal contents (Ash, 1964; Mylrea, 1966b), and the rate of flow into the duodenum reaches a peak in the first hour after feeding; whereas, flow is greatest in the jejunum during the first 4 to 5 hours after feeding. There is no marked effect on feeding on the pattern of flow in the ileum (Porter, 1969). Ternouth et al. (1974a) 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.

An increased outflow of undigested protein during the first hour after feeding was reported 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 even whey powder (Gorrill and Nicholson, 1972a). These results suggest a lower abomasal proteolysis in over-heated milk protein or replacers containing non-milk protein. Guilloteau et al. (1979) concluded that suppressing the transit of digesta into the abomasum (by infusion into the duodenum), as well as accelerating the rate of passage, have an adverse effect on nitrogen and lipid digestibilities. In contrast, the slowing down of the rate of passage allows digestibility to increase.

On the other hand, Christison and Bell (1976) observed that a more digestible pea protein (60% crude protein digestibility) left the abomasum faster than a poorly digested pea product (41% crude protein digestibility), suggesting that the rate of passage of crude protein through the abomasum was not a major factor in determining the crude protein digestibility.

There is also evidence that abomasal secretory activity might be lower when calves are fed diets containing "severely" preheated skimmilk powder, soybean flour or fish protein concentrate as protein sources. Lowered abomasal secretions have been also associated with a high incidence of digestive disturbances in young calves on milk substitute diets (Shillam et al., 1962; Tagari and Roy, 1969; Ternouth and Roy, 1973; Ternouth et al., 1974b; Ternouth et al., 1975; Williams et al., 1976).

Protein Digestion in the Small Intestine

After pre-digestion in the abomasum, the hydrolysis of dietary protein to small peptides and amino acids continues in the small intes-

tine by action of trypsin, chymotrypsin and carboxypeptidase present in the pancreatic juice; and by various peptidases secreted into the small intestine or present in intestinal mucosa cells (Porter, 1969).

The anatomical relationships of the pancreatic duct, bile duct and duodenum vary between species (Hallenbeck, 1967). In adult cattle, the main pancreatic duct enters the duodenum about 300 mm caudal to the sphincter of Oddi (Sisson and Grossman, 1956). Wass (1965) demonstrated that an accessory pancreatic duct joins the bile duct in most cattle. Pancreatic trypsinogen and chymotrypsinogen are converted to active enzymes a relatively short distance from their entry into the upper small intestine at a pH lower (5.2 or less) than optimum "in vitro" conditions. There also appears to be rapid digestion of protein at a relatively low pH in the upper part of the small intestine (Gorrill and Nicholson, 1971).

Changes in pH in the duodenum reflect those in the abomasum, rising immediately after feeding and then falling. Feeding has little effect on pH values in the distal small intestine, but there is a gradual increase towards the ileum where values range between pH 7 and 8 (Porter, 1969). A more detailed discussion on small intestinal pH, its effects on enzyme activity, and the possible role of buffers will be presented later.

Emptying of zymogen granules within the pancreas is mainly under neural rather than entero-hepatic control (Ternouth et al., 1974a), i.e. the secretion of enzymes does not appear related to digestive requirements of the intestine. There is a large increase of trypsin and chymotrypsin activities in digesta just after feeding (Gorrill et al., 1967;

McCormick and Stewart, 1967; Gorrill and Nicholson, 1971; Ternouth et al., 1975). However, response of the pancreas to feeding in calves was much less than for non-ruminants such as dogs (Preshaw et al., 1966). This large output of enzymes from the pancreas during the first 2 hours after feeding does not coincide with the main outflow from abomasum of total nitrogen and lipids occurring 5 to 10 hours after feeding (Ternouth et al., 1975).

Ternouth et al. (1976) observed that the increased secretion of pancreatic fluid but not enzymes 6 to 12 hours after feeding was due to stimulation by secretin.

It was classically admitted that regulation of exocrine pancreatic secretion was controlled by neural and hormonal mechanisms (Thomas, 1967; Harper, 1972); however, a feedback mechanism may be involved. Taylor (1962) found that quantity of enzymes secreted by the pancreas decreased when these enzymes were prevented from entering the duodenum. Davicco et al. (1979) concluded that a pancreatic regulatory mechanism similar to that in rats and pigs exists in young calves. Such regulation acts through a feedback of trypsin which would modulate the release of secretin.

Gorrill and Thomas (1965) and Gorrill et al. (1967) reported that adequate amounts of trypsin and chymotrypsin were produced from birth and there was no marked increase in the concentration of these enzymes with age. However, increases in protease activity associated with increases in the flow-rate of pancreatic juice during the first week of calf's life were reported by Huber (1969). Ternouth et al. (1976)

fed Ayrshire calves whole milk and observed in absolute terms and in relation to milk intake or metabolic size, a large increase with age (7 to 63 days of age) in secretion of total protease but no change in trypsin activity. They (Ternouth et al., 1976) also fed Holstein calves milk replacers and concluded that secretion of pancreatic fluid, but not trypsin or total protease increased with age. Reasons for the observed differences were not apparent.

Diet quality may affect pancreatic and intestinal enzyme secretions. Gorrill and Nicholson (1972a) observed that proteolytic enzyme secretion from pancreas was about 2.5 times less for calves fed milk replacers containing 50% whey compared to 23% whey. Ternouth et al. (1974a) compared "mildly-" with "severely-" heated skimmilk and concluded that animals receiving "mildly" treated skimmilk secreted more pancreatic fluid, but quantity of trypsin did not differ significantly between diets.

Ternouth et al. (1975) reported that when skimmilk powder was partially replaced in the diets by soybean flour or fish protein concentrate, neither large reductions in secretion of pancreatic enzymes (Gorrill et al., 1967) nor changes in the pattern of duodenal outflow (Colvin et al., 1969; Smith et al., 1970) were found. Nevertheless, the small decrease in these parameters may have been sufficient to explain the lower growth rate observed for the first 3 weeks of life.

Calves fed soybean trypsin inhibitor (SBTI) showed a marked reduction in exocrine pancreatic secretion (Gorrill and Thomas, 1967; Gorrill et al., 1967; Gorrill and Nicholson, 1971). Gorrill and

Nicholson (1971) concluded that the reduced pancreatic secretion in calves fed SBTI was not caused by diarrhea because diarrhea induced by neostigmine methyl sulfate or magnesium sulfate did not affect enzyme activities. The diarrhea in calves fed SBTI could be due to protein and peptide accumulation in the large intestine, as postulated by Roy (1969). Indeed, accumulation of undigested protein in the lower intestine of animals fed SBTI was observed in calves (Gorrill and Nicholson, 1971), chicks (Coates et al., 1970) and rats (Carroll et al., 1952).

Buraczewski et al. (1967) found higher concentrations of free amino acids and small peptides in the intestinal contents of rats given severely-heated cod-fillet protein than in animals given undamaged proteins. Yet, concentrations of free amino acids in portal blood were lower. They postulated that the accumulation of "unavailable peptide" in the intestine, characteristic of rats fed heat-damaged protein, hinders absorption of amino acids by saturating mucosal transport sites. Shorrock and Ford (1978) showed that an extract containing "unavailale" small peptides from severely-heated cod-fillet strongly inhibited leucine uptake in rat intestine, but had no effect on uptake of glucose or its metabolism to lactate. Shorrock and Ford (1978) conjectured that the reduction in amino acid uptake was an allosteric effect resulting from an attachment of the peptides to binding sites adjacent to the sites for amino acid uptake. A further hypothesis was that "unavailable peptides" are transported into the mucosal cells and there interfere with normal cell function. Whatever the true explanation, it remains now to isolate representative "unavailable peptides" and to study their influence on amino acid transport.

The utilization of substitute proteins might result in modification of intestinal nitrogen digestion. The amount of apparently digestible nitrogen which disappears in the large intestine is only 2 to 4% with milk protein (Weerden et al., 1977) but may reach 8 to 10% with fish concentrate or soybean concentrate when dietary protein is less completely hydrolyzed (Thivend et al., 1980).

Thivend et al. (1980) observed that when ingested proteins came exclusively from milk, the amino acid composition of ileum contents differed greatly from that of milk, feces or fecal bacteria, but was similar to endogenous protein. Thus, when feeding milk protein, true digestibility is nearly complete, so protein appearing in the feces is mainly of endogenous origin. However, when casein is replaced by fish or soybean protein, a greater proportion of protein passing through the lower digestive tract and out the feces will be of dietary origin.

Soybean Products in Milk Replacers

Acceptable growth has been obtained in calves fed mixtures of plant and milk protein (Murley et al., 1957; Lassiter et al., 1963; Pettyjohn et al., 1963; Stone et al., 1963) but growth from plant protein alone was usually unsatisfactory (Stein et al., 1954; Noller et al., 1956; Fries et al., 1958). Among plant proteins, soybean products have been most successfully incorporated into milk replacers.

There are four main soybean products that have been tested. These are: soybean meal, soybean flour, soybean protein concentrate and soy protein isolate. They average 45, 60, 70 and 90% crude protein, and

45, 35, 25, and 5% carbohydrate, respectively. Results with preruminant calves vary widely, depending upon the soybean product and concentrations used in milk replacers (Table 1). Increased diarrhea has been reported at high levels of soy protein concentrate (Seegraber and Morrill, 1979; Coblentz et al., 1976). Others have found no diarrhea and some even showed improved fecal consistency (Morrill et al., 1971; Barr et al., 1978; Huber et al., 1978; Gaudreau and Brisson, 1978).

Poor performances have been reported for calves fed raw soybeans (Williams and Knodt, 1951; Kakade et al., 1974; Thompson et al., 1974). Reduced digestibilities of nitrogen, fat and ash of milk replacers containing soy protein concentrate or soybean meal were reported (Nitsan et al., 1971,1972; Pejic and Kay, 1979).

A hypothesis raised to explain these negative results relates to the presence of SBTI. Characteristics were severe decreases in pancreatic volume, enzyme concentrations, and specific activities of trypsin and chymotrypsin of 1-week-old calves receiving part of their protein from soybean flour (Gorrill et al., 1967; Gorrill and Thomas, 1967). The reduction in pancreatic secretions in calves directly 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; Mickelsen and Yang, 1966). Response of swine pancreas to raw soybeans (Hooks et al., 1965) was similar to that observed with calves.

Lepkovisky et al. (1971) suggested that impaired proteolysis was not the result of decreased activity of proteases in the intestine,

TABLE 1. Summary of dairy calves and lamb performances on different soy protein sources in milk replacers.

Authors	Products	Level of protein replacement	Age	ADG[a]	DMD[b]	PD[c]	Type of animal
		%	wk	kg	%	%	
Gorrill and Thomas(1967)	SPC[d]-50%	50	0-6	-.11			Calves
	SPC -71%	86	0-6	.33			
Gorrill and Nicholson(1969b)	SPC	0(milk)	0-7	.53	90.6	87.3	Calves
		70	0-7	.55	88.8	81.6	
Gorrill et al. (1971)	SPC	22	0-5	.51			Calves
Gorrill and Nicholson(1972a)	SPC	0(milk)	2-7	.377	90.0	85.0	Calves
		70	2-7	.348	85.0	77.0	
Gorrill and Nicholson(1972c)	SPC	50	0-5	.197	94.0	92.0	Lambs
Gorrill et al. (1974)	Full fat soybean flour	0(milk)	0-5		90.2	93.6	Lambs
		30	0-5		93.5	95.1	
		50	0-5		92.2	92.2	
Gorrill et al. (1975b)	Full fat soybean flour	0(milk)	0-4	.23			Lambs
		24	0-4	.20			
		30	0-4	.21			
Kakade et al. (1976)	Heated soybean	70	2	0	84.0	55.0	Calves
Roy et al. (1977)	Soy flour	0(milk)	0-3		95.0	94.0	Calves
		35	0-3		88.0	84.0	
		65	0-3		79.0	66.0	
Barr et al. (1978)	Modified soy protein	0(milk)	0-4	.50			Calves
		50	0-4	.46			
		72	0-4	.45			
Bringe and Barr (1979)	Modified soy protein	0(milk)	0-6	.67			Calves
		70	0-6	.64			
Jenkins (1981)	SPC	0(milk)	0-4	.443	93.6	90.8	Calves
		51	0-4	.198	91.4	85.8	

[a]Average daily gain.
[b]Dry matter digestibility.
[c]Protein digestibility.
[d]Soy protein concentrate.

but was due to the formation of a trypsin inhibitor-protein complex which resists proteolysis even in the presence of high concentrations of enzymes.

Ramsey and Willard (1975) concluded that fully-cooked soy flour contains an inactive form of trypsin inhibitor that is converted to the active form in the pH range of 7 to 9. This inhibitor can be destroyed by heating the soy flour in water, but the extent of destruction is partially dependent on concentration of flour in the water. They suggested that the presence of trypsin inhibitor in fully-cooked soy flour might explain why newborn calves performed poorly on milk replacers containing large quantities of the product.

Willard and Ramsey (1972) suggested that further removal of small amounts of trypsin inhibitor in heated soybean flour by acid or alkali improved its nutritional value, but this finding was not supported by Sissons and Smith (1976). Gorrill (1970) concluded that "in vitro" pepsin digestion of soybean protein was greater in .05N sodium hydroxide than in water. Opposite effects occurred with milk protein. Alkali treatment on the soybean protein had no effect on proteolysis by trypsin and chymotrypsin after subtracting pepsin digestion from total digestion. More recently, Kakade et al. (1976) studied the nutritional effects induced in calves by feeding SBTI, and did not find differences due to weight or enzymatic activities of pancreas. They concluded that SBTI plays a minor role, if any, in calf nutrition.

Toasting of soybean flour accompanied by acid or alkali treatment (Colvin and Ramsey, 1968, 1969; Colvin et al., 1969) or extraction with

alcohol (Nitsan et al., 1962; Smith and Sissons, 1975) has been claimed to overcome detrimental effects. Thermo-alkali treatment with lower temperatures inactivates SBTI more effectively than thermo-acid treatment (Wallace et al., 1971).

Colvin et al. (1969) analyzed digesta collected for 8 hours after feeding soybean flour and concluded that the treatment with acid or alkali had no effect on rate of passage through the abomasum, or on pH changes occurring in the abomasum. Thus, no explanation for the improved nutritional value of acid or alkali treatment of soy flour was found.

Kakade et al. (1971) fed rats lecithinated and non-lecithinated soy flours with and without acid treatment as the only protein and observed that rats receiving treated-lecithinated soy flour grew better than those fed the untreated. No such improvement was observed with treated non-lecithinated soy flour. They suggested that the beneficial effect of acid treatment involves the lecithin component of the soy flour.

Diets containing soybean flour reduced 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.

Improvement in the nutritive value of soybean products could involve the carbohydrate fraction. The residual carbohydrates in soy concentrates are pectin-like compounds, such as neutral arabinogalactans, acidic polyssacharides and arabinans (Kellor, 1974). Disappearance in digestibility studies of considerable carbohydrate from soy protein sources (Nitsan et al., 1971,1972; Kakade et al., 1976) is presumably

due to intestinal fermentation. However, replacement of 50% of milk protein with soy flour containing 30-40% carbohydrate should not be highly detrimental to calf performance because this fraction would only account for a maximum of 8% of the total solids in the milk replacer.

Enzymatic pre-digestion of soy flour did not stimulate growth, even though the carbohydrate fraction underwent extensive degradation (Colvin and Ramsey, 1968). Feeding of high chlorotetracycline to depress intestinal fermentation did not improve growth in calves fed untreated soy flour (Colvin and Ramsey, 1969). Moreover, removal of the water-soluble carbohydrates from soy flour did not improve growth and addition of the water-soluble carbohydrates to a replacer containing soy flour did not depress growth (Sudweeks and Ramsey, 1972).

Crude and refined soybean oils in milk substitutes severely retarded growth and increased diarrhea in young calves (Barker et al., 1952), but level of fat in soy protein concentrate is negligible and therefore presents no practical concern.

Methionine is the most limiting amino acid in soybean protein for rats, chicks and pigs (Almiquist et al., 1942; Berry et al., 1962; Hays et al., 1959). Lysine was lower in the alkali-treated than untreated soybean concentrate (Gorrill, 1970), and DeGroot and Slump (1969) reported that lysine, cysteine and serine were reduced when isolated soybean protein was mildly treated with .02N NaOH. Methionine supplementation (0.1% of dry matter) did not increase growth or nitrogen retention in young calves fed milk replacers containing 70% of the dietary protein from soybean protein concentrate (Gorrill and Nicholson, 1969b). On the other hand,

Porter and Hill (1964) reported improved calf performance when methionine supplementation of isolated soybean was increased from 1.5 to 2.3 g/100 g of protein.

The presence of hemaglutinates in calve's blood has been detected (Smith and Wynn, 1971), especially when milk protein was completely replaced by soybean flour, and when successive feedings of soybean flour was interpersed with feedings of normal milk diets. Smith and Sissons (1975) and Sissons and Smith (1976) observed digestive disturbances when soy products were fed to calves indicating a gastrointestinal allergic response. Serum antibody response was recorded 2 to 3 weeks after feeding milk replacers with soy protein (Kilsham and Sissons, 1979) and previously sensitized calves responded to reintroduction of soy protein in the replacer with marked increases in antibody level (Barratt et al., 1978). On the other hand, such evidence was not found by Ternouth et al. (1975) and Roy et al. (1977).

Extraction of soy protein sources with hot aqueous-ethanol rendered it non-immunogenic and seemed to eliminate digestive disturbances normally found with soy protein (Sissons et al., 1979). According to these authors, alcohol treatment denatures the specific antigenic proteins, glycinin and beta-conglycinin. However, other workers reported no benefit from alcohol treatment (Barratt et al., 1978,1979; Barratt and Porter, 1979). Differences in treatment method might be responsible for the conflicting results.

Seegraber and Morrill (1980) observed less villi and those villi present were short, blunted and convoluted when calves were fed soy

proteins. They concluded that the substitution of one-third of the milk protein with soy protein concentrate resulted in impairment of absorptive ability which was probably associated with morphological changes in intestinal structure. Stobo and Roy (1977) observed a slight thickening of the walls of the small intestine when dried bacterial cells or yeast supplied 22 or 42% of the dietary protein.

As mentioned, the maximum level of milk protein substitution by soy protein varies with the type of soybean product used. Roy et al. (1977) concluded that 30% replacement of milk protein by soybean flour was possible, but with some detrimental effects on the calf's health and growth. Higher levels of replacement (70%) were worse than lower levels.

Satisfactory growth was obtained when soybean protein concentrate supplied more than 50% of the total protein in milk replacers (Gorrill and Thomas, 1967; Gorrill and Nicholson, 1969b,1972a; Polzin, 1978). In contrast, Morrill et al. (1971) stated that soybean protein concentrate could successfully replace 25%, but not 44% of total protein in milk replacers.

More recently, Barr et al. (1978) and Bringe and Barr (1979) concluded that a "modified soybean protein" could replace 72% of the dietary protein without depressing calf growth, feed efficiency or increasing diarrhea.

When concentrate and hay were available to calves, in addition to milk replacers, the difference in growth rate of calves fed milk vs. soy proteins diminished (Nitsan et al., 1972).

Gorrill et al. (1974,1975a) concluded that specially processed full fat soybean flour could supply 50 to 60% of the total protein in a lamb milk replacer without adversely affecting growth and meat quality. Similar results on growth of lambs have been reported using soybean flour (Jordan et al., 1972) or soy protein concentrate (Gorrill and Nicholson, 1972c).

