THE EFFECT OF ENZYME-SUPPLEMENTATION OF MILK REPLACERS ON THE GROWTH OF CALVES

By

George F. Fries

AN ABSTRACT

Submitted to the School of Graduate Studies of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Dairy

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ABSTRACT

Two experiments were carried out to determine the effect of supplementing milk replacers with enzymes. In the first experiment, a vegetable milk replacer was predigested with malt diastase, papain, and a combination of these enzymes. Fifty-five pounds of milk were fed through the 20th day and 30.5 lb. of the treated milk replacer were fed through the 40th day. The experiment was terminated at 60 days. Decreased starter consumption and growth from 20 to 40 days and an increased mortality rate indicated that papain produced deleterious effects. Malt diastase produced no improvement in gain during the periods it was used.

In the second experiment, soybean flour-corn, soybean flour-cerelose, and skimmilk-cerelose milk replacers were supplemented with pepsin but were not predigested. All other aspects of the experiment were similar to the first experiment. Pepsin produced no improvement in growth with any of the replacers. The skimmilk-cerelose replacer produced significantly greater weight gains than the other replacers during the first 20-day period. In the second 20-day period the soybean flour-cerelose replacer produced significantly smaller gains than the other replacers. Starter consumption by this group was also significantly depressed.

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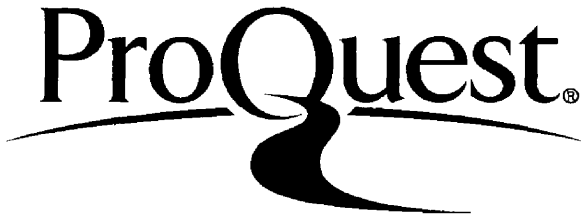

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF APPENDIX TABLES

INTRODUCTION

Milk is one of the most expensive items in the diet of the young calf. For this reason a large number of attempts have been made to develop feeding regimes which would reduce the amount of milk required for raising calves. Generally, these attempts have been successful with calves over 30 days of age. With calves under this age it has not been possible to develop a milk replacer which would support normal growth. The so-called milk replacers currently in use are composed to a large extent of milk by-products and, therefore, cannot be considered true milk replacers.

It has been shown that calves are unable to digest vegetable milk replacers at birth and that the ability to digest these replacers increases with age until it reaches the adult level at 30 to 35 days of age. Since growth was subnormal initially and improved as digestibility improved, it is reasonable to assume that the primary fault of these milk replacers is their indigestibility. The young of several species have been found to be deficient in one or more of the digestive enzymes. These enzyme deficiencies could explain the inability of the young calf to utilize vegetable milk replacers.

The following experiments were carried out to study the use of amyloytic and proteolytic enzymes and the use

of a simple carbohydrate instead of starch in vegetable milk replacer formulation.

REVIEW OF LITERATURE

The nutrient requirements of the young calf are influenced to a large extent by the degree of rumen development. At birth the rumen is nonfunctional and the nutrient requirements of the calf resemble the requirements of simple-stomached animals. Milk meets these requirements during this period. Replacement of milk with feeds of vegetable origin has not supported a normal growth rate. As the rumen becomes functional the requirements become less specific. At this time replacement of milk with various feeds of vegetable origin has generally been successful.

The current studies are concerned primarily with the period preceding the development of a functional rumen. The literature concerning the rumen development of the calf has been thoroughly reviewed by Noller (1955). This review includes discussions on the use of corn-soybean flour milk replacers, the effect of age on the utilization of various nutrients, the development of the enzyme systems in the young, and the effect of supplementing diets with various enzymes and hydrolysates.

Corn-Soybean Flour Milk Replacers

The milk replacer with which the present study concerns itself has been evolved from the formulas first described by Noller and Huffman (1953). All replacers contained finely-ground corn and rolled oats as the primary sources of carbohydrate and soybean flour as the primary source of protein. In addition all replacers contained 10% distillers solubles and 10% whey which was the only animal product used. A number of minor ingredients were used to supply the various vitamins and minerals. The ingredients used were considered to be the ones which had shown the most promise in previous studies (Noller, 1955). These milk replacers did not support satisfactory growth until the calves were approximately 30 days of age. After this time the growth rate was similar to the rate of the controls fed whole milk. The term "critical period" was applied to the period of low growth rate by these workers.

In later studies the milk replacer formulas were simplified and were similar to the basal replacer used in the current study. In comparison with a basal replacer that did not contain distillers solubles and whey, Huffman et al. (1954) found that inclusion of 5% brewers dried yeast or 10% distillers solubles did not improve the growth rate. The use of 5% whey improved growth rate and physical

appearance of the calves but the difference was not statistically significant. In a following study Noller et al. (1956a) confirmed the results concerning whey. Use of lactose in amounts equivalent to that contained in the whey did not produce these results. Fries et al. (1956) confirmed the results concerning distillers solubles and also found that supplemental vitamin B_{12} had no beneficial effect.

The results of digestion trials involving these types of milk replacers will be discussed in the following section.

The Effect of Age on the Utilization of Nutrients

While it appears to be general knowledge that very young animals (i.e., calves under 30 days of age or equivalent ages in other species) cannot utilize certain nutrients as well as older animals, there have been very few studies in which this point was specifically examined.

