UTILIZATION OF FABABEAN PROTEIN CONCENTRATE IN MILK SUBSTITUTE DIETS BY PRERUMINANT CALVES

by

Marie Katherine Wittenberg

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree Master of Science.

Department of Animal Science
Faculty of Agriculture
University of Manitoba

October, 1977

UTILIZATION OF FABAFEAN PROTEIN CONCENTRATE IN MILK SUBSTITUTE DIETS BY PRE-RUMINANT CALVES.

by

Marie Katherine Wittenberg

A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

Master of Science

TABLE OF CONTENTS

Table of Contents Page

ABSTRACT

Fababean protein concentrate (F.B.P.C.), containing 60-65% protein (N x 6.25) on a dry matter basis, was prepared from dehulled, ground fababeans using air-classification. Results of a 28 day P.E.R. assay with rats show that protein quality of methionine (meth.) supplemented F.B.P.C. is equivalent to casein and to meth. supplemented soybean protein concentrate.

The object of the first study was to determine the ability of the preruminant calf to utilize F.B.P.C. in milk substitute diets and to determine the influence of F.B.P.C. in the diet on the digestibility of other nutrients. Methionine (met.) supplemented F.B.P.C. supplied 80, 50, 25 and 0% of the protein in diets A, B, C and D respectively; milk proteins supplying the remainder. There were no significant differences among the diets in average daily gain and feed efficiency which were 216, 149, 306, 297 g/day and .33, .24, .46, .46 gain/intake for diets A, B, C and D respectively. Diet did not influence the digestibility coefficient of dry matter and fat, however, digestibility of crude protein decreased significantly ($P < 0.05$) as the level of F.B.P.C. in the diet increased. Using a regressional analysis, the crude protein digestibility of F.B.P.C. was estimated to be 57, 80 and 87% for calves aged 8-13, 15-20 and 22-27 days respectively.

A second trial, using male Holstein calves, was conducted to determine the optimal level of methionine supplementation for milk substitute diets in which 80% of the protein was supplied by F.B.P.C. Diets A, C and D differed only in the L-meth. content, which was 1.70, 2.38 and 2.84 g/16 g. N respectively. Diet B, containing .76 g. D-meth. and 1.53 g. L-meth/16 g. N, was included to determine whether calves can utilize D-meth. All diets contained .79 g. cystine/16 g. N. The differences between fasting and post-fasting plasma meth. levels indicate that the requirements for total sulfur amino acids by the preruminant calf is 2.5 to 3.1 g/16 g. N. Results also indicate that D-meth. is utilized by the calf.

ACKNOWLEDGMENTS

The author would like to thank Dr. J.R. Ingalls for his guidance, advice and inspiration during the course of this study.

The author also wishes to thank Dr. M.E. Seale, Head of the Department of Animal Science, for supplying the facilities and animals required to conduct these experiments.

Sincere thanks is extended to Mr. J. Woodhouse for his help in the barn. Help from fellow graduate students during the course of the experiments was also greatly appreciated. Acknowledgment is also due Mr. P.A. Mills for his excellent technical assistance during the course of this study.

The provision of financial support by the University of Manitoba fellowship has been gratefully appreciated. Additional financial help received from Dr. J.R. Ingalls is also appreciated.

Finally, the author wishes to extend deep gratitude to her husband, Eric, for his forebearance and encouragement during the course of this study.

LIST OF TABLES

List of Tables (continued)

List of Tables (continued)

LIST OF FIGURES

List of Figures (continued)

LIST OF APPENDICES

List of Appendices (continued)

LIST OF ABBREVIATIONS

FOR AMINO ACIDS

Ala	Alanine
Arg	Arginine
Asp	Aspartic Acid
Cys	Cystine
Glu	Glutamic Acid
Gly	Glycine
His	Histidine
Ile	Isoleucine
Leu	Leucine
Lys	Lysine
Met	Methionine
Phe	Phenylalanine
Pro	Proline
Ser	Serine
Thr	Threonine
Try	Tryptophan
Tyr	Tyrosine
Val	Valine

INTRODUCTION

The demand for protein to meet animal nutritional requirements is increasing and is expected to continue increasing. These high demands will result in protein scarcity and increased costs. It is desirable, therefore, to fully exploit any or all protein feeds that can be produced in Canada.

Fababeans (Vicia Faba L var minor) have attracted attention as a potential home-grown protein source. Selective breeding programs have made it possible to grow high yielding varieties, producing beans with crude protein contents ranging from 25 to 30%. When considering the whole bean, fababeans appear to have a protein concentration second only to whole soybeans and for this reason may be used successfully to partially replace imported soybeans as a protein supplement.

The importance of the protein fraction of milk substitute diets lies not only in its major contribution to growth of the preruminant calf, but also to the fact that minor deviations in protein quality and changes in concentration may have a deleterious influence on the health of the calf. Poor digestion and malabsorption of protein appear to be major factors in allowing bacterial overgrowth and adhesion of enterobacteria to the mucosa in the anterior region of the small intestine with subsequent diarrhoea. Bovine

milk proteins, when properly added to milk substitute diets, are of very high quality and are better digested than any other protein source of the young calf.

The high cost of milk proteins limits the profits of veal production. The situation has been made worse by an increasing demand for beef. Consequently, rearing calves for veal production has declined and is expected to continue to decline. One method of solving these problems would be the partial or total replacement of milk proteins with less costly substitutes. This would also be beneficial in reducing the costs of raising replacement animals in dairy herds.

Fish meal, whey and soybean protein have been tested in milk substitute diets as potential alternative sources of protein and have been found to be satisfactory if used as a partial replacement of milk proteins in milk replacers. The most promising studies, however, have been done on calves with body weight greater than 60 kg. (approximately four weeks of age) and not with the highly sensitive, very young calf.

The major objective of the studies that were carried out was to determine the utilization of fababean protein concentrate (F.B.P.C.) in milk substitute diets by preruminant calves. It was also attempted to determine the optimal level of methionine concentration for preruminant calves when F.B.P.C. contributes 80% of the protein in the diet.

No information was available on the utilization of D-methionine by preruminant calves. Various growth and blood para-

meters were studied to determine whether preruminant calves can utilize the D-isomer to meet the sulfur amino acid requirements.

REVIEW OF LITERATURE

Fababeans have been introduced into Canada in the past decade as a potential protein supplement for human consumption and for animal and poultry feeds. The major objectives of Canadian plant breeders working with fababeans is to develop cultivars that will permit it to be an economical "home-grown" protein supply. The major agronomic improvements sought are earlier maturity, reduced shattering and disease resistance. Methods to improve weed and pest control practises are also being studied. There are also quality improvements that would increase the value of fababeans as a protein source, namely an increase in the level of sulfur containing amino acids.

Utilization of fababeans as poultry and animal feed has been restricted due to the presence of heat-labile growth depressing factor(s). These may be in the form of a trypsin inhibitor, a chymotrypsin inhibitor, an amylase inhibitor and/or hemagglutinins (Leiner and Kakade, 1969; B.J. Wilson *et al.*, 1972; Marquardt *et al.*, 1975). Studies are being conducted to determine what these anti-nutritional factors are and to find processing methods which will eliminate these factors or minimize their effects.

FABABEAN PROTEIN

A review of the chemical composition of fababean seeds by Sjödin (1973) gave a description of fababean protein. In the seed, the majority of the nitrogen is present as protein and only about 4% as free amino acids, mainly arginine and histidine. The protein is made up of two types, metabolic and storage proteins. The amount of metabolic protein is not constant throughout growth but will increase during periods of intense metabolic activity. The majority of storage proteins (globulins) are formed in the cotyledons. These globulins are formed as two groups of proteins, legumin (350,000 M.W) and vicilin (180,000 M.W.) during the late stages of the vegetation period. The amount of vicilin remains relatively constant from flowering to maturity whereas there is a steady increase in the level of legumin up to maturity. The amino acid composition for the two globulins is different; legumin containing considerably more methionine than vicilin and the reversed relationship for lysine.

The major constituents of the fababean seed are protein, starch and fiber (Craig, 1974). Fababeans, to be competitive as a protein supplement, must be processed to form a protein isolate or concentrate. Fababean flour made by grinding whole fababeans would have approximately 25 to 28% crude protein content.

A protein isolate powder containing 91% protein (N x 6.25) on a dry matter basis can be prepared by extracting flour from dehulled and ground beans in a dilute aqueous alkali, followed by acid precipitation, neutralization and spray-drying (Duthie et al., 1972). On the basis of net protein utilization and protein efficiency ratio assays with weanling rats, Duthie et al. (1972) found that

fababean isolate is a useful protein which compares favourably with a commercial soybean protein isolate, both being considerably improved with the addition 0.25% D,L-methionine. On the basis of a five-day collection period, the nitrogen digestibility of fababean and soybean isolates are 93.5 and 94.8% respectively for growing male rats.

Weight and size differences between the starch and protein particles in fababean flour have made it possible to separate the two fractions with 90% protein recovery by a simple process called air classification (Craig, 1974). The dehulled fababean is pin-milled and air classified (Figure 1). A high protein fraction containing approximately 70% crude protein is obtained. The remaining starch fraction is then reground in a pin mill and passed a second time through the air classifier resulting in a starch fraction (4% crude protein) and a 65% protein fraction. The result is a high protein concentrate of approximately 68% crude protein. The process lends itself readily to the preparation of graded levels of protein content in the final product by making simple adjustments to the equipment.

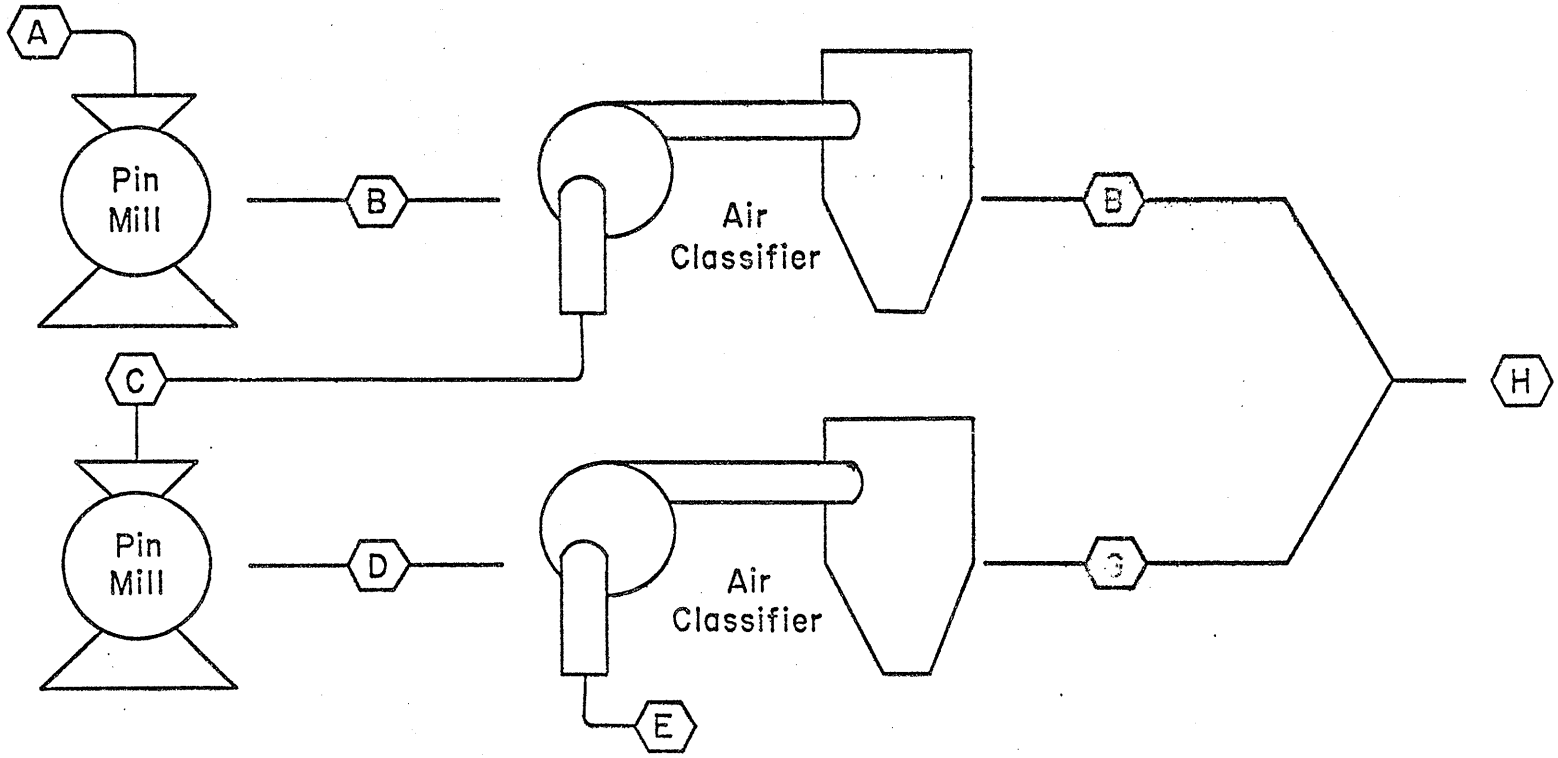
Little work with F.B.P.C. has been done in animal nutrition. Physically, this concentrate is a fine white powder which mixes well with water and remains in suspension (Latrille et al., 1975). F.B.P.C. has the natural raw flavor of the bean.

Using data collected from pigs initially weighing approximately 15.3 kg., Maltman (1976) found some differences in the individual amino acid availabilities for F.B.P.C. and soybean meal (44%). Availability of methionine from the two protein sources did not

FIGURE 1

Flowsheet and Yields of Products Produced by Pinmilling and Air Classification of Fababeans

Reference: Craig, 1974



A 100 lbs. dehulled (31 % protein)
B 100 lbs. bean flour (31 % protein)
C 78 lbs. bean starch (20 % protein)
D 78 lbs. bean starch (20 % protein)
E 59 lbs. bean starch (4 % protein)
F 22 lbs. bean protein (70 % protein)
G 19 lbs. bean protein (65 % protein)
H 41 lbs. bean protein. (68 % protein)

differ significantly, however, availability of cystine was greater for soybean meal than that derived from F.B.P.C. The most available essential amino acids in F.B.P.C. were arginine, histidine, leucine and lysine, then isoleucine, phenylalanine, threonine and valine, with methionine being the least available (88%).

Sarwar et al. (1977), on the contrary, found the availability of methionine and cystine to be significantly greater ($P > 0.05$) for soy protein (84%) than for fababean protein (70%). They also found methionine to be the least available of the essential amino acids in fababean protein when fed to growing rats. Supplementation of the diet with methionine or wheat flour caused improved availability of methionine from the fababean protein source.

The low availability of methionine from fababean flour may be improved through the use of heat treatment (Wilson and McNab, 1972; Marquardt and Campbell, 1974). They observed a greater growth response by chicks due to autoclaving when the diet consisted of nonsupplemented fababean flour as compared to diets in which methionine and cystine levels were adequate.

Studies (Marquardt et al., 1976; Wilson and McNab, 1972; Wilson et al., 1972) conducted with broiler chicks demonstrated that autoclaving improved the utilization of fababeans due to the effect on components associated with the protein and hull fractions. However, performance data from studies on growing pigs, where F.B.P.C. constituted 7 to 29% of the diet, suggested that there did not appear to be any appreciable amount of growth inhibiting substances in the protein concentrate (Maltman, 1976). Macdonald (1974) demonstrated that autoclaving fababean flour (at 115°C for 12 or 15 min.) had no

effect on the growth performance of rats fed diets in which fababean flour was the sole protein source. Marquardt et al. (1974) found that autoclaved (120°C for 15 or 20 min.) as compared to raw fababeans in diets of chicks and rats resulted in significant ($P < 0.05$) improvements in the respective weight gains (9 and 6%) and feed:gain ratios (14 and 6%). The response by rats was not as great as that of chicks suggesting that there may be differences among species of animals in terms of utilization of the raw fababeans (Marquardt et al., 1974).

Results obtained, when F.B.P.C. replaced the soybean meal in a normal wheat and soybean meal prestarter-starter ration for weanling pigs, indicated a greater (11%) feed efficiency value as compared to the control soybean-wheat diet (Maltman, 1976). These results contradict those of Sarwar et al. (1975) who found that feed intake, weight gain, true protein digestibility and PER values were lower for fababean diets than for soybean diets when fed to growing rats. There was a substantial improvement when a fababean-wheat blend was fed to rats.

With the exception of some preliminary studies done at the University of Manitoba (Latrille et al., 1975), F.B.P.C. has not been studied as a protein source for milk substitute diets for pre-ruminant calves. In these studies, fababean protein concentrate made up (I) 50% and (II) 80% of the protein in the milk substitude diets, the remainder being provided by skim milk powder and dried whey. Results indicated an improvement with age for digestible dry matter, crude protein and fat. A few cases of diarrhoea and loose feces were observed, fecal consistency in general

being looser in calves fed diet I. There were several cases of steatorrhea. This may have been due to malabsorption associated with disturbances of protein or carbohydrate digestion (Roy, 1974; Radostits and Bell, 1970).

Duthie et al. (1974) did some preliminary studies on the suitability of field bean (Vicia faba L.) protein isolate on lambs from 0-14 days of age. Fababean isolate as the sole protein source in the diet was well accepted by the lambs and no adverse reactions were observed. In the second experiment conducted with veal calves from 0 to 14 weeks of age, fababean protein isolate made up 50% and 100% of the protein in the diet. As with the lambs, there was no significant difference among the diets in liveweight gain, food intake or the food conversion ratio. Similarly for the calves there were no differences in acceptability, performance, killing-out percentage, meat color, conformation or finish as compared to calves fed diets containing dried skim milk.

VEGETABLE PROTEINS IN MILK SUBSTITUTE DIETS

(I) Soybean Protein: Numerous attempts have been made to replace part or all of the milk protein in calf milk replacers with vegetable proteins. Soybean protein has a complete and reasonably well-balanced amino acid composition (Nitzan et al., 1972) and is available as a by-product of the oil industries (Porter, 1969). Raw soybeans contain a heat-labile trypsin inhibitor and other factors causing a depression in food intake, poor growth, increased secretion of pancreatic enzymes and hypertrophy of the pancreas in

growing monogastric animals (Sambeth et al., 1967; Gorrill and Thomas, 1967). There are many contradictory results from experiments in which soybean flour, meal or isolate have been used as a protein source, generally however, the cruder the product the more unsatisfactory the results have been (Roy, 1970).

Preruminant calves fed diets containing more than 30 to 40% of the protein in the form of heated, fat-extracted but otherwise untreated soybean flour had relatively poor growth, weight loss, reduced digestibility of fat and protein, and reduced nitrogen retention and frequently had diarrhoea (Colvin and Ramsey, 1968); Gorrill et al., 1967; Gorrill and Thomas, 1967; Nitzan et al., 1971). Unlike other monogastrics, preruminant calves showed marked reductions of trypsin and chymotrypsin activity and no hypertrophy of the pancreas when fed raw or partially heated soybean flour as compared to calves fed either an all-milk replacer or a soybean protein concentrate having a very low content of soybean trypsin inhibitor (S.B.T.I.) (Colvin and Ramsey, 1968; Gorrill and Thomas, 1967). This response appears to be found in preruminant calves; no similar studies have been done with other preruminants.

The role of S.B.T.I. in calf nutrition was questioned by Kakade et al. (1976) when it was determined that there were no differences in weight gain or feed efficiency for calves fed isolated raw S.B.T.I. and heated S.B.T.I. in milk substitute diets. Studies conducted by Gorrill et al. (1971), however, showed that when S.B.T.I. was added to whole milk the activity of trypsin and chymotrypsin were reduced, total nitrogen increased and the proportion of total

nitrogen as nonprotein nitrogen decreased in the duodenum. It was also concluded that it is not the diarrhoea induced by S.B.T.I. but some other effect of the inhibitor which reduces enzyme secretion in the calf pancreas.

Results from four trials conducted by Colvin and Ramsey (1968) indicate that the nutritive value of soy flour for young calves can be improved by exposing the flour to an acid environment, pH 4.0 for five hours at 39°C, prior to its inclusion in the milk replacer. Acid treated soy flours making up the sole source of protein in milk replacers have resulted in growth rates up to 0.57 Kg/day in the first eight weeks of life. Similar results were obtained when calves were fed milk substitute diets containing alkali-treated (pH 10.6) fully cooked soy flour as the sole protein source (Colvin and Ramsey, 1969).

Soybean flour consists of approximately 30% carbohydrates which are poorly utilized by the newborn calf (Ramsey and Willard, 1975). However, the improved nutritive value of acid or alkali treated soy flour for calves does not appear to be due to acid hydrolysis of carbohydrates (Colvin and Ramsey, 1968), or destruction of a water soluble growth inhibitor (Colvin and Ramsey, 1968). Fully cooked soybean flour does contain an inactive form of trypsin inhibitor that can be converted to the active form in the pH range of seven to nine and subsequently destroyed by heating soybean flour in water (Ramsey and Willard, 1975).

Smith, Hill and Sissons (1970) reported that when calves were, for the first time, given a liquid feed in which all the protein was provided by soybean flour, movement of digesta along the

alimentary tract was fairly normal. After several such feeds, however, stomach emptying was inhibited, transit times through the small intestine were decreased and abnormally large volumes of digesta left the ileum. The increased rate of passage of total nitrogen and dry matter, when calves were fed soybean flour, has been verified by other studies (Sambeth et al.,1967; Smith and Wynn, 1971). This may be the result of the development of a gastro intestinal allergy, the initial feeds having sensitized the calf to a constituent in the soybean product. The production of antibodies may arise following the ingress of partially degraded protein through the damaged mucosa caused by excessive proliferation of some micro-organisms (Roy, 1974). Serums from calves aged four to six weeks, given diets mainly of whole milk but with 30% of the nitrogen intake supplied by soybean meal (55% C.P., heat treated) or soy protein isolate showed mean titres for circulating antibodies which were low after two weeks on the supplemented diets but approached maximum values of 20,000 $\pm$ 10,000 and 140 $\pm$ 100 respectively when calves were six to ten weeks of age (Smith et al., 1970). Calves fed alcohol-extracted soybean concentrate had no similar antibody levels (Smith and Sissons, 1975).

When soy protein in the form of a protein concentrate (60 - 65%) or soybean meal (44 - 48%) contributed 50 to 88% of the total protein in a milk replacer diet, digestibility of the food components increased from the first to third week on the diet, increasing more for the soybean components than for the milk proteins (Nitzan et al., 1971). The inclusion of soybean protein in these diets resulted in a reduced absorption of fat and ash from the diet.

Digestibility of the actual soybean protein was between 72 and 82% for the protein concentrate and 50% for the soybean meal. Nitzan et al. (1971) demonstrated that heating improved the digestibility of the raw soybean protein when included as a 60 - 65% concentrate in a milk substitute diet from 72% for raw to 79 - 88% for heated soybean concentrate.

Nitzan et al. (1971) also found blood urea levels to be higher in calves fed raw or partly heated soybean protein concentrate in the milk substitute diets than those fed a heated soybean replacer or a commercial milk replacer. This may be due to a possible temporary shortage or imbalance of food components such as the essential amino acids. This could be caused by a different pattern of absorbed amino acids from milk and soybean protein as was shown by Paturea-Mirand and Prugnaud (1971), or to differences in the rate of breakdown of the different proteins.

In trials run by Gorrill and Thomas (1967) calves were fed a 71% soybean protein concentrate providing 86% of the protein in a milk replacer. Although weight gains were equal to those of calves fed whole milk, the level of feeding was such that the calves given whole milk gained weight at a rate of 0.20 Kg/day and the soybean fed calves at 0.33 Kg/day (Roy, 1970). A similar experiment conducted by Gorrill and Nicholson (1969) demonstrated no significant difference of nitrogen retention (41% of that consumed) due to protein source.

Porter (1969) cited work done by Hill and Porter using isolated soybean proteins, ADM assay protein and α-protein. Trials with one to two week old calves given milk substitute diets based

on these proteins and supplemented with methionine gave values for percentage retention and apparent nitrogen digestibility with ADM assay protein of 30 and 75 and with ∝-protein of 14 and 72.

Some experiments were conducted by the Ministry of Agriculture in Northern Ireland (1972) to determine the utilization of calf milk replacer diets, containing various protein isolates. Some of the proteins studied included hydrolyzed soybean protein isolate, protein isolate derived from field beans and yeast cultured on N-paraffins. Each test protein provided 50% of the total dietary protein and was compared to a control diet based on spray-dried skim milk. All treatments resulted in lower nitrogen retention values than the milk control diet. The dry matter and apparent nitrogen digestibilities of the field bean protein diets were similar to the control, the other diets having lower values. When the field bean and yeast diets were supplemented with D,L-methionine at a level of .04% there were marked improvements in both nitrogen retention and digestibility.

(II) Cooked Potato Flour: The replacement of skim milk by cooked potato flour (C.P.F.) in milk substitute diets was studied by Hinks et al. (1974). Milk proteins were replaced by levels of 7, 14 and 21% C.P.F. and fed to calves under 25 days of age. Liveweight gains were depressed 20% and firmness of feces was reduced by 14% for each 10% inclusion of C.P.F. in the diet. After the calves were 25 days of age, these effects became less pronounced.

(III) Pea Protein: Although pea protein concentrate (P.P.C.), when supplemented with methionine is equal to egg, fish protein concentrate or methionine-supplemented casein in biological value, Bell et al. (1974) found that P.P.C. was only 25% digestible by calves under two weeks of age and 65 to 70% digestible by calves three weeks of age. Milk substitute diets in which P.P.C. made up half of the protein showed increases in digestibility of dry matter, protein, energy and the ether extract fraction with age of the calf. By using enzyme hydrolysis, Bell et al. (1974) were able to convert 80% of the starch in pea flour to glucose which could be utilized as an energy source by the calf. This glucose was easily digested by the calf but hydrolysis did not improve dry matter or crude protein digestibility. The apparent digestibility of methionine appears to be influenced by the level of P.P.C. in the milk substitute diet, being 91% for the all milk control diet and 59% digestible when P.P.C. made up 23.1% of the dry matter in the diet.

ABOMASAL PROTEASES

In the young calf fresh milk coagulates in the abomasum within three to four minutes of ingestion. The whey appears in the duodenum within five minutes of feeding, but the casein clot is slowly degraded and the products are discharged into the duodenum (Myleea, 1966). Degradation of casein on the abomasum is brought about as a result of the actions of rennin, pepsin or both under acidic conditions due to the secretions of HCl (Roy, 1974). During the first six hours, release of the products is slow and

then as the curd disintegrates the peptides and non-protein nitrogenous substances are released into the duodenum more quickly. Toullec et al. (1974) showed that there is reduced digestibility and growth rate due to suppression of coagulability when the calf is under one month of age.

Garnot et al. (1974) suggested that the nature of dietary proteins influence the enzyme production in the calf vell. As casein was replaced by whey proteins there was a reduction in the amount and activity of rennin in the calf vell, while the level of pepsin remained constant. From the results of these experiments Garnot et al. (1974) theorized that more secretory cells may be stimulated to secrete rennin or alternatively, if the number of secretory cells remains constant, their rate of secretion may be stimulated by the presence of casein. Therefore, substituting skim milk proteins with other types of proteins in milk substitute diets may reduce the quantity or activity of rennin secreted.

CARBOHYDRATE DIGESTION IN PRERUMINANT CALVES

Starch is composed of two fractions: a linear fraction, amylose, and amylopectin which is the branched fraction. Amylose, consisting of α 1-4 linked glucose units makes up 34 to 44% of the starch fraction in fababeans (Bhatty, 1974). Amylopectin is composed of α 1-4 linked glucose units and α 1-6 branch links which comprise 4 to 5% of the total number of linkages. This branch chain polysaccharide is the major component in fababean starch, comprising 55 to 62% depending on the cultivar of Vicia faba minor (Bhatty, 1974).

The complete breakdown of starch to glucose in the digestive tract requires enzymes capable of splitting both the α 1-4 and α 1-6 glycosidic linkages. The amylose fraction of starch is broken down by pancreatic amylase to maltotrisoe and maltose, which are further hydrolyzed to glucose by maltase activity. For complete hydrolysis of the amylopectin fraction, the animal requires an intestinal α 1-6 glycosidase such as isomaltase.

