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Title: ALFALFA PROTEIN CONCENTRATE IN MILK REPLACER FORMULAS FOR
DAIRY CALVES

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Abstract approved:

Dr. J. E. Oldfield

To reduce the loss of feeding calves during the nursing period and to conserve milk for human consumption research has been conducted to develop milk replacer formulas which result in calf performance comparable to that of milk. In the first section of this investigation different aspects of utilization of fats, carbohydrates and protein by the calf are discussed. The review of literature showed that the calf can adequately utilize a number of different fats as alternatives to butterfat. As regarding carbohydrates the only ones that are satisfactory for young calves are lactose and glucose. The young calf shows poor ability to digest starch and sucrose.

The protein used in milk replacers is generally provided by skim milk and various sources of protein have been tried to substitute for it. The review indicated that results were widely different due to the source of protein, rate of substitution, age of the calf and physical and chemical effects on the product. However, provided any toxic factors are extracted from the protein source and amino acid deficiencies rectified, many proteins can substitute for skim milk at levels between 25-50%. Even 70% substitution is possible with satisfactory performance of the calf.

In the second section an investigation was undertaken to evaluate alfalfa protein concentrate (APC) as a partial substitute for milk protein in milk replacers for calves. Alfalfa has a high potential as a protein source and methods have been developed to extract the protein from other less nutritious leaf constituents for use by monogastric animals as well as pre-ruminant calves. Three milk replacers were formulated. The control had all the protein from milk. The other two contained 30 and 60% of the total crude protein from APC. These were fed to three groups of 5 calves each for six weeks and the growth rate, feed efficiency and health of the calves were assessed. The results showed that the growth rates for the calves on the three replacers were not significantly different. The growth rate of the three groups was poor during the first three weeks and incidence of diarrhea was high during the same period. Failure of coagulation as a reason for this was discussed. Feeding efficiency was not significantly different. The calves in the three trials were healthy and APC was well accepted by the calves. With increasing levels of APC substitution the cost of feed per weight gain was markedly reduced. Conclusions were that APC can substitute for a major part of the skim milk after the third week. For automated feeding systems more research should be done with APC to improve its solubility characteristics.

Alfalfa Protein Concentrate
in Milk Replacer Formulas for Dairy Calves

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TABLE OF CONTENTS

	<u>Page</u>
INTRODUCTION	1
LITERATURE REVIEW - Milk Replacers for Dairy Calves	3
Fat in Milk Replacers	3
Carbohydrates	5
Proteins	6
Substitution of Milk Protein by Alternative Sources of Protein	8
Soy Products	8
Fish Products	9
Meat Byproducts	10
ALFALFA PROTEIN IN MILK REPLACERS FOR DAIRY CALVES	
Abstract	13
Introduction	14
Materials and Methods	16
Results and Discussion	20
Conclusions	22
References	27
SUMMARY AND CONCLUSIONS	28
BIBLIOGRAPHY	30

LIST OF TABLES

<u>Table</u>		<u>Page</u>
1	Composition of Milk Replacers	18
2	Chemical Composition of Milk Replacers	19
3	Average Performance of Calves	23
4	Average Weight Gain of Calves	24
5	Visual Evaluation of Calves	25
6	Fecal Score in Weeks	26

ALFALFA PROTEIN CONCENTRATE IN MILK REPLACER FORMULAS FOR DAIRY CALVES

INTRODUCTION

With increasing demand for milk for human use, it has been necessary to look for milk substitutes for calf feeding. Experiments have been conducted for over 30 years investigating the possibility of constituting satisfactory milk substitutes for calves based on skim milk with added cheap fats of animals and plant origin. After the different aspects of fat utilization by the calf were understood many sources of fat have been used as successful substitutes for butterfat. Various carbohydrates have also been tried as a source of energy but none of them except glucose were as satisfactory for the young calf as lactose, the natural carbohydrate of milk. Nevertheless, processing could render other carbohydrates like starch more useful, especially for older calves.

The protein source generally used in milk replacers is still skim milk and it would be of great advantage if cheaper sources of protein could substitute for skim milk proteins. Many studies have been done on this possibility using animal as well as plant proteins with various results.

Proteins of plant origin are more abundant and generally less expensive and their use could result in a great reduction of cost of milk replacers if a suitable plant protein could substitute for skim milk successfully. Extraction of protein from leafy plants, thus

eliminating fiber problems is one of the ways to obtain cheaper but digestible proteins. Alfalfa has been the plant most used as a raw material in more recent leaf protein research. It is a major cultivated forage crop in most temperate countries and it has a high productivity per hectare per year and high protein content and quality. Methods of recovering the protein from alfalfa have been developed for both human and animal use.

The objectives of this study are:

1. Review important factors in the formulation of milk replacers for dairy calves emphasizing energy and protein considerations.
2. Investigate the possibility of substituting alfalfa leaf protein for part of the milk protein in milk replacers for dairy calves and to determine the rate of growth, feed efficiency and survival abilities of the calves as a result.