Fish Products in Milk Replacers

One of the major problems in comparing literature results on fish product utilization by calves is the diversity in type and quality of the products tested. Several investigators have observed marked variability in nutritional quality of products obtained from different fish species and processing procedures (Miller, 1956; Morrison and McLaughlan, 1961; Morrison et al., 1962; Makdani et al., 1971a).

Processing Fish Proteins

Fish meal was originally obtained after collection and treatment of the filleting offal by drying and grinding to a meal. Increased demands for such a protein supplement in stock feeds were met by the drying of whole white fish, such as herring. Processing included cooking, followed by pressing to remove most of the oil (Geiger and Borgstrom, 1965).

Production of fish flour or fish protein concentrate involves use of the entire fish and yields a dry powder edible by humans. The two processes cleared by the Food and Drug Administration are: a) Isopropanol extraction and b) Ethylene dichloride as the initial solvent, followed

by subsequent extraction with isopropanol (Wilcke, 1969). In this paper, products from both processes will be referred to as fish protein concentrate (FPC). For human consumption (Geiger and Borgstrom, 1965), the finely-ground product is washed repeatedly with alcohol to remove solvent residue and to deodorize.

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. The pulp is dried and made into fish meal. The stickwater exhibits properties characteristic of both fish muscle extracts and the gelatin- and glue-containing extracts of bone, cartilage, skin and other connective tissues. It is customary to discard this stickwater after removing the oil by "settling tanks," or centrifugation. Stickwater, which contains about 30% of the original fish protein, is then evaporated to yield dried fish solubles (Lassen, 1965).

Nutritional Value of Fish Protein Products

Morrison and Campbell (1960) confirmed previous results obtained by Sure (1957) in which fish protein concentrate was an excellent source of high quality protein for rats, particularly in diets deficient in lysine. Fish protein concentrate also improved the biological value of cereal based diets for growing infants when used as the main protein source (Yanes et al., 1969). Tavill and Gonik (1969) obtained similar results in Morroco with infants fed diets containing 3% FPC (w/w).

Performance of calves on milk replacers containing FPC depends on

age, level of FPC, level of crude protein in the replacer, and the use of dry feed in the feeding program (Table 2). Poor performance was obtained when calves were fed high levels of FPC in milk replacers which served as the only source of nutrients (Huber and Slade, 1967; Matre, 1970,1971,1973,1977; Gillespie, 1971; Sleiman and Huber, 1971; Raven, 1972; Ramsey, 1975; Makdani et al., 1971c). Most of the growth depression occurred during the first 3 weeks.

Average daily gain and feed efficiency were not significantly depressed when FPC furnished up to 40 to 50% of dietary protein in calves fed milk replacer (Huber and Slade, 1967; Huber and Sleiman, 1971; Sleiman and Huber, 1971; Gorrill et al., 1972; St. Laurent and Brisson, 1972; Ramsey, 1975; Matre, 1977). Good results were also obtained with milk replacers containing higher FPC, but fed with hay and starter (Gelwichs, 1965; Harshberger and Gelwichs, 1965; Williams and Rust, 1968; Danell, 1970; Gorrill, 1970; Sleiman and Huber, 1971; Opstvedt et al., 1978).

Huber et al. (1978) obtained poorer calf performance when 33% of dietary protein was replaced by fish hydrolysate compared to all milk. However, relatively good gains were obtained when 33% of milk protein was replaced by equal parts of fish hydrolysate and soy protein.

There are some reports showing that calves fed milk replacers containing 70% of dietary protein from FPC grew as fast as those receiving all milk protein (Van Hellamond, 1967; Sorensen and Lykkeaa, 1968; Danell, 1970). In these experiments, however, milk replacers contained 24 to 26% crude protein and calves were 3 weeks of age or older. The higher total protein and older age of calves may explain why results

TABLE 2. Summary of dairy calves performance on different fish protein sources in milk replacers

Authors	Products	Level of replacement	Age	ADG[a]	DMD[b]	PD[c]
		%	wk	kg	%	%
Raven (1972)	Fish meal	0(milk)	0-8		93.0	86.2
		15	0-8		90.7	83.8
Gorrill et al. (1975)	Herring meal	65	0-3		92.6	81.6
	FPC	62	0-3		92.7	84.8
Huber and Slade (1976)	FPC	0(milk)	1-6	.397	90.2	90.3
		33	1-6	.360	88.7	84.3
		67	1-6	.273	89.0	85.4
Gorrill et al. (1972)	FPC	0(milk)	0-3	.365	90.0	79.0
		25	0-3	.283	89.0	76.0
Roy et al. (1977)	FPC	35	0-3	.88	87.0	83.0
		65	0-3	.66	83.0	75.0
Opstvedt et al. (1978)	FPC	0(milk)	1-9	.514	98.3	96.1
		36	1-3	.142	94.5	84.4
		36	4-5	.337	97.0	93.7
		96	1-9	.303	95.3	90.5
Huber et al. (1978)	Predigested FPC	0(milk)	0-6	.416		
		33	0-6	.303		
	SDFS[d]	16.5	0-6	.356		

[a]Average daily gain.
[b]Dry matter digestibility.
[c]Protein digestibility.
[d]Spray dried fish solubles.

were different than obtained in most studies.

After weaning, fish protein seems to be good for lambs (Orskov et al., 1971; Fraser and Orskov, 1974; Folman and Eyal, 1978) and calves (Preston et al., 1960,1965; Whitelaw et al., 1961,1963; Kay et al.,1967), probably due to its low degradability in the rumen.

The extraction procedure used in FPC production can influence nutritional value of the product. The importance of complete removal of solvent residues was emphasized by Morrison et al. (1962).

Isopropanol-extracted fish protein concentrate (IP-FPC) was shown equal or superior to casein as the only protein source for rats and pigs (Power, 1964; Sidwell et al., 1970; Pond et al., 1971). The IP-FPC was also better than dichloroethane-extracted fish protein (DCE-FPC) (Munro and Morrison, 1967b; Ershoff and Rucker, 1969; Makdani, 1969; Makdani et al., 1971a,1971c) and hexane-heptane-extracted fish protein concentrate (Makdani et al., 1974). Increased biological value was obtained when DCE-FPC was washed with ethanol, suggesting removal of certain polar-soluble substances that adversely affected protein quality (Makdani et al., 1971a).

Munro and Morrison (1967a) concluded that extraction with dichloroethane resulted in formation of chlorocholine chloride (CCC), a choline derivative toxic to rats. The CCC is a potent inhibitor of acetyl choline esterase (Friess and McCarville, 1954). Later, Munro and Morrison (1967b), suggested that toxicity of DCE-FPC in rats could not be explained by CCC formation alone. Although the toxic factor(s) were extracted by methanol, the resultant material still did not support growth, even when supplemented with cystine and histidine. The authors suggested that methanol did not remove all of the toxic substances from DCE-FPC.

Makdani et al. (1971b) suggested that FPC extracted with isopropanol was more desirable for humans than DCE-FPC. Surprisingly, calves fed DCE-FPC grew better than those fed IP-FPC (Makdani et al., 1971c). Reasons for this discrepancy were not apparent.

Protein digestibility and nitrogen retention were depressed when FPC constituted the most of the dietary protein for calves (Huber and Slade, 1967; Matre, 1970; Sleiman and Huber, 1971; Gorrill et al., 1975; Huber, 1975; Roy et al., 1977; Opstvedt et al., 1978). Protein digestibilities for FPC have been 10 to 30% lower than milk protein. Ether extract digestibilities were also depressed by inclusion of FPC in milk replacers, but carbohydrate utilization appeared unaltered (Huber and Slade, 1967; Matre, 1970; Roy et al., 1977). Reduced fat digestibility might be explained by the high ash content of FPC which may have increased fecal excretion of calcium and magnesium soaps (Raven and Robinson, 1959). Negative results on fish protein may also be due to relatively indigestible long chain fatty acids in fish oil (Flatlandsmo, 1972).

Sleiman and Huber (1967) observed higher digestibilities in young calves when FPC was combined with whey instead of casein. For rats, best nutritive values were obtained when FPC was combined with wheat flour or rice.

Gorrill et al. (1972) observed reduced nitrogen retention in the absence of a change in protein digestibility when calves were fed FPC. This finding suggests an amino acid imbalance.

Generally, FPC compares favorably with whole egg in terms of amino acid composition, but it is lower in tryptophan and cysteine (Sidwell et al., 1970). Compared to skimmilk, FPC is slightly lower in tyrosine, phenylalanine, leucine, isoleucine and valine, but higher in arginine and lysine (Raven and Robinson, 1959). Under certain conditions, an excess of arginine and lysine were detrimental to chick growth (Huston

and Scott, 1966).

"In vitro" studies conducted by Ellinger and Boyne (1965) showed that heat treatment (105°C for 36 hr) of cod muscle caused significant losses of serine (8%), tyrosine (6%), lysine (11%), histidine (12%), and cysteine (64%). Alanine and methionine appeared the most stable amino acids.

Marked differences of FPC sources in availability to rats of certain amino acids such as methionine and histidine, were reported (Morrison, 1963). However, Morrison and Sabry (1963) concluded that unavailability of methionine could not explain entirely the reduced nutritional value of FPC since methionine supplementation did not improve growth.

Stillings et al. (1969) conducted a series of experiments with rats to determine the sequence of limiting amino acids in IP-FPC. Grouped according to limitation from most to least limiting were: 1) methionine; 2) histidine, tryptophan and threonine; 3) valine, isoleucine and phenylalanine; 4) leucine, lysine and arginine. Makdani et al. (1971a) also concluded that methionine and histidine were co-limiting in FPC regardless of fish species or extraction processes. Histidine or methionine supplementation alone were equal in increasing growth of rats fed FPC diets. Whereas, histidine plus methionine were additive. On the other hand, methionine supplementation to a milk replacer containing DCE-FPC did not improve calf growth (Makdani et al., 1971c). More recently, Matre (1977) suggested that isoleucine might be the limiting amino acid for calves fed protein from mackarel flour.

Morrison and Munro (1965) demonstrated that 1,2-dichloroethane can react with free sulfhydryl groups of cysteine to yield a thioether, which

they hypothesized reduces susceptibility of proteolytic attack (action of pancreatin). Specifically, the dihalide reacts with sulfhydryl groups on adjacent peptide chains and a stable thioether bridge forms which might bind peptide chains more tightly, thus reducing ease of proteolysis. Available histidine was also reduced, but a direct reaction of histidine with ethylene dichloride is unlikely. Rather, the histidine may occur adjacent to cysteine and be indirectly affected.

Other explanations for the lower performance of calves fed fish protein concentrate than milk protein were contaminants from extraction with DCE causing aplastic anemia (Pritchard et al., 1952); and passage of the diet into the rumen causing bloat (Makdani et al., 1971c).

Fish meal has also been tested in milk replacers for calves (Rupel and Wilson, 1962; Wendlandt et al., 1968; Genskow et al., 1968; Gorrill et al., 1975) but performance was generally poorer than for FPC.

White muscle disease (WMD) has been associated with calves fed fish products (Genskow, 1969; Michel et al., 1972). Calves are most susceptible to WMD from birth to 6 months of age (Poukka, 1966), probably because of rapid changes in tissue fatty acid composition.

According to Horwitt (1956), nutritional WMD in mammals occurs only when certain of the polyunsaturated fatty acids in the muscle lipids reach a high degree of "peroxidizability" in a given time. Therefore, increased linoleic and arachadonic acids in muscle predispose young calves to WMD.

The pathogenesis of WMD is not clear and divergent views are found in the literature; but the three factors most often discussed in

connection with the disease are vitamin E, selenium and unsaturated fatty acids. Fish fats contain high percentages of unsaturated fatty acids (Medwaddowski et al., 1967; Lovern, 1969) and played an important role in experimentally induced WMD (Adams et al., 1954; Blaxter and McGill, 1954). Dam nutrition may also predispose calves to WMD, notably when fed milk high in polyunsaturated fatty acids (Hidiroglou et al., 1968,1977).

Vitamin E and selenium function as biological antioxidants, but selenium possesses 50 to 500 times the antioxidant activity of alpha-tocopherol (Hamilton and Tappel, 1963). White muscle disease is cured by vitamin E (Blaxter et al., 1953; Harris and Embree, 1963; Roy, 1964), but amount needed in milk replacers is debatable.

Michel et al. (1972) observed muscular degeneration in 8-week-old calves fed fish protein concentrate as the sole protein source despite supplementation with 46 mg vitamin E/kg of dry ration. In a second experiment, 46 mg of vitamin E/kg of dry ration prevented the histopathologically-detectable degeneration in FPC-fed calves. The conflicting results may have been due to different batches of FPC in the two experiments. Despite the absence of muscle degeneration in calves of the second experiment receiving 46 mg/kg alpha-tocopherol, supplementation of 92 mg E/kg increased growth above that at lower E additions.

Other Protein Sources in Milk Replacers

Bacterial sludge (containing about 60% crude protein and 30% lactose) in combination with skimmilk and whey did not affect incidence

of diarrhea, dry matter and nitrogen digestibilities, and nitrogen retention when substituted for up to 35.6% of the milk protein in milk replacers (Bouchard et al., 1973).

Raven (1972) reported decreased nitrogen retention when 13.5% meat meals were substituted for milk protein. Polzin et al. (1976) also reported lower nutrient utilization in calves fed meat meals compared to those fed casein. They observed that meat meals contain mainly collagen, which is approximately 13% hydroxy-proline and is largely unavailable to the calf.

Weight gains and apparent digestibilities of nitrogen, ether extract and energy showed significant linear declines when increasing levels of distillers dried solubles were substituted for dried skimmilk and lactose (Bryant et al., 1967). However, the authors concluded that distillers dried solubles could replace up to 35% of the digestible protein in milk substitute diets for herd replacements without severely impairing growth.

Blood meal and blood flour were of comparable value in milk replacers, resulting in slightly lower growth when compared to all-milk protein (Brubaugh and Knodt, 1952).

Fababean (up to 25%; Wittenberg and Ingalls, 1979), a rapeseed (70 to 30%; Gorrill et al., 1976), alfalfa (up to 50%; Alpan et al., 1979) and crab meal (up to 20%; Patton et al., 1975) are other protein sources giving acceptable calf performances.

Addition of Enzymes to Milk Replacers

As mentioned before, one of the hypothesis raised to explain lower digestibilities of non-milk constituents in milk replacers was low concentration of enzymes in the gastrointestinal tract of calves. To be effective, an enzyme fed to young calves must be active in the acid conditions of the abomasum, or must escape destruction in the abomasum to be active in the small intestine.

Since calves secrete a limited amount of amylase (Huber et al., 1961a; Morrill et al., 1970a), the inclusion of amylolytic enzymes in milk replacers containing high levels of starch or inclusion of pre-digested starch, has been successful in some studies (Morrill et al., 1970b; Thivend et al., 1979) and unsuccessful in others (Raven and Robinson, 1965; Bell et al., 1974).

Chow and Bell (1976) showed that in "in vitro" conditions, the amount of trichloroacetic acid soluble protein could be greatly improved by enzymatic treatment of pea products. Later, Bell et al. (1979) fed young calves milk replacers containing 0 to 75% field pea protein with low or high levels of supplemental proteolytic and amylolytic enzymes. They concluded that gross energy and protein digestibilities were higher for the control replacer than the enzyme-treated rations.

Jenkins et al. (1980) tested "in vitro" hydrolysis of milk, soybean and fish proteins by several enzymes. They concluded that all enzymes studied hydrolyzed the milk proteins more extensively than the non-milk proteins, both at their optimum pH and at pH 6.1, which they suggested was comparable to calf abomasal contents immediately after feeding. They

also showed that papain and pronase preparations were quite efficient in degrading almost all the milk and non-milk proteins tested. However, enzyme addition or pre-digestion of milk replacers containing soybean protein (Fries et al., 1958; Lassiter et al., 1959; Jenkins, 1981) or fish protein (Toullec et al., 1972; Wilson, 1973; Soliman et al., 1976; Dodsworth et al., 1977; Huber et al., 1978; Petchey et al., 1979) did not improve nutrient digestibilities or calf performance.

Limestone As a Buffering Agent

Much of the current knowledge about post-abomasal pH has been extrapolated from information available for monogastrics with the assumption that digestion in monogastrics and ruminants differs only in the forestomach. However, species differences may extend to the lower tract (Wheeler, 1980a).

Gastric contents from the human stomach are rapidly neutralized in the duodenum (Borgstrom et al., 1957), while limited information for ruminants suggests that the acidic digesta entering the small intestine is slowly neutralized. Harrison and Hill (1962) found that pH of duodenal digesta in sheep increased slowly from 2.7 at the proximal duodenum to around 4.0 beyond the entry of the common bile and pancreatic ducts. Low pH values caudal to these ducts suggest that duodenal secretions of ruminants have a limited neutralizing capacity. This conclusion is supported by Lennox and Garton (1968) and Lennox et al. (1968), who found that conditions in the upper jejunum of sheep were notably acidic (pH of 2.0 or 3.0) and that pH did not reach values of 6.0 or 7.0 until

the lower jejunum. Several other workers (Smith, 1962; Phillipson and Storry, 1965; Topps et al., 1968) indicate a possible limit to the capacity of the small intestine of ruminants to neutralize acidic digesta from the abomasum.

The persistent acidity of digesta in the small intestine of ruminants may be attributed to the continuous secretion of hydrochloric acid by the abomasum (Phillipson, 1970) and/or to the weak alkaline nature of secretions entering the small intestine. Reports have indicated that from 300 to 400 ml of pancreatic juice (Magee, 1961; Taylor, 1962) and about 700 ml of bile (Hill, 1970) are secreted daily into the small intestine of sheep. However, these intestinal secretions have little buffering ability compared to duodenal secretions from monogastrics such as dog and man (White et al., 1959).

Wheeler (1980a) suggested that reductions in digestibility and physiological abnormalities in ruminants may be associated, at least in part, with changes in the gastrointestinal environment. The use of buffers in diets for dairy cows is extensive, although it is not well understood how and when buffers are beneficial (Muller, 1981). Dietary buffers are often fed to milk cows to counteract acidity and maintain a near normal pH in the digestive tract, especially the rumen.

Compounds such as limestone and dolomitic limestone exert a buffering influence in the small intestine of ruminants (Wheeler and Noller, 1976; Wheeler, 1979,1980a,1980b). These products add alkaline reserves to the lower digestive tract and help raise the pH in the small intestine. Fecal pH has been considered as a good indicator

of intestinal pH (Wheeler and Noller, 1977; Wheeler, 1980a). Limestone instead of sodium or potassium bicarbonate better buffer the intestine, since sodium and potassium are readily absorbed from the forepart of the digestive tract (NRC, 1978). Magnesium oxide has also been shown to increase fecal pH and may function as a buffer in the small intestine (Erdman et al., 1980).

The source of particle size of limestone influences its reactivity and effectiveness as a buffer (Wheeler, 1980b). Present recommendation for limestone addition to lactating cow diets is .5 to .8% of the total ration (Muller, 1981).

The use of limestone or sodium bicarbonate in pre-ruminant diets has not been extensively studied. Beneficial effects of bicarbonate addition to colostrum were associated with higher immunoglobulin absorption due to pH changes in the small intestine (Foley et al., 1978) and a bacteriostatic action (Harrison and Peat, 1972). Sodium bicarbonate has also been used routinely as fluid therapy for calves with diarrhea (Church, 1971).

Summary of the Literature Review

From the literature it may be concluded that the substitution for milk protein of soybean or fish products has to be partial, and even in this situation, poorer calf performance may be expected when compared to all-milk protein.