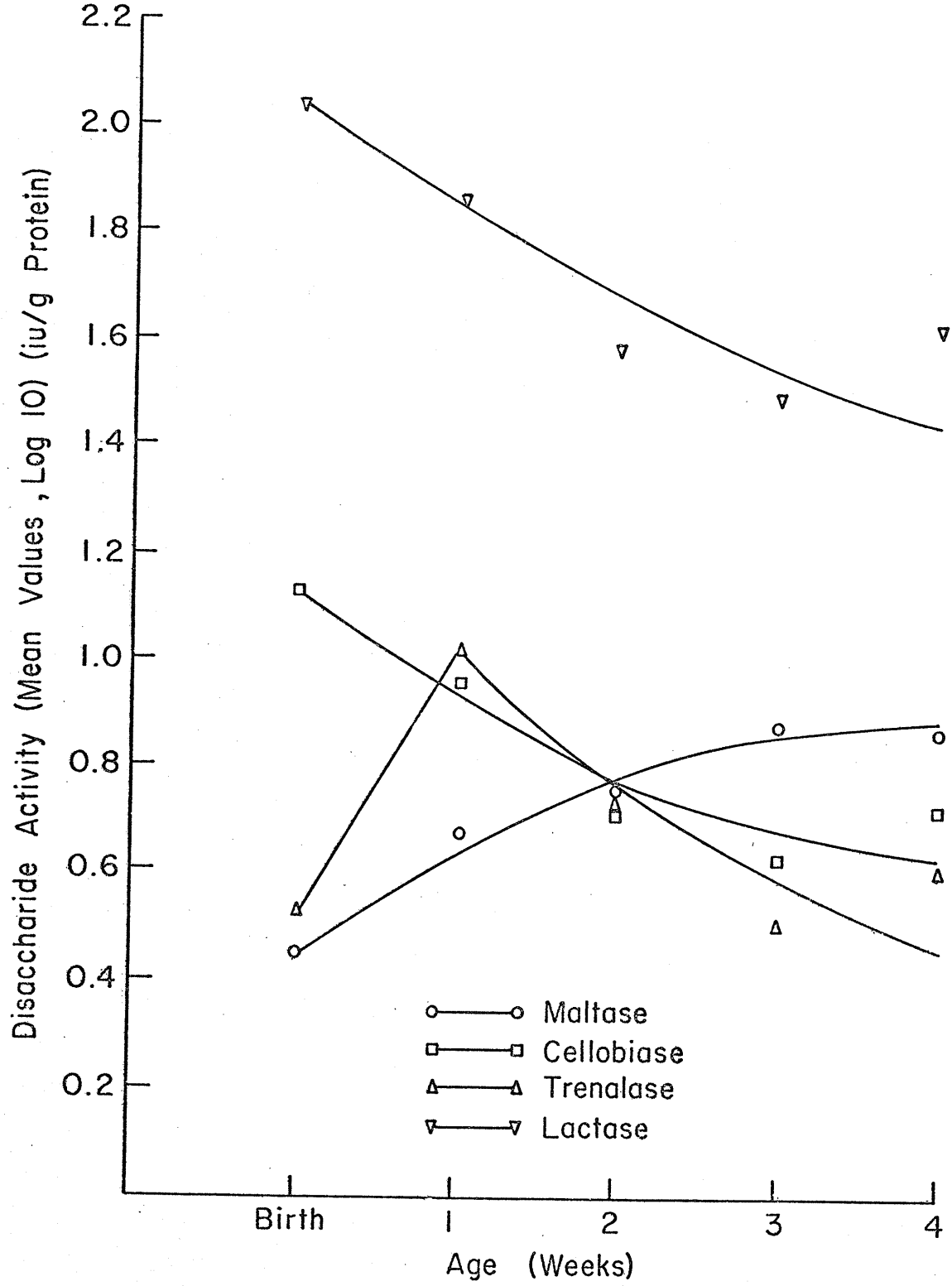
During the first four weeks of life, the digestive tract of the calf functions similar to that of other newborn monogastric mammals (Roy, 1970). The dietary needs are furnished mainly by liquid feeds which by-pass the rumen and are digested in the abomasum and small intestine by enzymes secreted therein. Limited quantities and activities of digestive enzymes limit the ability of the calf to utilize all forms of nutrients, for example, it does not have the ability to hydrolyze starch as an energy source (Burt and Irvine, 1970). Figure 2 illustrates the development of the intestinal disaccharidases in the calf.

Although pancreatic amylase and intestinal maltase and isomaltase are present in the post-ruminal digestive tract of the young calf, they are secreted in small amounts and at a low level of activity (Coombe and Siddons, 1973). Studies (Coombe and Siddons, 1973; Toofanian et al., 1974) have shown that there is an increase in activity of maltase and isomaltase during the first one to four weeks of life; thereafter the values are similar to those in adult ruminants. The activity of isomaltase is approximately half that of maltase (Coombe and Smith, 1974). Low pancreatic amylase secretion, however, is probably the limiting factor for starch utilization by the preruminant. From six weeks of age on, the

FIGURE 2

Postnatal Development of Intestinal Disaccharidases in the Calf

Reference: Toofanian *et al.*, 1974


Disaccharide Activity (Mean Values , Log 10) (iu/g Protein)
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
Maltase
Cellobiase
Trenalase
Lactase
Birth
1
2
3
4
Age (Weeks)

activities of amylase, maltase and isomaltase are balanced, however, the quantities of these enzymes secreted are not sufficient to release a significant amount of energy from starch (Morrill et al., 1970).

Further studies (Natrajan et al., 1972; Burt and Irvine, 1970) show no evidence of an adaptive response to the previous feeding of milk substitutes containing large amounts of starch. Huber et al. (1961) found that the type of starch used also does not influence the blood reducing sugar levels.

Despite the fact that very little of the starch is hydrolysed to form glucose, some digestion trials show a high level of disappearance of starch from the intestinal tract (Natrajan et al., 1972; Shaw et al., 1918). This has been attributed to microbial fermentation in the calf's cecum and colon (Liang et al., 1967). Fermentation of starch and sugars results in the production of organic acids and alcohols. The volatile fatty acids produced due to this fermentation may be absorbed and utilized by the preruminant calf (Liang et al., 1967).

In calves there is a balance (eubacteriosis) between the saccharo-proteolytic organisms, whose main activity is the breakdown and utilization of proteins, and sacchardytic organisms which are associated with the fermentation of sugars (Roy, 1970). If either group of microorganisms becomes dominant (dysteriosis) the animal is likely to be affected with diarrhoea, either putrefactive or fermentative. One sign of poor utilization of starch is feces of loose consistency (Burt and Irvine, 1970) due to a milk fermentative condition which is caused by the presence of undigested

starch in the intestine (Hinks et al., 1974). Under this condition even a mild dietary upset could result in proliferation of pathogenic organisms in the lower intestine resulting in depressed food intake, severe dehydration and possibly death (Hinks et al., 1974).

Dietary starch also has a depressing effect on apparent protein digestibility (Huber et al., 1968). This is probably due to the combined effect of increased fecal passage of undigested or partially digested starch (Blaxter and Mitchell, 1948) and increased microbial activity. The digestibility of other nutrients also appears to be influenced by the presence of starch in the diet. Huber et al. (1968) reported that a linear decrease ($P < .01$) in apparent digestibilities of dry matter, carbohydrate and crude protein as the level of starch in the diet increased from 0 to 29%.

Suggestions for the maximum allowances for starch in the diet of preruminant calves have been approximately 10% of the diet on a dry matter basis (Coombe and Siddons, 1973; Huber et al., 1968). These studies generally agree that young calves can not utilize polysaccharides such as starch or its break down products until the fifth or sixth week of age. The work of Liang et al. (1969) has resulted in speculation that starch may be used as an energy source by preruminant calves because of their ability to absorb and utilize volatile fatty acids which are produced by microbial fermentation in the intestinal tract.

GENERAL STUDIES DONE ON AMINO ACID LEVELS IN PLASMA

During digestion, proteins are hydrolyzed to free amino acids that are absorbed rapidly from the small intestine and pass, via the mesenteric and portal veins, to the liver before entering the general circulation (Graham, 1974). The naturally occurring L-amino acids are transported across the intestinal wall by an active process which may be upset by metabolic inhibitors or by a lack of oxygen (Munro, 1970).

There are two sources of plasma free amino acids from the gastrointestinal tract, the amino acids supplied by dietary proteins and by endogenous proteins. The endogenous protein consists largely of serum albumin in digestive secretions, mucoproteins and digestive enzymes. Nasset and Ju (1961) observed that ingested amino acids may be diluted as much as six fold by amino acids derived from endogenous protein. In a normal adult man, endogenous nitrogen has been estimated to be approximately one half of the daily protein intake in the diet (Munro, 1964). Nasset and Ju (1961) theorized that the hydrolysis of endogenous protein may serve to protect the animal from the immediate effects of ingestion of an imbalanced protein.

However, in a subsequent review Fauconneau and Michel (1970) concluded that endogenous protein has little influence on the plasma amino acid pattern shortly after a meal because the rate of digestion of endogenous protein is slower than that of exogenous protein. Therefore, an abnormal dietary pattern of amino acids in a meal is reflected in the free amino acid pattern of the portal blood, and if the meal is large enough, throughout the entire blood supply for several hours after the meal (McLaughlan *et al.*, 1963).

The digestion of endogenous nitrogen occurs along the length of the intestinal tract and is continuous throughout the day. Much of the digestion of this protein source takes place in the ileum and cecum through the joint action of bacteria and the host's enzymes (Fauconneau and Michel, 1970). In contrast, the majority of the dietary protein is digested in the upper part of the small intestine at a rapid rate. The digestion of dietary protein is discontinuous and related to meal frequency and the rate of gastric emptying. The continuous digestion of endogenous protein allows the digestive tract to exercise a significant regulatory action over protein metabolism by ensuring stable amino acid concentrations in the blood of an animal in the postabsorptive state (Fauconneau and Michel, 1970).

Some amino acids derived from the intestine are removed for the regeneration of intestinal proteins during absorption (Nordstrom et al., 1970), the remainder are absorbed from the intestine and directed to the liver. In the liver, amino acids are withdrawn from the blood for the synthesis of liver and plasma proteins. Studies (Porter and Williams, 1963; Nordstrom et al., 1970) have indicated that increases in systemic free amino acid levels are less than in portal blood plasma after ingestion of a meal.

Surplus amino acids undergo transamination or deamination in the liver to yield: 1. nonessential amino acids such as glycine, serine, alanine, aspartic acid and glutamic acid; 2. α-keto acids which may be used in the synthesis of fat, glucose, and other substances which are oxidized directly to yield energy; 3. ammonia which is converted to urea and excreted in the urine (Harper, 1974).

Animals differ in their ability to carry out in vivo synthesis of amino acids. Consequently amino acids have been distinguished as indispensible or dispensible. An amino acid is considered to be indispensible if it must be included in the diet for optimal growth of an immature animal or for the maintenance of nitrogen balance in the mature organism (Mahler and Cordes, 1971). The essential feature of this class of amino acids is their carbon group. For the calf the essential amino acids are the same as those of the rat; isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, histidine and arginine (Harper and Rogers, 1965).

Harper developed a definition for amino acid imbalance and denotes it as:

> "a change in the proportions of amino acids in the diet which results in a depression in food intake or growth rate that can be completely alleviated by a supplement of the essential amino acids present in the least amount in a diet in relation to the amount required for optimal growth." (Harper and Rogers, 1965)

To create imbalances, the total quantities of amino acids added may be greater than the quantities causing toxicity. Generally no single amino acid is included in the diet in an amount that, by itself, would be considered toxic.

An amino acid deficiency is related to an inadequate intake of an amino acid (Munro, 1970). The first limiting amino acid in a diet for an animal's consumption is that amino acid supplied in the lowest amount by the product in proportion to the animal's requirements (Keis et al., 1975).

The plasma contains a very small proportion of the total free amino acid pool, varying from 0.2 to 6% for the individual amino acids (Munro, 1970), however, plasma amino acid concentrations can be used in protein quality evaluations (Rogers and Leung, 1973). If reference diets to produce baseline or reference plasma amino patterns are not used, then it appears essential to know the effect of fasting prior to time of withdrawal of blood samples (Typpo et al., 1970). If concentrations of plasma free amino acids are to provide meaningful information about the protein in the diet, blood samples should be taken at times that will reflect differences due to source and level of dietary protein, intakes of amino acids and availabilities of amino acids (Typpo et al., 1970).

The following theory has been accepted regarding the influence of diet on plasma free amino acids (Nordstrom et al., 1970; Lewis and Speer, 1973; Whitaker and Homer, 1971; Zimmerman and Scott, 1965; Munro, 1970). A rise in plasma free amino acid levels during absorption is counterbalanced by removal of amino acids, largely to provide for tissue protein synthesis. The pattern of amino acid requirements for the latter does not alter with the diet, but has a constant spectrum. If one essential amino acid is present in relatively low concentrations in the dietary protein, it will tend to be removed by the tissues rapidly as it is absorbed from the intestine to allow maximal protein synthesis to proceed. The plasma level of a limiting amino acid generally remains at a low, relatively constant plasma level until the dietary requirement is met, after which it increases rapidly. There is a linear decline in plasma levels of almost all other essential amino acids and urea

with increasing levels of the limiting amino acid. This illustrates an improvement in amino acid balance and as a consequence, improvement in utilization as the amino acid deficiency is corrected (Lewis and Speer, 1973). Lewis and Speer (1973) found that using criteria such as nitrogen balance, nitrogen retention, weight gain, plasma essential amino acids minus the first limiting amino acid and plasma urea were all equally informative estimates of the optimal level of the dietary requirements for the first limiting amino acid.

Eggum (1973) cited earlier work in which discrepancies have been found between the dietary concentrations of amino acids and increases in plasma concentrations during the absorptive period. Several factors may cause this discrepancy. Denton and Elvehzin (1953) subjected beef casein and zein proteins to three hour hydrolysis with pepsin followed by hydrolysis with dessicated whole pancreas and duodenal powder. Generally they found increased liberation of amino acids with increased time of hydrolysis. However, the results indicated that all amino acids were not released at uniform rates from the same protein.

There are differences in the rate of absorption of amino acids once they have been made available in the gut (Eggum, 1973) due to the transport mechanisms. The availability of individual amino acids in various proteins differ (Windels et al., 1971). This was illustrated with the data collected by Maltman (1976) when growing pigs were fed soybean meal and fababean protein concentrate. Maltman (1976) cited Liener who stated that in their natural state, plant proteins can be shown to be resistant to proteolytic enzymes.

Nitzan and Liener (1976) observed that amino acid absorption of raw soybean flour was delayed to a more distal portion of the small intestine whereas absorption from heated soy flour began in the jejunum of rats. Abidi (1976) proposed that the ileum is less efficient in absorbing amino acids than the upper parts of the small intestine, resulting in a reduced nitrogen retention for raw soybean flour.

Cantoly and Nasset (1975) demonstrated that the form of amino acids influences the time of appearance in plasma. It was found that even when free dietary methionine and protein bound methionine left the stomach at the same rate, free methionine was absorbed from the intestine more rapidly. Once partially digested dietary protein reaches the small intestine the rate that protein bound amino acids are released influences the rate of their absorption from the gut. Therefore, for several hours after ingestion of the meal, the rate of release of protein bound amino acids will influence the concentrations and molar ratios of free amino acids in the plasma.

The state of excitement or stress of the experimental animals may affect plasma levels of individual amino acids (Eggum, 1973). Surgical trauma in rats produced changes in total plasma amino acid levels which were characterized by an initial drop followed by a transient recovery, then a further drop followed by slow recovery to levels before surgery (Plow, et al., 1976). It appears that such trauma reduces protein synthesis by reducing the plasma precursor pools of free amino acids, but does not alter the rate of catabolism.

Other ingredients in the diet, such as the level of carbohydrates, and the source and level of fats may influence plasma free amino acid ratios (Canolty and Nassat, 1975). Insulin secretion is more enhanced by a meal containing carbohydrate and protein than by eating carbohydrates or protein seperately (Canolty and Nasset, 1975).

STUDIES OF BLOOD UREA LEVELS IN MONOGASTRICS

The two major metabolic fates of amino acids in the liver are incorporation into proteins and catabolism to urea and CO_2 (Graham, 1974). The amino acid degrading capacity of animals, particularly the capacity to degrade essential amino acids is highly responsive to protein intake (Graham, 1974). Amino acid degrading enzymes do not undergo adaptation as rapidly or extensively in animals fed low protein diets as happens in animals fed a high protein diet, presumably because conservation of a limited supply of amino acids in a well-balanced pattern has evolved as a mechanism contributing to the survival of animals receiving an inadequate diet (Munro, 1970). Harper (1970) postulated that when a surplus of amino acid arises, from ingestion of a diet having an amino acid imbalance or one containing a large excess of one amino acid, the amount of balanced protein ingested remains low and activities of enzymes of amino acid catabolism would tend to be lower.

Eggum (1970) did several experiments to determine the validity of using blood urea measurements as a technique for assessing protein quality. Three factors which influence blood

urea content were investigated: the protein content in the diet, the time after feeding and protein quality. Results from experiments on rats demonstrated a high positive correlation (r = .95) between the protein content in the diet and blood urea content. Eggum (1970) found that this is in agreement with observations made by Munchow and Bergner (1968) on pigs and horses, Fonnesbech and Symons (1969) in horses and Pastuszewska (1967) in suckling pigs.

Blood urea levels are also dependent on the time interval after feeding (Nasset and Ju, 1961). Tests with pigs showed an increase in blood urea levels for the first three to four hours after feeding and thereafter reached a plateau. By standardizing the protein content of the diet and time of sampling Eggum was able to determine the influence of protein quality on blood urea levels. Forty-two feedstuffs of widely differing quality were used in nitrogen balance trials with rats. The results showed an inverse relation between blood urea content and the biological value of the diet (coefficient of variation = 53%). Eggum (1970) cited several other studies by Munchow and Bergner (1968), Pracker (1971) and Bergner et al. (1971) who obtained similar relationships between protein quality and blood urea content.

PLASMA AMINO ACID AND PLASMA UREA STUDIES DONE WITH CALVES

As the preruminant calf has been shown to be as dependent on its dietary supply as simple stomached animals (Blaxter and Wood, 1952) it would seem likely that its amino acid requirements could be determined by methods used for monogastrics. These

methods include the changes in plasma free amino acid concentrations and plasma urea levels in response to dietary supplementation. Studies in the past have shown that such measurements can give a valid assessment of amino acid requirements for the preruminant calf (Boling et al., 1972; Williams and Smith, 1975; Foldager and Huber, 1975; Patureau-Mirand et al., 1971). A number of factors appear to influence plasma composition in the young calf.

Williams and Smith (1975) fed four calves equal amounts of whole milk twice daily at 10:00 and 17:00 hours. Data indicated a small decrease in plasma urea (P.U.) concentrations and a larger decrease in total plasma amino acids (P.A.A.) for three to four hours post-feeding. P.U. then showed a slight increase from four to six hours after feeding, while total P.A.A. remained constant. There was no significant change in either P.U. or total P.A.A. after the evening feed. Patureau-Mirand et al. (1971) also found, for calves fed milk, that the concentration of most individual P.A.A. decreased after feeding. Nitzan et al. (1971) and Patureau-Mirand et al. (1971) also observed decreases in P.U. for three to four hours post-feeding, although to different extents.

These findings are in contrast to the usual changes in simple stomached animals, normally responding with increased total P.A.A. and P.U. concentrations from zero to three hours after a meal (Nasset and Ju, 1961; Nortstrom et al., 1970). Williams and Smith (1975) postulate that the difference may be related to the slow and uniform flow of nitrogen compounds from the abomasum to the duodenum after a calf is given a milk feed. Patureau-Mirand et al. (1971) found that calves given milk replacers containing

proteins which do not form a clot in the abomasum showed a different pattern of P.A.A. with time after feeding, compared with calves fed milk. Rony et al. (1975) found that milk replacers containing milk proteins also resulted in a different total P.A.A. response as compared with whole milk in preruminant calves. In both cases total P.A.A. increased for several hours after feeding.

Both Patureau-Mirand et al. (1971) and Nitzan et al. (1971) did not find appreciable differences in P.U. responses with time after feeding due to protein source. Reece and Wahlstrom (1972) found urea nitrogen concentration to be higher in the plasma of calves fed whole cow's milk vs. the milk replacer diets.

Dietary fat sources appear to influence the levels of plasma free amino acids (Rony et al., 1975). When whole milk (control) was fed to calves the concentrations of all amino acids, except asparagine, glutamic acid and glutamine, were at a maximum at the time of feeding, rapidly decreasing for four hours after the meal and there was a general tendency to increase up to 13 hours post-feeding. For the experimental diets butter oil, lard, a mixture of lard and corn oil (2:1), hydrogenated corn oil or corn oil were incorporated into the respective milk replacers at a rate of 24.8% on a dry matter basis. Soya lecithin was added as an emulsifier at a rate of 6.6% of the fat portion of the diets. The protein was supplied by skim milk powder such that each experimental diet contained 24.8% crude protein on a dry matter basis.

The concentrations of all amino acids, hydroxy proline and glycine excepted, increased sharply during the first hour after feeding and reached a maximum two hours after feeding when calves

were fed milk replacer containing lard. This was followed by a general decrease in P.A.A. up to 13 hours after the meal. In the case of butter oil, corn oil, hydrogenated corn oil and the lard-corn oil mixture, the concentration cycles for the amino acids were intermediate between those of lard and whole milk, though in general they were closer to milk. The influence that the source of dietary fat has on the postprandial patterns of P.A.A. in young calves suggests that it influences amino acid metabolism in these animals (Rony et al., 1975).

Chiou and Jordan (1973) found that although dietary fat levels in milk replacers for neonatal lambs did not influence apparent digestibility, nitrogen retention and plasma urea levels, it had a significant effect on the feed:gain ratio, protein efficiency ratio and plasma free amino acid ratios. Concentrations of P.A.A. increased curvilinearly with increased fat levels with the exception of phenylalanine which increased linearly with increased dietary fat level. This response may be due to an increased transport of dietary amino acids into the blood stream or due to a decrease in the amount of plasma free amino acids transported into the tissue (Chiou and Jordan, 1973). Chiou and Jordan cited Emery who found that as the level of dietary fat is increased the process of emulsification of water-insoluble fat into micelles is retarded and thus delays gastric emptying. This delay of gastric emptying and of amino acid transit when lambs were fed high levels of dietary fat resulted in high P.A.A. concentrations at two and a half hours instead of two hours post-feeding.

Leibholz (1965) proposed that individual P.A.A. concentrations decrease between birth and four weeks in calves. Williams and Smith (1975) found little effect due to age on total P.A.A. or methionine concentrations in calves during an age period of two to nine weeks. Decreases in P.A.A. occur mainly during the first two weeks after birth.

The influence of age on P.U. concentrations has not been fully established for the preruminant calf. Early studies (Leibholz, 1965) indicate considerable fluctuation in P.U. concentrations with age for calves, up to four weeks of age. More recent studies (Williams and Smith, 1975; Reece and Wahlstrom, 1972) showed little change in the P.U. concentration with age. Chiou and Jordan (1973) found that concentrations of P.U. increased linearly ($P < .005$) between 17 and 31 days of age in neonatal lambs. The results of Nitzan et al. (1972) indicated a drop in P.U. concentration with age for the calf.

STUDIES ON METHIONINE REQUIREMENTS OF THE PRERUMINANT CALF

Muller and Rodriguez (1974) cited the results of several studies which suggested that methionine may be a limiting amino acid in ruminants. In growing calves methionine has been shown to have the lowest concentration of any amino acid in the plasma which indicates that it may be most limiting in growing calves (Muller and Rodriguez, 1974). Boling et al. (1972) studied the relationship of amino acid concentrations in the colostrum of Angus cows 24 hours postpartum and the calves' plasma after suckling. On a molar percentage basis, concentrations of glutamic acid and proline

were highest among amino acids in both colostrum and plasma. Concentrations of glycine and alanine were higher in plasma than in the colostrum. However, the relative concentration of methionine in the calves' plasma with that in the colostrum (.28 vs. 1.99%) was the lowest of all amino acids studied.

A study by Radostits and Bell (1968), using a milk replacer containing milk proteins, showed that with the exception of methionine and cystine the digestibility coefficients of amino acids were high, averaging 70 - 86% in calves aged six to 24 days. There was an improvement in the digestibility of most amino acids during the first week of the experiment. The apparent digestibility of methionine, however, was consistently low (averaging 11%) throughout the entire experiment, often showing negative coefficients during the first half of the experiment. Cystine was only moderately well digested, averaging 59% during the experimental period.

Sources of methionine used to supplement diets for calves include methionine hydroxy analogue (MHA) (Muller and Rodriguez, 1974), L-methionine (Williams and Smith, 1975; Foldager and Huber, 1975), and D,L-methionine (Gorrill and Nicholson, 1971). According to trials conducted and studies cited by Muller and Rodriguez (1974), MHA can be successfully used to supplement calf rations. The addition of MHA results in reduced acceptability and palatability of rations (Muller and Rodriguez, 1974; Lane and Leighton, 1973) which can be minimized by the addition of molasses at a level of 5% to the ration. Muller and Rodriguez (1974) calculated the methionine requirements for growing calves to be approximately four to five grams per day.

Methionine supplementation of a liquid diet containing isolated soybean protein as the only protein source was reported to improve nitrogen retention of calves (Porter and Hill, 1964), but no determination of the optimum requirements was made. Based upon responses to dietary L-methionine supplementation of either plasma methionine or P.U. concentrations, Williams and Smith (1975) estimated the mean values of methionine requirements for the preruminant calf to be 4.5 $\pm$ .02 and 3.9 $\pm$ .4 g/day respectively. The calves had an initial liveweight of 50 to 60 Kg. and were growing at a rate of .25 Kg/day. The cystine intake was approximately 0.3 g/day.

Foldager and Huber (1975) used numerous response criteria to determine methionine requirements of the preruminant calf, i.e. average daily gain, digestibility of dry matter and crude protein, nitrogen balance, plasma methionine and plasma urea nitrogen levels. Plasma methionine was the most sensitive method when poor health due to factors other than treatments were encountered. The experimental diets contained 1.05 g. cystine/16 g. nitrogen. The estimated requirements of total sulfur amino acids ranged from 3.8 to 4.0 g/16 g. nitrogen.

Williams and Smith (1975) compared their results with those of Patureau-Mirand and Pion (1973) and Patureau-Mirand *et al*. (1973) who supplemented milk protein diets with graded amounts of methionine. Methionine and total sulfur amino acid requirements were estimated to be .59 g/KgB.W.$^{\cdot 73}$/day and .66 g/KgB.W.$^{\cdot 73}$/day respectively. These values are much higher than those of Foldager and Huber (1975) or Williams and Smith. The higher level of crude pro-

tein in the milk replacer (26.4%) and the rapid growth of the calves (1.0 kg/day) may account for the higher total sulfur amino acid requirements.

Methionine supplementation in excess of the calf's requirements results in significantly depressed feed intake (Reece and Wahlstrom, 1972; Muller and Rodriguez, 1974) and reduced feed efficiency (Foldager and Huber, 1975). A moderate excess did not influence feed intake but weight gain was depressed (Foldager and Huber, 1975). This may be related to an amino acid imbalance which affects utilization of other essential amino acids but does not depress feed intake (Muller and Rodriguez, 1974). Benevenga (1974) postulated that the reduced growth with excessive amounts of methionine is not due to the effect of methionine on the transport and utilization of other amino acids but is due to an aberrant metabolism of the methyl group. Once the methionine requirements of the calf are met, additional L-methionine supplementation results in marked increases in plasma methionine concentrations and an increase in P.U. concentrations (Williams and Smith, 1975; Foldager and Huber, 1975; Williams and Smith, 1974).

UTILIZATION OF D-METHIONINE

Some animals can utilize the D-isomer of certain amino acids and such utilization can be sufficiently great to permit growth as a substitute of the corresponding L-isomer (Meister, 1965). Inversion would probably take place by an oxidative conversion of the D-isomer to the analagous α-keto acid followed by L-specific reamination. In general, the nutritive value of the D-isomers that can produce growth is less than that of the corresponding

α-enantiomorph , although experimental conditions have been found in which both isomers produce almost equal effects (Meister, 1965).

Economic considerations would suggest the use of a mixture of D- and L-isomers of methionine if they are equally utilizable. Also, L-methionine has distinct flavor and odor characteristics that may adversely alter the palatability of the products to which it is added (Kies et al., 1975). The L-isomer has a distinct bitter taste and the D-isomer, in contrast, is sweet (Graham, 1974). A combination of the two may partially overcome problems of palatability in feed products which require methionine supplementation.

Early studies cited by Meister (1965) indicate that D-methionine is utilized by the mouse, rat and man. Wretlind and Rose (1950) observed that D- and L-methionine supported nearly the same growth rate in rats as when only L-amino acids were fed in their diet. When a racemic mixture of amino acids was fed, the D-methionine was less effective than the L-form. The slightly lower efficiencies of D-methionine may be due to a slower rate of absorption of D-methionine from the gastrointestinal tract (Edwards et al., 1963).

Camien et al. (1951) have concluded that the D- and L-isomers of methionine are equally available to humans; however, results from other investigators suggest the opposite (Kies et al., 1975). Kinsell et al. (1947) demonstrated by microbiological methods that up to 35% of the D-methionine administered intravenously as a racemic mixture was excreted in three hours by normal human subjects. Under the same conditions L-methionine excreted in the urine was negligible, suggesting that D-methionine may be less efficiently

utilized than L-methionine by man. Albanese et al. (1944) reached the opposite conclusion from experiments in which urinary levels of methionine, cystine and inorganic sulfur were measured. No significant differences were found between ingested L- and D,L-methionine in inducing changes in the excretion of these substances. Rose et al. (1955) found D-methionine was as effective as D,L-methionine in the maintenance of nitrogen equilibrium in man.