LITERATURE REVIEW

MILK REPLACERS FOR DAIRY CALVES

Under natural conditions the calf sucks its dam about six to eight times a day at first and gradually this decreases to about three times daily. The calf can start eating solid food from about one week of age and can be weaned at five weeks onto a high concentrate diet. Nevertheless, if its supply of milk is abundant, the calf can continue nursing up to nine months of age. However, when milk is needed for human use, calves are usually separated from their dams as soon as they are born, given colostrum for two or three days and then given only a limited amount of milk twice a day by pail or bottle.

In the 1950's technological advances in the developed countries made it possible to constitute milk substitutes made up of cow's milk in which milk fat was replaced by more economical sources of energy e.g. fats of animal or vegetable origin and these were fed to calves as early as two days after birth. More recently, cheaper sources of protein have been considered to replace part of the skim milk for reasons of economy as well as conserving the maximum amount of milk for human use.

Fat in Milk Replacers:

In the neonatal calf, milk and milk replacers pass directly to the abomasum by way of the esophageal groove (Orskov 1975). Reviews of fat digestion by the calf by Raven (1970) and Toullec et al (1980) indicated that triglycerides undergo partial hydrolysis in the abomasum by the

action of a lipolytic enzyme known as pregastric esterase, secreted by the salivary glands, which is considered to act in the same manner as pancreatic lipase. The absorption of long chain fatty acids depends on the formation of micelles by bile salts. Bile plays an important role in fat digestion through its emulsifying action.

The digestibility of fat by the calf varies with the nature of fat e.g. tallow is less digestible than butter fat. The reason for this is the great difference in digestibility of the long chain fatty acids palmitic and stearic in the two fats, as well as the higher percentage of stearic acid, which is least absorbed, in tallow (Raven 1970). Rape-seed oil was found to be far less digestible by the calf than lard (69% and 94% respectively) due to its high percentage of erucic acid which is poorly absorbed by the calf (Bell and Sandra 1974).

The way fats are incorporated into milk replacers is another factor determining their usefulness. Fats are usually more digestible when homogenized in concentrated skim milk (in liquid form) than when melted and then mixed with spray dried skim milk (Toullec *et al.*, 1980). However the digestibility of the fat in the latter form is greatly improved by addition of emulsifiers e.g. lecithin and saccaroglycerides (Toullec and Mathieu 1970). When alternate sources of protein to skim milk protein are used in milk replacers, digestibility of fat decreases (Raven 1970) suggesting a positive correlation between digestibilities of protein and fat. High levels of divalent minerals lower the digestibility of fat due to the formation of insoluble soap complexes. The study by Raven and Robinson (1964) when feeding calves milk replacers containing 28% tallow and 4% dicalcium phosphate showed that the

digestibility of total fat was lowered.

Fat is used in milk replacers as a concentrated source of energy and the level is determined by the cost of the fat compared to lactose in terms of energy yield and by the level of energy required. In considering the optimum level of fat in milk replacers it should be noted that as less digestible fats are incorporated, indigestible portions of them may interfere with absorption of other nutrients e.g. forming soaps with divalent minerals. Replacement dairy calves need less fat unlike calves raised for veal which are given only milk replacers since increased levels can lead to reduced consumption of the dry concentrates (Olsen and Williams 1959).

Toullec et al. (1980) reviewed the digestion of fats by the calf and showed that the young calf is inherently capable of digesting other sources of animal fat than butterfat (tallow, lard and hydrogenized herring oil) as well as fats of plant origin (coconut oil, palm oil and peanut oil) with digestibilities ranging from 90-96%.

Carbohydrates:

Lactose is found in a high percentage in cow's milk (38% of dry matter) and is usually included in milk replacers at the rate of 38-50% of the dry matter. The neonatal calf can easily digest it by the action of intestinal lactase which is present in high quantities at birth and decreases between then and the 8th week (Huber et al., 1958). Lactose in milk replacers is mainly supplied by skim milk and whey. Glucose is also well utilized by the young calf and these two sugars are superior

to other carbohydrates such as maltose and starch which are poorly utilized in milk replacers (Huber et al., 1961). When starch replaced 27% of lactose in milk replacers, the result was a lower rate of gain by the calves and starch digestibility was 70% compared to 98% for lactose (Huber et al., 1968). Digestibility of starches varies according to the processing to which they have been subjected. Heat treatment resulted in improved digestibility of various starches (Huber et al., 1961) while dextrinization improved the digestibility of potato but not corn starch (Mathieu et al., 1970). In the latter study the overall digestibility of different dextrans was found to be high, varying with the type and amount of dextrans given. It decreased with increased level of dextrans. Malt extract was most digestible followed by corn dextrans and the poorly soluble potato dextrans.

Sucrose is poorly utilized by the calf and caused severe diarrhea in calves of all ages (Huber et al., 1961). However, the review of Toullec et al. (1980) indicated that there is an important function of caecum and colon microorganisms in the digestion of sucrose and starchy substances which produce volatile fatty acids and lactic acid. This digestion is useful unless the level of sucrose and starch become excessive which will then cause diarrhea.