Several reasons might explain these negative results of substitute protein sources. First, protein quality of soybean and fish products

is generally lower than milk protein. Also, industrial procedures may further alter the quality of these non-milk proteins by denaturation or introduction of toxic compounds during the process.

In general, non-milk proteins have resulted in impaired curd formation in the abomasum, perhaps due to reduced HCl and rennin secretions. In some experiments, secretion of pancreatic proteolytic enzymes has been reduced when non-milk proteins were fed resulting in a faster flow of protein through the digestive tract with consequent accumulation of undigested protein in the lower gut. This lowered protein utilization and greater fermentation of undigested nutrients in the lower gut might explain the poor calf performance.

With soybean products, the presence of SBTI, problems caused by the polyssacharide and oil fractions, and low levels of certain essential amino acids (methionine and lysine) were frequently claimed as reasons for reduced growth on milk replacers. Industrial processing and amino acid supplementation has improved the quality of soybean products used in milk replacers. More recently, allergic-type reactions in calves have been observed and were associated with changes in the intestinal mucosa structure and reduced nutrient absorption.

Quality of the fish products varies widely, depending on the processing method used. Denaturation of protein, presence of toxic compounds, low levels of essential amino acids, high ash content and polyunsaturated fatty acids have all been linked with the negative results observed when these products were fed.

The addition of enzymes or hydrolysis of non-milk proteins before

incorporation into milk replacers has not, as yet, improved calf performance.

For adult ruminants, it has been suggested that reductions in digestibility and physiological abnormalities may be associated, at least in part, with the persistent acidity of digesta in the upper small intestine. Limestone has been shown to raise the pH of the small intestine and improve nutrient utilization. Until now, no information was available on addition of limestone to milk replacer diets for calves.

Spray dried fish solubles (SDFS) is a new industrial product, high in soluble protein, desirable features for a milk replacer. Questionable results in a previous experiment at Michigan State (Huber et al., 1978) with SDFS were shown.

Experiment 1 was to further test SDFS in a large experiment involving three different locations. Experiment 2 was designed to explain results observed in Experiment 1. Experiment 3 tested limestone as a buffering agent at the small intestine of young calves. Possible beneficial effects on nutrient utilization of milk replacers containing a high level of soybean protein concentrate were investigated.

MATERIALS AND METHODS

Materials and Methods will be divided into three experiments which had different objectives, different animals and were chronologically separate.

Experiment 1

A total of 168 Holstein calves at three locations -- Michigan State University (62 males), Kansas State University (37 males and 13 females), and Cornell University (56 males) -- were fed milk replacers containing different protein sources and levels. The objective of this experiment was to study in baby calves the effect of the partial substitution of spray-dried fish solubles and/or soybean protein concentrate for milk protein on growth, health, plasma amino acid concentration and xylose absorptive capability of the small intestine.

At Cornell, calves were bought through a calf auction; at Kansas State, five calves were purchased and the remainder were from the Kansas State herd; and at Michigan State all calves were purchased from a large commercial herd and transported to the experimental facilities of Kellogg Farm at Battle Creek, Michigan, when 3 to 8 days old.

Preliminary meetings were held among leaders of this project to establish standard procedures and management specifications to be followed

at the different locations.

The day calves started on the experiment they were identified by an ear tag and navels were disinfected with iodine solution. Each calf received an injectable solution containing 500,000 IU of vitamin A, 75,000 IU of vitamin D, and 50 IU of vitamin E, and was randomly assigned to experimental diets. All animals were kept indoors in tie stalls or individual pens bedded with straw.

Each calf received the designated milk replacer as the only source of nutrients at 8, 9, 10, 11, 12 and 12% body weight from first to sixth week. Solids content of all milk replacers after mixing with water was 14%. Calves were fed twice daily (12 hr apart) from open pails. Fresh water was available at all times. Experimental diets according to protein percent and sources were:

Treatment 1: 13% crude protein (CP); 13% milk protein (MP) (negative control)

Treatment 2: 19% CP; 19% MP

Treatment 3: 19% CP; 13% MP; 6% spray-dried fish solubles (SDFS)

Treatment 4: 19% CP; 13% MP; 6% soy protein concentrate (SPC)

Treatment 5: 19% CP; 13% MP; 3% SDFS, 3% SPC

Treatment 6: 23% CP; 23% MP

Treatment 7: 23% CP; 13% MP; 10% SDFS

A sample was taken from each 22.7 kg sack of milk replacer used and composited for laboratory analyses. Samples of the original ingredients were also obtained. Ingredients and replacers were analyzed for dry matter (forced air oven at 65^{o}C), crude protein (macro-Kjeldahl),

ether extract and ash as described by A.O.A.C. (1980). Ingredient composition of milk replacer diets is shown in Table 3 and chemical

TABLE 3. Ingredient composition of milk replacer diets (%), Experiments 1 and 2

Ingredient	Diet Number						
	1	2	3	4	5	6	7
Protein fat mix (12/50)[a]	41.00	40.00	40.00	40.00	40.00	40.00	40.00
Dried skimmilk	24.60	42.00	24.00	24.00	24.00	54.00	24.00
Spray dried fish solubles	-----	-----	10.00	-----	5.00	-----	17.00
Soy protein concentrate[b]	-----	-----	-----	9.00	4.50	-----	-----
Dextrose	17.40	17.00	17.00	17.00	17.00	5.00	17.00
Lactose	16.40	-----	8.00	9.00	9.50	-----	1.00
Vitamin-mineral premix[c]	.63	.63	.63	.63	.63	.63	.63
Chlorotetracycline	.01	.01	.01	.01	.01	.01	.01
Lysine[d]	-----	-----	.15	.15	.15	-----	.26
Methionine[d]	-----	-----	.09	.09	.09	-----	.15
TOTAL	100.04	99.64	99.88	99.88	100.88	99.64	100.05

[a]Spray-dried mixture prepared to contain 12% milk protein and 50% fat (45% animal fat and 5% soy licithin).
[b]Mixture in equal amounts of three commercial soy protein concentrates: Procon, Promocalf and Ardex.
[c]Containing (per kg), 33,000 IU vitamin A, 6,600 IU vitamin D, 22 mg of vitamin E, Mg, Cu, Co, Zn, Mn and I.
[d]Added to equal the level of these amino acids in the milk protein at each particular percent of protein studied.

composition of ingredients and diets in Table 4.

TABLE 4. Chemical composition and pH of protein substitutes and formulated diets[a] (Experiments 1 and 2)

Item	DM(%)	CP(%)	EE(%)	Ash(%)	pH[b]
Whey	92.55	13.80	.72	8.94	5.90
Fish solubles	84.34	61.60	14.44	18.86	4.70
Ardex	90.98	66.25	.99	3.65	6.55
Dextrose	90.76	.06	1.21	.02	6.50
Lactose	96.52	.12	.07	.18	6.30
Promocalf	91.56	67.20	.37	5.95	6.70
Procon	89.39	67.80	.13	5.97	6.70
Diet 1	94.50	13.73	19.91	4.39	6.52
Diet 2	94.43	20.25	21.34	5.75	6.55
Diet 3	94.02	20.30	20.48	6.43	6.05
Diet 4[c]	94.69	20.14	20.46	4.67	6.60
Diet 5[c]	94.60	20.08	23.14	5.46	6.27
Diet 6	94.54	23.96	22.86	6.61	6.49
Diet 7	93.24	24.15	24.64	7.12	5.89

[a]CP = crude protein; EE = ether extract and ash are presented on dry matter basis.
[b]The pH was determined after diluting with water to 14% solids.
[c]Equal portions of Ardex, Promocalf and Procon were mixed in diets 4 and 5.

When a calf assigned to the experiment died prior to completion of treatment, the first calf available thereafter was the replacement. All animals which died during treatment at Michigan State were submitted for necropsy at the Veterinary Diagnostic Laboratory. The number of treatment replicates by location is shown in Table 5.

Calves were weighed individually for 2 consecutive days at the beginning and at the end of the trial. Weights were also taken when calves were 3 weeks old.

TABLE 5. Number of calves per treatment in each location (Experiment 1)

Diets	Locations		
	Michigan State	Kansas State	Cornell University
1	9	6	8
2	9	7	8
3	9	8	8 (7)[a]
4	9	7	8
5	9	7	8
6	9	8	8
7	8	7	8 (7)[a]
Total	62	50	56 (54)

[a]At Cornell University one calf from diet 3 and one other from diet 7 were lost when 5 and 3 wk old, respectively. Those calves were not replaced.

Daily observations were made on each animal for degree of scouring by rating fecal consistency on an index of 1 to 4 (Larson et al., 1977).

Rectal temperatures were taken daily (just before the morning feeding) for each animal during the first 2 weeks of trial.

Treatment for illnesses were recorded on weekly basis. When a calf was treated for an illness it was listed as a single treatment eventhough the treatment may have been repeated for more than 1 day in the same week.

At Michigan State, blood samples were taken from the jugular vein for immunoglobulin and plasma protein analyses at 24 and 48 hours after arrival of calves. Serum immunoglobulin (zinc sulphate turbidity) and

protein (macro-Kjeldahl) were determined, respectively, as described by Pfeiffer et al. (1977) and A.O.A.C. (1980).

At Michigan State, jugular blood samples were taken 8 hours after the morning feed from four randomly assigned calves on each diet when 3 and 6 weeks old. Blood was processed and analyzed for plasma amino acids as described by Foldager et al. (1977).

The xylose absorption test was used at Michigan State State to evaluate intestinal absorptive capacity of calves. Xylose tests were performed for all 62 calves at the termination of the feeding trial. Calves were fasted for 24 hours before administering xylose via nipple pail at .5 g/kg of body weight in a 10% aqueous solution. Jugular blood was sampled just before and .5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 and 5.0 hours after xylose ingestion. Plasma xylose was analyzed by the orcinol/ferric chloride spectrophotometric method as described by Seegraber and Morrill (1979).

Except for plasma amino acid concentrations, xylose absorption capability, and mortality, all variables were analyzed as completely randomized designs. Plasma amino acid and xylose absorption capability were analyzed as split-plot designs (treatments as plots and time as subplots). Mortality data were analyzed as contingency tables. F-test was used when only two means were compared; tests involving more than two means were as indicated in the table footnotes. Analysis of variance for all variables in this experiment are shown in Table A.1 (Appendix) and were processed as described by Gill (1978a, 1978b, 1978c).

Experiment 2

This experiment was a digestibility trial conducted at Michigan State University from October 26 to December 13, 1979, involving diets 1 to 4 from experiment 1. Our objective was to determine differences due protein sources in apparent dry matter and organic matter digestibilities and nitrogen retentions.

Calves (4 per treatment) were placed on experiment between 3 and 8 days of age and were fed and managed similarly to trial 1. Blood samples were obtained at calve's arrival for plasma protein determinations. All animals were kept in metallic metabolism cages for 2 weeks.

Calves were weighed at the beginning and end of the trial, and fecal consistencies were rated from 1 to 4 (Larson et al., 1977). Rectal temperatures were taken daily just before the morning feeding.

During the last 5 days of treatment, feces and urine were totally and jointly collected, weighed and homogenized in a blender. Composite samples of the 5-day collections were made by mixing 10% of each day's excreta. Samples were kept at -20°C until analyzed for dry matter (forced-air oven at 65°C), ash (for organic matter), and nitrogen (macro-Kjeldahl) according to A.O.A.C. (1980).

When a calf died prior to completion of treatment, the first calf available thereafter was used as a replacement. Mortality data were analyzed as contingency tables and all other variables as completely randomized designs (Table A.2) as described by Gill (1978a,1978b,1978c). Tests chosen for comparison of means are indicated as footnotes in each table.

Experiment 3

Thie experiment was conducted at the Dairy Research and Teaching facilities of Michigan State University from November 23, 1981 to January 25, 1982. The main objective was to study limestone as a potential buffer in the small intestine of preruminant calves fed milk replacers in which 50% of the milk protein was replaced by soy protein concentrate. The experimental treatments were as follows:

Treatment A: 19% CP; 19% MP

Treatment B: As A, but containing .8% limestone

Treatment C: 19% CP; 9.5% MP; 9.5% SPC

Treatment D: As C, but containing .8% limestone

A sample was taken from each 22.7 kg sack of milk replacer used and composited for dry matter (forced-air oven at 65°C), crude protein (macro-Kjeldahl), ether extract and ash determinations as described by A.O.A.C. (1980). Ingredient composition of milk replacers is shown in Table 6, and chemical composition in Table 7.

Sixteen male Holstein calves were purchased at 3-4 days of age from a commercial dairy farm and randomly allotted to treatments. Initial care of calves after arriving at Michigan State was the same as Trial 1.

For 42 days each calf received its designated milk replacer as the only source of nutrients at 8, 9, 10, 11, 12 and 12% of body weight from weeks 1 to 6, respectively. Total solids of all milk replacers after mixing with water was 14%. Replacers were offered to calves twice daily (12 hr apart) in open pails. Fresh, clean water was available at all times. Animals were kept in individual pens bedded with straw except

TABLE 6. Ingredient composition of milk replacers (%) (Experiment 3)

Ingredients	Diets			
	A	B	C	D
Dried whey	19.5	19.34	19.5	19.34
Sodium caseinate	8.2	8.13	----	-----
Skimmilk	20.3	20.14	14.0	13.89
7/60 High fat mixture[a]	33.3	33.03	33.3	33.03
Soy protein concentrate	----	-----	14.2	14.09
Dextrose	17.4	17.26	17.4	17.26
Premix(minerals and vitamins)[b]	1.37	1.36	1.37	1.36
L-lysine	----	----	.09	.09
DL-methionine	----	----	.15	.15
Limestone	----	.80	----	.80
TOTAL	100.07	100.06	100.01	100.01

[a]Spray-dried mixture prepared to contain 7% milk protein and 60% fat (lecithin included).
[b]Containing (per kg) 33,000 IU vitamin A, 6,600 IU vitamin D, and 22 mg vitamin E. Diets 1 and 3 were calculated to contain 1% calcium, .7% phosphorus and .1 ppm of selenium.

TABLE 7. Chemical composition of diets[a] (Experiment 3)

Items	Diets			
	A	B	C	D
Dry matter (%)	96.39	96.58	96.43	95.64
Crude protein (%)	18.53	18.15	18.42	18.33
Ether extract (%)	13.24	14.21	14.17	14.25
Ash (%)	6.52	7.06	6.44	7.12

[a]Crude protein, ether extract and ash are presented on dry matter basis.

during collection periods when they were placed in metallic metabolism cages.

Animals were weighed individually for 3 consecutive days at the beginning and at the end of the trial and at weekly intervals during treatment.

Scour scores and rectal temperatures were recorded as described in trial 1, except that rectal temperatures were taken throughout the entire 6 weeks of the experiment.

Calves were moved from individual pens to metabolism cages on days 14 and 35 of experiment. If a particular calf showed diarrhea or was under medication at the beginning of the third week, its transport to the metabolism cage was postponed for 1 week. For this reason, the average age of calves at the beginning of the first collection period was 17, 20, 19 and 20 days for treatments 1, 2, 3 and 4, respectively.

Total feces and urine were collected in separate containers for 4 days at 7 AM. At collection, 10% of the total feces and 5% of the total urine were saved, composited and kept in the freezer at -20^{o}C until analyses for dry matter (forced-air oven at 65^{o}C), crude protein (macro-Kjeldahl), and ash (for organic matter) as described by A.O.A.C. (1980). Separate samples of fresh feces were taken for pH determination after dilution with distilled water. The xylose absorption test was performed in all calves on days 21 and 40 of treatment, exactly as described for experiment 1.

Jugular blood samples were taken from each calf 5 hours after the morning feed on days 23 and 42 for plasma urea nitrogen determinations as described by Fawcet (1960).

At the end of the trial, two calves, randomly chosen from each treatment, were sacrificed (by electrocution) at the Veterinary Diagnostic

laboratory of Michigan State University. After removal of the entire digestive tract, weights were recorded for liver, pancreas, rumen, abomasal, cecal and large intestinal contents. Small intestines were divided in three equal sections (from anterior to posterior) and weights were recorded separately for contents. Samples of all contents were kept in freezer until laboratory analysis for pH, dry matter, crude protein (macro-Kjeldahl), and ash as described by A.O.A.C. (1980).

When a calf died prior to completion of treatment, the first calf available thereafter was a replacement. All dead animals were submitted for necropsy at the Veterinary Diagnostic Laboratory at Michigan State.

Initial body weights were analyzed as a completely randomized design. All other variables were analyzed as split-plot designs. F-test was used when only two means were compared; tests involving more than two means were as indicated in the table footnotes. Analysis of variance for all variables was as described by Gill (1978a,1978b,1978c), and these analyses are shown on Table A.3 (Appendix).

RESULTS AND DISCUSSION

Experiment 1

Initial body weights were higher (P<.10) at Kansas (43.6 ± .5 kg) than at Cornell (42.1 ± .6 kg) or Michigan (41.0 ± .5 kg). This same order for weight continued throughout the experiment.

At Michigan State, there was no difference (P>.10) between treatments in initial body weights or plasma protein and immunoglobulin levels (Table 8) of calves at arrival. Initial body weights were also not

TABLE 8. Initial mean body weight, immunoglobulin and protein levels in plasma of calves at Michigan State (Experiment 1)

Treatment	No. of calves	Initial body weight	Immunoglobulin	Plasma protein
		kg	mg %	%
1. 13% MP	9	41.1[a]	7.6[a]	5.03[a]
2. 19% MP	9	42.3[a]	7.9[a]	5.23[a]
3. 13% MP, 6% SDFS	9	39.7[a]	10.7[a]	5.41[a]
4. 13% MP, 6% SPC	9	42.6[a]	9.1[a]	5.26[a]
5. 13% MP, 3% SDFS, 3% SPC	9	40.4[a]	8.8[a]	5.33[a]
6. 23% MP	9	40.1[a]	9.4[a]	5.19[a]
7. 13% MP, 10% SDFS	8	40.9[a]	7.5[a]	4.92[a]
SEM		±1.5	±1.2	±0.18

[a]Means in columns not sharing the same superscript are different at P<.10 using Tukey's test.

different (P>.10) between treatments when data from all three locations

were analyzed together, being 41.9±.9, 42.3±.9, 41.3±.8, 43.0±.9, 41.9±.9, 41.9±.8 and 41.5±.9 kg for treatments 1 through 7, respectively. These results suggest homogenity among treatment groups.

Least square means of body weight gains for diets and locations are in Table 9. There was a difference ($P<.05$) among locations with higher gains at Cornell and Kansas than at Michigan. During the first 3 weeks of trial treatments 2 and 6 produced higher gains ($P<.05$) than all others. Treatment 7 gave the worst performance although not significantly different ($P>.05$) from 1, 3 and 5. During the last 3 weeks, treatments 2 and 6, containing higher amounts of milk protein, were again superior ($P<.05$) to all others. Treatment 4, containing 6% SPC, was inferior ($P<.05$) to 2 and 6 but superior ($P<.05$) to the negative control and to treatments containing SDFS. For the total period (0-6 weeks), treatments were in approximately the same order as for 0 to 3 and 3 to 6 weeks. However, an interaction ($P<.01$) between diet and location was observed, probably because diet 5 gave poorest gains at Michigan but relatively better gains at Kansas.

Detrimental effects of SDFS seemed greater during 0 to 3 weeks when animals were more sensitive to diet quality (Huber, 1975; Roy et al., 1977).