In studies by Camien et al. (1951) in which normal human subjects ingested D,L- or L-methionine, the methionine excretion did not appear to be significantly increased as a result of ingesting an additional four grams of L-methionine daily. D-methionine excretion was greatly increased by the daily ingestion of 7.5 g. of D,L-methionine. Kies et al. (1975) studied the comparative value of L-, D,L- and D-methionine supplementation of an oat based diet for humans. L-methionine did improve nitrogen retention while no effect was noted as a result of D-methionine supplementation. The highest concentrations of methionine in urine were found when subjects were fed the D-isomer.

In poultry it has been reported that the D-isomer is as well utilized as the L- form when tested with purified diets in which all other amino acids in the L- form (Marrett et al., 1964), but not when the diet contained other amino acids in the D- form (Marrett and Sunde, 1965). Bruggeman et al. (1965) showed that D-methionine was less potent than L-methionine added to a diet in which the other amino acids were supplied by proteins. Sugahara et al. (1967), however, reported that chicks fed D-methionine grew

at a rate almost comparable to the group fed L-methionine. Comparisons showed that chicks fed the L- form produced more antibodies against a challenge of Newcastle disease virus than did an equal amount of D-methionine.

No studies were found in the literature in which the ability of calves to utilize D-methionine was determined. Generally, the studies conducted to determine the sulfur amino acid requirements of preruminant calves have been done using L-methionine (Foldager and Huber, 1975; Williams and Smith, 1975).

PROTEIN EFFICIENCY RATIOS

Protein efficiency ratio (P.E.R.) can be defined as the gain in body weight per gram of protein or nitrogen consumed (Gitler, 1964). This test recognizes the fact that animals receiving diets of low protein quality will show some loss of appetite. In order that data from various experiments may be compared, factors other than protein source which have been found to influence the P.E.R. assay have been standardized (Gitler, 1964; Campbell, 1963). The test protein must be incorporated at a level of 10% by weight in a synthetic diet providing excess of all other nutrients. The diets must be fed ad libitum during the four week assay period. Finally, only male weanling rats can be used to do P.E.R. determinations. Munro cited Derse (1960) who found that tests carried out in different laboratories, using these standardized procedures, gave average P.E.R. values of 2.8 for casein and 2.4 for soy protein.

There are three major criticisms of the P.E.R. procedure in the evaluation of protein quality (Gitler, 1964): (1) It has not been proven that any gains in body weight are constant in composition. McLaughlan and Campbell (1969) cited several studies indicating that body composition will not vary to any significant degree within a four week period. (2) The results of the assay may vary with the level of protein in the diet. High quality proteins have a maximum P.E.R. value at a level of 7.8% crude protein in the diet whereas poor quality proteins have maximum P.E.R. values when the level of protein in the diet is greater. Peak P.E.R. values for casein are at 7% protein in the diet as compared to 15% for plant protein. (3) P.E.R. determinations make no allowance for maintenance, assuming that all protein consumed is used for growth. Growth rate is a sensitive index of the balance and supply of essential amino acids in a protein. However, because maintenance requirements of the animal are not taken into account, P.E.R. determinations are sometimes considered inappropriate for low quality proteins. This is not always a critical point, for if a protein will not support growth in young rats, then its ability to support growth in other growing animals will also be poor.

MATERIALS AND METHODS

PRELIMINARY RAT TRIAL

Preliminary trials were carried out on weanling rats to estimate the protein quality of protein sources used in calf milk substitute diets and to compare the effects of autoclaving and methionine supplementation on protein efficiency ratio (P.E.R.) values of fababean and soybean protein concentrates. The rats used were Sprague-Dawley. The method outlined by Campbell (1963) was followed for the P.E.R. determinations.

Seven diets, differing only in protein sources, were fed: Diet A represented the control in which casein was the protein source; Diet B contained raw fababean protein concentrate (F.B.P.C.); Diet C contained raw F.B.P.C. supplemented with methionine; Diet D contained autoclaved (120^{o}C for 30 minutes) F.B.P.C.; Diet E contained methionine supplemented, autoclaved F.B.P.C.; Diet F contained soybean protein concentrate; and Diet G contained methionine supplemented soybean protein concentrate (Table 1). All diets contained approximately 10% crude protein and were fed in a loose form.

EXPERIMENTAL DESIGN: Two days following their arrival, the rats were weighed and divided into ten groups according to body weight, each group being referred to as a block. The average initial weights

Table 1: COMPOSITION OF THE EXPERIMENTAL DIETS FOR PRELIMINARY RAT TRIAL

ITEMS	DIETS (g/100 g) A	B	C	D	E	F	G
INGREDIENTS							
Casein (96.75% C.P.)	10.34	-	-	-	-	-	-
Soybean Protein Concentrate[1] (66.61% C.P.)	-	-	-	-	-	15.01	15.01
Fababean Protein Concentrate (65.06% C.P.)	-	15.37	15.00	15.37*	15.00*	-	-
Corn starch	69.57	64.63	64.68	64.63	64.68	64.99	64.81
Corn oil	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Cellulose[2]	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Mineral Mix[3]	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin Mix[4]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
D,L-Methionine	-	-	0.32	-	0.32	-	0.175
ANALYSIS							
Crude Protein (%)	9.79	10.10	10.30	9.69	9.97	10.34	10.30
Methionine & Cystine (g/100 g. C.P.)	2.09	1.58	4.63	1.32	4.17	2.07	3.55
% of rat's Sulfur Amino Acid Requirements	41.8	31.6	92.6	26.4	83.4	41.4	71.0

Table 1: COMPOSITION OF THE EXPERIMENTAL DIETS FOR PRELIMINARY RAT TRIAL

[1] Promosoy

[2] Alfafloc

[3] (USP-XlV) contains, in g/Kg: NaCl - 139.3; KI - 0.79; KH_2PO_4 - 389.0; $MgSO_4$ $7H_2O$ - 162.9; $CaCO_3$ - 381.4; $FeSO_4$ $7H_2O$ - 27.0; $MnSO_4$ H_2O - 4.01; $CaSO_4 \cdot 5H_2O$ - 0.477; $CoCl_2 \cdot H_2O$ - 0.023; $ZnSO_4 \cdot 7H_2O$ - .548.

[4] contains - Vit.A - 1000 I.U.; Vit.D - 200 I.U.; Vit.E - 10 I.U.; menadione - .5 mg.; choline - 200 mg.; inositol - 25 mg.; niacin - 4.0 mg.; Ca-D-pantothenic acid - 4.0 mg.; folic acid - 0.2 mg.; Biotin - 0.02 mg.; B_{12} - 2 mg.; para-amino benzoic acid - 10 mg.; cellulose to make 1000 g.

* autoclaved 120°C for 30 minutes.

Table 2: COMPOSITION OF A MILK SUBSTITUTE DIET FED TO PRERUMINANT CALVES IN STUDY A

	DIETS (g/Kg)			
INGREDIENT	A	B	C	D
Fababean Protein Conc.	250.85	156.77	78.38	-
Skim milk[1]	115.85	288.77	430.38	540.05
Whey[1]	15.15	37.77	56.69	143.82
Tallow	163.20	163.20	163.20	163.20
Coconut Oil	40.80	40.80	40.80	40.80
Lecithin[2]	6.12	6.12	6.12	6.12
Glucose[3]	377.01	283.47	207.62	91.07
D,L-methionine	2.95	2.38	0.91	-
Vitamins[4]	7.10	7.10	7.10	7.10
Minerals[5]	11.18	11.18	11.18	11.18
$CaCl_2 \cdot 2H_2O$	9.88	-	-	-
Aurofac[6]	2.55	2.55	2.55	2.55

[1] Spray dried

[2] Lecithin-Soybean (oil not removed)

[3] Cerelose

[4] Mixed such that for every 45.35 Kg. milk replacer - units of the following vitamins were added: Vitamin A (6,000,000 I.U.); Vitamin D_2 (2,000,000 I.U.); Vitamin E (4,000 I.U.); Vitamin B_{12} (3,000 m.c.); Thiamine (1,000 mg.); Riboflavin (2,000 mg.); Pantothenic Acid (2,000 mg.); Niacin (2,000 mg.); Pyridoxine (450 mg.); Folic Acid (15,000 mg.); Choline (146.6 g.).

[5] Mixed such that for every 45.35 Kg. milk replacer - units of the following minerals were added: Na_2SeO_4 (0.04 g.); Na_2MoO_4 (0.57 g.); MgO (189.50 g.); Calcium Iodate (0.47 g.); Copper Sulphate (1.02 g.); Iron Carbonate (16.86 g.); Iron Sulphate (31.97 g.); Manganese Sulphate (36.34 g.); Zinc (1.87 g.); Cobalt Sulphate (1.27 g.).

[6] 10 g. auromycin/454 g.

of the rats in each block in the order of heaviest to lightest were: 58.5, 55.9, 53.8, 51.8, 51.2, 50.6, 48.4, 45.8, 43.6 and 40.9 grams. The rats were then randomly assigned to the seven diets such that each diet was assigned once to every block and every block contained all seven diets. The mean initial weights of rats within each of the seven diets ranged from 49.7 to 50.3 grams.

For statistical analysis, the randomized complete block design was used, and comparisons of diets were made using orthogonal contrasts (Snedecor and Cochran, 1976).

MANAGEMENT PROCEDURES: The rats were housed individually in suspended wire bottomecages. The assigned diets and distilled water were supplied ad libitum at all times. Weekly records of food consumption and changes in body weight were kept for each rat during the four week assay period. P.E.R. values were determined by measuring the weight gain of the test animal and the protein consumed.

CALF STUDIES

Two studies, using male Holstein calves, were conducted to look at the potential use of F.B.P.C. in milk substitute diets for preruminant calves. Factors such as the degree of utilization of F.B.P.C. as a function of the calf's age and effects on the digestibility of other dietary nutrients due to substitution of milk proteins by F.B.P.C. were studied. Also, attempts were made to determine the optimal level of methionine supplementation in milk replacers, the major protein source being from fababeans. A

preliminary trial was also run to determine the efficiency of D-methionine in satisfying the sulfur amino acid requirements of the preruminant calf.

EXPERIMENTAL ANIMALS: Holstein male calves, two to four days of age, were purchased from local farmers or obtained from the University of Manitoba dairy herd for these studies. Efforts were made to use healthy male calves having a minimum body weight of 40 Kg.

All calves received colostrum within 24 hours of birth. On arrival each animal was weighed and received an intramuscular vitamin injection (500,000 I.U. of Vit. A; 50,000 I.U. of Vit. D and 50 I.U. of Vit. E). Then the rump and tail were shaved and three strips of velcro tape were glued (Cattle Back Tag Cement, Kemco) around the tail area. During periods of fecal collection, a polyethylene bag fitted with four strips of the matching side of velcro tape attached to the calf.

Calf Study A:

EXPERIMENTAL DIETS: In the four experimental diets, the milk proteins were replaced at varying levels with F.B.P.C. such that the percentage of protein provided by fababeans was 80, 50, 25 and 0 for diets 1, 2, 3 and 4 respectively (Table 2). Methionine was supplemented at the rate of 1% of F.B.P.C. to bring the level of methionine in F.B.P.C. equivalent to that of milk. $CaCl_2 \cdot 2H_2O$ was added to diet 1 as an additional source of calcium as F.B.P.C. does not supply a sufficient amount for the growing calf. Glucose was used as a filler as well as an energy source. Chromium oxide was mixed into the dry milk replacer at a rate of .46%.

As previously mentioned (page 6), the F.B.P.C. was processed using the air classification procedure at Newfield Seeds Ltd., Nipawin, Saskatchewan. The fababeans were of the Diana variety, grown at the University of Manitoba research station in 1974.

MANAGEMENT PROCEDURES: The calves were housed in individual pens, wood shaving used as bedding, for the duration of the experiment. Calves were randomly assigned to one of the four experimental diets, each diet being fed to six animals.

The calves were fed an equal quantity of diet 4 (all milk protein, control) and their assigned diet for the first two feedings to allow time for adjustment to their diets. The diets were prepared at 37°C and 13% solids, and fed twice daily, at 8:00 A.M. and 5:00 P.M. The calves were fed at a rate of 9 g. crude protein per kg. of metabolic body weight. Dietary intake adjustments were made for weight gains but were not altered in event of weight losses. In case of total or partial (greater than 50%) refusal of feed, calves were fed one half ot the diet allowance for two feedings and thereafter were slowly brought up to the original level. Calves were weighed at the start of the experiment and after the completion of each collection period. Daily records of health and feed consumption were kept.

The study lasted 28 days (30-31 days of age) for each calf, during which time three five-day fecal collections were made. Calf ages during the three collection periods were: 8 to 13, 15 to 20 and 22 to 27 days. Feces collected during each period were frozen (-20°C) until time of analysis. At that time all feces collected from a particular calf during a specific collection period were combined and dried in a forced draft oven at 60°C and then ground

in a Wiley Mill. From this a subsample was taken for analysis.

Due to an outbreak of enzootic pneumonia, the experiment was stopped after data from the first 19 calves was collected. The pens were left vacant for one month and disinfected before the remaining calves were brought in for completion of the study. Treatment for enzootic pneumonia consisted of an initial injection of 2 cc. chlorampenicol followed by 1 cc. injections twice daily for five days. Five calves, numbered 105, 292, 104, 24-75 and 106 were treated for enzootic pneumonia at some time during the trial (Appendix Table 2).

EXPERIMENTAL DESIGN: The experiment was conducted as a split plot design (Gills and Hafs, 1971). Twenty-four calves were randomly assigned to one of the four diets for the duration of the experiment. Response criteria included growth rate and apparent digestibilities of dry matter, crude protein and the ether extract fraction of the diet. Apparent availabilities of the nutrients were determined by calculations based on the levels of chromic oxide in feed and feces. The equation used was:

$$\text{Digestion Coefficient} = 100\left(1-\left(\frac{Ci - No}{Co - Ni}\right)\right)$$

where: Ci = % indicator in feed (d.m. basis)

Co = % indicator in feces (d.m. basis)

Ni = % nutrient in feed (d.m. basis)

No = % nutrient in feces (d.m. basis)

Due to illness, there were three missing observations for which pseudovalues were calculated (Cochran and Cox, 1970). Mean comparisons were done using the Student Newman Keul procedure (Snedecor and Cochran, 1976).

Calf Study B

EXPERIMENTAL DIETS: In this study four isonitrogenous and isocaloric milk substitute diets were used, the major protein source (80%) supplied by F.B.P.C. and the remainder being contributed by skim milk powder and whey (Table 3). The milk substitute diets were formulated to contain 20% crude protein and 20% fat on an air dry basis. Diets A, C and D varied only in the dietary levels of L-methionine which were formulated to be 1.9, 2.5 and 3.6 g. per 16 g. nitrogen respectively. The fourth diet, diet B, contained .76 g. D-methionine and 1.74 g. L-methionine per 16 g. nitrogen. All diets contained .79 g. cystine per 16 g. nitrogen.

MANAGEMENT PROCEDURES: Calves were housed in individual stalls on rubber mats and were confined to metabolic crates only during the periods of urine and feces collection. A two-foot strip of rubber matting was also laid on the front portion of the floor of the metabolic crate to minimize stress to the animals while calves were on the nitrogen balance study.

The calves were fed equal quantities of a commercial milk replacer and their assigned diets for the first two feedings to allow time for adaptation to the diets. The prepared milk substitute diet (37^{o}C, 13% solids) was fed at a daily rate of 10% of the body weight in two equal meals, twelve hours apart. As before, diet allowances were increased in accord with weight gains but were not decreased due to losses in body weight.

Calves were fed the diets six days before being placed on nitrogen balance trials. Fresh water was provided ad libitum. Daily records were kept on feed consumption and calf health.

Table 3: COMPOSITION OF MILK SUBSTITUTE DIETS FED TO CALVES IN STUDY B

	DIETS (g/Kg)			
INGREDIENT	A	B	C	D
Fababean Protein Conc.	254.78	252.84	252.84	250.91
Skim milk[1]	113.40	113.40	113.40	113.40
Whey[1]	14.51	14.51	14.51	14.51
Tallow	159.91	159.91	159.91	159.91
Coconut Oil	39.98	39.98	39.98	39.98
Lecithin[2]	6.00	6.00	6.00	6.00
Aurofac[3]	2.50	2.50	2.50	2.50
Glucose[4]	350.53	351.25	351.25	352.60
L-methionine	1.82	-	3.01	4.22
D,L-methionine	-	3.01	-	-
L-lysine monohydrochloride	3.25	3.25	3.25	3.25
Co-I-NaCl[5]	6.30	6.30	6.30	6.30
$CaCl_2$ $2H_2O$	9.67	9.67	9.67	9.67
Dicalcium PO_4	17.25	17.25	17.25	17.25
Mineral Premix[6]	0.504	0.504	0.504	0.504
Sodium Selinite (mg)	0.001	0.001	0.001	0.001
Sodium Molybdate	0.013	0.013	0.013	0.013
Vitamin Premix[7]	32.244	32.24	32.24	32.24
Folic Acid	0.005	0.005	0.005	0.005
Pyridoxine Hydrochloride	0.010	0.010	0.010	0.010
Menadione	0.250	0.250	0.250	0.250

Table 3: COMPOSITION OF MILK SUBSTITUTE DIETS FED TO CALVES IN STUDY B (continued)

---

[1] Spray dried

[2] Lecithin-Soybean (oil not removed) ICN #102148

[3] 10 mg. auromycin / 454 gm.

[4] Cerelose

[5] Cobalt - iodized salt

[6] Trace Minerals: Iodine - 0.30%; Copper - 0.40%; Iron - 6.3 - 12.50%; Manganese - 6.2 - 13.0%; Zinc - 0.25%; Cobalt - 0.25%

[7] One Kg. contains: Vitamin A - 13,200,000 I.U.; Vitamin D_3 - 2,200,000 I.U.; Vitamin E - 8,800 I.U.; Vitamin B_{12} - 6.6 mg.; Thiamine - 2,200 mg.; Riboflavin - 4,400 mg.; Niacin - 4,400 mg.; Calcium Pantothenate - 4,400 mg.; Choline - 44,000 mg.; Ascorbic Acid - 33,000 mg.; Stabilizer (E tox) - 5,000 mg.

Body weight was determined on arrival and on three consecutive days at the beginning and end of each period. To avoid variation in body weight due to time of day, all weighings were done at 6:00 P.M.

Collection Procedures: There was total collection of the feces for the duration of each nitrogen balance trial. Daily collections of feces were immediately frozen (-20°C). At the end of the experiment feces collected during each period were thawed, mixed and a subsample was taken for further analysis. These subsamples were then dried in a forced air dryer at 60°C, ground in a Wiley Mill and kept in cool storage (4°C) until further analysis.

Urine was collected in brown coloured glass jugs containing approximately 10 ml. of 6 N HCl. Daily volumes were recorded and a sample was taken and frozen immediately at -20°C till further analysis.

A series of blood samples, collected before feeding and one, two, four and six hours postprandial were taken on the first and last day of each period. Blood samples were collected into 15 ml. lithium hepar nized vacutainer tubes using 21 G, thin wall vacutainer needles. The blood was kept at 4°C for approximately three hours and then centrifuged at 2,500 g. for twenty minutes. The resulting plasma samples were then immediately frozen (-20°C) for approximately two months, until further analyses were performed.

EXPERIMENTAL DESIGN: Twelve male Holstein calves were employed in a two-period change over design with four diets. In the six two-by-two latin squares, two calves represented rows and two periods (9 - 15 and 21 - 27 days) represented columns. The blocks were

randomized and calves were assigned to diets accordingly (Table 4). Response criteria included average daily gain, digestibility of dry matter and crude protein and urea nitrogen levels before and at various intervals after feeding on the first and last day of each period. Also, urine methionine and urea nitrogen were determined for each period.

ANALYTICAL PROCEDURES

Analyses for ash, crude fiber, dry matter, ether extract and nitrogen were carried out according to A.O.A.C. (1971) procedures. The levels of chromic oxide in feed and feces were determined according to the atomic absorption spectrophotometry methods of Williams et al. (1962).

Plasma and urine urea nitrogen levels were determined using the Technicon auto analyser II. The method employed was a modification of the procedure of Marsh et al. (1965). A colored product is formed when urea, in relatively weak acid solution, reacts with diacetyl-monohime. The presence of thiosemicarbazide and ferric ion intensifies the color of the reaction. The colored product is heated to 90°C and the percentage of light transmittance of the analytical stream can then be measured at 520 r.m. in a 15 mm flow cell.

Deproteinization of plasma and urine samples prior to amino acid analysis were done according to the method of Folin and Wu (1919). To 2 ml. of blood plasma or urine, 1 ml. .67N $H_2 30_4$ and 1 ml. 10% solution of sodium tungstate were added. The precipitate was removed by spinning at 2,500 r.p.m. for 20 minutes. As the urine samples

Table 4: BALANCED TWO-PERIOD CHANGEOVER DESIGN FOR FOUR TREATMENTS USED IN CALF STUDY B

TREATMENTS WITHIN BLOCKS

BLOCK	PERIOD 1	PERIOD 2
1	A*	C
	C	A
2	A	B
	B	A
3	A	D
	D	A
4	C	B
	B	C
5	B	D
	D	B
6	C	D
	D	C

RANDOMIZATION OF BLOCKS

3, 2, 5, 6, 4, 1

* letters refer to the experimental diets (Table 3)

were too dilute to analyse for amino acids, a known volume of the deproteinized sample was freeze-dried, and the amino acids redissolved in a diluting buffer of pH 1.80 at the time of analysis.

Amino acid analysis of the experimental diets were carried out according to procedures outlined by Bragg et al. (1966) with modifications as described by Giovannetti et al. (1970) on a model 116-Beckman amino acid analyser. For normal hydrolysis 50 mg. of the sample combined with 6 N HCl were autoclaved for 16 hours at 121°C, the tubes being evacuated. To analyse for methionine and cystine the method as described by Hirs (1967) was used. First a mixture of 1.0 ml. 30% hydrogen peroxide and 9.0 ml. 88% formic acid is allowed to stand at room temperature for an hour to form performic acid. Two ml. performic acid are added to approximately 50 mg. of the sample which is then placed in ice for 20 hours to form a homogenous mixture. Next, 0.3 ml. hydrobromic acid is added to destroy the excess reagent. The sample is then evaporated to dryness and the tube containing the sample is evacuated by autoclaving for 16 hours at 121°C.

Amino acid analysis of the plasma and urine samples were carried out according to the method of Spackman et al. (1958). Analysis was performed on the long column using sodium citrate buffers. All amino acids up to and including leucine were recorded.

RESULTS

PRELIMINARY RAT TRIAL:

Chemical analysis showed that the crude protein levels were relatively uniform in the seven experimental diets (Table 5). The first limiting acid of all diets, with the exception of diets C and E, was methionine. The growing rat's requirement for total sulfur amino acids is 5.0 g. per 100 g. crude protein and for methionine, specifically, is 3.35 g. per 100 g. crude protein (National Academy of Sciences, 1972). The level of cystine varied among the diets, however, lysine content was fairly uniform (APPENDIX Table 1).

Results from the experiment showed that rats fed unsupplemented F.B.P.C. (raw and autoclaved) had the lowest ($P<0.05$) overall feed intake levels. Supplementation with D,L-methionine (.32 g/100 g. of diet) doubled the overall feed intake levels. Feed intake was greatest ($P<0.05$) for rats fed unsupplemented soybean protein concentrate, followed by rats fed diets E and A (Table 6). Rats fed unsupplemented, autoclaved F.B.P.C sorted out and spilled much of their feed during the 28 day trial. All spilled feed was collected and weighed on a weekly basis and feed intake records were corrected accordingly. A similar situation, to a lesser degree, was observed with rats fed the control diet.

Table 5: SOURCE AND LEVEL OF PROTEIN USED IN THE RAT DIETS AND THE FIRST AND SECOND LIMITING AMINO ACIDS OF THESE DIETS.

	DIET						
	A	B	C	D	E	F	G
PROTEIN SOURCE	CASEIN	RAW F.B.P.C.	RAW F.B.P.C.[2]	AUTOCLAVED F.B.P.C.	AUTOCLAVED F.B.P.C.[2]	SOYBEAN PROTEIN CONCENTRATE	SOYBEAN PROTEIN CONCENTRATE[2]
Crude Protein Level	9.79	10.10	10.30	9.69	9.97	10.34	10.30
First Limiting Amino Acid	methionine	methionine	phenyl-alanine	methionine	threonine	methionine	methionine
% of Rat's Requirement[1]	52.2	14.6	82.4	15.8	81.3	30.4	70.4
Second Limiting Amino Acid	arginine	phenyl-alanine	threonine	phenyl-alanine	phenyl-alanine	lysine	lysine
% of Rat's Requirement[1]	71.6	84.2	83.9	85.6	84.2	90.3	87.2

[1] National Academy of Sciences NRC, 1972

[2] Supplemented with methionine

Table 6: FEED INTAKE, WEIGHT GAIN AND PROTEIN EFFICIENCY RATIOS (P.E.R.) FOR RATS IN PRELIMINARY TRIALS[2]

	FEED INTAKE (gr.)							
DIET	Wk.1	Wk.2	Wk.3	Wk.4	TOTAL[1]	TOTAL PROTEIN INTAKE (gr.)	WEIGHT GAIN (gr.)	P.E.R. VALUES
A	63.0	74.8	78.3	92.3	307.3^{B}	29.53	85.15^{C}	2.87^{A}
B	39.2	36.8	38.2	42.1	156.3^{D}	15.78	10.30^{D}	$.65^{C}$
C	58.1	72.1	63.8	83.6	278.2^{C}	29.00	79.05^{C}	$2.70^{A,B}$
D	42.1	40.2	40.2	40.8	163.3^{D}	15.24	9.40^{D}	$.62^{C}$
E	42.6	79.9	89.5	95.4	309.3^{B}	30.65	90.75^{B}	2.82^{A}
F	72.1	88.5	95.0	102.8	358.6^{A}	37.67	96.50^{A}	2.57^{B}
G	51.9	67.3	85.0	94.8	297.4^{B}	31.20	$89.50^{B,C}$	2.87^{A}
S.E.					4.67		1.77	0.029

[1] Means in the same row with the same superscript do not differ significantly ($P < 0.05$)

[2] Values for feed intake, weight gain and P.E.R. are means of ten observations

Rats fed unsupplemented soy protein concentrate had the highest ($P < 0.05$) weight gains, followed by rats fed methionine supplemented, autoclaved F.B.P.C. and supplemented soybean protein concentrate. The lowest body weight gains were recorded for rats fed unsupplemented F.B.P.C. (raw and autoclaved) (Table 6).

The control diet, containing casein, and the methionine supplemented vegetable proteins had similar protein efficiency (P.E.R.) ratios. Rations A, B, D, F and G were significantly greater ($P < 0.05$) than the unsupplemented F.B.P.C. diets. Statistical analysis showed that there was little variation in P.E.R. values due to blocks, that is, the initial weight of the rat. Diet did significantly influence ($P < 0.05$) P.E.R. values.

A comparison of the P.E.R. means resulted in the following conclusions. There is no significant difference ($P < 0.05$) between raw and autoclaved F.B.P.C. as a protein source for growing rats. D,L-methionine supplementation of soybean protein concentrate and F.B.P.C. (raw and autoclaved) resulted in significant ($P < 0.05$) increases in P.E.R. values. Although unsupplemented soybean protein was a better protein source ($P < 0.05$) than unsupplemented fababean protein, there were no significant differences between the vegetable protein sources when they were supplemented with methionine.

CALF STUDY A:

Proximate analyses of the four experimental diets containing increasing levels of F.B.P.C., showed uniform levels of crude protein (Table 7). The level of fat (ether extract) was variable. Fat content of diet A was low, 16.27% on an air dry basis, as

Table 7: PROXIMATE, AMINO ACID AND CHROMIC OXIDE ANALYSIS OF MILK SUBSTITUTE DIETS USED IN STUDY A

DIETS LEVEL OF F.B.P.C. (%)	A 80	B 50	C 25	D 0
ITEMS				
Dry matter (%)	95.40	95.19	95.75	96.17
Protein (N x 6.25) (%)	22.78	24.86	24.01	23.79
Ether Extract (%)	16.27	22.22	21.18	19.70
Chromic Oxide (%)	.46	.46	.46	.46
Methionine (%)	.51	.55	.48	.46
Cystine (%)	.60	.94	1.30	1.66
Total Sulfur Amino Acids (%)	1.11	1.49	1.78	2.12

compared to 22.22, 21.18 and 19.70 in diets B, C and D. Although no explanation was given for this variability, it may be that at high levels of F.B.P.C. fat can not be properly incorporated into the milk substitute diet. This is further discussed on page 74. The F.B.P.C. used in formulating the rations contained 65.06% crude protein on an air dry basis (Table 8). Levels of the sulfur amino acids, methionine and cystine, showed some variation among the diets. Although methionine content decreased as the proportion of crude protein supplied by milk proteins increased, total sulfur amino acids content increased.

As was found in diets prepared by Dr. Latrille (personal communication) the milk substitute diets containing F.B.P.C. mixed well with water and would remain in suspension. All diets appeared palatable.

Five calves, numbered 105, 292, 104, 24-75 and 106 were treated for enzootic pneumonia at some time during the trial (APPENDIX Table 2). The treatment is described in Materials and Methods, page 48. Fecal samples collected and data from these calves were used.