Proteins:

The digestion of protein by the young calf has been reviewed by Roy (1979), Stobo and Roy (1978) and Toullec et al. (1980). These reviews showed that once milk is in the abomasum it is soon (1-10 min.) acted upon by hydrochloric acid and rennin causing the clotting of casein and incorporating the milk fat into a firm clot. On the other

hand, fluid whey containing lactose and whey proteins including a small quantity of immunoglobulins and minerals will pass to the small intestine. The casein clot in the abomasum is gradually degraded by the action of hydrochloric acid, rennin, and pepsin and the partially digested protein and the fat are continuously released into the small intestine where digestion is then completed by the action of the pancreatic enzymes trypsin, chymotrypsin and carboxypeptidase followed by the absorption of the resulting amino acids. However, this is not the case when skim milk has been subjected to severe preheating (74°C for about 30 min.) during the drying process as opposed to 77°C for only 15 sec. In this "severely" preheated skim milk the clotting properties are reduced, resulting in the escape of casein from the abomasum without proteolysis taking place. This was suggested to cause qualitative and quantitative changes in the bacterial flora which result in diarrhea (Roy 1979). Shillman and Roy (1963) reported that such "severely" preheated skim milk resulted in 66% lower rate gain in calves compared to a spray dried skim milk subjected to a mild heat treatment (77°C for 15 sec.) and they discussed the possibility that this may be related to denaturation of the non-casein proteins. Excessive heat treatment of skim milk as well as replacement of milk protein by other sources of protein are believed to have unfavourable effects on the secretion of hydrochloric acid and proteases by the abomasum (Williams, Roy and Gilles, 1976) resulting in undigested casein escaping into the duodenum.

The effect of coagulation on digestibility of milk replacers was studied by Toullec et al. (1974a) when partial and complete failure of coagulation of milk replacers containing 67% skim milk was obtained by

adding sodium citrate and hydrochloric acid respectively at the moment of feeding. Digestibility was reduced until three weeks of age after which there was no effect of absence of coagulation. The study suggested that absence of coagulation per se should not be an obstacle to total replacement of milk proteins in milk replacers.

Substitution of milk protein by alternative sources of protein:

For any protein source to be considered for the replacement of milk proteins, it should be easily digested, well balanced as regards to essential amino acids or easy to supplement and devoid of poorly digestible or undigestible constituents e.g. complex carbohydrates. Physically, the product should have good suspension properties in water, good storage properties and must be palatable. Palatability is believed to be of great importance because if the calf is not keen to drink its milk the reflex closure of the esophageal groove may not occur and liquid diet will find its way to the rumen resulting in bloat which may be fatal (Stobo and Roy 1978).

Soy Products:

Soy products have been tried extensively as substitutes for milk protein with varying results due to the physical and chemical treatments they receive. Early reports showed that soy flour as a sole source of protein for calves resulted in poor performance and high mortality (Shaptaw 1936). Since then a lot of work has been done to determine the detrimental factors which resulted in such poor performance. Acid or alkali predigestion of soy flour was found to result in marked improve-

ment in growth of calves fed a milk replacer containing only soybean protein (Calvin and Ramsey 1969). Soy flour containing antitryptic activity which was shown to be present in inactive form in fully cooked soy flour and can be converted to active form within the pH range of 7-9 (Ramsey and Willard 1975). They suggested that this antitryptic activity would reduce protein digestibility if the secretion of trypsin by the calf is marginal. Soybean meal (oil and antitryptic factors removed) which provided 70% of the protein in milk replacer resulted in unsatisfactory growth when fed to calves (Paruelle et al., 1972). However, soybean protein concentrate, prepared by extraction of fat and carbohydrates from soy flour, when similarly incorporated in milk replacers for calves (constituting 70% of the total protein) gave good results in terms of growth rate and nitrogen digestibility (Gorril and Nicholson 1969).

Other sources of plant protein have been tried as a substitute for milk protein in milk replacers for calves. Some of these sources are: field beans, peas, potatoes and rapeseed; results were satisfactory but the digestibility of the protein was markedly lowered up to the first month (Stobo and Roy 1978).

Fish Products:

These are variable in composition due to the nature of the raw material, extent of boning or removal of offals and the methods for oil extraction. Satisfactory growth of calves was reported when 40% of the milk replacer protein was supplied by fish flour prepared by dichlorethane extraction of fish meal but poor performance and even death sometimes resulted when it was the only source of protein (Huber and Slade 1967). Isopropanol-extracted fish protein concentrate, however, brought

about improved calf performance which was similar to that on whole milk and milk replacer with only milk protein when it provided 50% of the protein of the milk replacer (Gorril et al., 1972). Subjecting fish meal to partial digestion by enzymatic action resulted in satisfactory substitution of two thirds of the milk protein in milk replacers for calves (Dodsworth et al., 1977). Fish protein concentrate and whey protein seem to complement each other resulting in increased nitrogen retention (Sleiman and Huber 1971).

Vitamin E deficiency represents a problem in diets with fish protein concentrate providing all the protein of milk replacers. Michel et al. (1972) confirmed the need for higher supplemental vitamin E on fish than on milk protein diets, particularly for dichloroethane extracted fish protein concentrate. The deficiency was believed to be associated with the 2-4% unsaturated fish oil remaining after the extraction with dichlorethane.