Among diets containing SDFS, treatment 5 in which 31% of the replaced protein was shared equally by SPC and SDFS showed the greatest weight gains, although they were not different ($P>.05$) from the negative control. As the amount of SDFS in the diet was increased, performance was reduced. Huber et al. (1978) concluded that SDFS successfully replaced SPC at 8 and 16% of the total dietary protein. These results

TABLE 9. Least square means of daily weight gains (g/animal) for calves fed milk replacers varying in protein content and source by location, diets, and age (experiment 1)

Age	Treatments	Cornell	Kansas	Michigan	Mean	SEM
weeks						
0-3	1	27	-22	-50	-15^{bc}	26
	2	59	160	-10	70^{a}	25
	3	-67	-19	- 5	-30^{bc}	25
	4	54	- 9	-29	5^{b}	26
	5	-16	77	-84	8^{bc}	26
	6	65	186	60	104^{a}	25
	7	9	-98	-92	-60^{c}	26
	Mean	$19^{A}\pm17$	$39^{A}\pm18$	$-30^{B}\pm16$		
4-6	1	389	238	286	304^{d}	28
	2	597	605	478	560^{a}	27
	3	361	394	288	345^{cd}	27
	4	510	447	403	453^{b}	27
	5	383	435	310	376^{c}	27
	6	529	664	482	558^{a}	26
	7	352	296	375	341^{cd}	41
	Mean	$446^{A}\pm18$	$439^{A}\pm19$	$375^{B}\pm17$		
0-6[e]	1	208^{bc}	108^{c}	118^{c}	145^{cd}	19
	2	328^{a}	383^{a}	221^{ab}	315^{a}	19
	3	151^{c}	181^{bc}	142^{bc}	160^{cd}	19
	4	282^{ab}	219^{b}	187^{ab}	229^{b}	19
	5	184^{c}	256^{b}	104^{c}	184^{c}	19
	6	297^{ab}	425^{a}	271^{a}	331^{a}	18
	7	181^{c}	99^{c}	142^{bc}	140^{d}	19
	Mean	$232^{A}\pm12$	$240^{A}\pm13$	$172^{B}\pm11$		

[abcd] Means in columns within each age not sharing a common superscript are different at P<.05 using Tukey's test.

AB Means in the same row not sharing a common superscript are different at P<.05 using Tukey's test.

e Interaction between diets and locations was significant (P<.01).

were not repeated in the present work, perhaps due to differences in the two SDFS products. Negative effects on growth of young calves fed milk replacers containing fish protein were reported by others (Rupel and Wilson, 1962; Genskow et al., 1968; Gillespie, 1971; Sleiman and Huber,

1971; Huber et al., 1978).

Digestibility of fish protein incorporatedinto milk replacers was 10 to 30% lower than milk protein (Huber and Slade, 1967; Sleiman and Huber, 1971; Huber, 1975). Ether extract digestibility was also depressed but carbohydrate utilization was unaltered (Huber and Slade, 1967). Roy et al. (1977) realized that protein digestibility of non-milk sources was low, so diets were formulated with higher protein to compensate for the low digestibility. This rationale was the reason for diet 7, which contained 23% CP, with 10% from SDFS. Because calf growth was inferior and mortality higher on this than on diet 3 (19% CP with 6% from SDFS), we concluded that lack of digested protein was not the primary problem with diets containing high levels of SDFS.

Retention of digested nitrogen was lower for calves receiving fish than milk protein, suggesting poor availability of essential amino acids (Sleiman and Huber, 1971). At all locations, lysine and methionine were added to fish and soy protein diets to equal concentrations of these amino acids in the respective milk diets.

Diet and age effects on plasma free amino acids are presented separately in Tables 10 and 11 since the interaction between diet and age was not significant for most amino acids (Table A.1 Appendix). An increase in dietary CP from 13 to 19% in diets containing only milk protein resulted in higher ($P<.10$) plasma total essential amino acids (TEAA). Total nonessential amino acids (TNEAA), sulfur amino acids (SAA) and branched chain amino acids (BCA) and most individual amino acids also increased, but not significantly ($P>.10$). A further increase

TABLE 10. Effect of diets on plasma free amino acids (μmoles/100 ml) for calves fed milk replacers varying in protein level and source (Experiment 1)

Amino acids	Treatments							
	(1) 13% MP	(2) 19% MP	(3) 13% MP+ 6% SDFS	(4) 13% MP+ 6% SPC	(5) 13% MP+ 3% SDFS +3% SPC	(6) 23% MP	(7) 13% MP+ 10% SDFS	SEM
No. calves	4	4	4	4	4	4	4	
Thr	5.7[b]	13.8[a]	7.2[ab]	7.2[ab]	9.7[ab]	8.9[ab]	9.0[ab]	2.4
Val	11.2[b]	18.3[ab]	12.1[b]	14.6[ab]	14.5[ab]	20.0[a]	15.8[ab]	1.8
Met	1.8[a]	2.8[a]	2.2[a]	1.6[a]	1.7[a]	2.5[a]	3.0[a]	.4
Ile	4.6[b]	7.0[ab]	4.8[b]	6.4[ab]	4.9[ab]	9.2[a]	6.1[ab]	1.0
Leu	6.5[b]	9.8[ab]	6.6[b]	8.9[ab]	8.3[ab]	13.0[a]	8.5[ab]	1.3
Phe	4.8[a]	6.5[a]	4.6[a]	6.3[a]	6.0[a]	6.6[a]	6.3[a]	.8
Lys	5.6[b]	10.2[ab]	8.3[ab]	9.2[ab]	8.7[ab]	14.2[a]	12.9[a]	1.7
His	5.0[a]	7.8[a]	6.7[a]	5.9[a]	5.7[a]	7.0[a]	7.1[a]	.7
Arg	11.0[b]	15.7[ab]	13.7[ab]	12.9[ab]	12.7[ab]	15.6[ab]	18.7[a]	1.7
SAA[c]	2.7[a]	4.2[a]	2.8[a]	2.8[a]	2.8[a]	3.6[a]	3.6[a]	.5
BCAA[d]	22.2[b]	35.0[ab]	23.6[b]	29.7[ab]	28.7[ab]	42.3[a]	30.4[ab]	3.9
TEAA[e]	54.7[b]	92.4[a]	66.2[ab]	72.6[ab]	71.8[ab]	97.2[a]	87.3[ab]	9.0
TNEAA[f]	83.7[a]	119.7[a]	95.2[a]	89.9[a]	82.4[a]	101.2[a]	103.3[a]	9.3
N/E[g]	1.5[a]	1.3[a]	1.4[a]	1.2[a]	1.2[a]	1.0[a]	1.2[a]	.1

[ab]Means in each row not sharing a common superscript are different at P<.10 using Tukey's test.

[c] Sulfur amino acids (sum of Met and Cys).

[d] Branched chain amino acids (sum of Val, Ile and Leu).

[e] Total essential amino acids (sum of Thr, Val, Met, Ile, Leu, Phe, Lys, His and Arg).

[f] Total nonessential amino acids (sum of Asp, Ser, Glu, Pro, Gly, Ala, Cys, Nle, and Tyr).

[g] Nonessential:essential amino acid ratio.

TABLE 11. Effect of the animal age (weeks) on plasma free amino acids in young calves (μmoles/100 ml) (Experiment 1)

Amino acids	Age (week)		SEM	(P <)
	3rd	6th		
No. calves	28	28		
Threonine	8.8	8.8	.8	NS
Valine	16.9	13.5	.7	.01
Methionine	2.5	2.0	.2	NS
Isoleucine	6.8	5.8	.4	.05
Leucine	9.9	7.7	.7	.05
Phenylalanine	6.6	5.1	.3	.01
Lysine	12.3	7.5	.6	.01
Histidine	7.0	5.9	.3	.05
Arginine	15.8	12.8	.8	.05
SAA[a]	3.4	3.0	.3	NS
BCA[b]	33.6	27.0	1.5	.05
TEAA[c]	86.5	69.1	3.2	.05
TNEAA[d]	95.7	97.2	4.9	NS
N/E[e]	1.1	1.4	.1	.05

[a]Sulfur amino acids (sum of Met and Cys).
[b]Branched chain amino acids (sum of Val, Ile and Leu).
[c]Total essential amino acids (sum of Thr, Val, Met, Ile, Leu, Phe, Lys, His and Arg).
[d]Total nonessential amino acids (sum of Asp, Ser, Glu, Pro, Gly, Ala, Cys, Nle and Tyr).
[e]Nonessential:essential amino acid ratio.

in CP to 23%, with only milk protein (diet 6), did not increase plasma TEAA, TNEAA, BCA, SAA or individual amino acids.

Within diets containing 19% CP, higher concentrations for TEAA, TNEAA, BCA, SAA or individual amino acids were observed in calves receiving only milk protein, although differences were not significant ($P>.10$). The apparent decrease in plasma amino acids when milk was partially replaced by other protein sources could be due to their lower digestibility. On the other hand, calves fed high SDFS (diet 7) showed lower ($P<.05$) weight gains and higher mortality than those fed milk protein (diet 6), but plasma amino acid concentrations for both diets containing 23% protein were similar. These contradictory results are in agreement with Bergen (1979) who concluded that static measurements of plasma amino acids have limited value as sensitive indicators of protein status because they do not reflect the magnitude of amino acid fluxes in and out of free amino acid pools. The amino acid profiles obtained in the present experiment do suggest, however, that amino acid imbalance in SPC or SDFS diets is unlikely to be the major factor inhibiting growth.

The lower concentrations of plasma amino acids at 6 weeks (Table 11) might be explained by a greater utilization, reflected by faster weight gains during this period compared to 3 weeks of age (Munro, 1970).

Poor results with fish protein in some experiments were associated with vitamin E deficiency precipitated by residual fish oil, which consists largely of polyunsaturated fats (Makdani et al., 1971c; Michel et al., 1972). Necropsy results at Michigan State of calves failing to complete the experiment (Table 12) showed one case of white muscle

TABLE 12. Calves omitted from experiment at Michigan State due to unexpected problems or death (Experiment 1)

Diet	Calf no.	Death days[a]	Cause of death or problem
1	56	22	Undetermined
1	57	3	Undetermined
2	45	7	Acute and subacute enterites and pneumonia
2	45'	10	Subacute fibrinous pneumonia
2	54	7	Severe chronic suppurative bronchopneumonia
4	55	14	Acute copper toxicity
5	31	20	Slight white muscle disease
5	46	21	Bronchopneumonia
6	40	17	Pasteurellosis
7	9	-	Broken leg when 2 weeks old
7	9"	2	Subacute fibrinous pneumonia
7	9"	17	Rupture of abomasal wall; pneumonia
7	9"	12	Undetermined
7	22	7	Rupture of abomasal wall
7	22'	6	Bronchopneumonia
7	33	35	Bronchopneumonia
7	51	32	Undetermined; ulceration of abomasal mucosa

[a]Days under experimental condition.

disease for treatment 5 (containing SPC and SDFS). Of seven dead animals fed the replacer containing 10% SDFS (diet 7), three had ulceration of abomasal mucosa, two of them showing rupture of the abomasum wall.

It is doubtful that a high level of polyunsaturated fatty acids was the main reason for poor growth on SDFS in this experiment because of the inconsistency of the results. For instance, treatment 3 containing 31% of the dietary protein from SDFS resulted in no animal losses, and treatment 5, also containing SDFS, resulted in only one of 11 calves which was diagnosed with white muscle disease. Moreover, there were none on treatment 7 which had highest SDFS.

Least square means for dry matter and protein intakes and for feed efficiency are in Table 13. Dry matter intake was higher ($P<.05$) for diets containing high milk protein and 6% SPC. Intakes were lower for the negative control and diets containing fish solubles. Differences among treatments in dry matter intake were from the feeding criterion, which adjusted weekly the amount of replacer fed to each animal to body weight of the preceeding week.

As expected, crude protein intake was higher ($P<.05$) for diets containing 23% CP, intermediate for 19% CP, and lowest for the negative control. Differences among treatments within each protein percent were mostly due to dry matter intakes.

Feed efficiencies, in terms of dry matter or crude protein, were higher ($P<.05$) for diets containing high milk protein (treatments 2 and 6), intermediate for the SPC diet, and lowest for negative control and diets containing SDFS. This suggests poor utilization for animal growth of nutrients from fish solubles.

TABLE 13. Least square means of dry matter (DM) and crude protein (CP) intakes, and feed efficiency for calves fed milk replacers of varying protein content and source averaged over time and location (Experiment 1)

Variables	Treatments													
	(1) 13% MP		(2) 19% MP		(3) 13% MP+ 6% SDFS		(4) 13% MP+ 6% SPC		(5) 13% MP+ 3% SDFS +3% SPC		(6) 23% MP		(7) 13% MP+ 10% SDFS	
	$\bar{x}$	SEM	$\bar{x}$	SEM	$\bar{x}$	SEM	$\bar{x}$	SEM	$\bar{x}$	SEM	$\bar{x}$	SEM	$\bar{x}$	SEM
No. animals	23		24		24		24		24		25		22	
DM intake (g/an/day)	560^{b}	16	606^{a}	15	547^{b}	15	597^{a}	15	572^{ab}	15	602^{a}	15	542^{b}	16
CP intake (g/an/day)	77^{f}	3	123^{c}	3	111^{e}	3	120^{cd}	3	115^{de}	3	144^{a}	3	131^{b}	3
Weight gain/DM intake	$.23^{c}$	.03	$.50^{ab}$	.03	$.28^{c}$	.03	$.38^{bc}$	.03	$.30^{c}$	.03	$.58^{a}$	.03	$.25^{c}$	.03
Weight gain/CP intake	1.78^{bc}	.16	2.60^{a}	.16	1.44^{cd}	.16	1.88^{bc}	.16	1.54^{cd}	.16	2.24^{ab}	.16	1.04^{d}	.17

^{abcdef}Means in the same row not sharing a common superscript are different at $P<.05$ using Tukey's test.

Xylose transport in the intestine is similar to glucose (Levitt et al., 1969), but normal levels in blood are much lower (Seegraber and Morrill, 1979). Hence, xylose uptake into blood after feeding a test meal has been used to evaluate malabsorption in the proximal intestine (Levitt et al., 1969; Seegraber and Morrill, 1979).

Plasma xylose concentrations for the different sampling times are in Table 14. They increased to a peak 2 hours after feeding xylose and then decreased after 3 hours. Large variations in xylose patterns for the different diets were observed, but no consistent trend was related to protein source. These results contrast with those of Seegraber and Morrill (1979) who concluded from their xylose uptake tests that absorptive capability was lower in calves fed soy protein concentrate than milk protein. Sissons and Smith (1976) also stated that calves fed heated soybean flour developed a severe disturbance in digestive function probably due to an allergic reaction in the gut. As mentioned, autopsy results from dead calves fed treatment 7 (containing 10% CP from SDFS) showed necrosis and even rupture in the digestive tract. Hence, it is plausible that high SDFS impaired intestinal absorption, although the xylose test did not indicate a malabsorption syndrome.

Plasma xylose concentrations from 2 to 5 hours after ingestion were lowest for diets 2 and 6, which contained only milk protein. The data suggest that animals fed inferior protein sources or low protein diets have greater difficulty in clearing xylose from their blood which may be due to a slower anabolism of ingested nutrients. It is also possible that less efficient kidney clearance might be part of the broad syndrome (higher rectal temperatures, diarrhea and consequent lower weight gains)

TABLE 14. Average plasma xylose concentration (mg/dl) after an oral dose of .5 g xylose/kg body weight for calves fed milk replacers varying in protein level and source (Experiment 1)

Treatments	No. of calves	Xylose concentration[f] (mg/dl of plasma)								
		.5 h	1 h	1.5 h	2 h	2.5 h	3 h	4 h	5 h	Means[g]
1. 13% MP	9	38.8	86.9	121.8	126.3	131.2	121.9	113.0	108.2	94.2[a]
2. 19% MP	9	41.5	94.0	125.7	124.8	118.6	109.9	96.0	68.2	97.3[a]
3. 13% MP, 6% SDFS	9	45.7	101.9	109.6	113.9	107.6	107.5	95.3	84.2	95.7[a]
4. 13% MP, 6% SPC	9	45.4	114.3	139.3	140.8	141.7	133.1	114.0	97.0	115.7[a]
5. 13% MP, 3% SDFS, 3% SPC	9	47.9	96.4	99.0	136.0	109.8	138.8	117.8	97.7	105.4[a]
6. 23% MP	9	59.1	113.2	113.3	136.3	122.9	120.2	105.8	72.5	107.9[a]
7. 13% MP, 10% SDFS	8	51.2	128.4	139.6	144.5	138.1	135.9	126.5	117.7	122.7[a]
Means[g]		46.8[e]	105.0[cd]	109.8[bc]	131.8[a]	124.3[ab]	123.9[ab]	109.8[bc]	92.2[d]	

[abcde] Means in same row or same column not sharing a common superscript are different at P<.10 using Tukey's test.

[f] Pre-administration xylose concentration in plasma (at time 0) were deducted from post-administration xylose concentration before data analysis.

[g] Standard error for hourly means = ±4.0 and for treatment means = ±16.5.

observed in calves fed non-milk protein.

The reason for contrasting results between this study and that of Seegraber and Morrill (1979) is not apparent, but initial xylose concentrations (0 hour) in our study were much higher (31 vs 12 mg %). Also, increases after feeding were considerably higher for our calves.

Least square means for fecal scores and treatments for sicknesses are in Table 15. Interaction of diets by location was statistically significant ($P<.01$). Fecal scores for diet 3 at Michigan were lower than diet 5, whereas the opposite was shown at Kansas and Cornell. Besides the subjectiveness of the measurement no other explanation for this apparent discrepancy is evident. At all locations, treatment 7 resulted in higher ($P<.05$) fecal scores than the other treatments. Treatment 3, also containing SDFS, was the second highest, although at Michigan it did not differ ($P<.05$) from others. Fecal indices of calves fed SPC were no higher than for calves fed milk protein. Increasing milk protein from 13 to 19% decreased scouring, but no change was observed between 19 and 23%.

There was no difference ($P>.05$) between diets for treatments of sicknesses at Cornell and Kansas. At Michigan, animals receiving diet 7 required more($P<.05$) treatment than other diets. The higher ($P<.05$) fecal scores and treatments for sicknesses during the first 2 or 3 weeks compared to the last 3 or 4 weeks show the greater sensitivity of the very young calf to illness.

Least square means for rectal temperatures during the first 14 days of trial are in Table 16. During the first week, calves fed diet 7 had higher ($P<.10$) rectal temperatures than those fed diets 1 and 3,

TABLE 15. Least square means of fecal score and treatments for sickness for calves fed milk replacers varying in protein content and source by location and diet[a] (Experiment 1)

	Cornell	Kansas	Michigan
	Fecal Score[g]		
Treatment			
1. 13% MP	2.1^{d}	2.2^{d}	1.5^{c}
2. 19% MP	1.6^{ef}	1.9^{de}	1.2^{d}
3. 13% MP, 6% SDFS	2.8^{c}	3.1^{c}	1.4^{cd}
4. 13% MP, 6% SPC	1.4^{f}	1.9^{de}	1.3^{cd}
5. 13% MP, 3% SDFS, 3% SPC	1.8^{de}	2.0^{de}	1.5^{c}
6. 23% MP	1.9^{de}	1.8^{e}	1.1^{d}
7. 13% MP, 10% SDFS	3.7^{b}	3.6^{b}	1.9^{b}
SEM	±.06	±.08	±.08
Week on Diet			
1	2.6^{b}	2.3^{c}	1.5^{b}
2	2.7^{b}	2.8^{b}	1.6^{b}
3	2.0^{c}	2.5^{bc}	1.5^{b}
4	1.8^{c}	2.1^{c}	1.4^{bc}
5	1.9^{c}	2.3^{c}	1.3^{c}
6	1.8^{c}	2.3^{c}	1.3^{c}
SEM	±.06	±.08	±.06
Treatment	Treatment for Sickness[h]		
1. 13% MP	.27^{b}	.22^{b}	.51^{c}
2. 19% MP	.22^{b}	.10^{b}	.28^{c}
3. 13% MP, 6% SDFS	.31^{b}	.14^{b}	.39^{c}
4. 13% MP, 6% SPC	.25^{b}	.26^{b}	.31^{c}
5. 13% MP, 3% SDFS, 3% SPC	.25^{b}	.21^{b}	.46^{c}
6. 23% MP	.27^{b}	.08^{b}	.24^{c}
7. 13% MP, 10% SDFS	.23^{b}	.04^{b}	.81^{b}
SEM	±.04	±.06	±.06

[a]Because of the significant (P<.01) interaction rations x locations, treatment means are compared within each location.