Due to navel ill, no data was obtained from calves 22-75 and 19-75 during the first collection period. Feces collected from calf 17-75 during the second collection period were misplaced. When the statistical analyses for apparent digestibility of dry matter, ether extract and crude protein fractions were done, pseudo values were calculated to replace these three missing observations (Anderson, 1946). Individual calf data and the pseudo values for digestible dry matter, ether extract and crude protein are given in the APPENDIX (Tables 3, 4 and 5).

Table 8: CHEMICAL COMPOSITION OF FABABEAN PROTEIN CONCENTRATE USED IN EXPERIMENTAL DIETS IN THE PRELIMINARY RAT TRIAL AND CALF STUDY A.

PRINCIPLE	AS IS (%)	DRY MATTER BASIS (%)
Dry Matter	97.18	
Protein (N x 6.25)	65.06	69.61
Ether Extract	2.40	2.47
Crude Fiber	2.27	2.34
Ash	5.49	5.65
Calcium	.13	.13
Phosphorous	.99	1.02

The average initial weights of calves assigned to diets A, B, C and D were 43.47, 45.32, 44.68 and 43.87 respectively. No significant differences due to diet were found in average daily gain (Table 9). There was much variation in average daily gain among calves within the same diet which could be partially accounted for by calf health. Calf #105, for example, had an average daily gain of 137 Kg./day up to the beginning of period 3, but lost 3.4 Kg. in body weight in the final eight days of the study due to illness. Calves fed diets C and D gained twice as much weight as those fed diet B in which F.B.P.C. made up 50% of the total protein. Calves fed ration A, in which 80% of the crude protein was supplied by F.B.P.C., had body weight gains between these two extremes.

Mean values of feed efficiency for rations A, B, C and D were .326, .242, .457 and .457 kg. gained per kg. fed (Table 10). Calves fed diets C and D were more efficient ($P < 0.05$) than calves fed the other two diets, diet B having the lowest values.

Fecal dry matter content was not influenced by diet, but as expected, increased with age (Table 11). It was observed for the majority of calves fed diets A and B, that for the first two or three days on these rations the calves had distended abdomens and did not defecate. Following this, the calves had a mild case of diarrhoea after which normal excretory patterns were observed.

Dry matter digestibilities based on total fecal dry matter collections and chromic oxide levels were 81.71, 79.86, 84.53 and 85.25% for diets A, B, C and D respectively. There was no significant difference due to diet, however, apparent dry matter digestibility increased significantly ($P < 0.05$) as a function of age (Table 12).

Table 9: AVERAGE DAILY GAINS OF CALVES FED THE FOUR MILK SUBSTITUTE DIETS OVER A 28 DAY PERIOD IN STUDY A (Kg/day)

Diet A		Diet B		Diet C		Diet D	
Calf	ADG	Calf	ADG	Calf	ADG	Calf	ADG
15-75	.181	14-75	.212	104	.327	106	.372
101	.361	22-75	.140	19-75	.345	24-75	.072
23-75	.300	297	.240	102	.288	17-75	.322
105	-.038	298	.193	16-75	.327	20-75	.348
292	.227	299	.008	291	.219	294	.332
306	.262	300	.100	296	.327	295	.336
Mean(1)		Mean		Mean		Mean	
.216 ± .026*		.149 ± .026		.306 ± .026		.297 ± .026	

(1) No significant difference in the average daily gain due to diet

* Mean ± S.E.

Table 10: FEED EFFICIENCY (F.E.)[1] OF THE FOUR EXPERIMENTAL MILK SUBSTITUTE DIETS FED TO CALVES OVER A 28 DAY PERIOD IN STUDY A.

DIET A		DIET B		DIET C		DIET D	
CALF	F.E.	CALF	F.E.	CALF	F.E.	CALF	F.E.
15-75	.293	14-75	.318	104	.589	106	.577
101	.521	22-75	.242	19-75	.507	24-75	.508
23-75	.423	297	.371	102	.442	17-75	.110
105	-.071	298	.288	16-75	.415	20-75	.565
292	.368	299	.013	291	.283	294	.497
306	.415	300	.217	296	.506	295	.485
Mean[2]		Mean		Mean		Mean	
$.326^{B} \pm .031$		$.242^{A} \pm .031$		$.457^{C} \pm .031$		$.457^{C} \pm .031$	

[1] Feed efficiency was measured as Kg. body weight gain/kg. diet fed (air dry basis).

[2] Means with different superscripts are significantly different ($P < 0.05$).

Table 11: MEAN VALUES OF FECAL DRY MATTER CONTENT (%) FOR CALVES ASSIGNED TO THE EXPERIMENTAL DIETS IN STUDY A.

	AGE (days)			
	8-13	15-20	22-27	AVERAGE
Diet				
A	14.9 (6)*	16.5 (6)	18.7 (6)	16.7
B	14.7 (5)	17.6 (6)	19.3 (6)	17.6
C	15.9 (5)	18.4 (6)	18.6 (6)	17.6
D	14.8 (6)	15.3 (5)	16.2 (6)	15.4
Average	15.1	17.0	18.2	

* number of observations

Table 12: MEAN DRY MATTER DIGESTIBILITY COEFFICIENTS (%) OF EXPERIMENT DIETS WHEN FED TO CALVES AGED 8-13, 15-20 AND 22-27 DAYS OF AGE.

	AGE (days)			
	8-13	15-20	22-27	AVERAGE[2,3]
Diets				
A	76.26[4]	81.04	87.84	81.71±1.36
B	74.91	80.35	84.30	79.86±1.36
C	79.10	84.37	90.13	84.53±1.36
D	84.77	83.83	90.49	85.25±1.36
Average[1,2]	77.93^{a}±1.01	82.40^{b}±1.01	88.19^{c}±1.01	

[1] mean of 24 observations ± S.E.

[2] averages with different superscripts are significantly different ($P<0.05$)

[3] mean of 18 observations ± S.E.

[4] average of 6 observations

Mean values of fecal fat content for calves fed diets A, B, C and D were 24.92, 28.26, 23.24 and 27.85% respectively (Table 13) although the dietary level of fat was lower for diet A, the fecal fat content did not appear to be depressed. Apparent digestibility of the ether extract fraction in the four diets improved as the portion of protein supplied by milk protein increased, however, these increases were not significant (Table 14). As expected, digestibility improved significantly ($P < 0.05$) with age. The greatest variation in digestibility among the diets was found when calves were 8 to 13 days of age, the range being 14.3 percentage units. This was reduced to 4.98 percentile units when calves aged 22 to 27 days. In some cases steatorrhea was observed, the individual calf data is shown in the APPENDIX Table 4.

Average levels of crude protein and apparent crude protein digestibility for calves fed diets A, B, C and D were 38.22, 35.64, 30.69, 36.69% and 70.11, 72.57, 78.65 and 79.29% respectively (Table 15 and 16). Although fecal crude protein levels were not influenced by diet, there was a significant influence ($P < 0.05$) on protein digestibility. As an increasing portion of the dietary protein was supplied by F.B.P.C., crude protein digestibility decreased. Also, protein digestibility increased significantly ($P < 0.05$) as a function of the calf's age. There was no diet x age interaction effect.

Using diet D as the control, the apparent digestibility of crude protein in skim milk and whey was found to be 73.25, 78.78 and 85.84 for periods 1, 2 and 3 respectively. Crude protein digestibility improved 17.2% over the four week period for the milk

Table 13: MEAN FECAL FAT CONTENT (%) OF FOUR GROUPS OF CALVES FED MILK SUBSTITUTE DIETS IN STUDY A.

	AGE (days)			
	8-13	15-20	22-27	AVERAGE
Diet				
A	32.30 (6)*	28.70 (6)	13.77 (6)	24.92
B	41.25 (5)	24.80 (6)	20.90 (6)	28.26
C	36.42 (5)	21.13 (6)	14.38 (6)	23.24
D	39.20 (6)	27.13 (5)	17.21 (6)	27.85
Average	37.15	25.27	16.57	

* number of observations

Table 14: MEAN FAT DIGESTIBILITY COEFFICIENTS (%) OF EXPERIMENTAL DIETS WHEN FED TO CALVES AGED 8-13, 15-20 AND 22-27 DAYS OF AGE.

	AGE (days)			
	8-13	15-20	22-27	AVERAGE
Diets				
A	50.62[4]	66.27	90.02	68.97±3.76
B	50.57	76.34	88.16	71.67±3.76
C	59.70	84.01	93.44	79.05±3.76
D	64.87[a]	81.45[b]	71.88[c]	79.40±3.76
Average[1,2]	56.43[a]±2.84	79.02[b]±2.84	90.88[c]±2.84	

[1] mean of 24 observations ± S.E.

[2] averages with different superscripts are significantly different ($P < 0.05$)

[3] mean of 18 observations ± S.E.

[4] average of 6 observations

Table 15: MEAN FECAL CRUDE PROTEIN CONTENT (%) FOR FOUR GROUPS OF CALVES FED EXPERIMENTAL DIETS IN STUDY A.

	AGE (days)			
	8-13	15-20	22-27	AVERAGE
Diet				
A	40.11 (6)[1]	39.38 (6)	35.47 (6)	38.32
B	37.31 (5)	37.46 (6)	32.43 (6)	35.64
C	34.56 (5)	34.83 (6)	35.89 (6)	30.96
D	35.22 (6)	40.58 (5)	34.91 (6)	36.69
Average	36.88	37.96	34.68	

[1] number of observations per mean

Table 16: MEAN CRUDE PROTEIN DIGESTIBILITY COEFFICIENTS (%) OF EXPERIMENTAL DIETS WHEN FED TO CALVES AGED 8-13, 15-20 AND 22-27 DAYS OF AGE.

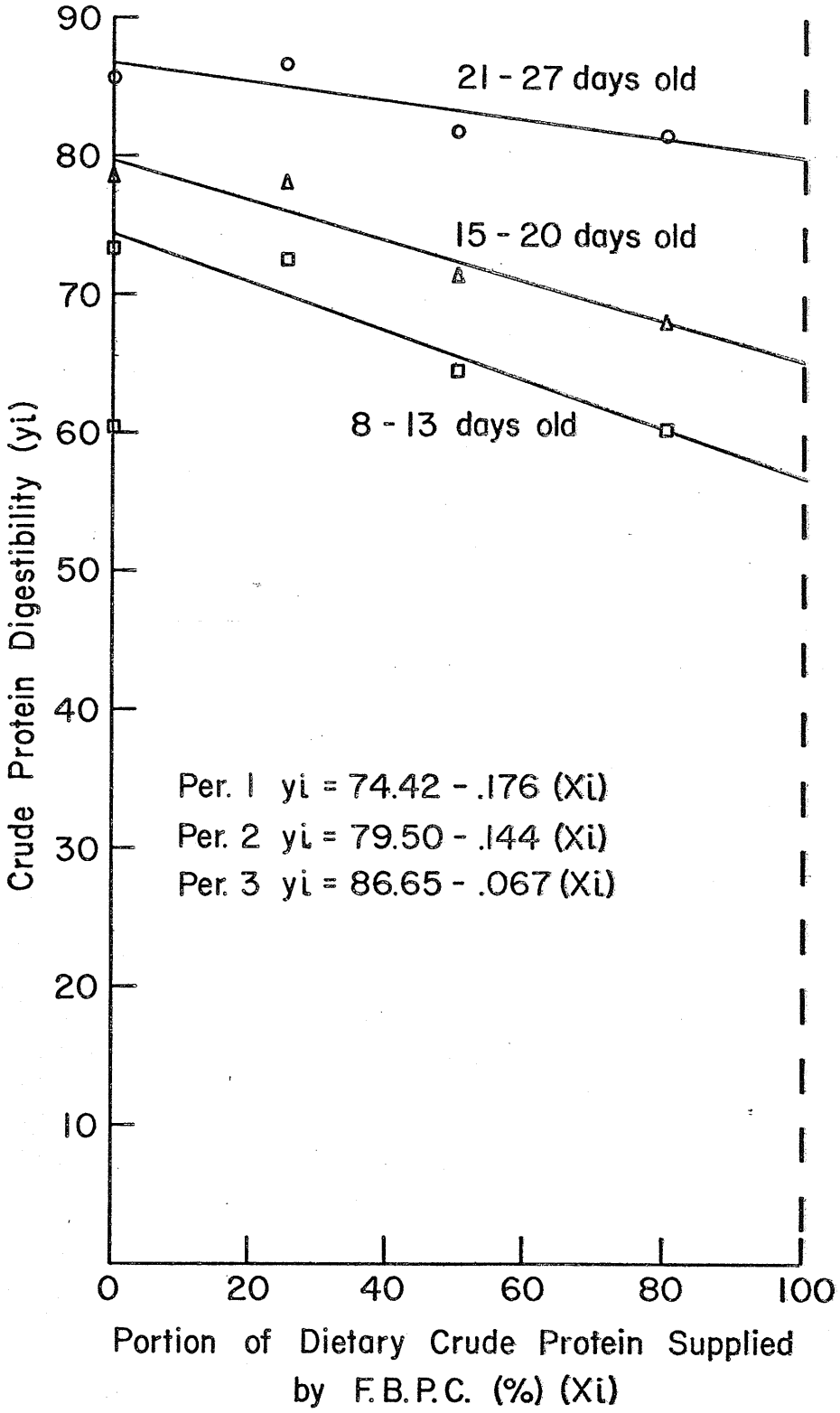
	AGE (days)			
	8-13	15-20	22-27	AVERAGE[2,3]
Diets				
A	60.49[4]	68.28	81.56	$70.11^{A} \pm 1.71$
B	64.16	71.58	81.96	$72.57^{AB} \pm 1.71$
C	72.48	78.13	86.84	$78.65^{BC} \pm 1.71$
D	73.25	78.98	85.84	$79.29^{BC} \pm 1.71$
Average[1,2]	$67.59^{a} \pm 1.21$	$74.19^{b} \pm 1.21$	$83.68^{c} \pm 1.21$	

[1] mean of 24 observations ± S.E.

[2] averages with different superscripts are significantly different ($P < 0.05$)

[3] mean of 18 observations ± S.E.

[4] average of 6 observations


90
80
70
60
50
40
30
20
10
21 - 27 days old
15 - 20 days old
8 - 13 days old
Per. 1 yi = 74.42 - .176 (Xi)
Per. 2 yi = 79.50 - .144 (Xi)
Per. 3 yi = 86.65 - .067 (Xi)
Crude Protein Digestibility (yi)
0
20
40
60
80
100
Portion of Dietary Crude Protein Supplied
by F.B.P.C. (%) (Xi)

proteins. Using a simple regression (Fig. 3) the digestibility of crude protein in F.B.P.C. when fed in a milk substitute diet to calves can be predicted to be 56.80, 65.10 and 79.95% at ages 8-13, 15-20 and 22-27 days respectively. Slopes of the regressions indicate that as the calf ages the ability to digest fababean protein becomes comparable to the ability to utilize milk proteins. Slopes of the regression lines are not significantly different ($P<0.05$).

CALF STUDY B:

The F.B.P.C. used in milk substitute diets for this study contained 61.03% crude protein on an air dry basis (Table 17). Ether extract, crude fiber and ash content were similar to that of F.B.P.C. used in the previous two experiments.

The texture of milk substitute diets in this study was sticky and more coarse compared to diets prepared in Study A. The nutritionist at Mutual Products Ltd. (personal communication) observed that when coconut oil was included in a spray dry form no problems were encountered while mixing. However, when tallow was injected into the mixer in a liquid form there was "balling" in the product. This balling effect was suspected to be due to the presence of one or more of the other ingredients in the milk replacer because fat has been injected in greater amounts than 20% of the diet without such problems. It was speculated that F.B.P.C. may have caused this balling effect.

Upon arrival, the diets were ground and remixed to break up all lumps. The milk substitute diets did not mix well with water and unlike rations fed in Study A, they did not remain in suspension.

Table 17: CHEMICAL COMPOSITION OF FABABEAN PROTEIN CONCENTRATE USED IN EXPERIMENTAL DIETS FOR CALF STUDY B.

PRINCIPLE	AS IS (%)	DRY MATTER BASIS (%)
Dry Matter	95.29	
Protein (N x 6.25)	61.03	63.47
Ether Extract	2.65	2.76
Crude Fiber	1.88	1.96
Ash	5.87	6.10

The diets were, however, palatable and readily consumed by the calves.

A proximate analysis of the diets showed that the crude protein levels were uniform, varying only 1.13% among the four diets (Table 18). Fat content was higher than expected and less uniform. In all diets, the energy requirements at 20% crude protein were met.

The actual levels of methionine in the rations were 1.70, 2.29, 2.38 and 2.76g/16 g. N for diets A, B, C and D respectively. Levels of cystine were fairly uniform at 0.84, 0.77, 0.78 and 0.77 g/16 g. N for diets A, B, C and D respectively. Diet B contained approximately .76 g. D-methionine and 1.53 g. L-methionine/16 g. N.

Average daily gain (A.D.G.) did not change significantly due to diet or age (Table 19). Adjusted means of A.D.G. for diets A, B, C and D were -39.47, -45.34, -39.84 and -31.73 g/day. There was a significant difference due to blocks, that is, variability among calves (APPENDIX Table 8). The great animal-to-animal variation may be partially explained by calf health during the trials and the ability of calves to adjust to the metabolism crates. The majority of calves lost weight due to stress while in the crates (APPENDIX Table 9).

Dry matter digestibility was not influenced by diet, the adjusted means being 80.51, 80.96, 77.46 and 74.93% for diets A, B, C and D respectively (Table 20). Unlike results from Study A, there was no improvement in dry matter digestibility with age, being 77.29 and 79.65%for calves aged 9 - 15 and 21 - 27 days respectively.

Table 18: PROXIMATE AND AMINO ACID ANALYSES OF FOUR MILK SUBSTITUTE DIETS FED TO CALVES IN STUDY B.

ITEMS (on a Dry Matter Basis)	DIETS			
	A	B	C	D
Crude Protein (N x 6.25) (%)	20.45	20.79	21.41	20.28
Fat (Ether Extract) (%)	24.17	25.16	22.98	25.54
Ash (%)	5.40	4.99	5.59	5.39
Energy, cal/gm.*	5315	5387	5237	5299
Methionine (%)	.35	.48	.51	.56
Cystine (%)	.17	.16	.17	.16

* Gross energy obtained by bomb calorimetry

Table 19: THE EFFECTS OF GRADED LEVELS OF METHIONINE IN MILK SUBSTITUTE DIETS ON AVERAGE DAILY GAIN (A.D.G.) IN PRERUMINANT CALVES

DIET (g. meth/16 g. N)	CALF	PERIOD[1]	A.D.G. g/day	ADJUSTED A.D.G. MEANS[2]
1.70	1	1	-120	
	3	1	- 70	
	12	1	-236	
	2	2	25	
	4	2	-217	
	11	2	0	
				-39.47
2.29	4	1	- 80	
	5	1	-170	
	10	1	160	
	3	2	-192	
	6	2	- 12	
	9	2	25	
				-45.34
2.38	7	1	- 10	
	9	1	10	
	11	1	30	
	8	2	42	
	10	2	200	
	12	2	-182	
				-39.84
2.74	2	1	- 60	
	6	1	- 40	
	8	1	120	
	1	2	-142	
	5	2	- 67	
	7	2	16	
				-31.72

[1] Period 1 refers to calves aged 9-15 days; Period 2 refers to calves aged 21-27 days.

[2] Standard error is 12.53.

Table 20: THE EFFECTS OF GRADED LEVELS OF DIETARY METHIONINE ON THE DIGESTIBILITY OF DRY MATTER (D.M.) AND CRUDE PROTEIN (C.P.) IN CALVES.

	DIET				PERIOD		
Digestibility	A	B	C	D	1	2	S.E.
D.M.	80.51	80.96	77.46	74.93	77.27	79.65	4.83
C.P.	52.98	54.30	45.47	44.5	46.73	53.67	4.01

Adjusted means for crude protein digestibility of diets A, B, C and D were 52.98, 54.30, 45.47 and 44.50 respectively (Table 20). Diet and age did not significantly influence crude protein digestibility (APPENDIX Table 8). Also, there was no significant animal-to-animal variation. Individual calf data for dry matter and crude protein digestibility are given in the Appendix (Table 10).

Nitrogen utilization, calculated as grams gained per gram of nitrogen intake was found to be -2.3, -2.6, -1.5 and -2.5 for diets A, B, C and D respectively (Table 21). Diet and age did not influence nitrogen utilization, however, there was much animal-to-animal variation ($P < 0.05$).

The adjusted means for nitrogen balance of diets A, B, C and D were -0.23, 4.24, 2.01 and 0.67 g/day respectively (Table 21). Age and diet did not significantly influence nitrogen balance. Individual calf data of nitrogen utilization and nitrogen balance are given in APPENDIX Table 11 Mean intake of nitrogen from diets A, B, C and D was 82.7, 82.9, 83.4 and 80.1 grams respectively (Table 21). Urinary nitrogen output was influenced by diet ($P < 0.05$), calves fed diet A secreting the greatest amount of nitrogen followed by calves fed diets D and B. Calves fed 2.38 g. methionine/16 g. N secreted the least amount of nitrogen via the urine. Diet did not influence nitrogen output via feces.

Results from blood samples taken on the first day of the nitrogen balance trial, six days after calves were initially introduced to their respective diets, will be referred to as data from "set 1". Results from blood samples taken on the last day of the nitrogen balance trial, eleven days after the calves were initially

Table 21: THE EFFECTS OF GRADED LEVELS OF DIETARY METHIONINE ON NITROGEN BALANCE AND RELATED PARAMETERS IN CALVES[2]

N-CONTENT (g)	DIET				PERIOD		S.E.
	A	B	C	D	1	2	
Milk repl.[1]	82.7	82.9	83.4	80.1	81.65	82.91	0.72
Urine[3]	32.46^{C}	18.00^{A}	19.22^{A}	24.63^{B}	24.79	22.35	1.30
Feces	47.65	54.34	44.02	55.16	52.18	48.40	3.63
N-Balance (g/day)	-.23	4.24	2.01	.67	.89	2.45	0.59
N-Utilization (gain/N intake)	-2.3	-2.6	-1.5	-2.5	-2.8	-1.6	0.60

[1] Milk substitute powder intake corrected for feed weigh back.

[2] All means are adjusted to take into account "within animal" variation

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

introduced to their diets, will be referred to as data from "set 2".

Plasma free methionine levels, from set !, for animals fed diets A, B, C and D were 6.34, 8.46, 4.39 and 7.85 mg/l at fasting state; 5.71, 13.54, 9.30 and 11.64 mg/l at two hours postfeeding and 6.71, 15.15, 12.61 and 16.85 mg/l at four hours postfeeding (Fig. 4). Diet significantly influenced plasma free methionine (P 0.05) at fasting, diet C being lower than diets B and D, but similar to diet A (APPENDIX Table 12).

At two hours postfeeding, plasma free methionine levels decreased by 0.59 mg/l for calves fed diet A whereas there was as increase of 5.08, 4.87 and 5.18 mg/l for diets B, C and D respectively (Fig. 5). Diet did influence (P 0.05) the extent of change in plasma free methionine levels at two hours postfeeding; levels for calves fed diet A being lower than for diets B, C and D; diets B and C being lower than diet D. Animal variation did not influence plasma free methionine concentrations (P 0.05).

Referring to absolute values of plasma free methionine levels, diet (P 0.01) and animal variation (P 0.05) influenced levels at two hours postfeeding. Diet A had the lowest plasma methionine content, followed by diet C, diet D and diet B in that order (APPENDIX Table 12).

Peak levels of plasma free methionine occurred at four hours after feeding. Calves fed 1.70 g L-methionine/16 g N had the lowest plasma free methionine values, foolowed by calves fed diets C and B; the greatest values occuring in calves fed diet D (P 0.05) (APPENDIX Table 12). Although not significant (P 0.05), calves aged 9-15 days had greater free meth. values

FIGURE 4

The Relationship Between Graded Levels of Dietary Methionine and Plasma Free Methionine Levels at 0, 2 and 4 Hours Post Feeding on the First Day of the Experimental Period

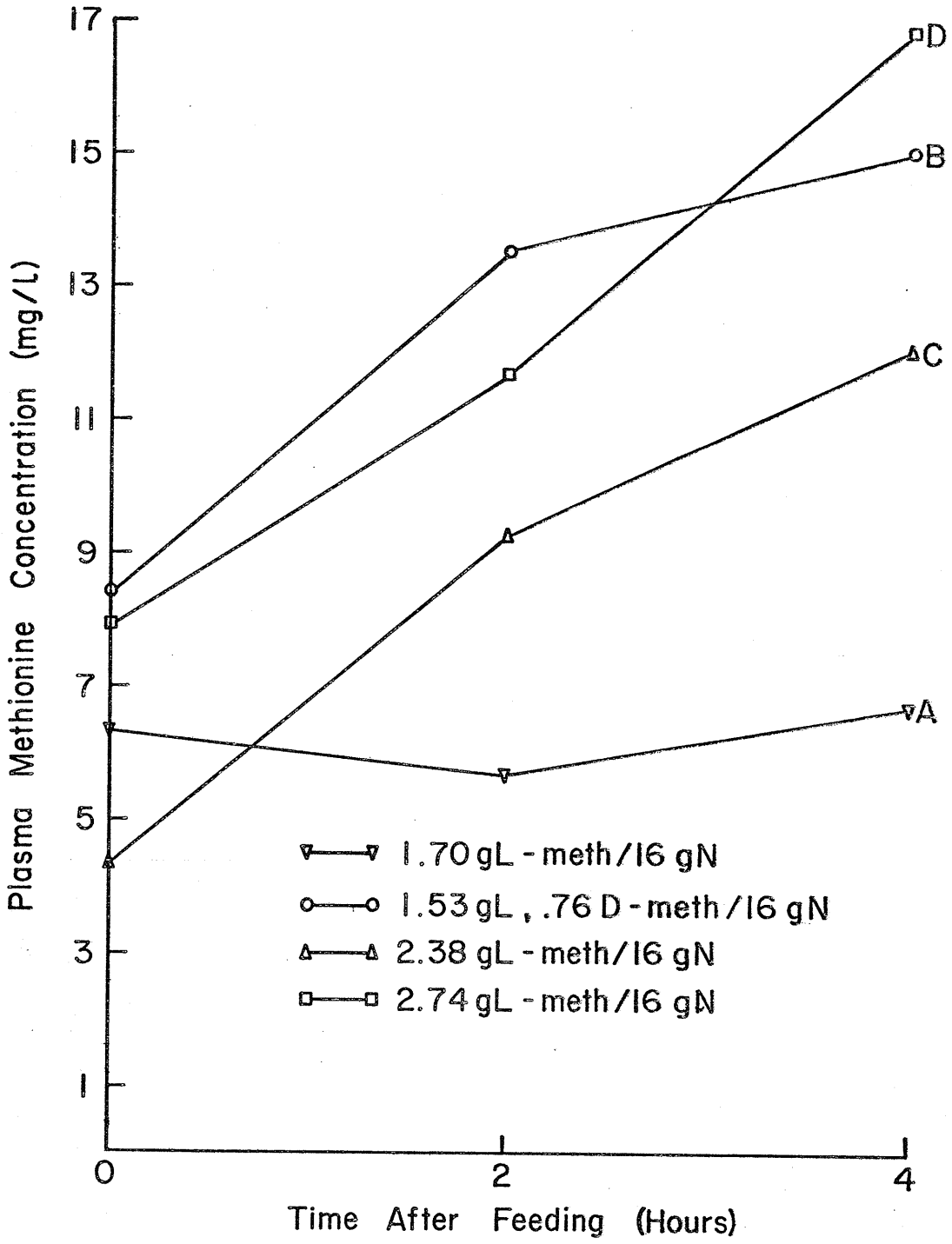
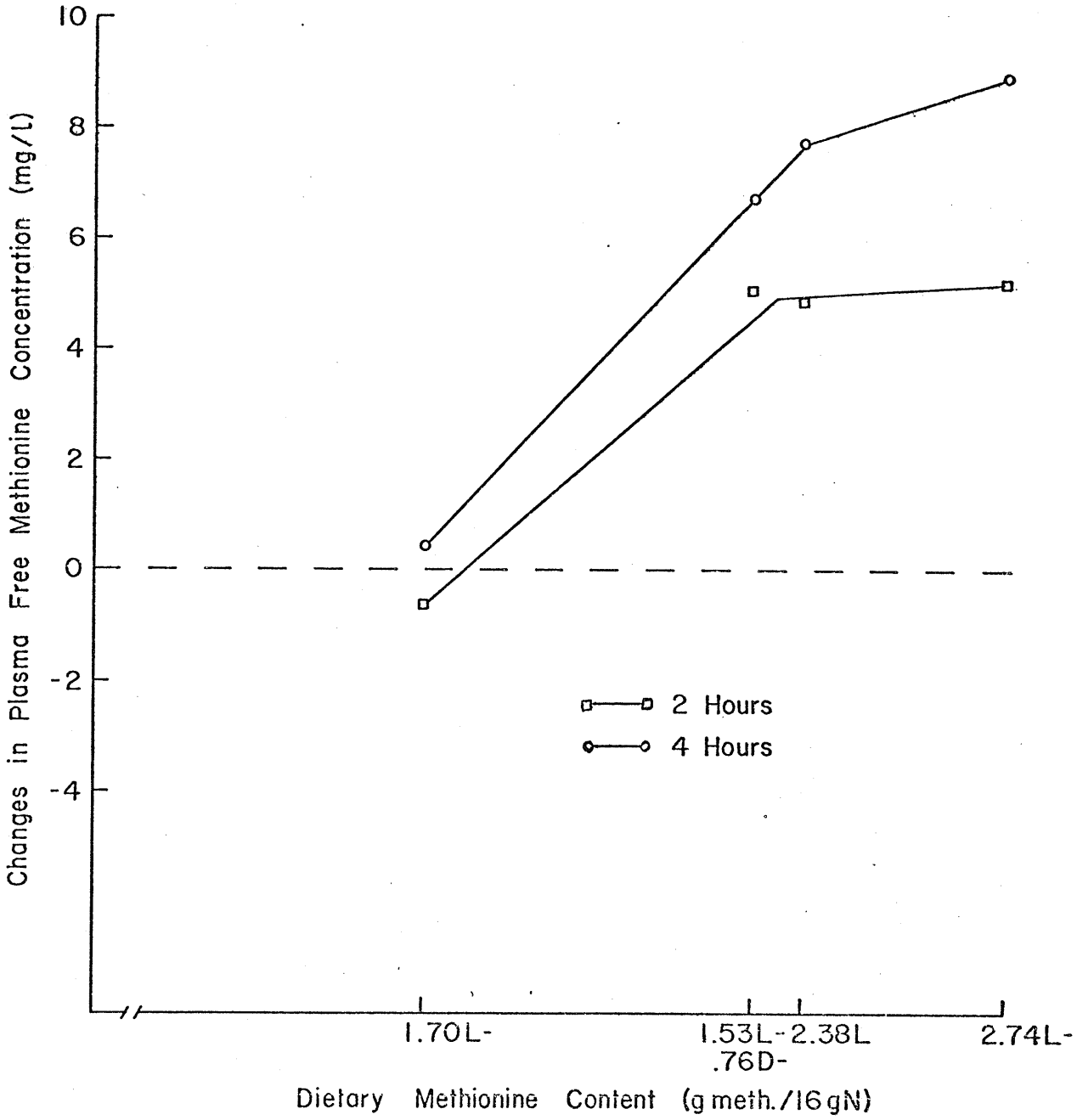

17
15
13
11
9
7
5
3
1
0
2
4
Plasma Methionine Concentration (mg/L)
Time After Feeding (Hours)
A
B
C
D
1.70 gL - meth/16 gN
1.53 gL, .76 D - meth/16 gN
2.38 gL - meth/16 gN
2.74 gL - meth/16 gN

FIGURE 5

The Relationship Between Graded Levels of Dietary Methionine and Differences Between Plasma Free Methionine Levels at Fasting and at 2 and 4 Hours After Feeding on the First Day of the Experimental Period


10
8
6
4
2
0
-2
-4
Changes in Plasma Free Methionine Concentration (mg/l)
2 Hours
4 Hours
1.70L-
1.53L-2.38L
.76D-
2.74L-
Dietary Methionine Content (g meth./16 gN)

(13.87 mg/1.) than older calves aged 21 - 27 days (11.62 mg/1.) (APPENDIX Table 10). There was also a significant amount of variation among animals.