Whey:

A considerable quantity of dried whey is produced as a byproduct of the cheese industry. With protein content as low as 11-13% it is often included in milk replacers when a non-milk protein source of high content is to be used so that the total protein content of the diet would not exceed 28%. Dried whey contains about 70% lactose, the carbohydrate of choice for young calves and it is mainly included as a source of lactose. However, dried delactosed whey which contains relatively higher protein has the disadvantage of being very high in ash content (24%) and so when included in milk replacers will tend to decrease intake of total organic

matter. Nevertheless, when used by Raven (1972) to replace half of the skim milk protein, its crude protein digestibility and biological value were comparable to milk protein. Whey proteins (lactoproteins) obtained by heat precipitation in the presence of hydrochloric acid, when replacing all skim milk protein of milk replacers, the calf performance was the same as in the case of skim milk protein in the first eight weeks and thereafter whey protein gave better growth rate and feed efficiency (Toullec et al., 1974).

Meat Byproducts:

Abattoir byproducts have been tried as a substitute for part of the milk protein in milk replacers. Raven (1972) reported that meat meal and dried blood providing 50% of the protein, resulted in poor digestibility of total dietary protein as well as poor nitrogen retention. Addition of proteolytic enzymes to the meal in this study did not affect its digestibility or subsequent nitrogen retention.

Meat byproduct solubles are low in essential amino acids and the high content of collagen makes the nitrogen largely unavailable; the result being low nitrogen digestibility, low nitrogen retention and low growth rate of calves when it constituted one third of the protein in milk replacers (Polzin et al., 1976).

Single cell protein (yeast and bacterial protein) has stimulated some interest for the use in diets of preruminant calves. Reports by Stobo and Roy (1977) and Hinks (1978) showed that it can supply up to 25% of the replacer protein with results comparable to all-milk protein diets. Limited data however, have indicated satisfactory growth when

the milk replacer contained 75% of the total protein from yeast (Paruelle et al., 1972). The particle size was an important factor in the favourable results obtained (50 μm diameter was better utilized than 200 μm).

Other protein sources like distiller's dried solubles have also been considered for replacing part of the milk protein. Iowa experiments feeding various levels of corn distillers dried solubles to calves showed that when allowances were made for the depression in the digestibility of the nutrients in the replacer, body weight gains were nearly equal with as high as 35% of milk protein replaced (Bryant et al., 1967).

ALFALFA PROTEIN IN MILK REPLACERS FOR DAIRY CALVES

ABSTRACT

Alfalfa protein concentrate (APC) was evaluated as a partial substitute for milk protein in milk replacer for calves. Milk replacer formulations (21% crude protein, 12% fat and equal in supplemental vitamins, minerals and antibiotics) contained all milk protein (A), 30% of the crude protein from APC (B), and 60% of the crude protein from APC (C). The three replacers were fed to calves (5 calves per treatment) 3-5 days of age for 6 weeks. No calf starter or hay were fed. Average daily gains (g), time scouring per calf and fecal scores on a scale of 1, (normal) to 4 (fluid) of calves on replacers A, B, C were 291, 272, 241 grams; 3.6, 3.6, 5 days and 1.48, 1.68, 1.83. The scores for appetite, calf demeanor and dehydration were close to 1 for the three treatments as rated on a scale 1(good) to 4 (poor). Total feed costs/Kg weight gain of calves were \$2.80, \$1.89 and \$1.36 for replacers A, B and C respectively. It was concluded that APC could supply a major portion of the protein in milk replacers for calves after 3 weeks of age.

INTRODUCTION

Alfalfa is a rich source of protein. Methods have been developed by the Western Regional Research Centre, U.S.D.A., Berkely, California (Kohler et al., 1978) to separate the protein from other leaf constituents. One method called the Proxan process consists of ammoniation of freshly harvested chopped forage to raise the pH to about 8.5 to inactivate the lipoxygenases and proteases. The forage is then pressed to remove the juice. The protein is coagulated from the juice by direct injection of steam to raise the temperature to 85°C and the coagulated protein is then pressed in a platen belt press. Finally the pressed curd is dried in a hot air drier. This forms the whole leaf protein and is called Proxan because it is a good source of both protein and xanthophylls. A study by Bickoff et al. (1975) showed that the crude protein content of alfalfa whole leaf protein concentrate is in the range of 45-60%. This study with other studies (Cheeke et al., 1977) indicated that methionine and lysine enhanced the nutritive value of alfalfa protein concentrate (APC) when fed to rats, resulting in increased growth. Freeze-dried alfalfa protein concentrate (APC) brought about better performance of pigs than a commercial preparation of Proxan (Cheeke et al., 1977) and the study suggested that with adequate processing methods which avoid heat damage to the protein, APC can equal soybean meal as a protein supplement for swine. In poultry rations APC was found to be an effective protein as well as metabolizable energy supplement for broilers (Kuzmicky and Kohler, 1977).

Substituting 50% of the milk protein in calf milk replacers with alfalfa leaf protein coagulum resulted in lower apparent nitrogen and dry matter digestibility as well as lower growth rate (Wheeler and Buchanan-Smith, 1978). Other studies with Proxan also showed poor growth rate when calves were fed only milk replacers with different levels of protein supplied by Proxan for the first 5 weeks (Alpan, unpublished).