[bcdef]Means in columns not sharing the same letter are different at P<.05 using Tukey's test.

[g]Fecal consistency was rated from 1 to 4 (1 being normal feces and 4 being very fluid feces).

[h]Average days each calf was treated from an illness.

TABLE 16. Least square means of rectal temperatures for calves fed milk replacers varying in protein content and source by location, diet and week on diet (Experiment 1)

Week	Treatments	Cornell	Kansas	Michigan	Mean	SEM
		----------°C----------				
1st	1	38.67	39.01	38.11	38.60^{b}	.05
	2	38.90	39.08	38.31	38.77^{ab}	.05
	3	38.69	39.02	38.09	38.60^{b}	.05
	4	38.90	39.13	38.10	38.71^{ab}	.05
	5	38.82	39.04	38.27	38.71^{ab}	.05
	6	38.94	38.97	38.21	38.71^{ab}	.05
	7	38.83	39.03	38.47	38.78^{a}	.05
	Mean	$38.82^{B}\pm.08$	$39.04^{A}\pm.09$	$39.22^{C}\pm.08$		
2nd	1	38.83	39.52	38.37	38.91^{a}	.07
	2	38.83	39.28	38.32	38.81^{a}	.07
	3	38.76	39.13	38.21	38.70^{a}	.07
	4	38.70	39.62	37.78	38.89^{a}	.07
	5	38.60	39.32	38.27	38.73^{a}	.07
	6	38.96	39.32	38.28	38.54^{a}	.07
	7	38.77	39.27	38.38	38.81^{a}	.07
	Mean	$38.78^{B}\pm.04$	$39.35^{A}\pm.04$	$38.11^{C}\pm.04$		

^{abc}Means in columns within each week not sharing a common superscript are different at P<.10 using Tukey's test.
^{ABC}Means in the same row not sharing the same superscript are different at P<.10 using Tukey's test.

but were similar to those fed diets 2, 4, 5 and 6. During the second week of trial there were no differences (P>.10) between treatments. Among locations, rectal temperatures were highest (P<.10) at Kansas, perhaps because of local factor such as environment or management. Rectal temperatures were higher (P<.10) at Cornell than Michigan, but temperatures at all locations were about normal.

Mortality of calves at Michigan State is shown in Table 17. The hypothesis of independence between diets and death cases may be rejected with 90% confidence. The analysis for pairs of treatments, in 2 x 2

contingency tables (by Bonferroni chi-square) indicates that mortality in treatment 7 was higher ($P<.10$) than in treatments 3, 4 and 6. If replacement calves are not considered as "alive calves" on Table 17, the calculated value for "q" changes from 10.8 to 18.0. In this situation, mortality in treatment 7 is clearly different, even when compared to treatment 2. Across all locations, mortalities for diets 1 through 7 were 14, 14, 8, 17, 11, 4 and 30%. About 90% of the deaths occurred during the first 3 weeks on treatment. These data indicate that 10% SDFS is excessive for inclusion in calf milk replacers. This statement is supported by growth, feed efficiency and fecal score data.

In conclusion, spray-dried fish solubles are inferior to milk protein or soy protein concentrate for inclusion in milk replacers for baby calves. The data further suggest that high fish solubles aggravate the health of the young calf.

TABLE 17. Mortality of calves at Michigan State fed milk replacers containing different protein levels and sources (Experiment 1)

Treatments	Number of calves		
	Dead	Alive	Totals
13% MP	2	9	11
19% MP	3	9	12
13% MP, 6% SDFS	0	9	9
13% MP, 6% SPC	1	9	10
13% MP, 3% SDFS, 3% SPC	2	9	11
23% MP	1	9	10
13% MP, 10% SDFS	7	8	15
TOTALS	16	62	78

The hypothesis of independence between treatments and death cases is rejected ($q = 10.792$; $\chi 2, 0.1, 6 = 10.64$).

Experiment 2

Average initial body weights and plasma protein levels when calves arrived at Michigan State were similar (P>.10) between treatments (Table 18).

Protein source affected (P<.10) weight gains during the 2 weeks of treatment (Table 18) with animals on diet 3 (6% protein from SDFS) showing lower gains (P<.10) when compared to animals fed diet 2. These data are in general agreement with those reported in Experiment 1 and by other workers (Rupel and Wilson, 1962; Gillespie, 1971; Sleiman and Huber, 1971; Ternouth et al., 1975).

There was no difference (P>.10) between treatments in rectal temperatures and all temperatures were within the normal range (Table 18). Scour scores were highest for diets containing SDFS and SPC, although not different (P>.10) from the negative control (Table 18). An impairment in milk coagulation (Tagari and Roy, 1969; Paruelle et al., 1972), followed by faster passage of undigested material through the lower gut (Colvin et al., 1969; Ternouth et al., 1975), might explain the higher incidence of diarrhea in calves fed non-milk protein which was observed in this and other studies (Gorrill and Thomas, 1967; Seegraber and Morrill, 1979).

Apparent dry matter and organic matter digestibilities were highest (P<.10) for diet 2 (19% CP as milk protein) although not different from the negative control (Table 18). Similar observations were made for apparent nitrogen retention. These results agree with those reported by other workers (Matre, 1970; Sleiman and Huber, 1971) and partially

TABLE 18. Influence of protein level and source on daily weight gain, rectal temperature, scour scores, apparent dry matter (DM) and organic matter (OM) digestibilities and apparent nitrogen retention in 2-week-old calves (Experiment 2)

	Treatments				
	(1) 13% MP	(2) 19% MP	(3) 13% MP+ 6% SDFS	(4) 13% MP+ 6% SPC	SEM
No. of calves	4	4	4	4	
Average initial body weight (kg)	36.1[a]	40.1[a]	34.9[a]	33.2[a]	2.5
Plasma protein(%)	5.4[a]	5.3[a]	5.9[a]	6.5[b]	.2
Average daily weight gain (g)	70 [a]	395 [a]	-167 [b]	18 [a]	182
Average rectal temperatures (^{o}C)	39.1[a]	38.5[a]	38.7[a]	38.9[a]	.2
Average scour scores	2.2[bc]	1.9[c]	2.8[ab]	3.0[a]	.3
Apparent DM digestibility (%)	82.5[a]	88.0[a]	71.8[b]	74.9[b]	3.4
Apparent OM digestibility (%)	84.6[a]	90.6[a]	75.7[b]	78.7[b]	3.2
Apparent N retention (g/day)	1.6[b]	7.6[a]	2.1[b]	.1[b]	1.4

[abc]Means in the same row not sharing the same superscript with the control (treatment 2) are different at P<.10 using Dunnett's test.

explain the lower weight gains observed for SDFS and SPC diets. The lower digestibilities of calves fed fish protein or soybean protein may be related to impaired curd formation in the abomasum resulting in faster flow of digesta through the gut and a reduction in proteolytic enzyme secretions in the tract (Gorrill and Thomas, 1967; Ternouth et al., 1975; Williams et al., 1976; Roy et al., 1977; Jenkins et al., 1980).

In this experiment, mortality rate (20%) and the number of calves leaving the experiment before completion (Table 19) was extremely high; probably because of cold stress during transport of baby calves by truck during the winter after which they were housed for 2 weeks in unbedded, metallic cages for fecal and urine colletions.

The hypothesis of independence between diets and number of deaths (Table 20) may be rejected with 90% confidence. The analysis for pair of treatments, in 2 x 2 contingency tables, indicates that diet 3 caused higher ($P<.10$) mortality. If replacement calves are not considered as "alive calves" on Table 20, the calculated value for "q" changes from 7.50 to 15.75. In this situation, the hypothesis of independence between diets and death cases may be rejected with 95% confidence.

An allergic-type reaction in calves fed SDFS was again suggested (as in Experiment 1) by necropsy reports from two calves (6872 and 6881) in which abomasal lesions were observed. Other possible causes of the lesions might have been the low pH, a tendency of the diet to adhere to the mucosa or an unknown irritant.

High mortality on diet 3 was not observed during the first experiment, but results of Experiment 2 emphasize the problem of SDFS incorporation in milk replacers for baby calves. Greater stress or a different

TABLE 19. Calves omitted from experiment due to unexpected problems or death (Experiment 2)

Treatment	Calf No.	Death (days)[a]	Cause of death or problem
2	6863	--[b]	strong diarrhea
2	6883	--	strong diarrhea, high body temperature
3	6860	5	undetermined
3	6872	5	gastroenterites, pneumonia
3	6881	4	gastroenterites, pneumonia
3	6884	6	undetermined
3	6903	--	not drinking the replacer
4	6862	--	strong diarrhea, high body temperature
4	6990	--	strong diarrhea, high body temperature

[a]Days under experimental conditions.
[b]Calves showing extremely poor health prior to collection period.

batch of SDFS might explain this appearance of the problem at a lower SDFS level.

In summary, lowered digestibilities of dry matter and organic matter, and less retained nitrogen were associated with decreased weight gains of young calves fed milk replacers containing SDFS or SPC as partial substitutes for milk protein. It is also possible that SDFS may cause an allergy in the young calf, with damaging effects on the mucosa of the digestive tract and a result in high mortality.

Experiment 3

The influence of protein source (milk or soy protein) and limestone (absent or present) on daily weight gain, intake of nutrients, feed efficiency, fecal score and rectal temperature of calves are shown in Table 21. Means are presented for main effects because the interaction of protein source x limestone was not significant (P>.10) for any variable listed. Initial body weights were similar (P>.10) for treatment groups.

Replacement of 50% of the milk protein with SPC resulted in 20% lower average daily gains. This difference was not significant (P>.10) probably due to the large variation and small number of animals within treatments. Since the initial body weight of calves fed SPC was slightly higher and observed average daily gains were lower, an analysis of covariance using initial weights as the covariant was realized. In this comparison, the F-test did not indicate significant differences among diets.

Dry matter and crude protein intakes were not significantly affected (P>.10) by protein sources. Although not significantly different (P>.10), feed efficiency was higher for calves fed only milk protein, probably

TABLE 20. Mortality of calves at Michigan State fed milk replacers containing different levels and sources of protein (Experiment 2)

Treatments	Dead	Alive	Totals
		No. of calves	
1. 13% MP	0	4	4
2. 19% MP	0	4	4
3. 13% MP, 6% SDFS	4	4	8
4. 13% MP, 6% SPC	0	4	4
Totals	4	16	20

The hypothesis of independence between diets and death cases if rejected ($q = 7.50$; $\chi^2_{.1,\ 3} = 6.25$).

because of higher weight gains during the experiment. Likewise, fecal scores and rectal temperatures were not affected (P>.10) by SPC incorporation into milk replacers.

TABLE 21. Influence of protein source and limestone on daily weight gain, intake of nutrients, feed efficiency, fecal score and rectal temperature of baby calves (Experiment 3)

Variables	Protein source		Limestone		SEM
	Milk	Milk+SPC	Present	Absent	
Initial body weight (kg)	38.0[a]	40.7[a]	40.7[a]	38.0[a]	1.7
Weight gain (g/an/day)	275 [a]	223 [a]	250 [a]	247 [a]	41
DM intake (g/an/day)	563 [a]	558 [a]	563 [a]	558 [a]	33
CP intake (g/an/day)	107 [a]	107 [a]	106 [a]	108 [a]	6
Weight gain/DM intake	.49[a]	.40[a]	.44[a]	.44[a]	.06
Fecal dry matter (%)[c]	23.98[a]	23.80[a]	24.70[a]	23.08[b]	.58
Fecal scores	2.2[a]	2.0[a]	2.2[a]	2.0[a]	.1
Rectal temperature (^oC)	38.9[a]	38.9[a]	38.9[a]	38.9[a]	.1

[ab]Means in rows (within protein sources and limestone) not sharing a common superscript are different at P<.10.

[c]Average dry matter in feces sampled during 4 days when calves were 3 and 6 weeks old.

The addition of .8% of limestone to milk replacers did not significantly change (P>.10) weight gains, intake of nutrients, feed efficiency or rectal temperature (Table 21). However, fecal dry matter of samples obtained when calves were 3 and 6 weeks old were higher (P<.10) in calves receiving limestone. The same tendency was observed in fecal score, although the difference was not significant (P>.10).

The effects of protein source and limestone on the apparent digestibilities of dry matter, organic matter and crude protein, and on apparent crude protein balance and plasma urea nitrogen are in Table 22. Once again the interaction of protein source x limestone was not significant (P>.10) for all above mentioned variables.

TABLE 22. Influence of protein source and limestone on the apparent digestibilities of dry matter (DM), organic matter (OM), crude protein (CP) on apparent crude protein balance and plasma urea nitrogen of baby calves (Experiment 3)

Variables	Protein source		Limestone		
	Milk	Milk+SPC	Present	Absent	SEM
Apparent DM digestibility (%)	87.64[a]	85.04[b]	85.20[a]	87.48[b]	.93
Apparent OM digestibility (%)	87.80[a]	85.95[a]	86.35[a]	87.40[a]	1.13
Apparent CP digestibility (%)	85.24[a]	76.81[b]	79.53[a]	82.51[a]	1.87
Apparent CP balance (g/day)	66.37[a]	59.68[a]	59.99[a]	60.06[a]	6.54
Apparent CP balance (% of ingested)	50.20[a]	44.95[a]	46.54[a]	48.61[a]	3.42
Plasma urea N (mg/100 ml)	5.63[a]	6.06[a]	5.35[a]	6.33[a]	.71

[ab]Means in rows (within protein sources and limestone) not sharing a common superscript are different at P<.10.

The substitution of 50% of the dietary protein with SPC resulted in significant (P<.10) reductions in dry matter and crude protein digestibilities. Apparent crude protein digestibility was 10% lower when SPC

was used. Organic matter digestibility was also reduced, although not significantly (P>.10). Similar results were obtained in experiment 2 and by other workers (Nitsan et al., 1971,1972; Ternouth and Roy, 1973; Pejic and Kay, 1979). Lower nutrient digestibilities may explain in part, the lower weight gains (Table 21) of calves fed SPC.

Apparent crude protein balances, expressed either as g/day or as percent of the protein ingested, was 10% lower in calves fed SPC than only milk protein. Although this difference was not significant (P>.10), it is a reflection of the depressed protein digestibility.

Plasma urea nitrogen (Table 22) was only slightly higher (P>.10) for calves fed SPC than only milk.

Limestone in the replacer slightly reduced nutrient digestibility and nitrogen balance (P>.10). Neither was plasma urea nitrogen significantly affected (P>.10) by limestone incorporation.

The interaction treatments x week was not significant (P>.10) for any variable in Tables 21 and 22, suggesting that there were no specific ages at which effects of SPC or limestone, or both, were greater.

Age effects on weight gains, intake of nutrients, feed efficiencies, fecal consistency and pH, rectal temperature, apparent nutrient digestibilities, apparent protein retention, and plasma urea nitrogen are in Table 23. As expected most variables were affected (P<.10) by age.

Weight losses during the first 2 weeks of treatment emphasizes the importance of good management and nutrition for these young and sensitive animals in order to avoid high mortality. Gains improved (P<.10) with age, but the relatively low gain observed in the sixth week was due to

TABLE 23. Effect of age on weight gain, intake of nutrients, feed efficiency, fecal score, fecal pH, rectal temperature, apparent nutrient digestibilities, apparent nitrogen retention and plasma urea nitrogen of young calves fed milk replacers as the only source of nutrients (Experiment 3)

Variables	Weeks						
	1	2	3	4	5	6	SEM
Weight gain (g/an/day)	-99^{d}	-39^{d}	206^{c}	446^{ab}	612^{a}	344^{bc}	44
DM intake (g/an/day)	403^{d}	445^{cd}	497^{c}	593^{b}	702^{a}	713^{a}	17
CP intake (g/an/day)	77^{d}	85^{d}	96^{c}	113^{b}	134^{a}	136^{a}	2
Weight gain/DM intake	$-.22^{c}$	$-.16^{c}$	$.45^{b}$	$.75^{ab}$	$.87^{a}$	$.49^{b}$	.10
Fecal dry matter (%)	-----	-----	24.07^{a}	-----	-----	23.71^{a}	.97
Fecal scores	2.3^{a}	2.1^{a}	2.2^{a}	1.9^{a}	1.8^{a}	2.1^{a}	.2
Rectal temperature (^{o}C)	38.9^{a}	38.7^{a}	38.9^{a}	39.0^{a}	39.0^{a}	38.9^{a}	.1
Apparent DM digestibility (%)	-----	-----	87.59^{a}	-----	-----	85.09^{b}	.84
Apparent OM digestibility (%)	-----	-----	86.82^{a}	-----	-----	86.92^{a}	.92
Apparent CP digestibility (%)	-----	-----	80.03^{a}	-----	-----	82.02^{a}	1.55
Apparent CP balance (g/day)	-----	-----	42.59^{b}	-----	-----	82.34^{a}	3.91
Apparent CP balance (% of ingested)	-----	-----	40.83^{b}	-----	-----	54.32^{a}	1.90
Fecal pH	-----	-----	6.79^{b}	-----	-----	7.06^{a}	.07
Plasma urea N (mg/100 ml)	-----	-----	7.79^{a}	-----	-----	3.90^{b}	.85

^{abcd}Means on the same line not sharing the same superscript are different at P<.10 using Tukey's test.

stress of keeping the animals in metabolism cages. Feed intakes and efficiencies increased (P<.10) with age. Since no significant weigh back of replacer was observed, intakes reflect the adopted feeding criterion. Nutrient intakes for weeks 3 and 6 were slightly lower than expected because animals were fasted for 24 hours for the xylose absorption tests. The poorer efficiencies for weeks 3 and 6 might also have been due to stress of the metabolism cages. Scour scores and fecal dry matter % were not affected (P>.10) by age. Although fecal scores tended to decrease with age, they were higher during the collection periods. Rectal temperatures were not affected (P>.10) by age, all being within the normal range.

Apparent dry matter digestibility was lower (P<.10) in older animals (6 vs 3 weeks of age). Instead of decreasing, dry matter digestibility was expected to remain the same or increase with age. Apparent crude protein retention was significantly (P<.10) higher for older animals, which supports the higher weight gains in older animals.

The higher plasma urea nitrogen (PUN) at 3 than 6 weeks (P<.10) agrees with weight gains and crude protein retention. The higher PUN at 3 weeks might be associated with greater catabolism of muscle protein as reported by Leibholz (1970). As calves became older, protein intake increased and digestibility did not change, but crude protein retention increased, apparently resulting in less gluconeogenesis.