The actual increases in plasma free methionine values from fasting state to four hours postfeeding were 0.40, 6.74, 7.74 and 8.90 mg/1. for diets A. B. C and D respectively (Fig. 5). Both diet ($P<0.01$) and age ($P<0.05$) influenced the extent of these changes. Calves aged 9 - 15 days had greater overall change in plasma methionine after feeding (10.02 mg/1.) than calves aged 21 - 27 days (7.87 mg/1.).

Plasma free methionine levels, from set 2, for animals fed diets A, B, C and D were 4.72, 8.37, 6.02 and 8.42 mg/1. at fasting state; 7.34, 11.43, 8.63 and 12.04 mg/1. at two hours postfeeding and 5.4, 12.89, 11.60 and 14.58 mg/1. at four hours postfeeding respectively (Fig. 6). Diet did not significantly influence plasma free methionine values at fasting or two hours after feeding. There was an increase of 2.63, 3.05, 2.61 and 3.54 mg/1. over the two hour period for diets A, B, C and D respectively (Fig. 7). The overall change in plasma free methionine values from fasting state to four hours postprandial were 0.69, 4.51, 5.57 and 6.67 mg/1. for diets A, B, C and D respectively (Fig. 7). Peak levels for diet A occurred at two hours after feeding, whereas peak levels for the other diets occurred at four hours postfeeding. Diet did influence ($P<0.05$) plasma free methionine values at four hours postfeeding (APPENDIX Table 10). Analysis of variance for the data from the second set of blood samples is located in the Appendix (Table 12).

FIGURE 6

The Relationship Between Graded Levels of Dietary Methionine and Plasma Free Methionine Levels at 0, 2 and 4 hours Post Feeding on the Last Day of the Experimental Period

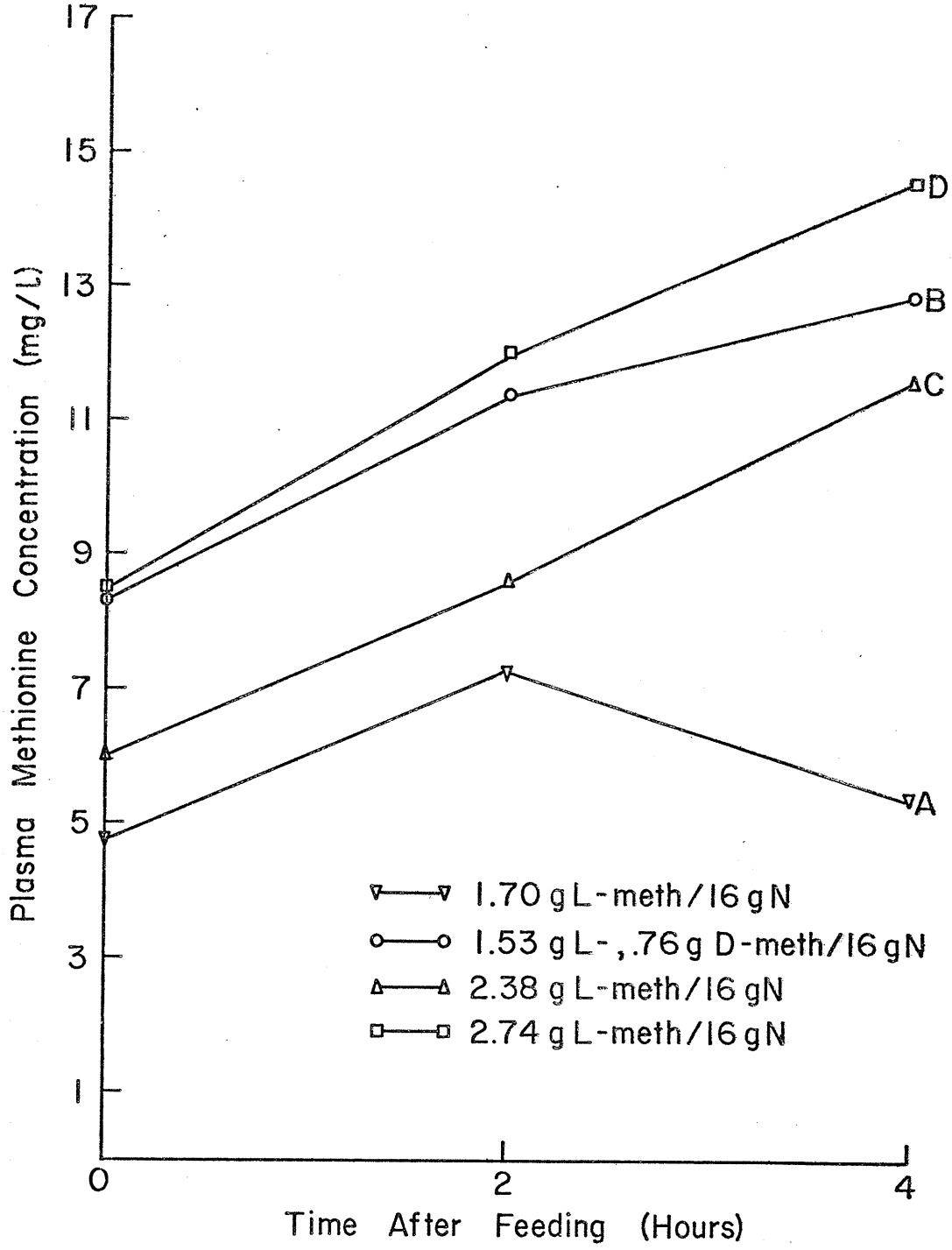
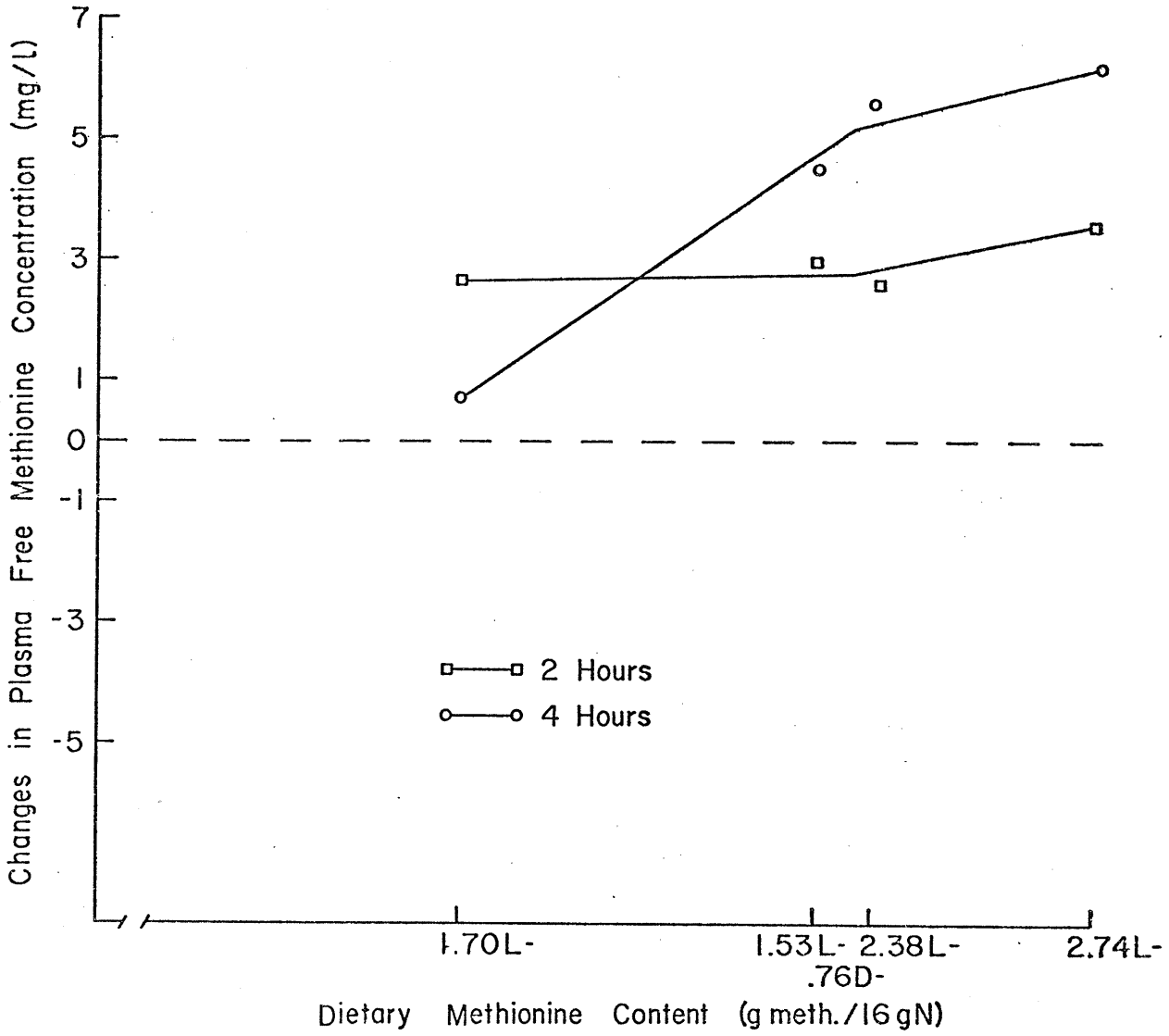

17
15
13
11
9
7
5
3
1
Plasma Methionine Concentration (mg/L)
D
B
C
A
1.70 g L-meth/16 gN
1.53 g L-, .76 g D-meth/16 gN
2.38 g L-meth/16 gN
2.74 g L-meth/16 gN
0
2
4
Time After Feeding (Hours)

FIGURE 7

The Relationship Between Graded Levels of Dietary Methionine and Differences Between Plasma Free Methionine Levels at Fasting and at 2 and 4 Hours After Feeding on the Last Day of the Experimental Period


Changes in Plasma Free Methionine Concentration (mg/l)
7
5
3
1
0
-1
-3
-5
2 Hours
4 Hours
1.70L-
1.53L- .76D-
2.38L-
2.74L-
Dietary Methionine Content (g meth./16 gN)

No significant differences were found among means of plasma urea nitrogen values for calves fed the four experimental diets (Table 22). Age did not appear to influence urea nitrogen levels with the exception of samples taken at two hours after feeding in set 2. In this case plasma urea nitrogen increased with age ($P < 0.05$). Plasma urea nitrogen content for samples taken at zero, one, two, four and six hours after feeding were averaged to determine the overall urea nitrogen values of the calves. The results from the first set of blood samples were 9.03, 6.66, 7.90 and 8.18 mg/100 ml. for diets A, B, C and D respectively. Results from the second set of blood samples were 9.27, 6.68, 7.26 and 9.07 mg/100 ml. for diets A, B, C and D respectively. Although diet did not significantly ($P < 0.05$) influence plasma urea nitrogen, the lowest levels occurred in calves fed diet B, containing 2.29 g. methionine/16 g. N.

The effect of feeding graded levels of methionine in milk substitute diets of preruminant calves at 0, 2 and 4 hours after feeding on plasma free glutamic acid, glycine, alanine, valine and isoleucine is illustrated by Figures 8, 9, 10, 11 and 12 respectively. Plasma free cystine levels were consistently recorded in trace amounts and could not be recorded accurately. Aspartic acid, threonine, serine, proline and leucine were also recorded as trace amounts for many plasma samples and consequently no statistical analysis was done for these amino acids.

Results from plasma samples in set 1 and 2 showed that age influenced ($P < 0.05$) plasma free glutamic acid levels when the calf is at fasting state. Values were 29.87 and 22.98 mg/1. for calves aged 9 - 15 days and 15.14 and 10.65 mg/1. for calves aged 21 - 27

Table 22: THE EFFECT OF GRADED LEVELS OF DIETARY METHIONINE ON PLASMA UREA-NITROGEN (mg/100 ml) IN PRERUMINANT CALVES AT 0, 2 AND 4 HOURS AFTER FEEDING[1]

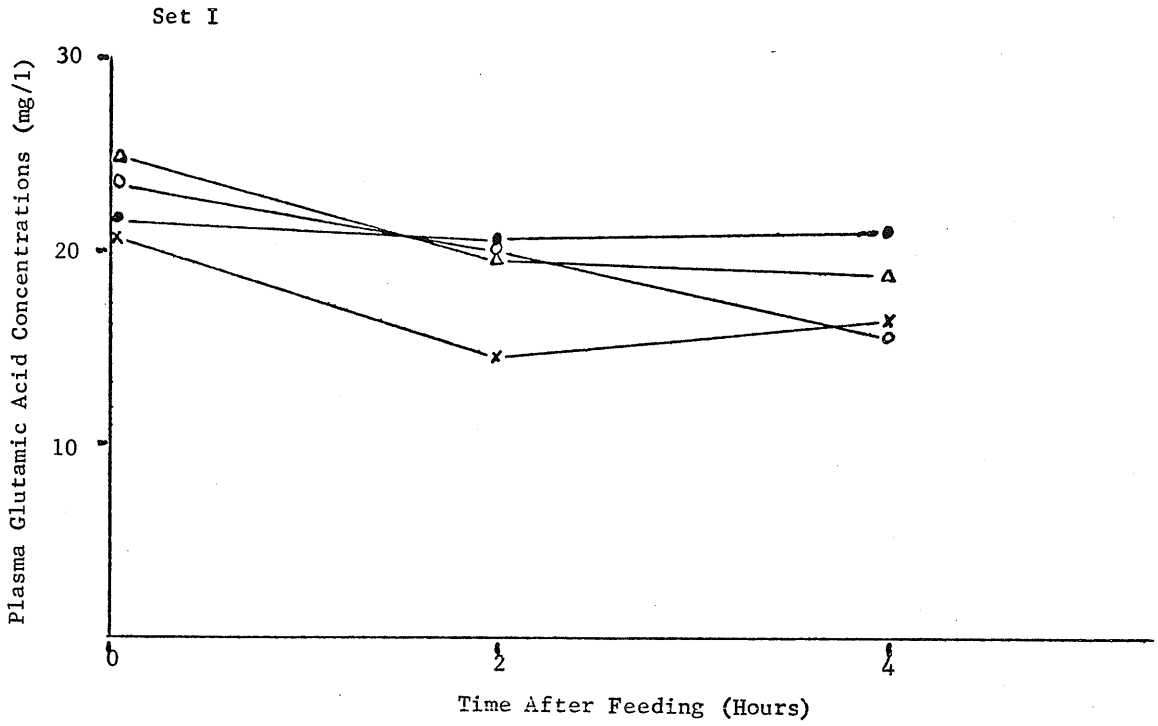
TIME AFTER FEEDING (HRS)	DIET				PERIOD[2]		S.E.
	A	B	C	D	1	2	
Set 1							
0	9.15	6.76	7.02	8.16	7.85	7.69	±.62
2	9.15	6.41	7.79	7.82	7.73	7.86	±.28
4	8.53	7.09	7.94	7.79	7.95	7.91	±.52
Set 2							
0	7.62	7.31	9.16	9.16	7.68	8.94	±.52
2	7.24	7.78	10.93	9.77	7.49[a]	10.37[b]	±.73
4	7.62	7.73	8.96	9.27	7.86	8.95	±.58

[1] All means are adjusted to take into account "within animal" variation.

[2] Means with the same superscript do not differ significantly ($P < 0.05$)

FIGURE 8

The Relationship Between Graded Levels of Dietary Methionine and Plasma Free Glutamic Acid Levels in Preruminant Calves at 0, 2 and 4 Hours After Feeding



A. Δ——Δ 1.70 g. L-meth/16 g. N
B. o——o 1.53 g. L-,.76 D-meth/16 g. N
C. ●——● 2.38 g. L-meth/16 g. N
D. x——x 2.74 g. L-meth/16 g. N

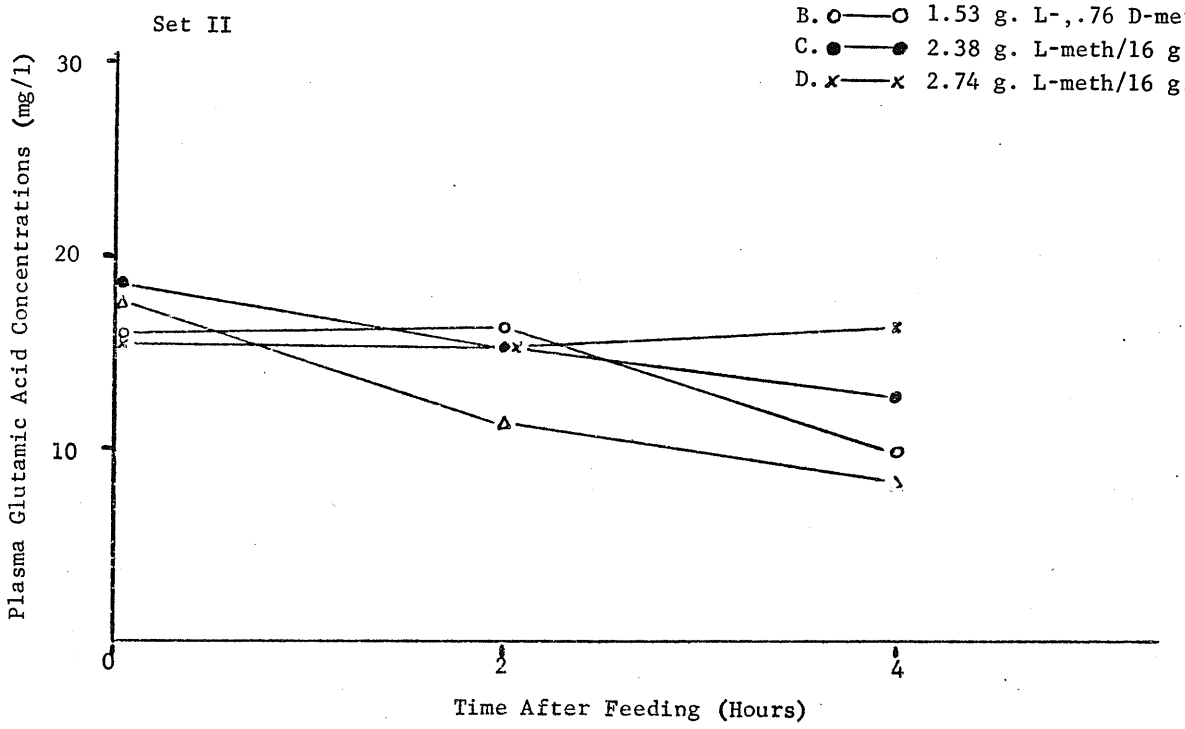
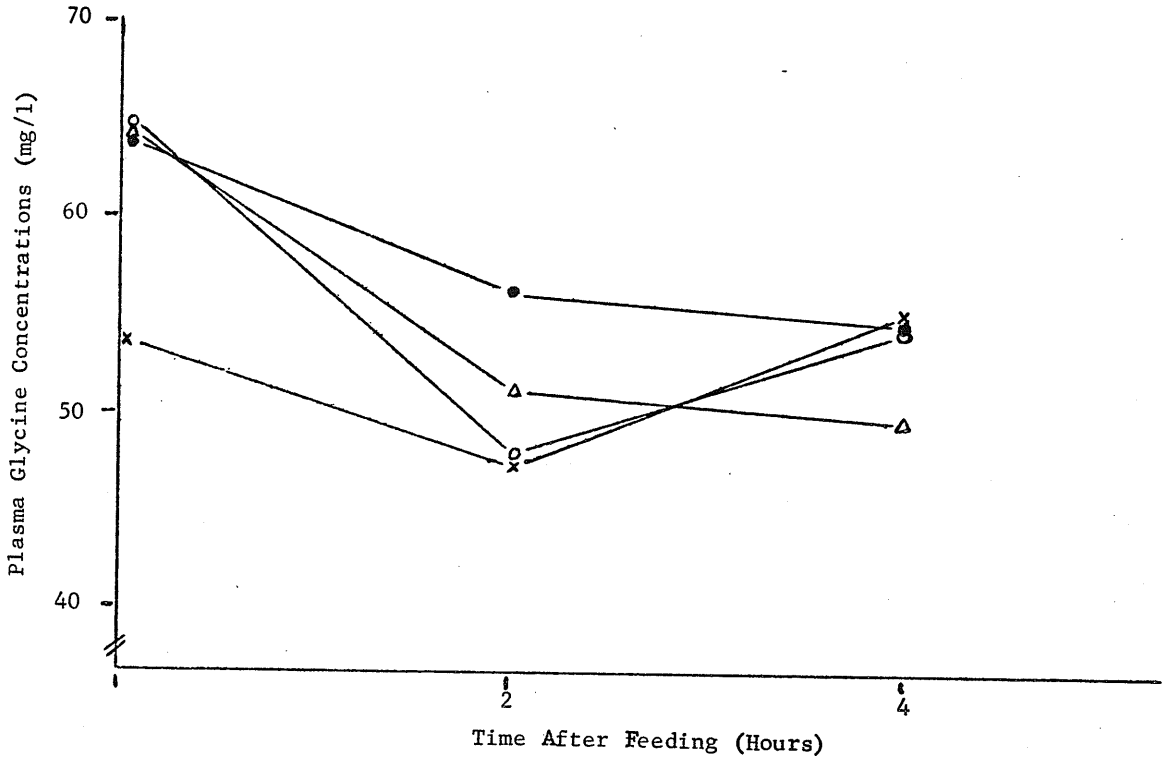


FIGURE 9

The Relationship Between Graded Levels of Dietary Methionine and Plasma Free Glycine Levels in Preruminant Calves at 0, 2 and 4 Hours After Feeding

Set I


70
60
50
40
Plasma Glycine Concentrations (mg/l)
2
4
Time After Feeding (Hours)

Set II

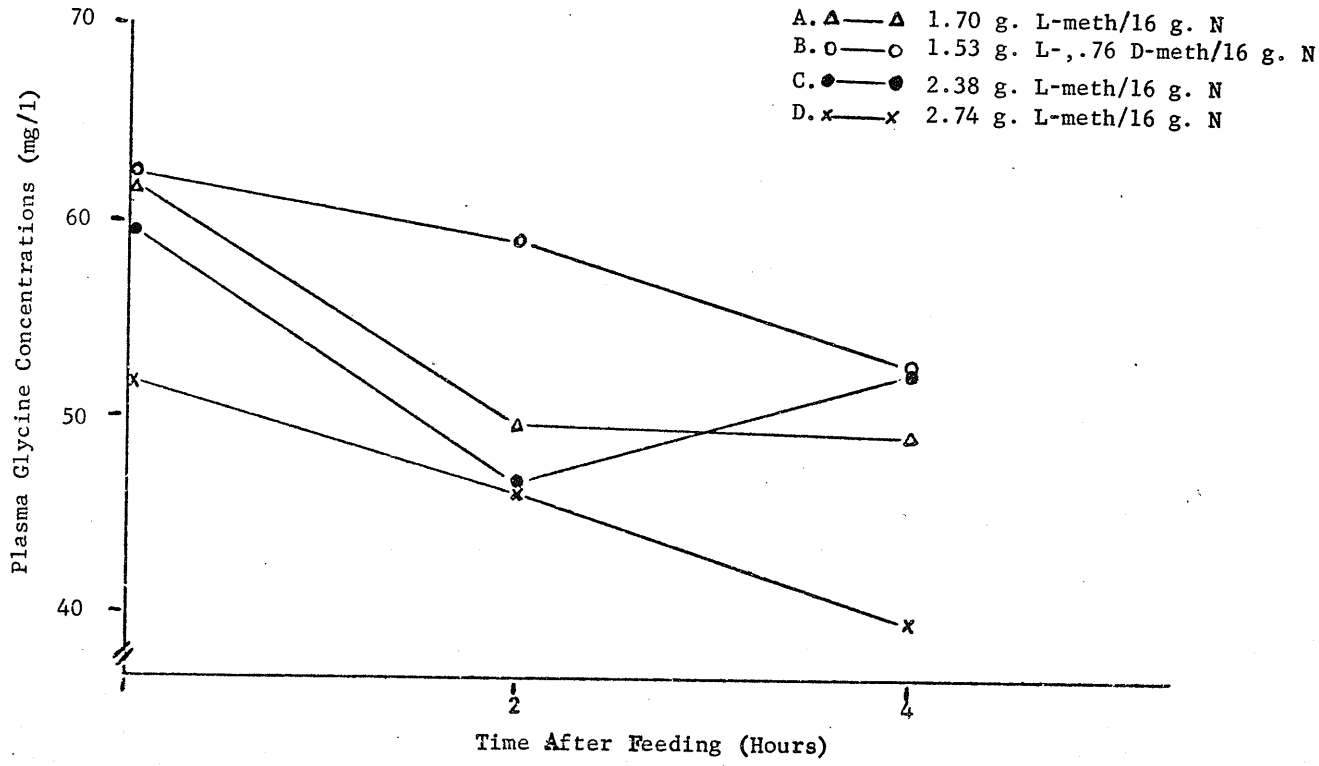
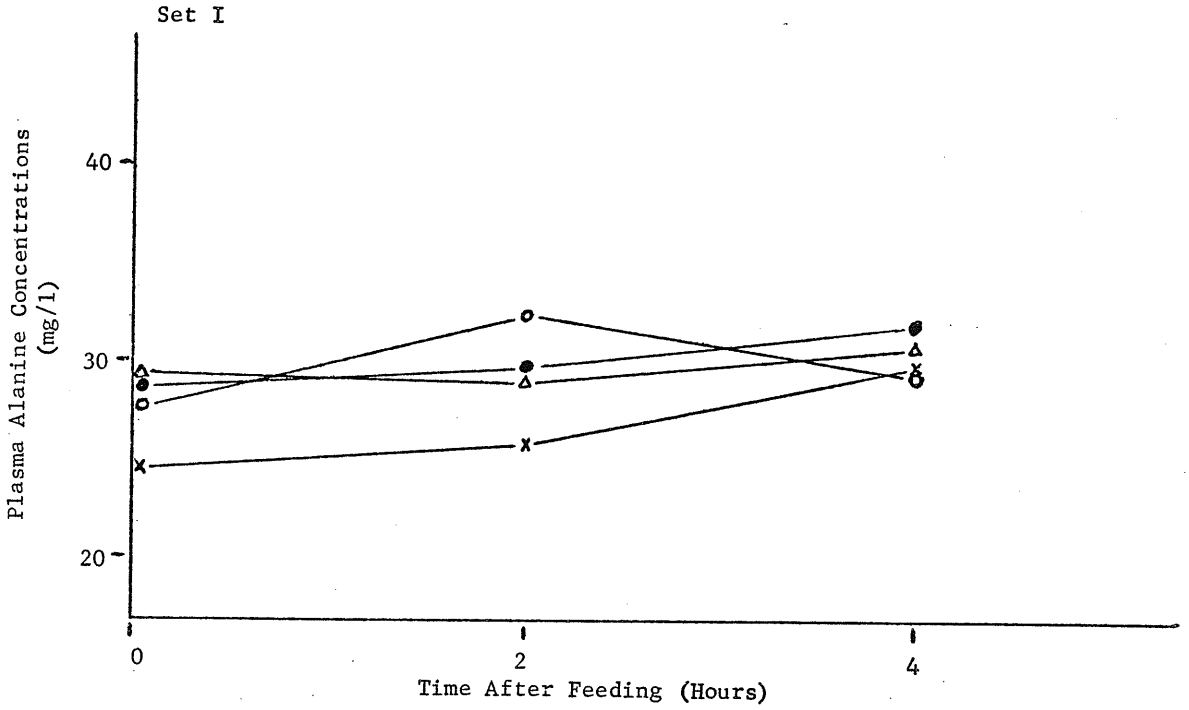
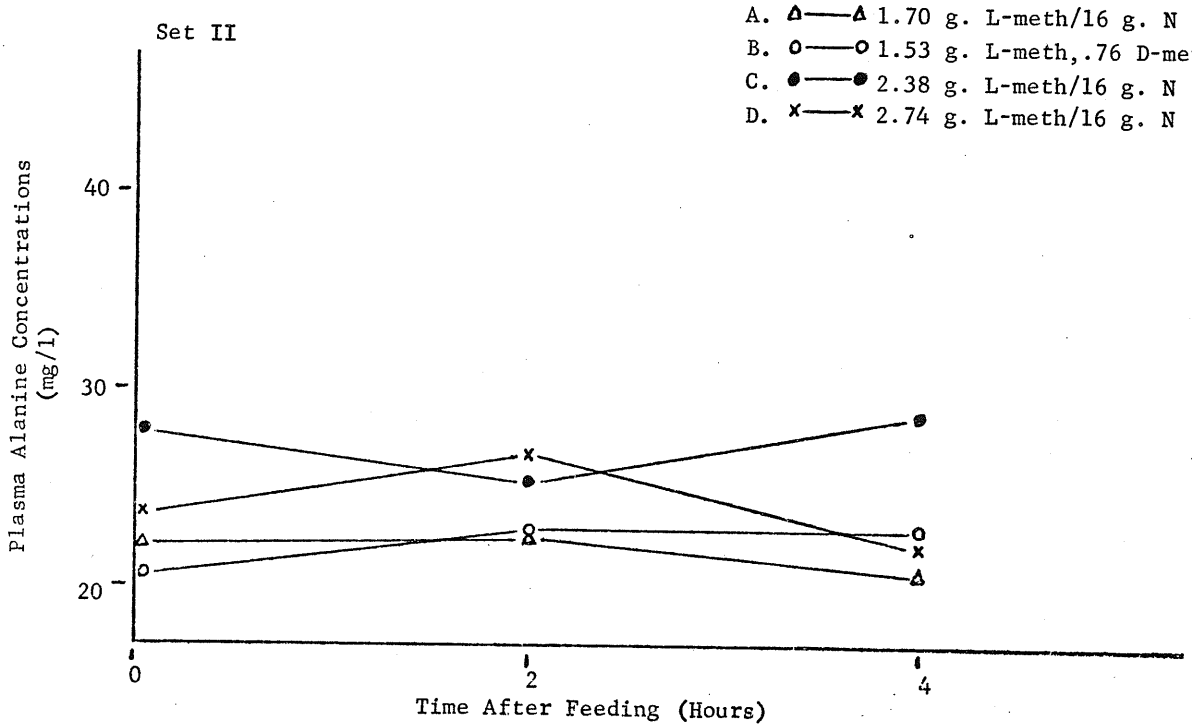