The objective of this study is to investigate the possibility of replacing part of the milk protein by alfalfa protein concentrate (APC).

MATERIALS AND METHODS

Fifteen Holstein bull calves between 3 and 5 days of age were randomly allotted, as they became available, to three treatments. The liquid diets compared were milk replacers containing all protein from milk (A), 30% of the protein from alfalfa protein concentrate (APC) (B), and 60% of the protein from APC (C). The ingredients and chemical composition of the milk replacer diets are given in Table 1 and 2 respectively. The calves were housed in individual pens under a shelter. The pens were bedded with wood shavings. Milk replacers were fed at a concentration of 15% solids and the liquid diet was prepared by adding water at about 37°C to the dry milk replacer just before feeding. During the first week on test milk replacers were fed at 8% of initial body weight. The replacers were given by bottle in two equal feedings at 9 am and 5 pm. No hay or started was offered and water was not available. Body weights were taken weekly and adjustment made in feed allowances accordingly until the termination of the experiment at six weeks.

General health and appearance of each calf and consistency of feces were recorded twice daily. A scoring system ranging from one to four was used to classify feces consistency, dehydration, calf demeanor and appetite. For feces consistency, rank one was given to normal while rank four was for fluid feces. For dehydration, demeanor and appetite one indicated good while four was given to poor.

In vitro rennet coagulation of the three replacers was measured in 100 ml of milk replacers containing 15% solids. A standard cheese making rennet of 0.2 ml was used. A sample of skim milk was used as a

control.

Data were analyzed by standard analysis of variance procedures and Tukey-HSD procedure was employed to identify the significant differences among the means (Steel and Torrie, 1960).

Table 1 . Composition of milk replacers

Ingredient	Ration		
	A	B	C
	(%)	(%)	(%)
Dried Skim Milk	40	18.5	-
Pro-Xan ^a	-	10.76	21.7
Fat Premix ^b	40	34.0	27.0
Dried Whey	19.5	36.24	50.8
Vitamin Premix ^c	.40	.40	.40
Mineral Premix ^d	.05	.05	.05
Antibiotics ^e	.05	.05	.05
	100.00	100.00	100.00

^a Alfalfa protein concentrate kindly supplied by Valley Dehydrating Company, Sterling, Colorado. Analysis (% as is basis) Solids, 96.4; Crude Protein, 56.9; Ether Extract, 12.6; Crude Fiber, 2.5; Ash 7.84.

^b FM 30-70 purchased from Land-O-Lakes, Minneapolis, Minnesota; 30% fat 70% dried whey.

^c Turkey Starter Premix; manufactured by Hoffman-LaRoche, Inc., Fresno, California; Contains the following/454g: Vitamin A, 1,800,500 IU; Vitamin D₃, 600,000 IU; Vitamin E, 200,000 IU; Vitamin B₁₂, 1.2 mg; Riboflavin, 1.2 g; Niacin, 1.3 g, Calcium Pantothenate, 2.8g; Choline, 120g; Vitamin K, 400 mg; Folic acid, 200 mg; Biotin, 20mg; Pyridoxine, 600 mg; Thiamine, 200mg.

^d The premix contains the following in mg/kg FeSO₄, 270; Mn SO₄, 109; ZnSO₄, 97 and CuSO₄ 25.

^e Oxytetracycline

Table 2. Chemical composition of milk replacers (dry matter basis)

	A	B	C
Dry matter, %	93.40	93.36	93.57
Crude protein, %	21.39	21.68	21.19
Fat, %	12.1	11.8	12.0
Ash, %	8.30	8.62	9.15
Gross energy (cal/g)	4376.91	4329.21	4342.01

RESULTS AND DISCUSSION

Since the change from colostrum or milk to the milk replacers was abrupt, severe digestive disturbances resulted in some cases. One calf on the 60% APC level died of scouring on the fifth day and another calf on the 0% APC level developed severe diarrhea associated with high fever on the third day. Both calves were replaced.

The performance data of the calves throughout the six weeks feeding trials are summarized in Table 3. There were no significant differences due to the nature of diets for all the variables considered ($P > .05$). The average daily gains (ADG) by calves on the three diets were slightly lower than those reported for other experiments where calves were fed concentrates and hay together with milk replacers (Gorril and Nicholson 1969; Dodsworth et al., 1977) but were higher than those in comparable experiments where calves were just fed milk replacers (Wheeler and Buchanan-Smith 1978; Alpan unpublished). Table 4 shows the growth in the six weeks of the experiment. The calves on the control diet (A) tended to grow better ($P > .05$) in the first two weeks than those on the 30%, and 60% (APC) (B) and (C), respectively. Growth rate started to improve from the third week onwards. On the sixth week calves on diet (C) showed greater compensatory growth than those on (A) and (B) diets ($P < .01$).

Visual observations of the calves are presented in Table 5. None of the variables showed statistically significant differences due to the nature of the diet. Dehydration score, demeanor score and appetite score were very close to one which shows that the calves on the three treatments were in good health and that the calves on diet B and C

readily accepted APC in milk replacers. Days scouring/calf for the three diets averaged four in forty two days and fecal score averaged 1.75. However, the data on Table 6 indicates that incidence of loose feces is highest in the first three weeks for the calves in the three diets. The reason for this is not known. Wallace et al. (1951) reported that milk replacers containing as much as 60% dried whey caused a high incidence of diarrhea. Feces voided by the calves on the high whey diets in this study were no more fluid than those of calves that consumed the least whey.