Fecal pH was higher (P<.10) in older calves and may be related to less fermentation in the large intestines due to more efficient nutrient absorption. The xylose absorption tests support this hypothesis.

Treatment effects on plasma xylose concentrations averaged for age and time of sampling are in Table 24. Analysis of variance (Table A.3) indicates differences ($P<.10$) for sampling time and age, and for the interaction between treatment and age. Differences due to time of sampling was expected. Xylose concentrations in plasma tend generally to increase up to 1.5-2.5 hr after ingestion and then decrease as observed in Experiment 1. In this experiment, plasma xylose concentrations (mg %) were: 82.2, 143.7, 162.0, 166.8, 163.5, 153.0, 123.2, and 112.9, respectively for .5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 and 5.0 hr after xylose ingestion. The standard error of these means was $\pm$8.2.

The interaction between treatment and age may be explained by a differential response to limestone within each protein source for each age (Table 24). When calves were 3 weeks old, average plasma xylose dropped from 134.6 to 96.5 mg % when limestone was added to replacers containing only milk protein. At this same age, plasma xylose concentrations did not change (138.2 vs 144.8 mg %) due to limestone addition to SPC diets. On the other hand, when calves were 6 weeks old, plasma xylose was slightly (151.2 vs 165.8 mg %) increased when limestone was added to only milk protein. Results for limestone incorporation into SPC diets at 6 weeks were similar to those observed for younger calves. Another way of understanding this significant interaction is shown in Table 25 where sampling times are combined and means comparing protein source and limestone addition at the two ages are given.

When animals were 3 weeks old, the partial replacement of milk protein by SPC resulted in higher ($P<.05$) plasma xylose which exactly contradicts the data reported by Seegraber and Morrill (1979). On the other

TABLE 24. Average plasma xylose concentrations (mg/100 ml) after an oral dose of .5 g xylose/kg of body weight, for calves fed milk replacers varying in protein sources (Experiment 3)

Treatments	No. of calves	Xylose concentration[a] (mg/100 ml of plasma) 3 week								
		.5 h	1.0 h	1.5 h	2.0 h	2.5 h	3.0 h	4.0 h	5.0 h	Mean
1. Milk	3	65.5	101.5	177.6	164.1	184.4	173.5	110.5	99.3	$134.6^{bc} \pm 8.2$
2. Milk+limestone	3	39.9	86.7	116.0	124.2	117.2	124.0	85.9	78.1	$96.5^{c} \pm 8.2$
3. SPC	3	93.6	128.3	143.4	149.2	171.4	175.1	131.7	113.3	$138.2^{bc} \pm 8.2$
4. SPC+limestone	3	66.0	164.7	164.7	155.0	184.6	157.6	143.8	122.0	$144.8^{b} \pm 8.2$
Means		66.3	120.3	150.4	148.1	164.4	157.6	118.0	103.2	$128.5^{A} \pm 4.1$

Treatments	No. of calves	Xylose concentration[a] (mg/100 ml of plasma) 6 week									Treatment means
		.5 h	1.0 h	1.5 h	2.0 h	2.5 h	3.0 h	4.0 h	5.0 h	Mean	
1. Milk	3	100.6	195.2	196.3	184.3	171.0	146.2	125.3	90.3	$151.2^{b} \pm 8.2$	$142.9^{b} \pm 5.8$
2. Milk+limestone	3	96.1	156.8	177.7	204.8	176.0	199.1	156.0	159.1	$165.7^{b} \pm 8.2$	$131.1^{b} \pm 5.8$
3. SPC	3	92.5	158.3	170.1	167.4	139.2	119.9	102.9	124.6	$134.4^{b} \pm 8.2$	$136.3^{b} \pm 5.8$
4. SPC+limestone	3	102.9	158.4	150.8	184.8	163.9	128.6	129.6	116.5	$141.9^{b} \pm 8.2$	$143.4^{b} \pm 5.8$
Means		98.0	167.2	173.7	185.4	162.5	148.4	128.4	122.6	$148.3^{B} \pm 4.1$	

[a]Pre-administration xylose concentrations in plasma (at time 0) were deducted from each post-administration xylose concentration before data analysis.

[bc]Means in the same column not sharing a common superscript are different at $P<.01$ using Tukey's test.

[AB]Means in rows not sharing a common superscript are different at $P<.05$ using Dunnett's test.

hand, when animals were 6 weeks old, SPC incorporation in the diet tended to reduce plasma xylose concentration, although not significantly (P>.05).

TABLE 25. Average plasma xylose concentrations for treatment combination means of protein sources and limestone with calve's age (Experiment 3)

Treatments	Calve's age	
	3 weeks	6 weeks
Milk protein only	115.5^{b2}	158.5^{c1}
Milk protein + SPC	141.5^{c1}	138.1^{c2}
No limestone	136.4^{c1}	142.8^{c1}
Limestone	120.7^{b2}	135.8^{c1}

[a]Standard error for treatment combination means = ±5.8.
[bc]Means in the same row not sharing a common letter superscript are different at P<.01.
[1,2]Means in columns (within protein sources and limestone) not sharing the same numerical superscript are different at P<.01.

More interesting, however, is the effect of age on protein source. As animal becomes older, average plasma xylose concentration increased (P<.05) in calves fed milk protein but not those fed SPC. This observation might lead to the conclusion that absorption in the small intestine does not improve with age in calves fed SPC, but does when only milk protein is fed.

However, interpretation of averages of plasma xylose concentrations obtained in a 5 hour period after xylose ingestion are not that simplistic. For instance, higher plasma xylose might result from a greater capacity for absorption or from an impairment in clearing of xylose from blood.

One way for interpreting treatment effects on xylose absorption

would be through a curve obtained as the function of time after xylose administration. Bolton et al.(1976) and Hill et al. (1970) suggested that abnormal xylose absorption curves would be characterized by a low peak, a delayed peak, or a flat curve with no distinguishable peak and no marked decline.

Even with such criteria, the same restriction listed above persists. Perhaps the best way of interpreting these data is to consider responses during the first 2-3 hours after xylose ingestion as the absorption pattern characteristic, and the behavior during the last 2-3 hours as an indication of xylose clearing from plasma. Figure 1 shows that the ascending portion of the xylose absorption curves for both protein sources were similar. These results agree with those of Experiment 1 when SPC replaced 31% of the milk protein but contradict with those of Seegraber and Morrill (1979), who showed flat curves when SPC was included in the replacer.

The addition of limestone to the replacer did not change the xylose absorption pattern (Figure 2). It is interesting to note however, SPC and limestone resulted in higher xylose concentrations 4 to 5 hours after ingestion.

Figures 3 and 4 illustrate the already discussed interaction between treatment and age. When calves were 3 weeks old, SPC presence and limestone absence in the replacer resulted in slightly higher plasma xylose after peak concentrations were reached. When animals are 6 weeks old, the opposite trend was observed.

Figure 5 suggests that xylose absorption capability is higher ($P<.05$) in older calves. A similar conclusion was made by Seegraber and Morrill (1979) and agrees with higher weight gains, feed efficiencies and lower

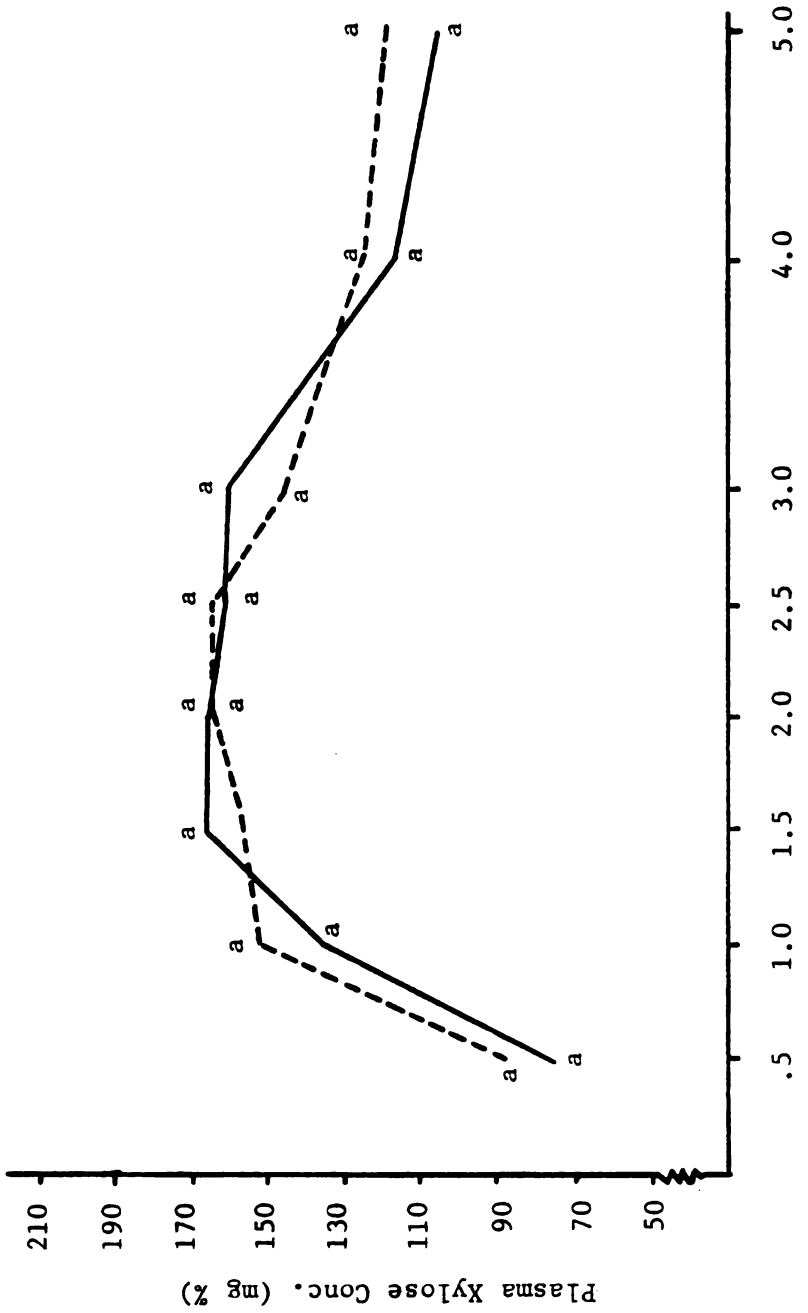

FIGURE 1. Mean xylose concentration in plasma of calves fed milk replacers containing milk (—) or soy protein (---). For each hour, points not showing the same letter are different at P<.10 (SEM = ±11.58).

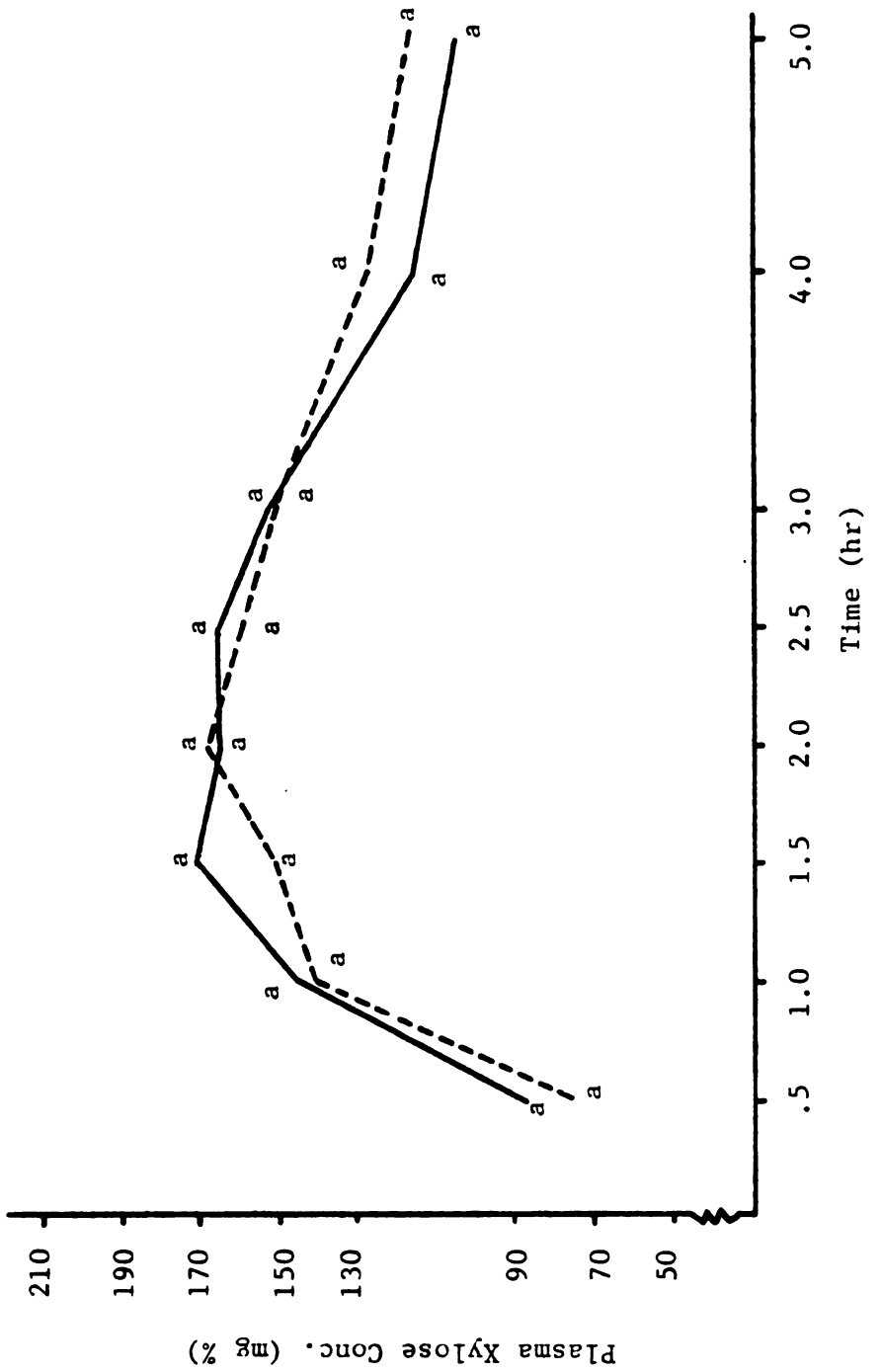

FIGURE 2. Mean xylose concentration in plasma of calves fed milk replacers with (---) or without (——) limestone. For each hour, points not showing the same letter are different at P<.10 (SEM = ±11.58).

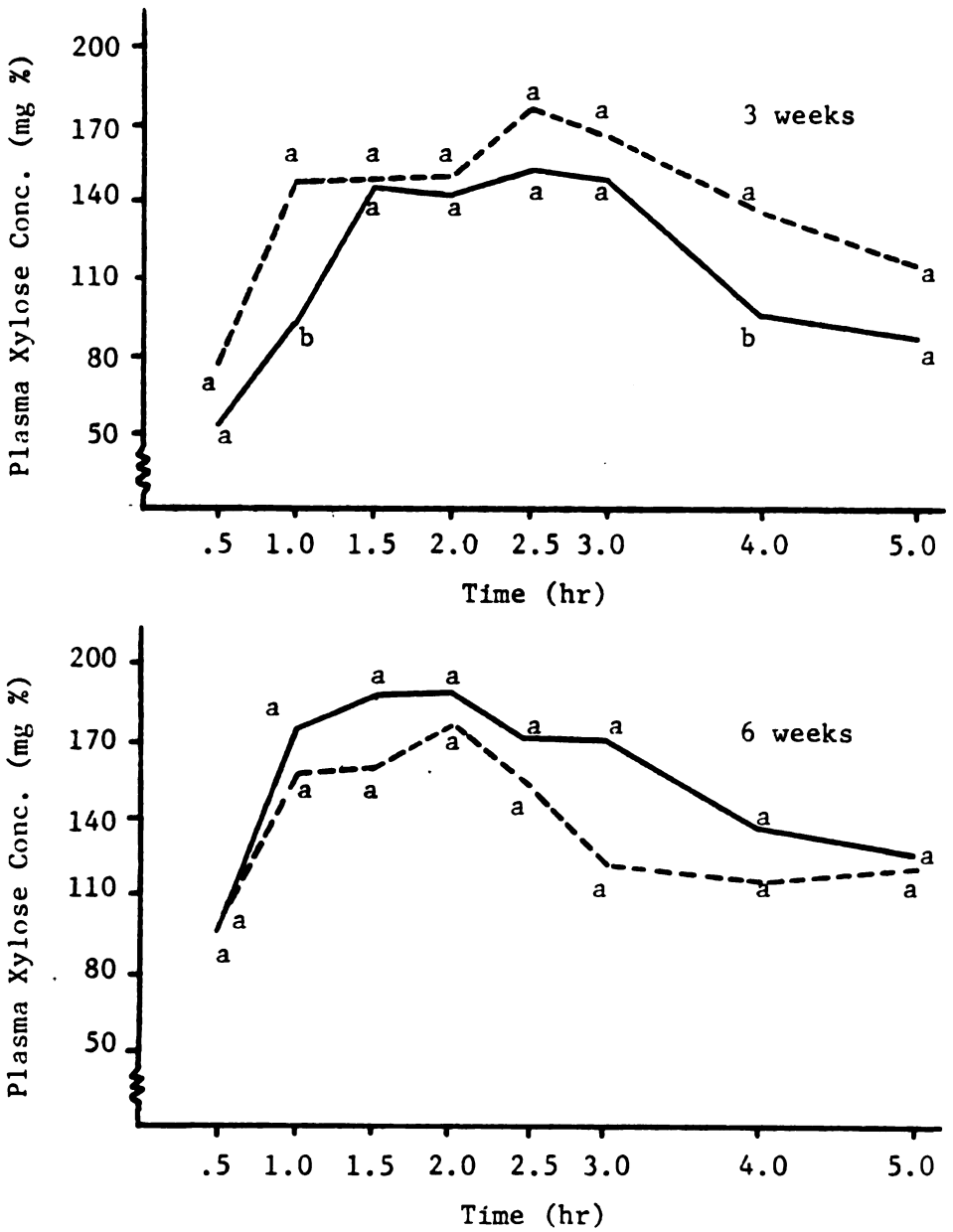

FIGURE 3. Mean xylose concentration in plasma of 3 and 6 weeks of age calves fed milk replacers containing milk (——) or soy (---) protein. For each hour, points in each graph not sharing the same letter are different at P<.01 (SEM = ±16.37).