A. Δ—Δ 1.70 g. L-meth/16 g. N
B. o—o 1.53 g. L-,.76 D-meth/16 g. N
C. ●—● 2.38 g. L-meth/16 g. N
D. x—x 2.74 g. L-meth/16 g. N
70
60
50
40
Plasma Glycine Concentrations (mg/l)
2
4
Time After Feeding (Hours)

FIGURE 10

The Relationship Between Graded Levels of Dietary Methionine and Plasma Free Alanine Levels in Preruminant Calves at 0, 2 and 4 Hours After Feeding





A. Δ——Δ 1.70 g. L-meth/16 g. N
B. o——o 1.53 g. L-meth,.76 D-meth/16 g. N
C. ●——● 2.38 g. L-meth/16 g. N
D. x——x 2.74 g. L-meth/16 g. N

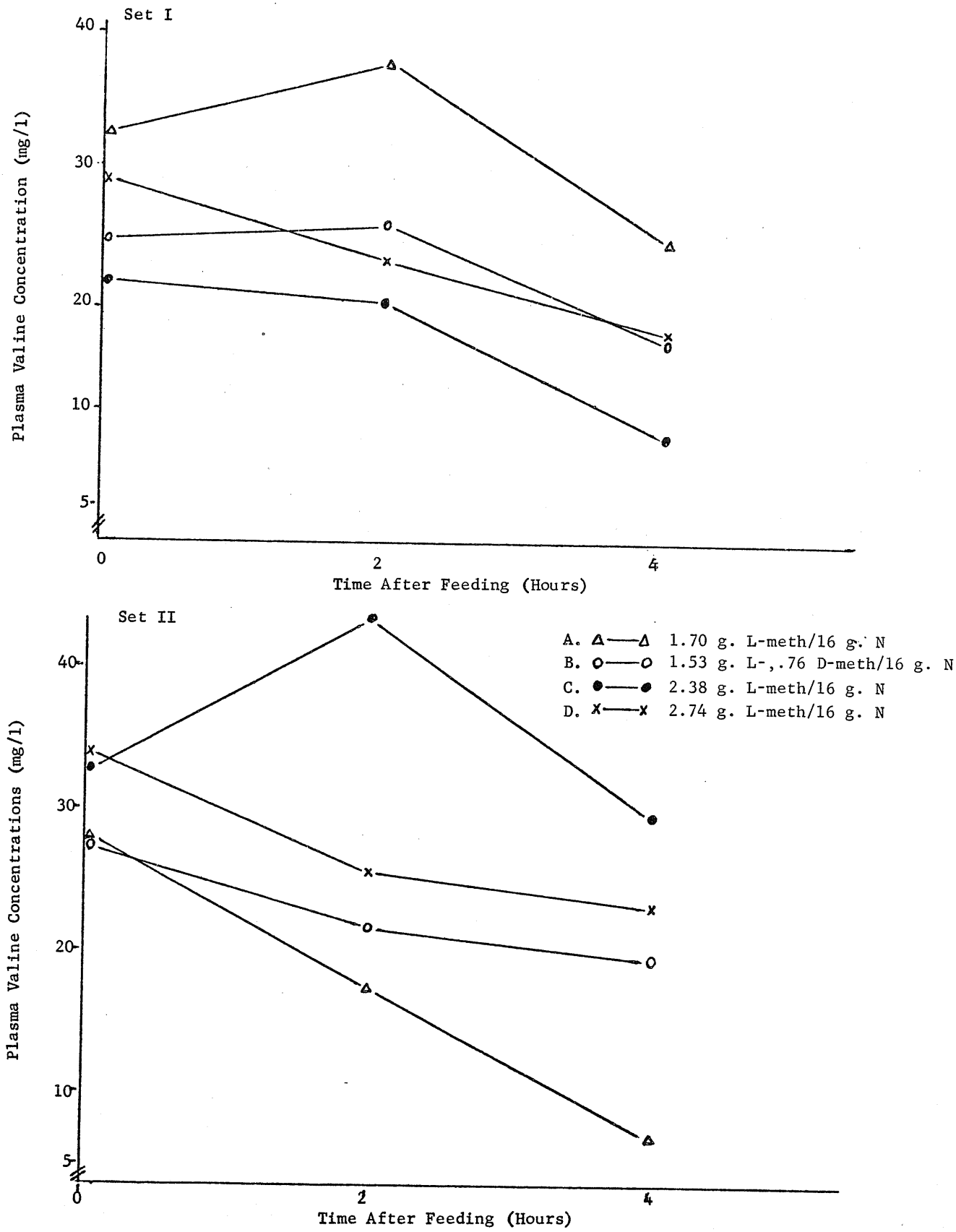
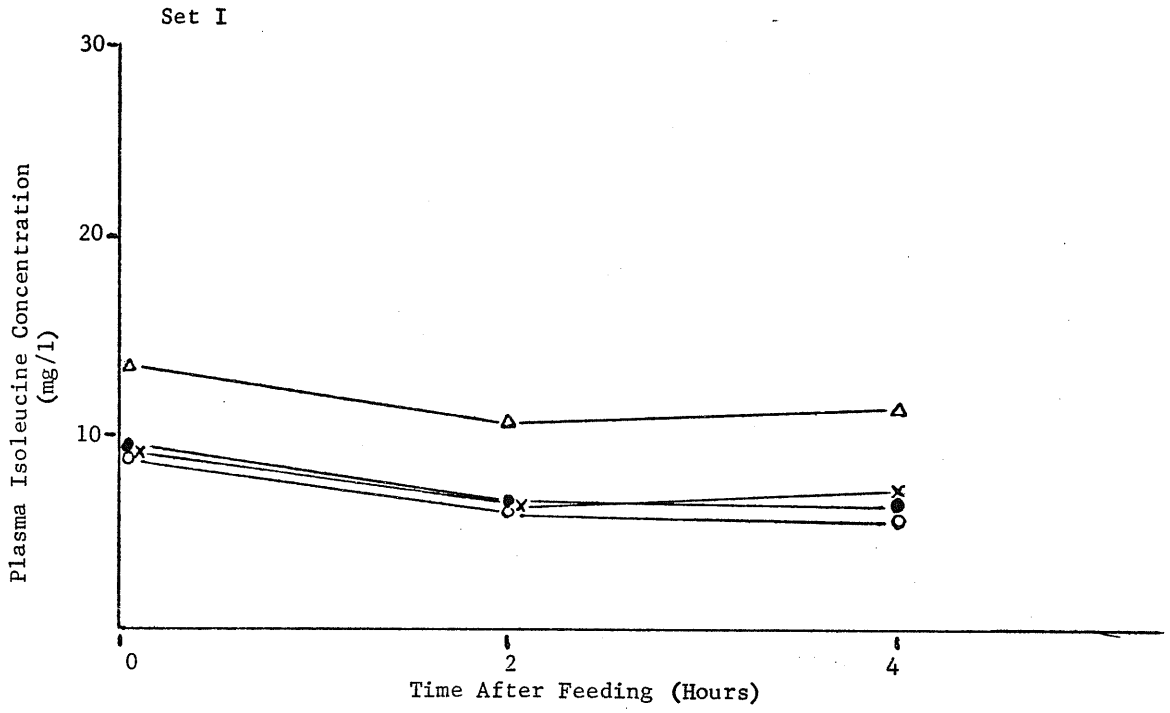
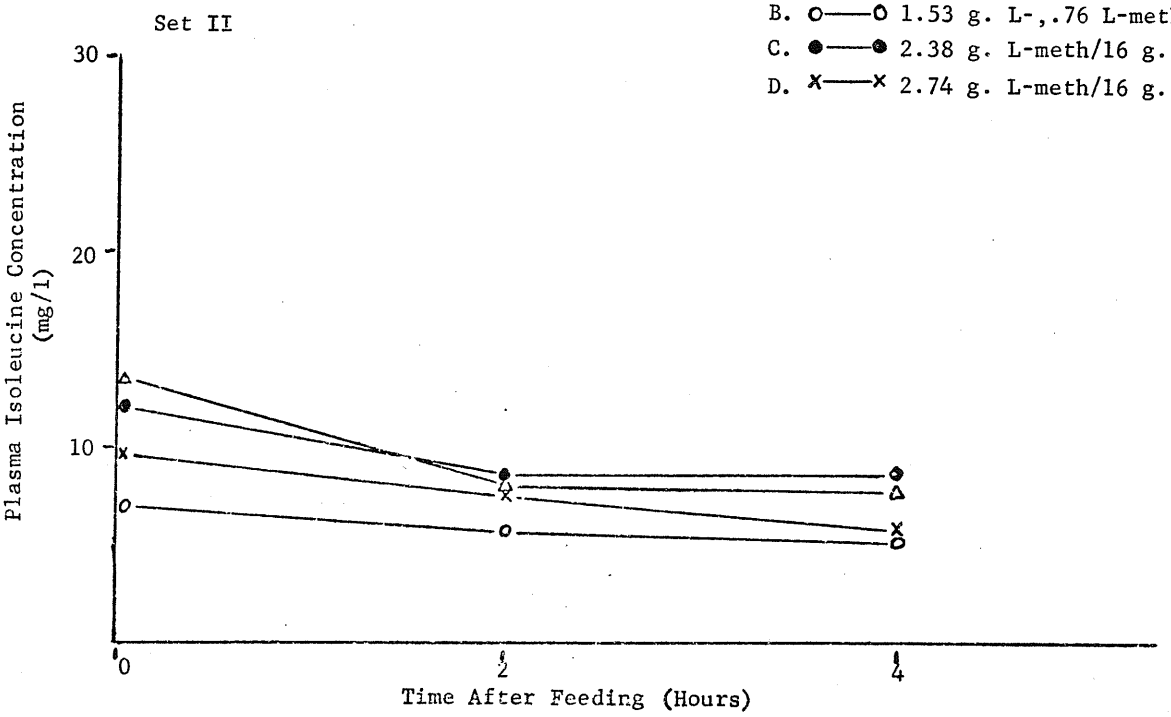

Set I
Plasma Valine Concentration (mg/l)
40
30
20
10
5
0
2
4
Time After Feeding (Hours)
Set II
Plasma Valine Concentrations (mg/l)
A. Δ—Δ 1.70 g. L-meth/16 g. N
B. o—o 1.53 g. L-,.76 D-meth/16 g. N
C. ●—● 2.38 g. L-meth/16 g. N
D. x—x 2.74 g. L-meth/16 g. N
Time After Feeding (Hours)

FIGURE 11

The Relationship Between Graded Levels of Dietary Methionine and Plasma Free Valine Levels in Preruminant Calves at 0, 2 and 4 Hours After Feeding



A. Δ——Δ 1.70 g. L-meth/16 g. N
B. o——o 1.53 g. L-,.76 L-meth/16 g. N
C. ●——● 2.38 g. L-meth/16 g. N
D. x——x 2.74 g. L-meth/16 g. N



days for set 1 and 2 respectively. Plasma free glutamic acid levels decreased with age of the calf (Table 23). Diet did not influence the fasting levels of plasma free glutamic acid in either set of samples. (Fasting levels for diets A, B, C and D were 24.84, 23.45, 21.52 and 20.22 mg/1. for set 1 and 17.39, 15.74, 18.12 and 15.47 for set 2 respectively.) Plasma free glutamic acid levels decreased with time after feeding (Fig. 8). In set 1, diet did influence ($P<0.05$) the change of plasma levels from zero to four hours after feeding (Table 25).

Plasma free glycine levels at fasting for diets A. B. C and D were 64.56, 64.88, 64.02 and 53.41 mg/1. for set 1 and 61.66, 62.33, 59.51 and 51.72 mg/1. for set 2 respectively. Plasma free glycine levels also decreased with time after feeding (Fig. 9). Fasting levels were not influenced ($P<0.05$) by diet or age for samples analysed from sets 1 and 2.

Diet and age did not influence the change in plasma free glycine levels at two hours after feeding, however, for samples taken four hours postfeeding in set 1, diet did influence ($P<0.01$) the decrease. Calves fed diets A, B and C had a greater decrease ($P<0.01$) in plasma free glycine than calves fed diet D (Table 23). A similar pattern was not observed for samples from set 2.

Fasting levels of plasma free alanine decreased ($P<0.05$) with age. Calves 9 - 15 days of age had levels of 36.33 and 31.70 mg/1. as compared to levels of 18.91 and 15.58 mg/1. for calves 21 - 27 days of age for sets 1 and 2 respectively (Table 23). Methionine content in the diet did not influence fasting levels.

FIGURE 12

The Relationship Between Graded Levels of Dietary Methionine and Plasma Free Isoleucine Levels in Preruminant Calves at 0, 2 and 4 Hours After Feeding

Table 23: THE EFFECT OF GRADED LEVELS OF DIETARY METHIONINE ON CHANGES IN PLASMA FREE GLUTAMIC ACID, GLYCINE, ALANINE, VALINE AND ISOLEUCINE IN PRERUMINANT CALVES[1]

AMINO ACID	TIME PERIOD	A	B	C	D	PERIOD 1	PERIOD 2	S.E.
Glutamic Acid (set 1)	0-2	- 5.42	- 3.58	- 1.45	- 6.12	- 2.83	- 5.38	±1.18
	0-4	- 6.46^{BC}	- 8.38^{C}	- .81^{A}	- 4.20^{B}	- 5.03	- 4.89	± .83
Glutamic Acid (set 2)	0-2	- 6.29	.29	- 3.10	.56	- 2.40	- 1.89	±1.27
	0-4	- 9.31	- 5.67	- 6.14	- .04	- 8.91	- 2.66	±1.48
Glycine (set 1)	0-2	-13.34	- 7.07	- 8.91	- 5.28	- 6.06	-10.23	±1.47
	0-4	-14.88^{B}	-10.61^{B}	- 9.64^{B}	1.58^{A}	- 6.55	-10.22	±2.65
Glycine (set 2)	0-2	-11.85	- 3.35	-12.72	- 8.40	- 7.28	- 9.42	±0.95
	0-4	-12.63	-10.01	- 7.35	-11.99	- 8.85	-12.15	±2.74
Alanine (set 1)	0-2	.07	5.47	.76	.88	3.73^{a}	- .13^{b}	±0.93
	0-4	1.56	1.61	3.05	4.84	2.02	3.51	±1.82
Alanine (set 2)	0-2	.13	1.33	- 2.64	3.05	- 1.32	2.25	±2.01
	0-4	- 1.89	2.07	.70	- 1.79	- 1.90	1.44	±1.61
Valine (set 1)	0-2	4.54	- 1.62	1.22	- 6.50	- 4.08	2.87	±2.38
	0-4	- 7.82	- 7.20	-10.85	- 9.64	-10.16^{a}	- 6.57^{b}	±3.82
Valine (set 2)	0-2	-10.84	- 5.79	10.64	- 8.61	- 4.97	- 2.32	±4.61
	0-4	-21.07	- 8.15	- 3.46	-10.98	-11.50	-10.34	±4.87
Isoleucine (set 1)	0-2	- 2.60	- 2.86	- 2.74	- 2.98	- 1.80^{a}	- 3.53^{b}	± .41
	0-4	- 2.01	- 3.38	- 2.82	- 2.18	- 2.55	- 2.65	± .57
Isoleucine (set 2)	0-2	- 4.12	- 1.23	- 3.50	- 2.19	- 3.11	- 2.45	
	0-4	= 5.40	- 1.82	- 3.52	- 4.10	- 4.31	- 3.11	± .60

[1] Means with different superscripts are significantly different ($P < 0.05$).

Results from the first set of samples taken at two hours after feeding indicated ($P < 0.05$) a greater increase in plasma free alanine levels for the younger calves. A similar trend, although not significant was observed for the second set of blood samples taken. Diet and age did not influence changes in plasma free alanine levels from zero to four hours postfeeding. Relative to other amino acids studied, levels of plasma free alanine showed little change with time after feeding.

Plasma free valine levels dropped with time after feeding (Fig. 11) although, in a few instances levels did increase up to two hours postfeeding before dropping. Fasting levels for set 1 indicate that plasma free valine levels decrease ($P < 0.05$) with age of the calf (Table 23). Also, calves aged 9 - 15 days had greater decreases ($P < 0.05$) in plasma free valine levels by four hours postfeeding as compared to older calves (Table 23). A similar trend although not significant was observed for the second set of samples.

Plasma free isoleucine levels did not differ due to diet when the calves were at fasting state or after calves were fed (Fig. 12). Plasma free isoleucine levels at fasting state decreased ($P < 0.05$) with the age of the calf for samples taken from set 1 (Table 23). Older calves showed greater decreases ($P < 0.05$) in plasma levels by two hours after the meal than did calves aged 9 - 15 days. A similar trend, although not significant was observed from samples from set 2.

Additional tests were conducted to determine whether metabolism of the D-isomer of methionine was similar to that of the L-

isomer in preruminant calves. Urine urea content was not influenced by diet or age of the calf. Adjusted means were 100.29, 89.52, 95.09 and 102.79 mg/100 ml. for diets A, B, C and D respectively.

Average values for urine methionine content for calves fed diets B and C were 6.71 and 5.09 mg/1. (Table 24). Values for calves fed diet B containing both the D and L isomers of methionine were consistent with one exception. Calf 3 during period 2 had urine methionine levels approximately six times greater (21.84 mg/1.) than values for the other five calves fed the diet. As data were not collected for urine methionine content for diets A and D, no statistical analysis could be performed.

Table 24: URINE METHIONINE CONTENT (mg/1.) OF CALVES FED DIETS B AND C IN CALF STUDY B.

DIET	B	C
L-methionine content (g/16 g. Nitrogen)	1.53	2.38
D-methionine content (g/16 g. Nitrogen)	.76	--

	Calf	Period	Urine Meth.(mg/1)	Calf	Period	Urine Meth.(mg/1)
	4	1	3.71	7	1	9.64
	5	1	3.59	9	1	4.30
	10	1	3.88	11	1	6.55
	3	2	21.84	8	2	1.43
	6	2	3.61	10	2	5.31
	9	2	3.61	12	2	3.32
Average			6.71			5.09

DISCUSSION

Fababean protein may be incorporated into the diet in three forms: as a flour containing 25 to 28% crude protein, as a protein concentrate containing 60 to 70% crude protein or as a protein isolate containing approximately 91% crude protein. Fababean protein concentrate (F.B.P.C.) is the most favorable form to be incorporated into a milk substitute diet because it has a relatively high crude protein content and low processing costs (pin milling and air classification). Fababean flour, processed from the whole bean, has a fiber content of 7.4 to 8.1% on an air dry basis (Marquardt et al., 1974). Using the flour in milk substitute diets would result in fiber content above recommended levels for the preruminant calf according to the Federal Feeds Act of 1960. Also, the starch content (54 to 58%) in fababean flour made from whole or dehulled beans could be detrimental as the preruminant calf is unable to digest starch (Burt and Irvine, 1970; Shaw et al., 1918).

QUALITY OF FABABEAN PROTEIN

In the preliminary study conducted with rats, it was demonstrated that a protein concentrate prepared from fababeans (Vicia Faba L var minor) compared favorably with soybean protein concentrate and casein as a protein source for growing rats. Both vegetable protein sources were supplemented with D,L-methionine. These results

are in agreement with those of Sarwar et al. (1975) who showed the protein efficiency ratios (P.E.R.) to be 2.11 and 2.43 for autoclaved fababean flour and autoclaved soybean flour. Both proteins were supplemented with 0.2% L-methionine. P.E.R. for the control casein diet was 3.10. Similarily, results of a 28 day P.E.R. assay on rats by Duthie et al. (1972) had P.E.R. values for fababean protein isolate plus 0.25% D,L-methionine as 3.08 as compared to 2.77 for soybean protein isolate plus 0.25% D,L-methionine and 3.06 for casein.

In this study, autoclaving the F.B.P.C. at 120°C for 30 minutes did not significantly improve P.E.R. or average daily weight gains of growing rats. Also, methionine supplementation did not influence the effect of autoclaving on P.E.R. values. There was, however, a significant improvement in P.E.R. values due to methionine supplementation of F.B.P.C.

Results of trials conducted to determine the effect of autoclaving on growth performance of experimental animals have been contradictory. MacDonald (1974) found no effect due to autoclaving (115°C for 12 or 15 min.) on the growth performance of young rats fed diets in which fababean flour was the sole protein source. The dehulled beans were ground to produce the flour. In a study conducted on growing pigs fed diets containing 9 to 29% F.B.P.C., Maltman (1976) found no differences in growth performance between the raw and autoclaved product. In comparison, two studies conducted by Wilson and McNabb (1971, 1972) demonstrated a beneficial effect on live weight gain and feed conversion efficiency due to autoclaving of fababeans prior to feeding to chicks at levels up to 95% of the diet. Supplementation of methionine resulted in increased responses

due to autoclaving. Hulls were not removed from these beans before being ground. Wilson and McNabb (1971) and Marquardt and Campbell (1974) suggested that autoclaving may increase the availability of fababean amino acids in general and methionine in particular.

Marquardt and Campbell (1973) reported improved feed efficiency and reduction in pancreas size in chicks when fababeans were autoclaved at 121°C for 15 minutes. Further work by Marquardt et al. (1974) with chicks showed significant improvements in weight gains and feed:gain rations: the response in rats being less dramatic than that of chicks. They also suggested that autoclaving of fababeans was most effective in improving efficiency of feed utilization and growth rate of chicks when the level of sulfur amino acids is limiting (Marquardt and Campbell, 1974). The diets prepared in these studies contained whole fababean flour.

Differences between authors in the results may be attributed to several factors. Firstly, there may be species differences which may be due to differences in the physiology of digestion. As indicated by Marquardt et al. (1974), growing rats appear to be less sensitive to the antinutritional factors than chicks. Secondly, trials conducted using flour from whole beans versus dehulled beans may have been different due to the hull constituents. Studies by Marquardt et al. (1976) have shown that a growth depressing factor is concentrated in the hull. This was later isolated and identified as condensed tannins (Marquardt et al., 1977). They found that the addition of condensed tannins to a chick diet decreased the retention of dry matter, protein (N x 6.25), amino acids and crude fiber.

The improved protein quality of F.B.P.C. with supplementation of methionine is consistent with results reported in the literature (Duthie et al., 1972; Wilson and McNabb, 1971; Sarwar et al., 1975) (Table 25). The feed intake levels and weight gains of rats fed unsupplemented raw and autoclaved F.B.P.C. were lower ($P < 0.05$) than for rats fed the supplemented diets. This is probably due to the low level of the first limiting amino acid, methionine, in diets B and D. Dietary methionine supplied only 14 to 16% of the rat's methionine requirements (Table 5). Reduced feed intake by rats fed these two diets were noticed within the first week of the study. This suggests, according to the definitions by Harper (Harper and Rogers, 1965), that poor growth of rats fed diets B and D was due to a deficiency or an imbalance of the essential amino acid, methionine. Poor utilization of the protein supplied by these diets may be due to low availability of methionine and cystine as well as low levels of these amino acids in the diet (Sarwar et al., 1977).

The amino acid composition of fababean protein concentrate used for calf studies A and B was similar to that reported by Marquardt and Campbell (1974) and Clarke (1970) (Table 26). The total sulfur amino acid content of the fababean protein analyzed was 2.2, 1.57, 1.83 and 1.56 g/16 g. nitrogen for Marquardt and Campbell, Clarke, calf study A and calf study B respectively. The amino acid composition of fababean protein was compared with results of an amino acid analyses conducted on soybean meal (44% crude protein) by Maltman (1975). Soybean protein was a superior source of methionine as compared with fababean protein and cystine levels were comparable for the two proteins. Lysine content was lower in soybean protein than in fababean protein.

Table 25: A COMPARISON OF PROTEIN EFFICIENCY RATIOS (P.E.R.) OF FABABEAN PROTEIN IN FABABEAN FLOUR, FABABEAN PROTEIN CONCENTRATE AND FABABEAN PROTEIN ISOLATE

SOURCE	PROTEIN FORM	P.E.R. VALUE		
		CONTROL PROTEIN (Casein)	NO METHIONINE SUPPLEMENTATION	METHIONINE SUPPLEMENTATION
Sarwar et al., 1975	Whole Fababean Flour	2.50	.52	2.11
This study	Protein Concentrate	2.87	.62-.65	2.70-2.82
Duthie et al., 1975	Protein Isolate	3.06	.99	3.08

Table 26: AMINO ACID PROFILES OF FABABEAN PROTEIN AND SOYBEAN PROTEIN[1]

AMINO ACID SOURCE	FABABEAN				SOYBEAN
REFERENCE	MARQUARDT & CAMPBELL 1974	CLARKE 1970	CALF STUDY A	CALF STUDY B	MALTMAN 1976
Essential					
Arg.	8.8	10.30	10.25	9.51	6.81
His.	2.6	2.55	2.78	2.40	2.48
Ile.	4.6	4.35	4.46	4.51	4.52
Leu.	7.8	7.87	8.04	7.68	7.62
Lys.	6.8	6.59	6.81	6.30	5.86
Met.	0.9	0.73	0.78	0.56	1.38
Phe.	4.6	4.63	4.57	4.37	4.95
Thr.	4.1	4.02	3.52	3.25	3.67
Val.	5.4	4.92	5.19	4.82	4.86
Non-Essential					
Ala.	4.6	4.20	4.22	3.99	4.24
Asp.	11.0	11.88	11.67	10.59	11.24
Cys.	1.3	0.84	1.05	1.00	1.30
Glu.	18.0	19.68	17.98	16.30	17.14
Gly.	4.8	5.57	4.25	3.89	4.10
Pro.	4.7	-	4.27	4.10	5.00
Ser.	5.5	5.48	4.50	4.13	4.71
Tyr.	3.0	3.86	3.13	2.74	2.67

[1] Values are reported as g/16 g. N.