With the rennet coagulation tests, the three milk replacers failed to coagulate while the skim milk sample formed a firm clot within two minutes. The fact that the control diet failed to coagulate may be due to the low level of skim milk, 40% compared to 60-70% in replacers in other studies where complete coagulation occurred (Toullec et al. (1974a), Alpan unpublished). Absence of clotting characteristics were shown to reduce digestibility during the first three weeks (Toullec et al. (1974a) and consequently reduction of live weight gains and diarrhea resulted (Stobo and Roy 1978). This might offer an explanation for the high incidence of diarrhea in the first three weeks of this study for the three diets since all failed to coagulate. Dodsworth (1977), however reported no difference in performance of calves in the first and second 3-week period feeding of milk replacers with different levels of fish protein hydrolysate. The very low level of replacer allowance in the first three weeks was thought to be the reason protecting the calf digestive system from overloading.

A good substitute for milk protein should possess good suspension characteristics in water. The Proxan used in this study was micropulverised and was very dusty. Stability of a suspension of Proxan was no problem since calves were hand-fed from bottles. Standing for as long as half an hour caused settlement of the Proxan particles in the bottom of the bottle. This might be a problem in an automated feeding system.

The Proxan is now available for commercial use. Comparing the 0, 30, and 60% Proxan milk replacers valued at \$1.30/Kg, 91¢/Kg and 55¢/Kg dry matter, total feed costs/Kg weight gain of calves were \$2.80, \$1.89 and \$1.36 respectively.

Conclusions:

Milk replacers with up to 60% of the protein from APC were readily consumed by calves and after the third week the calves grew well and were very healthy. With costs of feed per Kg. weight gain of \$1.36 compared to \$2.80 for replacers with all protein from milk, supplying 60% of protein from APC would be of economic importance. However, during the first two or three weeks it would be better if calves are given whole milk or milk replacers with 60-70% skim milk since the coagulation property of the diet is very important to health of calves during this period.

Further experiments need to be carried out to form a product with improved solubility characteristics especially for automated feeding systems.

Table 3. Average performance of calves

Criteria	Ration			SE	
	A	B	C		
Body Wt (Kg)					
Initial	44.9	42.2	44.9	2.5	NS
Final	57.1	53.6	55.0	2.6	NS
Body Wt gain (g/day)	292	272	241	26	NS
Daily milk replacer powder intake (g)	596	565	589	36	NS
Milk replacer powder intake /Kg Wt. gain	2.15	2.08	2.47	.21	NS

Table 4. Average weight gain of calves (Kg)

Ration	Weeks						Overall
	1	2	3	4	5	6	
A	1.72	.41	1.50	2.90	3.31	2.40 ^a	12.24
B	.84	0	2.46	2.85	3.19	2.10 ^a	11.44
C	-.45	.0	1.77	2.40	3.18	3.22 ^b	10.12

ab

Means with different superscripts are different ($P < 0.01$).

Table 5. Visual evaluation of calves¹

Observation	A	B	C	Standard Error	
Days scouring/calf	3.6	3.6	5.0	.55	N.S.
Fecal score	1.68	1.68	1.83	.08	N.S.
Dehydration score	1.08	1.08	1.09	.03	N.S.
Demeanor score	1.03	1.07	1.10	.02	N.S.
Appetite score	1.04	1.04	1.01	.02	N.S.

¹
For feces, score 1(normal) 4 (fluid)
for dehydration, demeanor and appetite 1 (good) 4 (poor).

Table 6. Fecal score by weeks¹

Ration	Weeks						Overall
	1	2	3	4	5	6	
A	2.07	2.35	1.61	1.43	1.43	1.21	1.68
B	1.97	2.39	1.86	1.37	1.29	1.29	1.68
C	2.43	2.90	2.08	1.40	1.07	1.20	1.83
	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

1 (normal) 4 (fluid)