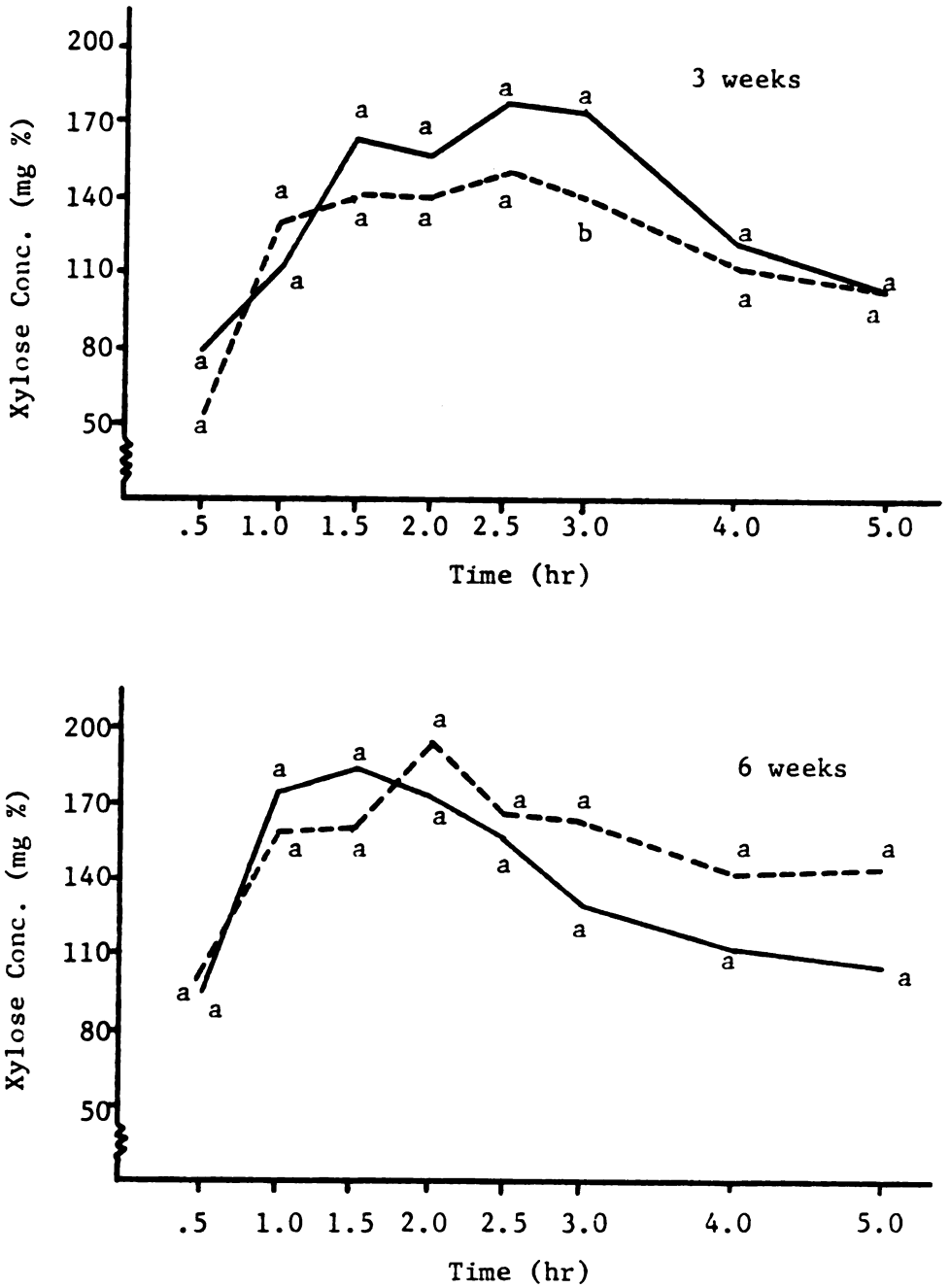

FIGURE 4. Mean xylose concentration in plasma of 3 and 6 weeks of age calves fed milk replacers with (---) and without (——) limestone. For each hour, points in each graph not showing the same letter are different at P<.10 (SEM = ±16.37).

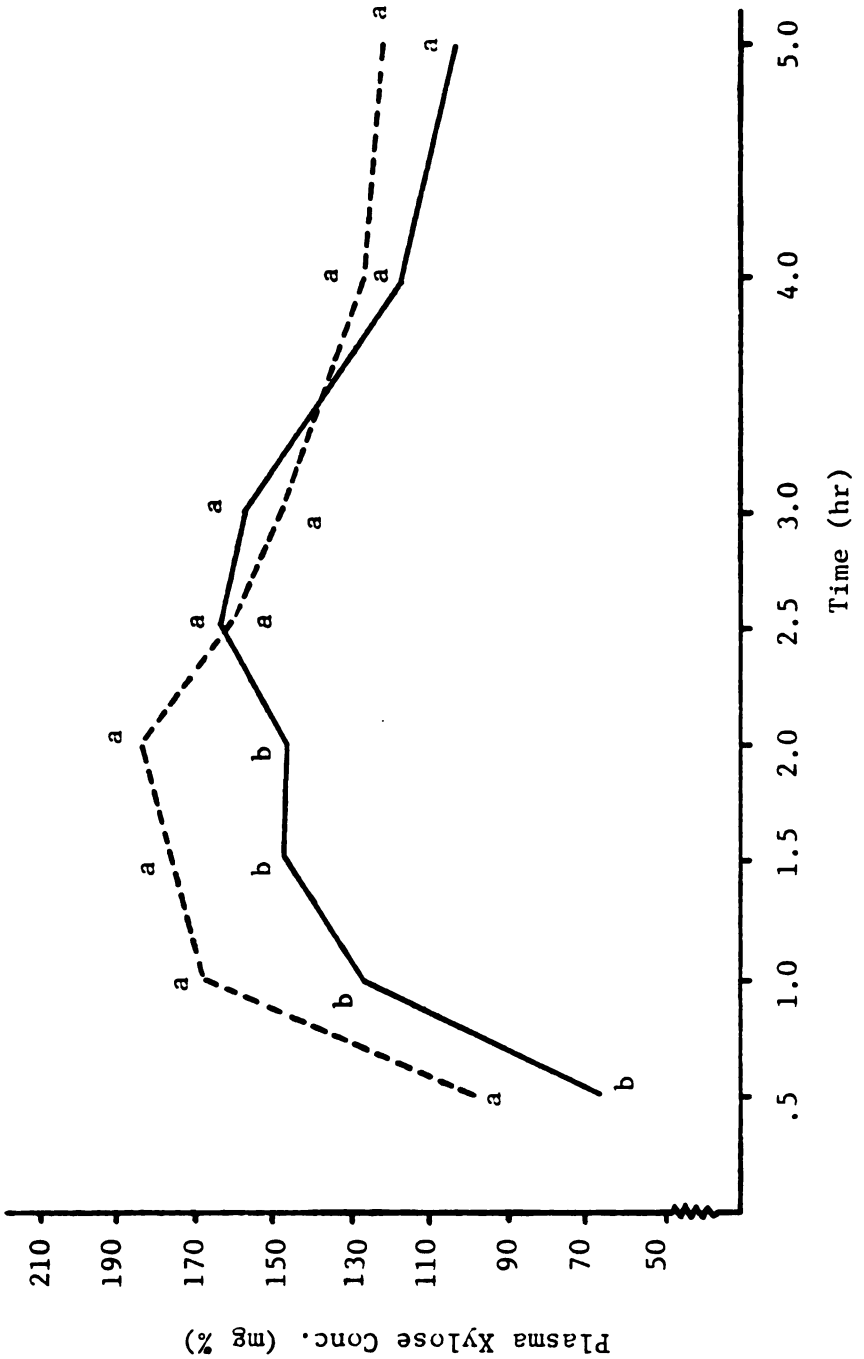

FIGURE 5. Mean xylose concentration in plasma of calves with 3 (——) or 6 weeks of age. For each hour, points not showing the same letter are different at P<.01 (SEM = ±11.58).

fecal pH at 6 than 3 weeks of age.

Weight of contents from different sections of the digestive tract and size of liver and pancreas of calves selected for slaughter at 6 weeks are in Table 26. Size of pancreas in these calves are similar to those reported by Gorrill and Thomas (1967) except for calf 7550 which had an extremely heavy pancreas. Liver size tended to be heavier in calves fed limestone (23.2 vs. 21.5 g/kg body weight). Reasons for this are not apparent and the difference is not great. Gross examination of rumen mucosa showed little papillary development, typical of calves receiving only liquid diets.

Dry matter, organic matter and crude protein contents, and pH values of digesta sampled in different sections of the gut are in Table 27. Data from Tables 26 and 27 were used to construct Table 28, which shows pH values and grams of dry matter, crude protein and organic matter present in different sections of the digestive tract of calves sacrificed 6 hours after a meal.

Mylrea (1966a) and Porter (1969) observed that pH of abomasal contents 5 hours after a meal reached pre-feeding levels (1.0 to 2.0) in calves fed whole milk. In the present work, samples of abomasal contents obtained 6 hours after feeding showed higher pH values (3.10) as observed by Gorrill and Thomas (1967). Milk replacers containing skimmilk and whey (diets A and B) or SPC (diets C and D) may have resulted in less HCl production than whole milk, as proposed by Tagari and Roy (1969) and Colvin et al. (1969). The higher abomasal pH may in part explain the lower crude protein digestibilities obtained in this experiment (Table 22) than those reported by Noller et al. (1956) and Jacobson et al. (1965)

TABLE 26. Weight of contents from different sections of the gastrointestinal tract, size of liver and pancreas, and weight at slaughter of 6 week old calves fed experimental milk replacers (Experiment 3)

Variables	Treatment											
	Milk			Milk + Limestone			SPC			SPC + Limestone		
	7546	7565	$\bar{x}$	7550	7567	$\bar{x}$	7556	7563	$\bar{x}$	7552	7564	$\bar{x}$
Body weight (kg)	66	50	58	44	58	51	42	42	42	52	48	50
R.R.O. content[a](g)	1,950	1,830	1,890	2,865	2,300	2,583	2,535	1,340	1,938	2,729	2,070	2,400
Abomasal content (g)	274	590	432	496	680	588	239	750	494	364	730	547
1st 3rd S.I. content[b](g)	129	186	158	125	117	121	88	255	171	170	24	97
2nd 3rd S.I. content[c](g)	326	195	260	277	377	327	369	359	364	409	433	421
3rd 3rd S.I. content[d](g)	243	155	199	333	547	440	237	273	255	240	349	294
Large int. content[e] (g)	455	237	346	262	280	271	318	153	235	424	390	407
Liver (g/kg body wt)	20.5	22.4	21.5	27.6	19.3	23.5	22.0	21.0	21.5	23.0	22.6	22.8
Pancreas (g/kg body wt)	.64	.64	.64	2.10	.98	1.54	.80	-	.80	.75	.79	.77

[a]Contents from the rumen-reticulum-omasum sections altogether.
[b]Contents from the first third of the small intestine (close to the pylorus).
[c]Contents from the second third of the small intestine
[d]Contents from the third third of the small intestine (close to the cecum).
[e]Contents from the large intestine.

TABLE 27. pH, dry matter, organic matter and crude protein in contents from different sections of the gastrointestinal tract of 6-week- old calves fed experimental milk replacers (Experiment 3)

Sections of the gut	Treatments											
	Milk			Milk + Limestone			SPC			SPC + Limestone		
	7546	7565	$\bar{x}$	7550	7567	$\bar{x}$	7556	7563	$\bar{x}$	7552	7564	$\bar{x}$
						pH						
R.R.O.[a]	6.33	6.30	6.32	6.20	6.40	6.30	6.84	6.12	6.48	6.32	6.37	6.35
Abomasum	3.93	2.42	3.18	3.12	2.57	2.85	2.19	4.04	3.12	3.68	2.81	3.25
1st 3rd S.I.[b]	6.41	7.59	7.00	5.91	6.03	5.97	6.08	6.05	6.07	5.92	6.26	6.09
2nd 3rd S.I.[c]	6.74	7.27	7.01	6.61	6.79	6.70	6.86	6.60	6.73	6.77	6.13	6.45
3rd 3rd S.I.[d]	7.76	7.27	7.52	7.03	7.69	7.36	7.27	6.66	6.97	7.62	7.38	7.50
Large intestine[e]	7.03	6.28	6.66	6.74	6.63	6.69	5.71	5.91	5.81	5.84	6.22	6.03
						Dry Matter (%)						
R.R.O.[a]	12.94	3.59	8.27	10.08	13.56	11.82	12.13	8.99	10.56	13.74	12.10	12.92
Abomasum	9.20	16.69	12.95	19.59	9.91	14.75	11.34	5.43	8.39	8.65	11.68	10.17
1st 3rd S.I.[b]	10.43	9.01	9.72	10.14	12.02	11.08	9.36	8.73	9.05	7.81	7.94	7.88
2nd 3rd S.I.[c]	10.46	6.64	8.55	22.38	7.90	15.14	7.60	8.24	7.92	8.66	8.28	8.47
3rd 3rd S.I.[d]	6.85	8.65	7.75	17.43	8.66	13.05	11.71	8.03	9.87	11.38	7.85	9.62
Large intestine[e]	16.58	15.65	16.12	18.96	19.54	19.25	19.30	15.32	17.31	19.04	19.12	19.08

TABLE 27 (continued)

Sections of the gut	Treatments											
	Milk			Milk + Limestone			SPC			SPC + Limestone		
	7546	7565	x̄	7550	7567	x̄	7556	7563	x̄	7552	7564	x̄
	Organic matter (%)[f]											
R.R.O.[a]	89.64	77.43	83.54	89.19	88.50	88.85	88.87	91.32	90.10	89.43	89.22	89.33
Abomasum	88.32	94.61	91.47	94.57	90.05	92.28	89.09	87.99	88.54	87.16	91.37	89.27
1st 3rd S.I.[b]	90.33	83.72	87.03	91.59	90.69	91.14	89.11	86.94	88.03	87.24	83.95	85.60
2nd 3rd S.I.[c]	89.87	82.41	86.14	86.63	84.58	85.61	86.49	87.40	86.95	85.99	86.32	86.16
3rd 3rd S.I.[d]	82.52	83.72	83.12	83.69	82.83	83.62	83.74	83.80	83.77	84.34	80.75	82.55
Large intestine[e]	83.00	86.78	84.89	81.01	87.18	84.10	85.52	81.50	83.51	85.83	83.72	84.78
	Crude protein (%)[f]											
R.R.O.[a]	9.51	14.76	12.14	10.22	8.55	9.39	8.08	10.01	9.05	8.59	10.91	9.39
Abomasum	17.39	10.66	14.03	22.66	18.36	20.51	29.45	36.28	32.87	19.19	20.89	20.04
1st 3rd S.I.[b]	62.70	25.31	44.01	58.58	52.08	55.33	64.74	53.15	58.95	54.54	-	54.54
2nd 3rd S.I.[c]	49.04	53.31	51.18	-	47.59	47.59	46.84	60.56	53.70	45.15	46.26	45.71
3rd 3rd S.I.[d]	39.27	57.69	48.48	25.99	32.33	29.16	27.24	40.35	33.80	23.64	20.64	22.14
Large intestine[e]	12.42	20.38	16.40	21.36	13.31	17.34	15.75	26.37	21.06	19.07	19.93	19.50

[abcde] As defined in Table 26.

[f] Organic matter and crude protein densities are expressed on dry matter basis.

TABLE 28. Effect of protein source (milk or milk + SPC) and limestone incorporation in milk replacers on pH, dry matter, crude protein and organic matter in contents from different sections of the gastrointestinal tract of 6-week-old calves (Experiment 3)

Contents from	Protein Source		Limestone		Mean	SEM
	Milk[k]	Milk + SPC[k]	Absent[k]	Present[k]		
			pH			
R.R.O.[a]	6.31	6.42	6.40	6.33	6.36[i]	.19
Abomasum	3.02	3.19	3.15	3.05	3.10[j]	.19
1st 3rd S.I.[b]	6.49	6.08	6.54	6.03	6.28[i]	.19
2nd 3rd S.I.[c]	6.86	6.59	6.87	6.58	6.72[hi]	.19
3rd 3rd S.I.[d]	7.44	7.24	7.25	7.43	7.29[h]	.19
Large intestine[e]	6.68	5.92[f]	6.24	6.36	6.30[i]	.19
Mean	6.11	5.89[f]	6.07	5.95[g]		.04
Feces	7.35	6.50[f]	6.94	6.91	6.93	.20
			Dry matter (g)			
R.R.O.[a]	230.	263	187	307[g]	247[h]	16
Abomasum	72	46	48	70	59[i]	16
1st 3rd S.I.[b]	14	11	15	10	13[i]	16
2nd 3rd S.I.[c]	35	32	26	41	33[i]	16
3rd 3rd S.I.[d]	34	26	20	40	30[i]	16
Large intestine[e]	54	60	49	65	57[i]	16
Mean	73	73	57	89[g]		8
			Organic matter (g)			
R.R.O.[a]	203	235	165	273[g]	219[h]	16
Abomasum	68	41	44	65	54[i]	16
1st 3rd S.I.[b]	13	10	13	10	11[i]	16
2nd 3rd S.I.[c]	30	28	23	35	29[i]	16
3rd 3rd S.I.[d]	28	22	17	33	25[i]	16
Large intestine[e]	46	49	40	55	47[i]	16
Mean	65	64	50	78[g]		7

TABLE 28 (continued)

Contents from	Protein Source		Limestone		Mean	SEM
	Milk[k]	Milk + SPC[k]	Absent[k]	Present[k]		
			Crude protein (g)			
R.R.O.[a]	23	24	18	29[g]	23[h]	2
Abomasum	12	12	10	15	12[i]	2
1st 3rd S.I.[b]	7	8	7	7	7[i]	2
2nd 3rd S.I.[c]	13	16	14	15	14[i]	2
3rd 3rd S.I.[d]	11	8	8	1	9[i]	2
Large intestine[e]	9	12	8	12	10[i]	2
Mean	12	13	11	15[g]		1

[abcde] As defined in Table 26.

[f] Different from milk at P<.05.

[g] Different from absence of limestone at P<.05.

[hij] Means in columns within each variable measured not sharing the same superscript are different (P<.05) using Tukey's test.

[k] Standard error for treatment means in each of the different sites of the gastrointestinal tract were respectively .26, 23, 22 and 2.4 for pH, dry matter, organic matter and crude protein.

with whole milk, since the optimum pH for pepsin activity was reported to be 2.0 by Tagari and Roy (1969). Neither the substitution of milk protein by SPC, nor the inclusion of limestone in the replacer significantly ($P<.05$) affected abomasal pH (Table 28).

Small intestinal pH increased from 6.28 to 6.72 from the proximal to the distal portion (Table 28). This result agrees with studies of Huber (1958) and Huber and Moore (1964). Harrison and Hill (1962), Lennox and Garton (1968) and Lennox et al. (1968) found that conditions in the upper jejunum of adult ruminants were notably acidic (pH of 2.0 or 3.0) and that pH did not reach values of 6.0 or 7.0 until the lower jujunum. However, our studies were with calves fed only milk replacer and the proximal section of the small intestine may have extended to the lower jejunum.

Other workers (Smith, 1962; Phillipson and Storry, 1965; Topps et al. 1968) indicated a limit to the capacity of the small intestine of adult ruminants to neutralize acid digesta from the abomasum. Results obtained in the present experiment do support these findings and tend to indicate that the neutralizing capacity of bile and pancreatic juice of pre-ruminant calves is similar to monogastrics. As a result, the inclusion of .8% limestone in milk replacer might not be expected to cause a significant change in small intestinal pH (Table 28). On the contrary, the presence of limestone resulted in a lower ($P<.05$) pH of gastro-intestinal contents, perhaps due to a stimulatory effect of limestone on HCl production by parietal cells of the abomasum in an effort to compensate for the added buffer. The replacement of one-half of the dietary protein with SPC resulted in a slightly lower small intestinal pH,

but the change was not significant (P>.05).

The interaction of protein source times the presence or absence of limestone was significant (P<.05), since limestone addition to replacers containing only milk protein decreased (P<.05) overall gut pH, whereas no changes (P<.05) were observed for calves fed SPC (Table 29).

TABLE 29. Effect of limestone addition on overall gut pH of calves fed milk replacers containing only milk protein or half of dietary protein from SPC (Experiment 3)

Limestone	Protein source	
	Milk	SPC
Absent	6.28[a]	5.86[a]
Present	5.98[b]	5.91[a]

[ab]Means in the same column with unlike superscript are different at P<.05.