Average values of amino acid composition of F.B.P.C. used in calf studies A and B were compared to the amino acid profile of whole bovine milk (Block and Weiss, 1956) (Table 27). Clearly, the main amino acid deficiency of fababean protein is the methionine content, equivalent to 28% of that found in milk protein. Second limiting amino acids are likely tryptophan and isoleucine. Threonine was also lower in fababean protein than in milk.

CALF STUDY A

AVERAGE DAILY GAIN: Calves were fed 9 g. crude protein/kg. $W^{0.75}$/day which is approximately equivalent to the requirements for 0.5 kg. of daily growth. Average daily gain (A.D.G.) of the calves was lower than calculated, although differences due to level of F.B.P.C. in the diet did not influence growth. This may be due to several factors. (1) Calves were kept under suboptimal management conditions required for collections to obtain digestibility data. Calf health may also have been a contributing factor as calves afflicted with viral pneumonia lost weight during periods of illness. (2) Although calves received 9 g. crude protein/kg. $W^{0.75}$/day, the actual intake of digestible protein was lower. Apparent crude protein digestibility of F.B.P.C. was estimated to be 56.8, 65.1 and 80.0% for calves aged 8-13, 15-20 and 21-27 days respectively (Figure 3).

DIGESTIBILITY VERSUS AGE: Digestibilities of dry matter, ether extract and crude protein improved with increased age of the calf. This is related to the changes in the kinds and amounts of digestive enzymes present in the calf. Improved fat digestibility is likely

Table 27: AMINO ACID COMPOSITION OF FABABEAN PROTEIN AND BOVINE MILK[1]

AMINO ACID	FABABEAN	MILK[1]	FABABEAN AS % OF MILK
His	2.59	2.7	94.8
Ile	4.49	6.5	69.1
Leu	7.86	9.9	79.4
Lys	6.56	8.0	82.0
Met (+ Cys)	.67 (1.80)	2.4 (3.2)	27.9 (53.1)
Phe (+ Tyr)	4.47 (7.21)	5.1 (10.0)	87.6 (72.0)
Thr	3.39	4.7	72.0
Val	5.03	6.7	75.1
Trp	80[2]	1.3	61.5

[1] Block and Weiss, 1956

[2] Estimated from the Literature

related to the marked increase in pancreatic lipase activity which is characteristic of calves 20 days of age (Huber et al., 1961).

Protein digestibility values of diets A and B were very close to values observed by Latrille et al. (1975) for calves in the three age groups. This trend of increased digestibility appears to be more dramatic for vegetable proteins than for milk proteins. Studies conducted on soybean protein with neonatal calves indicate that protein digestibility was low and variable during the first two weeks of age and there is a trend to improve with age (Nitzan et al., 1971, 1972; Erbersdobler and Gropp, 1973; Ramsey and Willard, 1975).

FAT DIGESTIBILITY: Fat content in feces of calves fed diet A was approximately twice as great as was observed for calves fed a similar diet by Latrille et al. (1975). Results for calves fed diets in which 50% of the total protein was supplied by F.B.P.C. were similar for both studies.

Steatorrhea or high levels of fecal fat was observed for some calves (APPENDIX Table 3). The production of large amounts of fecal fat is considered to be a reflection of malabsorption associated with disturbances of protein or carbohydrate digestion rather than a primary failure of fat absorption (Roy, 1974). This explanation was accepted by Latrille et al. (1975) to account for the cases of diarrhoea observed. However, the high levels of fecal fat for calves fed the control (casein) diet in calf study A cannot be explained by this theory. High levels of fecal fat observed in this study are probably due to the type of fat used or the mode of incorporation of the fat into the diet because the fecal fat levels were similar for all four diets.

Malabsorption of fat may result in the formation of calcium and magnesium soaps, rendering these minerals unavailable to the calf (Roy, 1974). No symptoms of mineral deficiencies were observed for calves in calf study A.

The majority of calves fed diets in which F.B.P.C. supplied greater than 50% of the total protein had digestive upsets after they were introduced to their diets. This was followed by diarrhoea after which normal excretory patterns were observed. Diets fed to calves in study B resulted in similar responses. Smith et al. (1970), Sambeth et al. (1967) and Smith and Wynn (1971) reported that calves introduced to a liquid diet containing soyflour experienced gastric stasis, followed by rapid passage through the intestine and diarrhoea. Conclusions derived from later trials by Smith and Sissons (1973) led to the hypothesis that these disturbances may be related to a gastro-intestinal allergy reaction. This hypothesis is supported by data showing dramatic increases in mean titres for circulating antibodies after preruminant calves were fed soybean meal or protein isolate for four weeks (Smith et al., 1970). In addition to the general immune responses there may be a more immediate tissue immunity response in the small intestine.

Another hypothesis is that digestive disturbances develop in calves that are not able to digest the diet. This may result in excessive proliferation of certain micro-organisms which may cause flattening of the villi. The production of antibodies may follow the invasion of partially digested protein through the damaged mucosa (Roy, 1974). The allergic reaction results in decreased transit time through the small intestine, abnormal water and salt exchange

in the small intestine and decreased nitrogen absorption up to the ileum (Smith and Sissons, 1974).

PROTEIN DIGESTIBILITY: Digestibility of F.B.P.C. was estimated to be 56.8, 65.1 and 80.0% for calves aged 8-13, 15-20 and 22-27 days respectively. Apparent protein digestibility of pea protein concentrate was 25% for calves under two weeks of age (Bell et al., 1974). Results of these two studies suggest that F.B.P.C. is a superior protein source for milk substitute diets as compared to pea protein concentrate.

Numerous studies have been conducted to determine the ability of the young calf to utilize soybean protein. For liquid diets containing soybean flour fed to calves under three weeks of age, the digestibility of the soybean protein ranged from 11 to 55% (Gorrill and Nicholson, 1969; Kalkade et al., 1976; Colvin and Ramsey, 1968; Nitzan et al., 1971). Gorrill and Nicholson (1969) reported that the nitrogen digestibility of the soy protein in a liquid diet, in which 70% of the total protein was supplied by soybean protein concentrate, was 79%. The data were collected from calves one to seven weeks of age. Porter and Hill (1973) reported 75 to 87% apparent nitrogen digestibility for isolated soybean protein when fed to calves one to two and four to five weeks of age respectively. Work completed with soybean protein indicates that use of refined protein sources results in improved apparent protein digestibility. A comparison of these studies with those of Latrille et al. (1975) and calf study A suggests that F.B.P.C. is comparable to soybean protein concentrate and superior to soybean flour.

Relatively little work has been conducted on plant protein utilization by neonatal calves (under two weeks of age), the majority of this work having been done with soybean protein. One of the major differences between milk proteins and vegetable proteins in the diet of preruminant calves is the rate of release of dietary nitrogen compounds from the abomasum. Calves fed diets prepared from milk proteins have a slow, steady release of nitrogen over a nine hour period after the feed. Smith and Sissons (1975) observed that the nitrogen hold up for soybean containing feeds was slight for the soybean flour but extremely marked for the protein isolate. The latter hold up was followed by several hours of rapid outflow of nitrogen from the small intestine. Fababean protein, like soybean protein, does not clot in the abomasum. The small intestine is probably overloaded at some time after the feed and as a result the protein is not properly digested.

CALF STUDY B

The sulfur amino acid studies conducted with the preruminant calves were similar to those conducted by Foldager *et al*. (1975). Protein sources used in the latter studies were milk proteins and crystalline L-amino acids (18.08% of total protein). The diet contained 17% crude protein on an air dry basis. The experimental diets formulated for calf study B were similar (with the exception of level of dietary methionine) to diet A in calf study A and diet 80 (80% of total protein was supplied F.B.P.C.) in the trial conducted by Latrille *et al*. (1975).

AVERAGE DAILY GAIN: Average daily gain (A.D.G.) of animals fed the four experimental diets were lower than A.D.G. of calves fed diet A in calf study A and diet 80 in trial conducted by Latrille et al. (1975). Differences in feed intake were taken into account. This was partially attributed to the stresses to which the calves were exposed while on the metabolism crates. The calves had problems when attempting to lie down or stand up due to slippery flooring in these crates.

In this study varying levels of dietary methionine were compared. The results showed that there were no effects due to level of dietary methionine on average daily gain. This is comparable with results of Foldager et al. (1975), who reported decreased A.D.G. when calves were fed diets containing greater than 4.33 g. methionine per 16 g. nitrogen. Levels this great were not fed to calves in calf study B.

DRY MATTER AND CRUDE PROTEIN DIGESTIBILITY: Results from the current experiment showed that apparent dry matter digestibility of the four diets was similar to values reported for diet A in the previous study. Apparent crude protein digestibility, however, was approximately 10 to 20% lower than values reported for diet A, calf study A. Reduced protein digestibility may also account for the lower A.D.G.

Statistical analysis indicated that dry matter and crude protein digestibilities were not influenced by level of dietary methionine or age of the calf. This is in contradiction with results from previous studies (Latrille et al., 1975; Radostits and Bell, 1968; Porter, 1969; Roy, 1970) or calf study A which showed an increase in both dry matter and crude protein digestibility with age

of the calf. This was shown for calves fed both milk proteins and vegetable proteins. No explanation was found for these results as digestibility tests do not give any direct information about the various processes taking place in the whole digestive system, namely clotting of proteins in the milk substitute, protein degradation in the small intestine or bacterial fermentation processes in the large intestine. High levels of fecal crude protein may be from endogenous sources which include sloughed cells of the intestinal mucosa lining, digestive enzymes and bacterial proteins.

One hypothesis for the reduced protein digestibility may be the development of a gastro-intestinal allergy as described earlier. This may have been aggravated by the stress placed on calves while on the metabolism crates and blood collections. Other metabolic disturbances and diarrhoea may have resulted from such an incompatibility reaction.

A second explanation may be that the tallow was not properly incorporated into the diet. Roy (1970) concluded from a review of the literature, that unless correctly emulsified and homogenized, dietary fat tends to cause diarrhoea. Poor quality fats incorporated into milk substitute diets results in reduced nitrogen retention (Blaxter and Wood, 1951; Raven and Robinson, 1964). Low quality dietary dietary fat can reduce nitrogen utilization in two manners. (1) Fats that are poorly digested may result in reduced protein digestibility (Bell, 1970). (2) Fat is a major energy source in a milk replacer and in event of fat malabsorption the level of energy received by the calf may be too low for optimal nitrogen utilization. If the available energy in the four experimental diets was low, it may explain the negative nitrogen utilization.

NITROGEN OUTPUT: Results of this study indicate that urinary nitrogen output was affected by the level of dietary methionine in the diet. Calves fed diet C (2.38 g. L-meth/16 g. nitrogen) had the lowest urinary nitrogen output, the greatest amounts being excreted by calves fed diet A. Diet B (1.53 g. L- & 0.76 g. D-meth/16 g. nitrogen) and diet D (2.74 g. meth/16 g. nitrogen) had intermediary levels of nitrogen output. These differences were not reflected in data of urinary urea concentrations. Average values for total urinary urea output were 105, 87, 108 and 93 g/period for diets A. B. C and D respectively.

PLASMA UREA LEVELS: Results from this study indicate that there was little change in plasma urea levels with time after feeding. This does not agree with results by Patureau-Mirand et al. (1971), Nitzan et al. (1971) and Williams and Smith (1975), which showed decreases in plasma urea nitrogen with time after feeding. In all three studies, the calves were fed milk proteins. Nitzan et al. (1971) also found that calves fed diets containing soybean protein, which does not clot in the abomasum, had similar plasma urea responses. Statistical analysis of plasma urea nitrogen concentration data from this study indicates that there was a high degree of variability among calves which may have masked trends influenced by time after feeding and diet.

PLASMA FREE AMINO ACID LEVELS: Results of this study showed a decrease in plasma free methionine, glutamic acid, alanine and isoleucine concentrations with increasing age of the calf. Plasma free valine concentrations also decreased according to results from the

first set of samples, although this trend was not apparent for the second set.

Leibholz (1965) reported that the concentrations of most of the free amino acids in the blood plasma of the calf decreased with increasing age from birth to four weeks. This effect was significant for serine, proline, methionine and isoleucine; concentrations of aspartic acid, glutamic acid, valine and cystine remained constant. Results for glutamic acid concentrations are contradictory for these two studies. The decreases in amino acid concentrations reported by Leibholz (1965) in calf study B occurred mainly within the first three weeks of the calf's life. Many other species have been found to show a similar decrease in plasma amino acid concentrations in the first few weeks after birth (Munroe, 1970). Williams and Smith (1975) indicated that little change occurred for plasma free methionine or total plasma amino acids once the calf was over two weeks of age.

PLASMA METHIONINE LEVELS: Peak concentrations of plasma free methionine occurred four hours after feeding, two hours later than expected according to the literature (Williams and Smith, 1975; Foldager et al., 1975). One explanation may be that the source of dietary fat influences the concentration pattern of plasma free amino acids in calves. Rony et al. (1975) demonstrated that peak concentrations of methionine in plasma occurred at two and one hour(s) after feeding when calves were fed lard and butteroil respectively. Calves fed whole milk showed peak methionine concentrations at the time of feeding. These results suggest that the source of dietary fat may influence digestion of dietary protein and/or the metabolism of amino

acids in preruminant calves. Fats used in trials by Williams and Smith (1975) and Foldager et al. (1975) were not specified.

Plasma free methionine concentrations were influenced by level of dietary methionine when calves were at fasting state and after the feed. The concentrations of plasma methionine at four hours post feeding in the order of highest to lowest were diets D, B, C and A. Results from calf study B and studies conducted by Foldager et al. (1975) indicate that plasma methionine levels are the most sensitive method of determining optimal methionine supplementation when poor health due to factors other than treatments are encountered.

D-METHIONINE UTILIZATION: Calves fed diet B (1.53 L- and 0.76 g. D-meth/16 g. nitrogen) and diet C (2.38 g. L-methionine/16 g. nitrogen had similar plasma free methionine levels at fasting and at two and four hours post feeding for sets one and two. If D-methionine could not be utilized by the preruminant calf, diet B would provide only 1.53 g. utilizable methionine/16 g. nitrogen. The D-methionine would be deaminated and excreted from the body as urea. Also, the requirements of other essential amino acids would be reduced and excesses of these amino acids would also be broken down in the liver. No differences were found in plasma and urinary urea nitrogen, urinary methionine concentrations or the nitrogen balance trials. These results suggest that D-methionine is absorbed from the intestinal tract into the blood stream and is utilized for protein synthesis in a similar manner as the L-isomer.

OPTIMAL METHIONINE SUPPLEMENTATION: The optimal level of methionine supplementation of a milk substitute diet in which 80% of the crude protein was supplied by F.B.P.C. was determined by observing the changes in plasma free methionine concentrations with time after feeding. Results from sets one and two indicate that diet A does not supply sufficient amounts of methionine to allow optimal utilization of amino acids other than methionine. In set one, plasma free methionine concentrations increased 0.40 mg/1. over four hours. In set two there was an increase of 1.5 mg./1. at two hours post feeding followed by a drop to concentrations slightly greater than when the calves were at fasting state.

The changes in plasma free methionine concentrations at four hours post feeding indicate that calves fed diets B, C and D were fed methionine in excess of their requirements. This was indicated by the sudden increase in circulating methionine at four hours post feeding. In set one a similar pattern was observed at two hours post feeding.

The conclusion derived from these results is that the optimal level of methionine supplementation is between 1.70 and 2.30 g/16 g. nitrogen. A more accurate value could be obtained by adding several more treatments with varying levels of dietary methionine in this type of study. Dietary cystine was approximately 0.79 g/16 g. nitrogen. Therefore, the total sulfur amino acid requirements of a preruminant calf fed a diet in which 80% of the protein is supplied by F.B.P.C. is 2.5 to 3.1 g/16 g. nitrogen (Table 28). This is equivalent to 0.76% of the dry matter of the diet. Based on these results we can calculate that a 40 kg. calf gaining 0.5 kg/day would

Table 28: THE REQUIREMENT OF TOTAL SULFUR AMINO ACIDS IN THE PRERUMINANT CALF[1]

SOURCE	TOTAL SULFUR AMINO ACIDS			
	g/16 g. N	g/Kg. B.W.$^{.73}$/day	g/day	% of D.M.
Foldager & Huber (1975)	4.00	0.26	.385	0.70
Patureau-Mirand et al. (1973)	3.50(2)	0.58	---	0.90
Williams & Smith (1975)	---	0.23-0.26	3.9-4.5	---
Tzeng et al. (1974)	---	---	---	1.15-1.65
Present Study (1977)	2.5-3.1	---	---	0.76

[1] Adjusted from table by Foldager et al., 1975

(2) Experimental diet contained 26.4% protein

require 3.90 g. methionine/day. This is similar to results obtained by Williams and Smith (1975).

According to a study by Williams and Smith (1975), the estimated optimal level of dietary methionine was 4.5 and 3.9 g/day respectively (Table 28). These results were based on plasma methionine and urea-nitrogen responses to graded levels of dietary L-methionine supplementation. Cystine intake was 0.3 g/day. The calves were from 50 to 60 kg. liveweight and grew at a rate of .25 kg/day.

Foldager et al. (1975) estimated total sulfur amino acid requirements to be 3.80 and 4.00 g/16 g. nitrogen (Table 28). Williams and Smith cited Patureau-Mirand and Pion (1973) and Patureau-Mirand et al. (1973), whose results indicated that the methionine requirement was 0.58 g/kg. $W^{0.73}$/day. These results were greater than results from studies already mentioned. Their experiments differed slightly from the others in that diets contained 26.4% crude protein and calves were fed to gain approximately 1.0 kg/day. Foldager et al. (1975) cited Tzeng et al. (1974) who reported that the total sulfur amino acid requirements for pre-ruminant calves was 1.15 to 1.65% of the dry matter.

CONCLUSIONS

1. Autoclaving fababean protein concentrate at 120°C for thirty minutes does not improve growth performance of weanling rats when fed as the sole protein source. No differences were found in feed intake, average daily gain or protein efficiency ratio for rats fed raw as compared to autoclaved fababean protein concentrate.

2. Fababeans have a low level of sulfur amino acids, particularly methionine. D,L-methionine supplementation of fababean protein concentrate at a level providing 3.20 g. methionine per 100 g. protein results in a protein that is equivalent to casein and to methionine supplemented soybean protein concentrate.

3. Milk substitute diets containing fababean protein concentrate as the major protein source mix well with water and will reamin in suspension. These diets were palatable.

4. Statistical analysis showed that there were no differences among calves fed an all milk replacer and a milk replacer in which 25% of the protein was supplied by fababean protein concentrate. Parameters considered were average daily gain, feed efficiency, dry matter, fat and crude protein digestibilities.

5. There was decreased protein digestibility as the level of fababean protein concentrate in the diet increased. Young calves are not able to digest this vegetable protein as efficiently as milk proteins. Crude protein digestibility improved with the age of the calf, the response being more dramatic for fababean protein than for the milk proteins. Using a simple regression analysis, the crude protein digestibility of fababean protein concentrate was estimated to be 56.8, 65.1 and 80.0% for calves aged 8-13, 15-20 and 22-27 days respectively. Comparisons with work done on other vegetable proteins suggest that fababean protein concentrate is a better protein source for milk substitute diets than pea protein concentrate and soybean flour and is equal to soybean protein concentrate.

6. The requirements for total sulfur amino acids by the preruminant calf is 2.5 to 3.1 g/16 g. nitrogen for calves between nine and 27 days of age. Plasma methionine level was a more sensitive measure than average daily gain, nitrogen balance and plasma urea nitrogen when health problems were occurring. These health problems were not directly related to dietary methionine levels, but were caused by stress and the low digestibility of other nutrients in the diet. The requirements determined by plasma methionine levels were approximately the same at five and eleven days after introduction to the treatments.

7. Factors affecting the concentration patterns of amino acids after a meal should be further investigated. These include the source of dietary proteins and fats and the mode of incorporation of fat into the diet. Also, the sparing effect of cystine on methionine in calves should be further investigated.

8. Results from the last study indicate that calves do utilize D-methionine to satisfy their methionine requirements. Supplementation of milk substitute diets with D,L- versus L-methionine is advantageous because it is lower in cost and more palatable.

BIBLIOGRAPHY

A.O.A.C. 1971. Official Methods of Analysis (11th ed.) George Banta Publishing Co., Menaska, Wis.

Abidi, S.A. 1967. Intestinal absorption of essential amino acids in man. Gastroenterology. 52:837

Albanese, A.A., Frankston, J.E. and Irby, V. 1944. The estimation of methionine in protein hydrolyses and human urine. J. Biol. Chem. 156:293

Bell, J.M., Royan, G.F. and Youngs, G.G. 1974. Digestibility of Pea Protein concentrate and enzyme-treated pea flour in milk replacers for calves. Can. J. Anim. Sci. 54:355

Bell, J.M. 1970. Pea factors in milk replacers for calves. In: Nutritional aspects of common beans and other legume seeds as animal and human foods. Proceedings of meeting held Nov. 6-9, 1973, Brazil.

Benevenga, M.J. 1974. Toxicities of methionine and other amino acids. J. Agr. & Food Chem. 22:2

Bhatty, R.S. 1974. Chemical composition of some fababean cultivars. Can. J. Plant Sci. 54:413

Blaxter, K.L. and Mitchell, H.H. 1948. A factorization of the protein requirement of ruminants and of the protein value of feces with particular reference to the significance of metabolic fecal nitrogen. J. Anim. Sci. 7:351

Blaxter, K.L. and Wood, W.A. 1952. The nutrition of the young Ayrshire calf. VII. The biological value of gelatin and of casein when given as the sole source of protein. Br. J. Nutr. 6:56

Block, R.J. and Weiss, K.W. 1956. Amino Acid Handbook, Charles C. Thomas, Springfield, Ill.

Boling, J.A., Bradley, N.W. and Willard, J.C. 1972. Amino acid patterns in the blood plasma of the young bovine. Int. J. for Vit. and Nutr. Res. 42:306

Bragg, D.B., Guy, D.A., Greene, D.E., Waldroup, P.W. and Stephenson, E.L. 1966. Comparison of amino acids in milo and corn. Poultry Sci. 45:1072

Brüggemann, J., Drepper, K. and Zucker, H. 1962. Quantitative bestimmung der Ververtung von D-, L-, DL-methionine und Ca-DL-2-Hydroxy-4-methyl thiobutyrat durch das Hunerkuken. Die Naturwissenschaften. 49:334

Burt, A.W.A. and Irvine, S.M. 1970. Carbohydrates in milk replacers for calves. J. Sci. Food Agric. 21:342

Camien, M.N., Malin, R.B. and Dunn, M.S. 1951. Urinary excretion of ingested L- and DL-methionine measured microbiologically. Archives of Biochemistry. 30:62

Campbell, J.A. 1963. Method for determination of PER and NPR. In: Evaluation of Protein Quality, National Academy of Sciences, NRC Publ. 1100

Canolty, N.L. and Nasset, E.S. 1975. Intestinal absorption of free and protein bound dietary methionine in the rat. J. Nutr. 105:867

Chiou, P.W.S. and Jordan, R.M. 1973. Ewe milk replacer diets for young lambs. I. Effect of age of lamb and dietary fat on digestibility of the diet, nitrogen retention and plasma constituents. J. Anim. Sci. 36:597

Clarke, H.E. 1970. The evaluation of the field bean (Vicia faba L) in animal nutrition. Proc. Nutr. Soc. 29:64

Cochran, W.G. and Cox, G.M. 1970. Experimental Designs, 2nd ed. J. Wiley and Sons, New York, 611 pp.

Colvin, B.M. and Ramsey, H.A. 1968. Soy flour in milk replacers for young calves. J. Dairy Sci 51:898

Colvin, B.M. and Ramsey, H.A. 1969. Growth of young calves and rats fed soy flour treated with acid or alkali. J. Dairy Sci. 52:270

Coombe, N.B. and Siddons, R.C. 1973. Carbohydrases in the bovine small intestine. Br. J. Nutr. 30:269

Coombe, N.B. and Smith, R.H. 1974. Digestion and absorption of starch, maltose and lactose by the preruminant calf. Br. J. Nutr. 31:222

Craig, B. 1974. The industrial potential of fababeans. In: Proceedings of the First National Fababean Conference 1974. E.H. Lange, Research Co-ordinator, Faculty of Agriculture, University of Manitoba.

Denton, A.E. and Elvehjim, C.A. 1953. Enzymatic liberation of amino acids from different proteins. J. Nutr. 49:221

Duthie, I.E., Porter, P.O. and Gadsby, B. 1972. Nutritional Value of a protein isolate from the Throws M.S. variety of field beans (Vicia faba L) grown in the U.K. Proc. Nutr. Soc. 31:80A

Duthie, I.F., Edwards, D.G. and Rogers, B. 1974. Preliminary studies on the suitability of field bean (Vicia faba L) protein isolate for lambs and calves. Proc. Nutr. Soc. 30:40A

Edwards, C.H., Gadsden, E.L. and Edwards, E.A. 1963. Utilization of methionine by the adult rat. II. Absorption and tissue uptake of L- and D,L-methionine. J. Nutr. 80:9

Eggum, B.O. 1970. Blood urea measurement as a technique for assessing protein quality. Br. J. Nutr. 24:983

Eggum, B.O. 1973. The levels of blood amino acids and blood urea as indicators of protein quality. In: Proteins in Human Nutrition (Porter, J.W.G. and B.A. Rolls, eds.) Academic Press, New York, 560 pp.

Erbersdobler, H. and Gropp, J. 1973. Aspects of protein quality in calf nutrition. Problems and possibilities of milk protein substitutes. Proc. Nutr. Soc. 32:223

Fauconneau, G. and Michel, M.C. 1970. The role of the gastro intestinal tract in the regulation of protein metabolism. In: Mammalian Protein Metabolism Volume 4. (Munro, H.N., ed.) Academic Press, New York, 764 pp.

Foldager, J. and Huber, J.T. 1975. Suflur amino acid studies in the preruminant calf. Presented at Rumen Function conference, Chicago.

Folin, O. and Wu, H. 1919. A system of blood analysis. J. Biol. Chem. 38:81

Garnot, P., Valles, E., Thapon, J.L., Toullec, R., Thomassone, R. and Ribadeau-Dumas, B. 1974. Influence of dietary proteins on rennin and pepsin content of preruminant calf vell. J. Dairy Res. 41:19

Gills, J.L. and Hafs, H.D. 1971. Analysis of repeated measurements of animals. J. Anim. Sci. 33:331

Gills, J.L. and Magee, W.T. 1976. Balanced two-period changeover designs for several treatments. J. Anim. Sci. 42:775

Giovannetti, P.M., Stothers, S.C. and Parker, R.J. 1970. Coprophagy prevention and availability of amino acids in wheat for the growing rat. Can. J. Anim. Sci. 50:269

Gitler, C. 1964. Protein digestion and absorption in nonruminants. In: Mammalian Protein Metabolism Volume L. (Munro, H.N., ed.) Academic Press, New York, 560 pp.

Gorrill, A.D.L. and Thomas, J.W. 1967. Body weight changes, pancreas size and enzyme activity, and proteolytic enzyme activity and protein digestion in intestinal contents from calves fed soybean and milk protein diets. J. Nutr. 92:215

Gorrill, A.D.L., Thomas, J.W., Stewart, W.E. and Morrill, J.L. 1967. Exocrine pancreatic secretion by calves fed soybean and milk protein diets. J. Nutr. 92:86

Gorrill, A.D.L. and Nicholson, J.W.G. 1969. Growth, Digestibility & Nitrogen Retention by calves fed milk replacers containing milk and soybean proteins, supplemented with methionine. Can. J. Anim. Sci. 49:315

Gorrill, A.D.L. and Nicholson, J.W.G. 1971. Effect of soybean trypsin inhibitor, diarrhoea and diet on flow rate, pH, protrolytic enzymes and nitrogen fractions in calf intestinal digesta. Can. J. Anim. Sci. 51:377

Graham, G.G. 1974. Effects of protein and animo acids. In: Improvement of Protein Nutriture. National Academy of Sciences; National Committee on Amino Acids, 201 pp.

Harper, A.E. and Rogers, A.R. 1965. Amino Acid Imbalance. Proc. Nutr. Soc. 24:173

Harper, A.E. 1970. Effects of disproportionate amounts of amino acids. In: Improvement of Protein Nutriture. National Research Council; Committee on Amino Acids.

Harper, A.E. 1974. Basic concepts. In: Improvement in Protein Nutriture. National Research Council; Committee on Amino Acids.

Hinks, C.E., Peers, D.G. and Armshaw, A.M. 1974. The replacement of skim milk by cooked potato flour. Anim. Proc. 19:351

Hirs, C.H.W. 1967. Determination of Cystine as Cysteic Acid, Methods in Enzymology XI:197. (Hirs, C.H.W., ed.) Academic Press, New York.