REFERENCES

- Bickoff, E. M., A. N. Booth, D. de Fremery, R. H. Edwards, B. E. Knuckles, R. E. Miller, R. M. Saunders and G. O. Kohler. 1975. Nutritional evaluation of alfalfa leaf protein concentrate - In: M. Friedman (Ed.) Protein nutritional quality of foods and feeds. Vol. 1, Part 2. pp. 319. Marcel Dekker, Inc.
- Cheeke, P. R., J. H. Kinzell, D. de Fremery and G. O. Kohler. 1977. Freeze dried and commercially-prepared alfalfa protein concentrate evaluation with rats and swine. J. Anim. Sci. 44:772.
- Dodsworth, T. L., J. B. Owen, T. M. Mackie, A. Ritchie and E. R. Orskov. 1977. Fish protein hydrolysate as a substitute for milk protein in calf feeding. Anim. Prod. 25:19.
- Gorril, A. D. L. and J. W. G. Nicholson. 1969. Growth, digestibility and nitrogen retention by calves fed milk replacers containing milk and soybean proteins supplemented with methionine. Can. J. Anim. Sci. 49:315.
- Kohler, G. O., S. G. Wildman, N. A. Jorgenson, R. V. Enochian and W. J. Bray. 1978. Leaf protein in relation to forage crop production and utilization. In: Protein Resources and Technology: Status and Research Needs. Ed. by Miller, M., N. S. Schrimshaw and D. I. C. Wang. Avi. Publ. Co. Inc., Westport Conn. pp.543.
- Kuzmicky, D. D. and G. O. Kohler. 1977. Nutritional value of alfalfa leaf protein concentrate (ProXan) for broilers. Poultry Sci., 56:1510.
- Steel, R. G. D. and J. H. Torrie. 1960. Principles and procedures of statistics. Mc Graw-Hill Book Co., New York.
- Stobo, I. J. F. and J. H. B. Roy. 1978. The use of non-milk proteins in milk substitutes for calves. World Animal Review 25:18.
- Toullec, R., J. F. Frantzen and C. M. Mathieu. 1974a. Effect of coagulation of milk protein on the digestibility of a milk replacer in preruminant calves. Annls. Zootech., 23:363.
- Wallace, H. D., J. K., Loosli and K. L. Turk. 1951. Substitutes for fluid milk in feeding calves. J. Dairy Sci., 34:256.
- Wheeler, E. E. and J. G. Buchanan-Smith. 1978. Utilization of alfalfa leaf protein coagulum by preruminant calves. ADSA Annual Meeting, July 9-13, 1978, Mich. State Univ., East Lansing. J. Dairy Sci., Supplement 1, p. 191.

SUMMARY AND CONCLUSIONS

The objectives of the present study were:

1. To discuss the utilization of fats, carbohydrates and proteins in milk replacers by the calf through the review of previous research in this area.

2. To determine if alfalfa protein concentrate (APC) can substitute for part of the milk protein in milk replacers for dairy calves with satisfactory performance by the calves.

The conclusions that appear warranted are:

1. The young calf is inherently capable of adequately utilizing a number of different fats of plant or animal origin as a substitute to butterfat provided these fats are stabilized, homogenized or with addition of emulsifiers in case of blended fats.

2. The carbohydrates that are well utilized by the young calf are lactose and glucose. Starches and sucrose are poorly utilized. However, processing of starch improves its digestion by older calves.

3. The protein in milk replacer is mainly supplied by skim milk. However, many protein sources can substitute for skim milk successfully at various levels after the third week since the coagulation property of casein is important for the calf prior to this.

4. Alfalfa protein concentrate could supply up to 60% of the protein in milk replacers for dairy calves successfully but with relatively poor performance in the first three weeks.

5. Including 60% of the protein of the replacer from alfalfa protein concentrate reduced the cost of feed per Kg by more than half.

6. Since the common practice in commercial systems of calf rearing is to bucket feed the calves automatically, further work is needed to improve the solubility of alfalfa protein concentrate in water.

BIBLIOGRAPHY

- Bell, J.M. and C.M. Adams Sandra. 1974. Digestibility of milk replacers containing rapeseed oil, fed to dairy calves under a month old. *Can. J. Anim. Sci.*, 54:331.
- Bickoff, E.M., A.N. Borth, D. de Fremery, R.H. Edwards, B.E. Knuckles, R.E. Miller, R.M. Saunders, and G.O. Kohler. 1975. Nutritional evaluation of alfalfa leaf protein concentrate. In: M. Friedman (Ed.) *Protein Nutritional Quality of Foods and Feeds*. Vol. 1, Part 2 pp. 319. Marcel Dekker, Inc. New York.
- Bryant, J.M., C.F. Foreman, N.L. Jacobson and A.D. McGilliard. 1967. Protein and energy requirements of the young calf. *J. Dairy Sci.*, 50: 1645.
- Cheeke, P.R., J.H. Kinzel, D. de Fremery and G.O. Kohler. 1977. Freeze-dried and commercially prepared alfalfa protein concentrate evaluation with rats and swine. *J. Anim. Sci.*, 44:772.
- Colvin, B.M. and H.A. Ramsey. 1969. Growth of young calves and rats fed soy flour treated with acid or alkali. *J. Dairy Sci.*, 52:270.
- Dodsworth, T.L., J.B. Owen, T.M. Mackie, A. Ritchie and E.R. Orskov. 1977. Fish protein hydrolysate as a substitute for milk protein in calf feeding. *Anim. Prod.*, 25:19.
- Gorril, A.D.L. and J.W.G. Nicholson. 1969. Growth, digestibility and nitrogen retention by calves fed milk replacers containing milk and soybean proteins supplemented with methionine. *Can. J. Anim. Sci.*, 49:315.
- Gorril, A.D.L., J.W.G. Nicholson and H.E. Power. 1972. Effects of milk, fish and soybean protein in milk replacers, and feeding frequency on performance of dairy calves. *Can. J. Anim. Sci.*, 52:321.
- Hinks, Charles E. 1978. Evaluation by digestibility, growth and slaughter with young milk fed calves of microbial cells as a source of protein. *J. Sci. Fd. Agric.*, 29:99.