Large intestinal and fecal pH was lower (P<.05) in calves fed SPC than milk protein (Table 28). Lower digestibility of nutrients may have resulted in accumulation of more undigested nutrients in the large intestine (Gorrill and Nicholson, 1971), which created conditions for greater fermentation of the organic matter. Gastrointestinal disturbances have been associated with lower nutrient digestibility in calves fed milk replacers containing severely heated skimmilk or non-milk proteins (Shillam et al., 1962; Tagari and Roy, 1969; Williams et al., 1976). Diarrhea in the present experiment however, was not increased by feeding SPC (Table 21).

Although data in Table 28 refer only to a particular time (6 hours) after feeding some conclusions can be made. Dry matter and organic

matter recovered at each site were not different (P>.05) for only milk protein or milk protein plus SPC. However, the amount of dry matter and organic matter present in the abomasum of animals fed SPC tended to be slightly lower than for calves fed only milk protein. This might be due to a shorter retention time of ingesta in the abomasum of non-milk proteins in preruminant calves, as postulated by Colvin et al. (1969) and Ternouth et al. (1975). However, quantity of protein in the abomasum of calves fed SPC was the same as those fed only milk protein (Table 28). According to Emmons et al. (1976) and Ternouth et al. (1980), milk protein (and milk fat) should remain in the abomasum longer than other nutrients.

The incorporation of .8% limestone in the replacers resulted in more (P<.05) dry matter, organic matter and crude protein in the digestive tract of calves, due to the larger (P<.05) amount present in the rumen-reticulum-omasum section. This observation suggests that calves fed limestone probably had higher intakes of bedding (straw), perhaps because they tried to compensate for the lower nutrient digestibilities and protein retention (Table 22).

The interaction of treatment x site in the gut (Table A.3, Appendix) was not significant (P>.10) for any digesta variable measured, suggesting that treatment effects followed the same trends throughout the different sections of the gut.

One calf from each treatment died during the experiment and necropsy results did not indicate a diet relationship.

In summary the replacement of one-half of dietary protein with SPC in milk replacers resulted in lower weight gains (20%) and decreased dry

matter and crude protein digestibilities. Organic matter digestibility and crude protein retention also tended to be reduced, but intake of nutrients, feed efficiencies and fecal scores were not affected. The incorporation of .8% limestone in milk replacers resulted in no significant changes in the above-mentioned parameters, although apparent nutrient digestibilities and protein retention tended to decrease when limestone was present.

The xylose absorption test showed differences in absorption due to calf age, but not between treatments. Similar results were obtained in Experiment 1.

Analysis of digesta from different sections of the gastrointestinal tract revealed a higher pH in the abomasum than previously reported for whole milk diets. Abomasal pH was not affected by protein source.

Small intestinal pH was always above 6, regardless of the dietary treatment. This observation suggests that digestion in preruminant calves is similar to monogastrics and different from adult ruminants. The high intestinal pH values might explain the ineffectiveness of limestone in improving nutrient digestibilities.

The replacement of milk protein by SPC resulted in a lower pH in the large intestine and feces, probably due to fermentation of accumulated organic matter in the large intestine because of a more rapid flow of digesta through the gut. Indeed, animals fed SPC showed lower amounts of dry matter and organic matter in the abomasum than those fed milk protein.

CONCLUSIONS

Replacing 31% of milk protein in calf milk replacers with soybean protein concentrate (SPC) or spray-dried fish solubles resulted in significantly lower weight gains, dry matter digestibilities and nitrogen retention. Increasing crude protein from 19 to 23% in a replacer containing 10% crude protein from spray-dried fish solubles did not improve the calf's performance. Furthermore, high mortalities indicated that 10% protein from spray-dried fish solubles was excessive. Necropsy results from animals fed large amounts of spray-dried fish solubles suggested that allergy problems may be involved since ulcerations, and in some cases, ruptures of the gastrointestinal wall occurred.

Replacing one-half of the milk protein with SPC reduced calf weight gain, dry matter and protein digestibilities, and nitrogen retention and pH in the large intestine and feces. The lowered pH may have been due to fermentation of more undigested material in the large intestine because of a more rapid flow of nutrients through the gut of calves fed SPC. Indeed, animals fed SPC showed lower amounts of digesta in the abomasum 6 hours after a meal than calves receiving only milk protein.

Samples from three sections of the small intestine obtained 6 hours after calves were fed, showed pH values above 6 independent of the protein source. These results suggest that unlike adult ruminants, bile and pancreatic juice exert adequate buffering in preruminant calves and

probably account for the ineffectiveness of limestone in the replacer fed in this study.

The xylose absorption test showed age differences in absorption capabilities of calves but did not show differences between protein sources.

APPENDIX

TABLE A.1. -- Analysis of variance for variables in experiment 1

Source of variation	d.f.	Mean square	F ratio	Significance level[a]
Initial body weight at Michigan State (kg)				
Treatment	6	10.6661	.51	NS
Error	55	20.8753		
Immunoglobulin (mg %)				
Treatment	6	11.0224	.84	NS
Error	55	13.1544		
Plasma protein (%)				
Treatment	6	.2494	.91	NS
Error	55	.2793		
Total weight gains - 0 to 3 wk (kg)[b]				
Location (L)	2	31.3531	4.70	**
Treatment (T)	6	35.1035	5.23	***
L x T	12	10.3144	1.54	NS
Error	146	6.7122		
Total weight gains - 4 to 6 wk (kg)[b]				
Location (L)	2	40.2621	5.33	***
Treatment (T)	6	115.7341	15.32	***
L x T	12	11.2236	1.49	NS
Error	145	7.5560		
Total weight gains - 0 to 6 wk (kg)[b]				
Location (L)	2	140.1698	9.61	***
Treatment (T)	6	265.9481	18.23	***
L x T	12	34.2053	2.34	***
Error	145	14.5875		
Dry matter intake - 0 to 6 wk (g)[b]				
Location (L)	2	202,208,068.9704	20.42	***
Treatment (T)	6	29,866,844.8616	3.02	***
L x T	12	10,574,208.3345	1.07	NS
Error	147	9,900,781.6781		
Crude protein intake - 0 to 6 wk (g)[b]				
Location (L)	2	8,843,517.9801	19.14	***
Treatment (T)	6	17,858,283.9987	38.65	***
L x T	12	465,579.3709	1.01	NS
Error	147	461,939.1200		

TABLE A.1. -- Continued

Source of variation	d.f.	Mean square	F ratio	Significance level[a]
Weight gain/dry matter intake[b]				
Location (L)	2	.0712	3.56	**
Treatment (T)	6	.3403	16.36	***
L x T	12	.0432	2.08	**
Error	145	.0208		
Weight gain/crude protein intake[b]				
Location (L)	2	.6800	1.11	NS
Treatment (T)	6	16.9000	27.70	***
L x T	12	1.0400	1.70	*
Error	145	.6100		
Rectal temperature - 1st week (^{o}F)[b]				
Location (L)	2	32.9601	84.49	***
Treatment (T)	6	.3875	.99	NS
L x T	12	.2281	.58	NS
Error	146	.3901		
Rectal temperature - 2nd week (^{o}F)[b]				
Location (L)	2	48.2704	69.63	***
Treatment (T)	6	.4698	.68	NS
L x T	12	.3188	.46	NS
Error	146	.6931		
Fecal scores[b]				
Location (L)	2	87.2610	296.47	***
Treatment (T)	6	43.5380	147.92	***
L x T	12	5.6225	19.10	***
Week (W)	5	8.5853	29.17	***
T x W	30	.7940	2.70	***
Error	946	.2943		
Treatment of sickness[b]				
Location (L)	2	6.1791	35.22	***
Treatment (T)	6	.4766	2.72	**
L x T	12	.8162	4.65	***
Week (W)	5	3.0410	17.34	***
T x W	30	.1452	.83	NS
Error	945	.1754		

TABLE A.1. -- Continued

Source of variation	d.f.	Mean Square	F ratio	Significance level[a]
Plasma xylose concentration (mg %)				
Treatment (T)	6	6,254.041	.32	NS
Animal/T (Error a)	55	19,697.388		
Sample time (S)	7	46,296.715	47.47	***
T x S	42	843.468	.85	NS
Error b	385	988.400		
Methionine (μmoles/100 ml)				
Treatment (T)	6	2.44	2.18	NS
Animal/T (Error a)	21	1.12		
Week (W)	1	3.92	2.48	NS
T x W	6	2.47	1.56	NS
Error b	21	1.58		
Leucine (μmoles/100 ml)				
Treatment (T)	6	38.93	2.72	**
Animal/T (Error a)	21	14.32		
Week (W)	1	63.60	5.41	**
T x W	6	6.47	.55	NS
Error b	21	11.76		
Valine (μmoles/100 ml)				
Treatment (T)	6	80.03	3.24	**
Animal/T (Error a)	21	24.73		
Week (W)	1	160.01	11.55	***
T x W	6	11.41	.82	NS
Error b	21	13.85		
Isoleucine (μmoles/100 ml)				
Treatment (T)	6	19.38	2.53	*
Animal/T (Error a)	21	7.65		
Week (W)	1	15.06	4.50	**
T x W	6	2.55	.76	NS
Error b	21	3.35		
Phenylalanine (μmoles/100 ml)				
Treatment (T)	6	5.40	1.00	NS
Animal/T (Error a)	21	5.39		
Week (W)	1	28.54	9.67	***
T x W	6	2.95	1.00	NS
Error b	21	2.95		

TABLE A.1 -- Continued

Source of variation	d.f.	Mean square	F ratio	Significance level[a]
Lysine (μmoles/100 ml)				
Treatment (T)	6	66.44	2.73	**
Animal/T (Error a)	21	24.31		
Week (W)	1	324.00	38.43	***
T x W	6	22.44	2.66	**
Error b	21	8.43		
Histidine (μmoles/100 ml)				
Treatment (T)	6	7.66	1.73	NS
Animal/T (Error a)	21	4.42		
Week (W)	1	15.92	5.34	**
T x W	6	2.19	.73	NS
Error b	21	2.98		
Arginine (μmoles/100 ml)				
Treatment (T)	6	47.68	2.15	*
Animal/T (Error a)	21	22.17		
Week (W)	1	97.94	5.18	**
T x W	6	50.38	2.66	**
Error b	21	18.93		
Threonine (μmoles/100 ml)				
Treatment (T)	6	54.33	1.72	NS
Animal/T (Error a)	21	31.53		
Week (W)	1	0.00	--	NS
T x W	6	15.15	.43	NS
Error b	21	35.40		
Total essential amino acids-TEAA (μmoles/100 ml)				
Treatment (T)	6	1,890.06	2.90	**
Animal/T (Error a)	21	651.79		
Week (W)	1	2,042.29	6.92	**
T x W	6	272.34	.92	NS
Error b	21	294.94		
Total non-essential amino acids-TNEAA (μmoles/100 ml)				
Treatment (T)	6	1,347.70	1.97	NS
Animal/T (Error a)	21	684.90		
Week (W)	1	30.01	.05	NS
T x W	6	261.06	.39	NS
Error b	21	665.46		

TABLE A.1. -- Continued

Source of variation	d.f.	Mean square	F ratio	Significance level[a]
Non-essential:essential amino acids ratio - N/E				
Treatment (T)	6	.26	2.17	NS
Animal/T (Error a)	21	.12		
Week (W)	1	1.26	5.48	**
T x W	6	.04	.17	NS
Error b	21	.23		
Branched chain amino acids-BCA (μmoles/100 ml)				
Treatment (T)	6	373.84	3.08	**
Animal/T (Error a)	21	121.30		
Week (W)	1	600.50	10.14	***
T x W	6	48.84	.82	NS
Error b	21	59.25		
Sulfur amino acids-SAA (μmoles/100 ml)				
Treatment (T)	6	2.80	1.28	NS
Animal/T (Error a)	21	2.18		
Week (W)	1	1.55	.64	NS
T x W	6	5.58	2.31	NS
Error b	21	2.42		

[a]NS = nonsignificant (P>.10)
* = P<.10.
** = P<.05.
*** = P<.01.
[b]Statistical analysis realized at Kansas State University.

TABLE A.2. -- Analysis of variance for variables in experiment 2

Source of variation	d.f.	Mean square	F ratio	Significance level[a]
Initial body weight (kg)				
Treatment	3	34.68	1.41	NS
Error	12	24.53		
Plasma protein (%)				
Treatment	3	.1906	1.44	NS
Error	11	.1328		
Daily weight gain (kg)				
Treatment	3	.1906	1.42	NS
Error	11	.1328		
Rectal temperatures (^{o}C)				
Treatment	3	.2680	1.38	NS
Error	12	.1939		
Scour scores				
Treatment	3	1.09	2.95	*
Error	12	.37		
Apparent dry matter digestibility (%)				
Treatment	3	.0215	4.50	**
Error	12	.0047		
Apparent organic matter digestibility (%)				
Treatment	3	.0174	4.34	**
Error	12	.0040		
Apparent nitrogen retention (g/day)				
Treatment	3	43.80	5.78	**
Error	12	7.58		

[a]NS = nonsignificant (P>.10).
* = P<.10.
** = P<.05.

TABLE A.3. -- Analysis of variance for variables in experiment 3

Source of variation	d.f.	Mean square	F ratio	Significance level[a]
Initial body weight (kg)				
Treatment (T)	3	23.1	1.00	NS
Error	11	23.0		
Weight gains (g/an/day)				
Treatment (T)	3	21,132.3666	.27	NS
Animal/T (Error a)	11	79,650.3879		
Week (W)	5	1,207,505.1794	39.43	***
T x W	15	28,168.6669	.92	NS
Error b	59	30,626.0820		
Dry matter intake (g/an/day)				
Treatment (T)	3	1,480.0028	.03	NS
Animal/T (Error a)	11	53,704.2871		
Week (W)	5	271,263.5080	61.68	***
T x W	15	2,032.8525	.46	NS
Error b	59	4,397.6160		
Crude protein intake (g/an/day)				
Treatment (T)	3	121.3167	.06	NS
Animal/T (Error a)	11	1,957.8288		
Week (W)	5	9,883.9058	148.48	***
T x W	15	42.4033	.64	NS
Error b	59	66.5687		
Weight gain/dry matter intake				
Treatment (T)	3	.0967	.06	NS
Animal/T (Error a)	11	.1907		
Week (W)	5	3.2827	19.39	***
T x W	15	.1449	.86	NS
Error b	59	.1693		
Fecal dry matter (%)				
Treatment (T)	3	13.2848	2.45	NS
Animal/T (Error a)	12	5.4319		
Week (W)	1	1.0047	.07	NS
T x W	3	12.9680	.86	NS
Error b	12	15.1504		

TABLE A.3. -- Continued

Source of variation	d.f.	Mean square	F ratio	Significance level[a]
Fecal scores				
Treatment (T)	3	.6020	.66	NS
Animal/T (Error a)	11	.9130		
Week (W)	5	.5480	1.39	NS
T x W	15	.3483	.88	NS
Error b	59	.3952		
Rectal temperature (^{o}C)				
Treatment (T)	3	.3767	.70	NS
Animal/T (Error a)	11	.5349		
Week (W)	5	.1865	.90	NS
T x W	15	.1961	.94	NS
Error b	59	.2076		
Fecal pH				
Treatment (T)	(3)	(1.9158)	(6.27)	***
Source (S)	1	5.7207	18.78	***
Limestone (L)	1	.0102	.03	NS
S x L	1	.0166	.05	NS
Animal/T (Error a)	12	.3056		
Week (W)	1	.5486	7.36	**
T x W	3	.0660	.88	NS
Error b	12	.0746		
Apparent dry matter digestibility (%)				
Treatment (T)	(3)	(45.2817)	(3.25)	*
Source (S)	1	53.7968	3.86	*
Limestone (L)	1	41.9323	3.01	NS
S x L	1	40.1160	2.88	NS
Animal/T (Error a)	12	13.9409		
Week (W)	1	49.6281	4.43	*
T x W	3	14.6532	1.31	NS
Error b	12	11.2059		
Apparent organic matter digestibility (%)				
Treatment (T)	3	43.6124	2.12	NS
Animal/T (Error a)	12	20.5429		
Week (W)	1	.0782	.01	NS
T x W	3	13.4667	.99	NS
Error b	12	13.5686		

TABLE A.3. -- Continued

Source of variation	d.f.	Mean square	F ratio	Significance level[a]
Apparent protein digestibility (%)				
Treatment (T)	(3)	(220.7409)	(3.96)	**
Source (S)	1	570.5574	10.23	***
Limestone (L)	1	72.6897	1.30	NS
S x L	1	18.9755	.34	NS
Animal/T (Error a)	12	55.7702		
Week (W)	1	31.8364	.83	NS
T x W	3	10.0297	.26	NS
Error b	12	38.3330		
Apparent protein retention (g/day)				
Treatment (T)	3	254.2522	.37	NS
Animal/T (Error a)	12	684.4508		
Week (W)	1	12,640.5040	51.81	***
T x W	3	113.3984	.46	NS
Error b	12	243.9874		
Apparent protein retention (% of ingested)				
Treatment (T)	3	109.2886	.58	NS
Animal/T (Error a)	12	187.0888		
Week (W)	1	1,454.8962	25.18	***
T x W	3	.8671	.01	NS
Error b	12	57.7742		
Plasma urea nitrogen (mg %)				
Treatment (T)	3	3.2069	.39	NS
Animal/T (Error a)	12	8.1549		
Week (W)	1	120.9113	10.44	***
T x W	3	16.2117	1.40	NS
Error b	12	11.5844		
Plasma xylose concentration (mg %)				
Treatment (T)	3	1,640.2619	.09	NS
Animal/T (Error a)	8	18,196.1625		
Week (W)	1	18,768.0571	11.67	***
Sample (S)	7	21,527.1529	15.25	***
T x W	3	14,092.3394	8.76	***
T x S	21	1,355.9258	.84	NS
W x S	7	2,212.3580	1.38	NS
T x W x S	21	270.2670	.17	NS
Error b	120	1,607.9258		

TABLE A.3. -- Continued

Source of variance	d.f.	Mean square	F ratio	Significance level[a]
Gastrointestinal pH				
Treatment (T)	(3)	(.4169)	(10.19)	**
Source (S)	1	.6912	16.90	**
Limestone (L)	1	.1850	4.52	NS
S x L	1	.3746	9.15	**
Animal/T (Error a)	4	.0409		
Gut (G)	5	14.4700	62.77	***
T x G	15	.1450	.52	NS
Error b	20	.2783		
Gastrointestinal dry matter content (g)				
Treatment (T)	3	4,050.3542	2.43	NS
Animal/T (Error a)	4	1,665.7292		
Gut (G)	5	60,199.2375	27.67	***
T x G	15	1,714.8042	.79	NS
Error b	20	2,175.6292		
Gastrointestinal organic matter content (%)				
Treatment (T)	3	3,221.8333	2.43	NS
Animal/T(Error a)	4	1,327.9584		
Gut (G)	5	47,869.6333	23.84	***
T x G	15	1,171.2667	.58	NS
Error b	20	2,007.6083		
Gastrointestinal crude protein content (g)				
Treatment (T)	3	68.7431	2.05	NS
Animal/T (Error a)	2	33.4583		
Gut (G)	5	266.3375	11.51	***
T x G	15	22.1931	.96	NS
Error b	18	23.1435		

[a]NS = nonsignificant (P>.10).
* = P<.10.
** = P<.05.
***= P<.01.

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