Hoover-Plow, J., Clifford, A.T., Hodges, R.E. and Halsted, C.G. 1976. Plasma amino acids, nitrogen balance, protein synthesis and degradation in traumatized rats. Fed. Proc. 35:343

Huber, J.T., Jacobson, N.L., McGillard, A.D. and Allen, R.S. 1961. Utilization of carbohydrates introduced directly into the omaso-abomasal area of the stomach of cattle at various ages. J. Dairy Sci. 44:321

Huber, J.T., Natrajan, S. and Polan, C.E. 1968. Varying levels of starch in calf milk replacers. J. Dairy Sci. 51:1081

Kakade, M.L., Thompson, R.D., Engelstad, W.E., Behrens, G.C., Yoder, R.D. and Crame, F.M. 1976. Failure of soybean trypsin to exert deleterious effects in calves. J. Dairy Sci. 59:1484

Kies, C., Rox, H. and Sprahamian, S. 1975. Comparative value of L-, DL- and D-methionine supplementation on an oat-based diet for humans. J. Nutr. 105:809

Kinsell, L.W., Harper, H.A., Barton, H.C., Micaels, G.D. and Wuss, H.A. 1947. Rate of disappearance from plasma of intravenously administered methionine in patients with liver damage. Science. 106:589

Lane, G.T. and Leighton, R.E. 1973. Response of young dairy calves to methionine hydroxy analog. J. Dairy Sci. 56:313A

Latrille, L., Ingalls, J.R. and Marquardt, R.R. 1975. Digestibility of faba bean protein concentrate by neonatal calves. 1975. Annual Meeting of the Canadian Society of Animal Science, Brandon, Manitoba.

Leibholz, J. 1965. The effect of age and dietary protein source on free plasma amino acids, ammonia and urea in the blood plasma of the calf. Aust. J. Agric. Res. 237:246

Leiner, I.E. and Kakade, M.L. 1969. Protease Inhibitors. In: Toxic constituents of Plant Foodstuffs. (Leiner, I.E., ed.) Academic Press, New York.

Lewis, A.J. and Speer, V.C. 1973. Lysine requirements of the lactating sow. J. Anim. Sci. 37:404

Liang, Y.T., Morrill, J.L. and Noordsy, J.L. 1967. Absorption and utilization of volatile fatty acids by the young calf. J. Dairy Sci. 53:72

Macdonald, B.E. 1974. Fababeans and Human Nutrition. In: Proceedings of the First National Faba bean conference 1974. E.H. Lange, Research co-ordinator, Faculty of Agriculture, University of Manitoba.

Mahler, H.R. and Cordes, E.H. 1971. Biological chemistry 2nd ed. Academic Press, New York, 1009 pp.

Maltman, J.M. 1976. The nutritive value of faba beans for young growing pigs. M.Sc. Thesis, University of Manitoba.

Marquardt, R.R. and Campbell, L.D. 1973. Raw and autoclaved faba beans in chick diets. Can. J. Anim. Sci: 741

Marquardt, R.R. and Campbell, L.D. 1974. Deficiency of methionine in raw and autoclaved faba beans in chick diets. Can. J. Anim. Sci. 54:437

Marquardt, R.R., Campbell, L.D. and Stothers, S.C. 1974. Growth responses of chicks and rats fed diets containing four cultivars of raw or autoclaved faba beans. Can. J. Anim. Sci. 54:177

Marquardt, R.R., McKirdy, J.A., Ward, T. and Campbell, L.D. 1975. Amino acid, hemagglutinin and trypsin inhibitor levels, and proximate analyses of faba beans (Vicia faba) and faba bean fractions. Can. J. Anim. Sci. 54:421

Marquardt, R.R., Campbell, L.D. and Ward, T. 1976. Studies with chicks on the growth depressing factor(s) in faba beans (Vicia faba L var minor). J. Nutri. 106:275

Marquardt, R.R., Ward, A.T. and Campbell, L.D. 1977. Further studies on the isolation of the thermolabile growth inhibitor from the faba bean. J. Nutr. 107:1213

Marrett, L., Bird, H.R. and Sunde, M.L. 1964. The effects of different isomers of methionine on growth of chicks fed amino acid diets. Poultry Sci. 43:1113

Marrett, L. and Sunde, M.L. 1965. The effect of the D- amino acid on the utilization of the isomers of methionine and its hydroxy analogue. Poultry Sci. 44:957

Marsh, W.H., Fingerhut, B. and Miller, H. 1965. Automated and manuel direct methods for determination of blood urea. Clin. Chem. 11:624

Meister, A. 1965. Biochemistry of the Amino acids, Volume I. Academic Press, New York, 119 pp.

Ministry of Agriculture. 1972. The utilization of calf milk replacer diets containing protein isolates from various sources. In: Annual Report on Research and Technical Work of the Ministry of Agriculture for Northern Ireland. Nelson and Knox (N.I.) Ltd., Belfast, Northern Ireland, 200 pp.

Morrill, J.L., Stewart, W.E., McCormick, R.J. and Fryer, H.C. 1970. Pancreatic Amylase Secretion by Young Calves. J. Dairy Sci. 53:72

Muller, L.D. and Rodrigues, A. 1974. Methionine hydroxy analog supplementation of low protein calf rations. J. Dairy Sci. 57:191

Munro, H.N. 1964. General Aspects of the regulation of protein metabolism by diet and hormones. In: Mammalian Protein Metabolism, Volume I. (Munro, H.N. and Allison, J.B., ed.) Academic Press, New York, 566 pp.

Munro, H.N. 1970. Free amino acid pools and their role in regulation. In: Mammalian Protein Metabolism, Volume 4. (Munro, H.N., ed.) Academic Press, New York, 764 pp.

Mylrea, P.J. 1966. Digestion of milk in young calves. I. Flow and acidity of the contents of the small intestine. Res. Vet. Sci. 7:333

McLaughlan, J.M., Noel, F.J., Morrison, A.B. and Campbell, J.A. 1963. Blood amino acid studies. IV. Some factors affecting plasma amino acid levels in human subjects. Can. J. Biochem. Physiol. 41:191

McLaughlan, J.M. and Campbell, J.A. 1969. Methodology of protein evaluation. In: Mammalian Protein Metabolism, Volume III. Academic Press, New York, 571 pp.

Nasset, E.J. and Ju, J.S. 1961. Mixture of endogenous and exogenous protein in the alimentary tract. J. Nutr. 74:461

National Academy of Sciences. National Research Council, 1962. Nutritional Requirements of Laboratory Animals. National Research Council Publication 990. Washington, D.C.

Natrajan, S., Polan, C.E., Chandler, P.T., Jahn, E. and Huber, J.T. 1972. Ruminal and post-ruminal utilization of starch in the young bovine. J. Dairy Sci. 55:238

Nitzan, Z., Volcani, R., Gordin, S. and Hasdai, A. 1971. Growth and nutrient utilization by calves fed milk replacers containing milk or soybean protein concentrate heated to various degrees, J. Dairy Sci. 54:1294

Nitzan, Z., Volcani, R., Hasdai, A. and Gordin, S. 1972. Soybean protein substitute of milk protein in milk replacers for suckling calves. J. Dairy Sci. 55:811

Nitzan, Z. and Leiner, I.E. 1976. Studies on the digestibility and retention of nitrogen and amino acids in rats fed raw and heated soybean diets. J. Nutr. 106:292

Nordstrom, J.W., Wirdels, H.F., Typpo, J.T., Mead, R.J. and Stockland, W.E. 1970. Influence of site of plasma free amino acids in the growing pig. J. Anim. Sci. 31:874

Patureau-Mirand, P. and Prugnaud, J. 1971. The influence of the milk replacer protein source on free amino acid concentration in the blood of the preruminant calf. European Ass. Anim. Prod. Xth Int. Congr., Paris, July 1971.

Porter, J.W.G. and Williams, A.P. 1965. Quality of dietary proteins and amino acid blood levels. Proc. Biochem. Soc. 87:7P

Porter, J.W.G. and Hill, W.G. 1964. Nitrogen balance trials with calves given synthetic milk diets. Nat. Inst. Res. Dairying Ann. Rep., p.124

Porter, J.W.G. 1969. Digestion in the preruminant animal. Proc. Nutr. Soc. 28:115

Radostits, O.M. and Bell, J.M. 1968. Nutrient digestibility by new-born calves fed milk replacer. Can. J. Anim. Sci. 48:293

Radostits, O.M. and Bell, J.M. 1970. Nutrition of the preruminant dairy calf with special reference to the digestion and absorption of nutrients. A review. Can. J. Anim. Sci. 50:405

Ramsey, H.A. and Willard, T.R. 1975. Symposium: Recent Advances in Calf Rearing. Soy protein for milk replacers. J. Dairy Sci. 58:436

Raven, A.M. and Robinson, K.L. 1964. Factors affecting the nutritive value of fat for calves. I. The mode of incorporation into milk diets. J. Sci. Fd. Agric. 15:214

Reece, W.O. and Wahlstrom, J.D. 1972. Variations in plasma composition of calves: Relationship of electrolyte, glucose and urea-nitrogen concentration to calf age, ration and feeding time. Dairy Sci. Abst. 35:226

Rogers, Q.R. and Leung, P.M.B. 1973. The influence of amino acids on the neuroregulation of food intake. Fed. Proc. 32:1709

Rony, D.D., Desmarais, M. and Brisson, G.J. 1975. Effects of various dietary fats on the post-prandial concentrations of blood plasma-free amino acids in young dairy calves. Can. J. Anim. Sci. 55:257

Rose, W.C., Coon, M.J., Lockhart, H.B. and Lambert, G.F. 1955. The amino acid requirements of man. XI. The threonine and methionine requirements. J. Biol. Chem. 215:101

Roy, J.H.B. 1970. The Calf - Nutrition and Health. Pennsylvania State University Press, U.S.A., 183 pp.

Roy, J.H.B. 1970. Protein in milk replacers for calves. J. Sci. Foods Agric. 21:346

Roy, J.H.B. 1974. Symposium on: Feeding the newborn: Comparative problems in animals and man. Problems in the nutrition of the preruminant calf. Proc. Nutr. Soc. 33:79

Sambeth, W., Nesheim, M.C. and Serafin, J.A. 1967. Seperation of soybean whey into fractions with different biological activities for chicks and rats. J. Nutr. 92:479

Sarwar, G., Sosulski, F.W. and Holt, N.W. 1975. Protein nutritive value of legume cereal blends. Can. Inst. Food Sci. Technol. J. 8:170

Sarwar, G., Sosulski, F.W. and Bell, J.M. 1977. Availability of amino acids in legumes and legume-wheat blends. Can. Inst. Food Sci. Technol. J. 10:32

Shaw, R.H., Woodward, T.E. and Norton, R.P. 1918. Digestion of starch in the young calf. J. Agr. Res. 12:575

Sjödin, J. 1973. Breeding for improved nutritional quality in field beans (Vicia faba L); First FAO/SIDA Seminar on Improvement and Production of Field Crops, Swedish Seed Assoc., Svalof, Sweden.

Smith, R.H., Hill, W.G. and Sisson, J.W. 1970. Effect of diets containing soya products on passage of digesta through alimentary tract of preruminant calf. Proc. Nutr. Soc. 29:6A

Smith, R.H. and Wynn, C.E. 1971. Effects of feeding soya products to preruminant calves. Proc. Nutr. Soc. 30:75A

Smith, R.H. and Sissons, J.W. 1975. The effect of different feeds, including those containing soya-bean products on the passage of digesta from the abomasum of the preruminant calf. Proc. Nutr. Soc. 33:329

Snedecor, G.W. and Cochran, W.G. 1976. Statistical Methods, Sixth Edition. Iowa State University Press, Aimes, Iowa, 593 pp.

Spackman, D.H., Stein, W.H. and Moore, S. 1958. Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem. 30:1190

Sugahara, M., Morimoto, T., Kobayashi, T. and Ariyosi, S. 1967. The nutritional value of D-amino acids in the chick nutrition. Agr. Biol. Chem. 31:77

Toofanian, F., Hill, F.W.G. and Kidder, D.E. 1974. The development of the intestinal dissacharidase activities in the fetal and newborn calf. Res. Vet. Sci. 16:375

Toullec, R., Frontzen, J.E. and Mathieu, C.M. 1974. Effect of coagulation of milk proteins on the digestibility of a milk replacer in preruminant calves. Annales de Zootechnit.

Typpo, J.T., Meade, R.J., Nordstrom, J.W. and Stockland, W.L. 1970. Influence of time of fast on concentrations of free amino acids in plasma and total amino acids in liver protein in young bovine. J. Anim. Sci. 31:885

Whitaker, T.R. and Gomer, P. 1971. A plasma amino acid method for determining protein quality. Bulletin. December 1971. :605

Williams, A.P. and Smith R.H. 1974. The amino acid requirements of the ruminating calf. Proc. Nutr. Soc. 33:35A

Williams, A.P. and Smith, R.H. 1975. Concentrations of amino acids and urea in the plasma of the preruminant calf and estimation of the amino acid requirements. Br. J. Nutr. 33:149

Wilson, B.J. and McNabb, J.M. 1971. The effect of autoclaving and methionine supplementation on the growth of chicks given diets containing field beans (Vicia faba L). Br. Poult Sci. 13:67

Wilson, B.J. and McNabb, J.M. 1972. The effect of autoclaving and methionine supplementation on the growth of chicks given diets containint field beans (Vicia faba L). Br. Poult. Sci. 13:67

Williams, B.J., McNabb, J.M. and Bentley, H. 1972. Trypsin inhibitor activity in the field bean (Vicia faba L). J. Sci. Food. Agric. 23:679

Williams, C.H., David, D.J. and Lismaa, D. 1962. The determination of chromic oxide in fecal sample by atomic absorption spectrophotometry. J. Agr. Sci. 59:381

Windels, H.F. 1971. Influence of source and level of dietary protein on plasma free amino acid concentrations of growing bovine. J. Anim. Sci. 32:268

Writland, K.A.J. and Rose, W.C. 1950. Methionine requirement for growth and utilization of its optical isomers. J. Biol. Chem. 187:697

Zimmerman, R.A. and Scott, H.M. 1965. Inter-relationships of plasma amino acid levels and weight gain in the chick as influenced by supoptimal and superoptimal dietary concentrations of a single amino acid. J. Nutr. 87:13

APPENDIX

APPENDIX Table 1: AMINO ACID REQUIREMENTS OF GROWING RATS AND AMINO ACID CONTENT OF THE SEVEN EXPERIMENTAL DIETS FED TO GROWING RATS IN THE PRELIMINARY EXPERIMENT

AMINO ACID	GROWING RAT'S REQ'T[1] (g/100 g. C.P.)	A	B	C	D	E	F	G
Lysine	.75	.82	.71	.70	.68	.68	.68	.65
Histidine	.25	.28	.27	.26	.26	.26	.25	.24
Arginine	.50	.36(72)[2]	1.01	1.01	.99	.96	.73	.69
Aspartic Acid		176	1.22	1.19	1.15	1.10	1.28	1.20
Threonine	.42	.40	.39	.35	.38	.34	.42	.39
Serine		.46	.42	.43	.45	.43	.44	.43
Glutamic Acid		2.26	1.89	1.84	1.91	1.79	2.02	1.94
Proline		1.01	.44	.30	.45	.49	.55	.50
Glycine		.18	.42	.40	.43	.39	.43	.43
Alanine		.30	.46	.40	.43	.39	.44	.45
Cystine (ox.)	.17	.03(21)	.11(64)	.11(64)	.08(48)	.09(57)	.11(64)	.12(70)
Valine	.50	.67	.60	.60	.59	.57	.61	.58
Methionine (ox.)	.34	.18(52)	.05(15)	.36(107)	.05(16)	.32(96)	.10(30)	.24(70)
Isoleucine	.46	.51	.46	.47	.48	.46	.50	.49
Tyrosine & Phenylalanine	.67	.44(65)	.52	.49(73)	.55	.52	.60	.68
Leucine	.63	.93	.86	.84	.85	.81	.84	.71

[1] National Academy of Sciences, 1972

[2] Numbers in brackets show the percentage of the rat's requirement for a particular amino acid that is provided by the diet

APPENDIX Table 2: INITIAL WEIGHT AND RECORD OF HEALTH OF CALVES USED IN STUDY A.

DIET	CALF	INITIAL WEIGHT (Kg.)	HEALTH RECORD
1	101	44.6	--
	15-75	38.8	--
	23-75	44.7	--
	105	46.4	got V.P.(1) just prior to 3rd collection period; lost 3.4 Kg. in last 8 days.
	292	48.3	showed some symptoms of V.P. at age 8 - 17 days.
	306	38.0	
2	14-75	46.6	--
	22-75	43.5	had navel ill - age 8 - 15 days; lost 2.9 Kg.
	297	45.2	--
	298	47.0	--
	299	43.2	severe scours at age 7 - 12 days; given ½ feed for 2 days.
	300	46.4	--
3	16-75	34.2	--
	19-75	48.0	had a navel infection at age 9 - 13 days; no determined weight loss.
	102	45.3	--
	104	55.0	had some symptoms of V.P. during last four days of experiment
	291	43.6	appeared to be in good health, but lost hair on back legs and rump.
	296	42.0	
4	17-75	38.8	--
	20-75	45.6	--
	106	42.5	had slight case of V.P. during final collection period
	294	43.5	had scours for first seven days of experiment
	295	45.8	--
	24-75	47.0	got V.P. at age 23 days; lost 1.1 Kg. in weight

(1) V.P. - viral pneumonia (enzootic pneumonia)

APPENDIX Table 3: INDIVIDUAL CALF DATA ON THE APPARENT DRY MATTER DIGESTIBILITY DURING COLLECTION PERIODS 1, 2 AND 3 IN CALF STUDY A.

DIET	CALF	D.M. DIGESTIBILITY IN COLLECTION PERIOD: (%) 1 8-13 DAYS	2 15-20 DAYS	3 22-27 DAYS	AVERAGE D.M. DIGESTIBILITY (%)
1	101	77.58	77.76	85.60	80.31
	15-75	74.16	78.41	86.90	79.82
	23-75	75.83	90.94	91.45	86.07
	105	70.17	82.46	88.03	80.22
	292	74.02	79.56	90.19	81.26
	306	85.80	77.08	84.88	82.59
2	14-75	76.75	87.33	89.55	84.54
	22-95	76.51*	79.64	88.80	81.65
	297	75.56	84.36	87.21	82.38
	298	86.19	83.71	78.45	82.78
	299	58.86	73.62	75.10	69.19
	300	75.61	73.43	86.71	78.58
3	16-75	82.48	94.88	90.57	89.31
	19-75	78.94*	79.90	88.01	82.22
	102	87.78	75.92	91.76	85.15
	104	92.17	86.81	88.38	89.12
	291	63.94	88.07	91.91	81.31
	296	69.27	80.86	90.16	80.10
4	17-75	82.72	88.57*	93.14	88.14
	20-75	83.18	89.28	91.10	87.85
	24-75	74.87	90.83	84.85	83.52
	106	88.66	63.27	91.83	81.25
	294	74.23	88.54	92.47	85.08
	295	84.94	82.51	89.57	85.67

* pseudo values (Anderson, 1946)

APPENDIX Table 4: INDIVIDUAL CALF DATA ON THE APPARENT DIGESTIBILITY OF FAT DURING COLLECTION PERIODS 1, 2 AND 3 IN CALF STUDY A.

DIET	CALF	FAT DIGESTIBILITY (%) IN PERIOD: 1 8-13 DAYS	2 15-20 DAYS	3 22-27 DAYS	AVERAGE FAT DIGESTIBILITY (%)
1	101	62.48	66.57	90.63	73.23
	15-75	44.34	63.44	70.60	66.13
	23-75	39.39	92.80	95.33	75.84
	105	34.75	58.37	82.09	58.40
	292	38.76	53.67	90.91	61.11
	306	84.08	62.74	90.55	79.12
2	14-75	60.33	89.67	92.40	80.80
	22-75	56.13*	79.59	92.65	86.12
	297	54.13	91.27	95.76	80.39
	298	84.21	78.94	65.04	76.06
	299	8.38	61.43	92.27	48.44
	300	56.62	57.12	90.85	68.20
3	16-75	72.74	97.16	93.70	87.87
	19-75	65.47*	69.42	93.13	81.28
	102	85.81	70.19	93.62	83.21
	104	93.17	81.93	90.17	88.42
	291	1.25	93.21	94.01	62.82
	290	39.77	92.15	96.00	75.97
4	17-75	63.21	90.37	93.71	78.46
	20-75	82.84	90.61	75.29	89.58
	24-75	46.74	94.08	93.93	78.25
	106	79.69	54.43	89.95	74.69
	294	43.54	79.75	94.63	72.31
	295	74.18	79.44	83.76	79.13

* pseudo values (Anderson, 1946)

APPENDIX Table 5: INDIVIDUAL CALF DATA ON THE APPARENT DIGESTIBILITY OF CRUDE PROTEIN DURING COLLECTION PERIODS 1, 2 AND 3 IN CALF STUDY A.

DIET	CALF	C.P. DIGESTIBILITY (%) IN PERIOD 1 8-13 DAYS	2 15-20 DAYS	3 22-27 DAYS	AVERAGE C.P. DIGESTIBILITY (%)
1	101	57.52	64.92	82.59	68.34
	15-75	60.44	67.92	77.41	68.92
	23-75	65.29	84.29	91.03	80.20
	105	58.70	73.20	82.08	71.33
	292	56.22	59.51	86.97	67.57
	306	64.75	59.81	69.26	64.61
2	14-75	72.33	83.63	86.84	80.93
	22-75	63.79*	69.29	86.48	77.87
	297	58.10	67.77	81.66	69.18
	298	77.26	74.08	73.23	74.86
	299	49.78	73.49	81.60	68.29
	300	63.68	61.22	81.96	68.95
3	16-75	78.14	93.49	85.78	85.80
	19-75	71.22*	75.64	80.72	78.18
	102	79.47	66.27	88.15	77.96
	104	88.28	77.93	79.92	82.04
	291	61.82	85.39	88.70	78.64
	246	55.94	70.04	88.82	71.60
4	17-75	81.72	86.80*	91.47	86.60
	20-75	73.56	81.64	87.34	80.85
	24-75	68.13	83.02	77.96	76.37
	106	81.83	72.40	83.98	79.40
	294	60.06	81.66	89.23	76.98
	295	74.22	67.17	85.06	75.48

* pseudo values (Anderson, 1946)

APPENDIX Table 6: ANALYSIS OF VARIANCE OF FOUR EXPERIMENTAL DIETS CONTAINING VARIABLE LEVELS OF F.B.P.C. FOR AVERAGE DAILY GAIN AND FEED EFFICIENCY IN CALF STUDY A.

SOURCE OF VARIATION	TREATMENTS	ERROR
DEGREES OF FREEDOM	3	20
ITEMS	(Mean Squares)	
Average Daily Gain	.033	.016
Feed Efficiency	.069	.022*

* Significant Difference due to treatment, $P < .05$

APPENDIX Table 7: ANALYSIS OF VARIANCE OF FOUR EXPERIMENTAL DIETS FED IN CALF STUDY A FOR DIGESTIBILITY COEFFICIENTS OF DRY MATTER, ETHER EXTRACT AND CRUDE PROTEIN.

SOURCE OF VARIATION	DIET	CALVES WITHIN DIET	AGE	AGE xx DIET	CALVES WITHIN AGE
DEGREES OF FREEDOM	3	20	2	6	37
ITEMS			(Mean Squares)		
Dry Matter Dig.	113.25	44.58	635.93*	5.22	47.30
Ether Extracts Dig.	497.78	258.72	7210.14*	809.22	370.77
Crude Protein Dig.	369.22*	70.28	1568.68*	26.92	67.55

* Significant Differences at $P < .05$

APPENDIX Table 8: ANALYSIS OF VARIANCE BETWEEN FOUR EXPERIMENTAL DIETS FOR AVERAGE DAILY GAIN, DRY MATTER DIGESTIBILITY, CRUDE PROTEIN DIGESTIBILITY, NITROGEN UTILIZATION, AND NITROGEN BALANCE IN CALF STUDY B

SOURCE OF VARIATION	BLOCKS (calves)	PERIODS (age)	TREATMENTS (diets)	ERROR
DEGREES OF FREEDOM		1	3	8
ITEMS			(mean squares)	
Average Daily Gain (g/day)	24748.20*	2340.24	12.57	3767.14
Dry Matter Dig. (%)	70.26	28.20	31.88	93.27
Crude Protein Dig. (%)	247.91	288.96	65.72	330.81
Nitrogen Utilization (g. gain/g. N intake)	54.20*	8.43	1.25	8.60
Nitrogen Balance (g/day)	11.58	14.59	15.09	8.22

* significant difference = .05

APPENDIX Table 9: INDIVIDUAL DATA ON WEIGHT CHANGES OF CALVES DURING THE FIRST SIX DAYS AFTER INTRODUCTION TO THEIR ASSIGNED DIETS AND WHILE ON METABOLISM CRATES FOR PERIODS 1 AND 2

CALF	PERIOD 1			PERIOD 2		
	DIET	WEIGHT CHANGES		DIET	WEIGHT CHANGES	
		1st 6 days Kg.	last 6 days* Kg.		1st 6 days Kg.	last 6 days* Kg.
1	A	0.8	-0.2	D	-1.5	-0.2
2	D	0.4	-1.0	A	0.5	-0.2
3	A	1.1	-0.8	B	6.0	-1.6
4	B	1.0	-0.5	A	-2.0	-1.0
5	B	-0.1	-1.4	D	-0.4	-0.5
6	D	0.2	-0.6	B	-0.3	0.7
7	C	0.2	-0.3	D	0.4	-0.2
8	D	0.9	0.3	C	-0.4	0.9
9	C	0	0.1	B	0.4	-0.1
10	B	1.3	0.5	C	0	2.6
11	C	0.2	0.3	A	-1.5	1.5
12	A	0.2	-2.3	C	-0.8	-1.4

* During the last six days of the period the calves were placed on metabolism crates.

APPENDIX Table 10: INDIVIDUAL CALF DATA ON DRY MATTER AND CRUDE PROTEIN DIGESTIBILITIES FOR CALF STUDY B

DIET	CALF	PERIOD	DRY MATTER DIG. (%)	CRUDE PROTEIN DIG. (%)
A	1	1	76.72	44.41
	3	1	62.71	21.89
	12	1	83.65	60.82
	2	2	86.85	64.14
	4	2	68.15	32.50
	11	2	82.45	56.49
B	4	1	76.89	36.39
	5	1	73.88	45.31
	10	1	88.78	66.40
	3	2	72.23	55.20
	6	2	86.34	69.00
	9	2	72.99	30.44
C	7	1	81.03	51.16
	9	1	84.33	62.78
	11	1	85.20	59.87
	8	2	80.73	52.81
	10	2	87.65	69.40
	12	2	75.18	46.58
D	2	1	62.51	23.16
	6	1	65.19	21.59
	8	1	88.38	66.99
	1	2	72.15	36.80
	5	2	85.13	63.79
	7	2	85.89	68.92

APPENDIX Table 11: INDIVIDUAL CALF DATA ON NITROGEN UTILIZATION AND NITROGEN BALANCE FOR CALF STUDY B

CALF	PERIOD	DIET	NITROGEN UTILIZATION (g. gained/g. N intake)	NITROGEN BALANCE (g/day)
1	1	A	- 6.84	4.32
	2	D	- 8.26	5.23
2	1	D	- 3.80	1.49
	2	A	1.61	2.84
3	1	A	- 3.88	0.67
	2	B	- 9.79	9.46
4	1	B	- 4.35	3.50
	2	A	-11.90	.73
5	1	B	-11.72	4.26
	2	D	- 4.60	4.95
6	1	D	- 3.22	.51
	2	B	- 0.71	11.85
7	1	C	- 0.66	5.94
	2	D	1.09	9.26
8	1	D	0.80	6.55
	2	C	2.79	6.78
9	1	C	0.77	5.64
	2	B	1.90	4.89
10	1	B	9.35	10.75
	2	C	11.72	11.66
11	1	C	1.62	8.78
	2	A	0	9.10
12	1	A	-12.08	6.30
	2	C	- 9.29	5.71

APPENDIX Table 12: ANALYSIS OF VARIANCE OF EXPERIMENTAL DIETS FED IN CALF STUDY B FOR PLASMA FREE METHIONINE CONTENT AT 0, 2 AND 4 HOURS AFTER FEEDING ON THE FIRST AND LAST DAY OF THE EXPERIMENTAL PERIOD

SOURCE OF VARIATION	AMONG CALVES	AGE	DIET	ERROR
DEGREES OF FREEDOM	11	1	3	8
ITEMS				
1st SET plasma free methionine:				
0 hrs.	7.30	0	13.47*	2.46
2 hrs.	13.62	5.16	14.48**	3.84
4 hrs.	26.58	27.36	79.24**	5.71
2nd SET plasma free methionine:				
0 hrs.	5.22	12.00	13.39	9.88
2 hrs.	14.68	3.01	20.13	12.56
4 hrs.	21.22	17.52	64.09**	9.54

* significant difference at = 0.05

** significant difference at = 0.01