- Huber, J.T., S. Natrajan and C.E. Polan. 1968. Varying levels of starch in calf milk replacers. *J. Dairy Sci.*, 51:1081.
- Huber, J.T., and L.M. Slades. 1967. Fish flour as a protein source in calf milk replacers. *J. Dairy Sci.*, 50:1298.
- Huber, J.T., N.L. Jacobson, A.D. McGilliard and R.S. Allen. 1961. Utilization of carbohydrates introduced directly into the omaso-abomasal area of the stomach of cattle of various ages. *J. Dairy Sci.*, 44:321.
- Huber, J.T., P.A. Hartman, N.L. Jacobson, and R.S. Allen. 1958. Digestive enzyme activity in the young calf. *J. Dairy Sci.*, 41:743.
- Kohler, G.O., S.G. Wildman, N.A. Jorgensen, R.V. Enochian and W.J. Berry. 1978. Leaf protein in relation to forage crop production and utilization. In: *Protein Resources and Technology: Status and Research Needs*. Ed. by M. Miller, N.S. Scrimshaw and D.I.C. Wang, Avi. Pub. Co. Inc., Westport, Conn. pp. 543.
- Kuzmicky, D.D. and G.O. Kohler. 1977. Nutritional value of alfalfa leaf protein concentrate (Pro-Xan) for broilers. *Poultry Sci.* 56:1510.
- Mathieu, C.M., P. Thivend and P.E. Barre. 1970. Digestion and utilization of feeds by fattening preruminant calf. V. Replacement of milk fat by dextrins. *Annls. Biol. Anim. Bioch. Biophys.*, 10:268.
- Michel, R.L., D.D. Makdani, J.T. Huber and A.E. Schulthrop. 1972. Nutritional myopathy due to vitamin E deficiency in calves fed fish protein concentrate as the sole source of protein. *J. Dairy Sci.*, 55:498.
- Olsen, W.A. and J.B. Williams. 1959. Effect of five levels of animal fat in calf milk replacers. *J. Dairy Sci.*, 42:918.
- Orscov, E.R. 1975. Physiological conditioning in ruminants and its practical implications. *World Animal Review (FAO)*, 16:31.
- Paruelle, J.I., R. Toullec, J.F. Frantzen and C.M. Mathieu. 1972. Utilization of proteins by the preruminant fattening calf. 1- Digestive utilization of soybean and alkane yeast proteins incorporated into the milk substitutes. *Annls. Zootech.*, 21:330.

- Polzin, H. W., D. E. Otterby, J. M. Murphy and J. B. Williams. 1976. Crude casein and meat solubles in milk replacers. *J. Dairy Sci.*, 50: 1842.
- Ramsey, H. A. and T. R. Willard. 1975. Soy protein for milk replacers. *J. Dairy Sci.*, 58:536.
- Raven, A. M., 1970. Fat in milk replacers for calves. *J. Dairy Sci. Fd. Agric.*, 21:352.
- Raven, A. M., 1972. Nutritional effects of including different levels and sources of protein in milk replacers for calves. *J. Sci. Fd. Agric.*, 21:346.
- Raven, A. M., and K. L. Robinson. 1964. Factors affecting the nutritive value of fat for calves. II - The system of feeding and the mode of incorporation into meal mixtures. *J. Sci. Fd. Agric.* 21:346.
- Schillam, K. W. G. and J. H. B. Roy. 1963. The effect of heat treatment on the nutritive value of milk for the young calf. *Brit. J. Nutr.*, 17:171.
- Shoptaw, L. 1936. Soybean flour as a substitute for cow's milk in feeding dairy calves. *J. Dairy Sci.*, 19:95.
- Steel, R. G. D. and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., New York.
- Stobo, I. J. F. and J. H. B. Roy. 1977. The use of microbial protein in milk substitute diet for calves. *Anim. Prod.*, 24:143.
- Stobo, I. J. F. and J. H. B. Roy. 1978. The use of non-milk proteins in milk substitutes for calves. *World Animal Review*, 25:18.
- Toullec, R., J. F. Frantzen and C. M. Mathieu. 1974z. Effect of coagulation of milk protein on the digestibility of a milk replacer in preruminant calves. *Annls. Zootech.*, 23:363.
- Toullec, R., M. Theriez, and P. Thivend. 1980. Milk replacers for calves and lambs. *World Animal Review*, 33:32.
- Sleiman, F. T., and J. T. Huber. 1971. Fish protein concentrate and whey protein in milk replacer diets. *J. Anim. Sci.*, 33:1170.

- Wallace, H.D., J.K. Loosli, and K.L. Turk. 1951. Substitutes for fluid milk in feeding calves. J. Dairy Sci., 34:256.
- Wheeler, E.E., and J.G. Buchanan-Smith. 1978. Utilization of alfalfa leaf protein coagulum by preruminant calves. ADSA Annual Meeting, July 9-13, 1978, Mich. State Univ., East Lansing. J. Dairy Sci., Supplement 1, p. 191.
- Williams, V.J., J.H.B. Roy, and C.M. Gillies. 1976. Milk substitute diet composition and abomasal secretion in the calf. Br. J. Nutr., 36:317.