Several studies have indicated that a number of carbohydrates which are utilized by older pigs are not utilized by the very young pig. Johnson (1949) found that glucose or lactose in purified diets fed beginning the second day after birth produced satisfactory growth in pigs. Sucrose resulted in acute diarrhea, kidney hemorrhage and death within a short time. Fructose was also found to be

unsatisfactory. The inability of the 2-day old pig to utilize sucrose has been confirmed by Becker et al. (1954a). In another study with pigs during the period from 0 to 9 days of age, Becker et al. (1954b) found that glucose or invert sugar produced satisfactory growth and survival rates. Pigs that received fructose or sucrose lost weight and had a very low survival rate. Diarrhea was severe with the use of fructose and sucrose, less severe with invert sugar, and there was none with glucose.

Two studies have been reported in which various carbohydrates were compared for the pig in the period from 7 to 35 days of age. Becker et al. (1954a) found that glucose, lactose, sucrose, dextrin or starch produced about equal gains during this period. However, less diarrhea was noted in the groups receiving lactose and starch. In contrast, Hudman et al. (1955) reported that lactose was superior to glucose, sucrose, corn syrup solids and corn. Corn starch, oat groats, corn flakes and gelatinized starch produced gains considerably lower than lactose.

Using 9-week old pigs Becker and Terrill (1954) found that glucose, sucrose, dextrin and starch produced equal gains when fed at the rate of 50% of the diet. A slight depression in growth occured when lactose was fed at this rate but the depression did not occur when the diet was composed of 25% lactose.

Shaw et al. (1917) have studied the effect of age on starch utilization by calves. Calves from 4 to 7 days of age were able to digest only 20% of the starch they were fed. At 3 to 4 weeks of age over 90% of the starch was digested.

Flipse and coworkers have published a number of studies on the ability of the young calf to utilize various carbohydrates. In 28-day trials calves which had received glucose as the carbohydrate increased 3.3% in body weight while calves receiving dextrin and starch lost 8.8% and 6.9% of their initial weight (Flipse et al., 1948). The calves which were fed glucose were in better condition than the other calves but showed a paralysis which was curable by either potassium or biotin.

In another study Flipse et al. (1950b) compared diets in which the carbohydrate used was glucose at the 60% level and in which part of the glucose was replaced by corn syrup or lactose. Lactose at the 5, 10 and 30% levels were about equal and were superior to glucose alone and glucose with various levels of corn syrup. Corn syrup at levels of 10 and 30% produced gains superior to glucose, but corn syrup at the 45% level resulted in a loss of weight during the experimental period.

The presence of 10% lactose in diets utilizing starch as the principle source of carbohydrate increased the rate

of gain to approximately double the rate for starch alone (Flipse et al., 1950a). After feeding test meals containing a single carbohydrate, it was found that glucose and lactose caused significant increases in blood sugar while starch did not. This would indicate that starch is not utilized to the same degree as the other carbohydrates studied.

Noller et al. (1956b) have studied the influence of age on the digestibility of vegetable milk replacers similar to those described in the preceding section. Digestibility of dry matter, crude protein and nitrogen-free extract was low at two weeks of age but increased with age. By five weeks of age the coefficients of digestibility of the various fractions had increased to near the levels that one would expect for adult animals. In the same experiment the digestibility of the various components of whole milk was high at all ages.

Development of Enzyme Systems in the Young

Most of the studies concerning the enzymes present in the contents and tissues of the alimentary tract have been of a qualitative nature. In quantitative studies a variety of methods for determining enzyme activity and for expressing units of activity have been employed. In addition, most methods of determining enzyme activity are not

specific for a given enzyme. For example, the term "trypsin" usually refers to all proteolytic activity of the pancreas and would include activity of proteolytic enzymes other than trypsin. Therefore, no attempt will be made to discuss these studies in other than the most general terms.

Pepsin. The appearance of pepsin in the development of the fetus appears to vary greatly depending upon the species. Pepsin has been found as early as the fourth month in the development of the human fetus (Keene and Hewer, 1929; Langendorf, 1879). It has been reported in cattle as early as the end of the third month (Moriggia, 1875) and in the stomachs of sheep embryos as small as 6 inches in length (Sewall, 1878). Langendorf (1879) has reported that pepsin is present in the stomachs of the rat and the rabbit fetuses. However, Wolffhugel (1876) was unable to find it in the rabbit fetus. Pepsin has not been detected or was found only in small traces in the fetus of the dog (Grutzner, 1875), the cat (Sewall, 1878), and the pig (Mendel, 1906; Grutzner, 1875; Langendorf, 1879; Sewall, 1878).

Zweifel (1874) and Schmidt (1914) reported that pepsin was always present at birth in the human. Hammarsten (1874) has reported that the amount present at birth may vary greatly. With a premature 7-months child which had been maintained 14 days, the gastric mucosa exhibited only traces of pepsin. Werner (1948) found that the pepsin activity

of full term infants was only 10% of the activity of the adult.

Hammarsten (1874) found no pepsin in the stomachs of dogs under one week of age. During the second week it began to appear in small quantities, but the amount was not significant until after 3 to 4 weeks of age. Results similar to this have been reported by Gmelin (1902). He found no pepsin at birth and significant quantities did not begin to appear until after 18 days of age. In pigs, Kvasnitskii and Bakeeva (1940) were able to detect the presence of pepsin in stomach contents at birth. However, it did not reach its highest activity until 45 days of age.

Pepsin has been reported to be present at birth in the rat (Langendorf, 1879), absent in the cat (Hammarsten, 1874), and present (Langendorf, 1879) and absent (Wolffhugel, 1876) in the rabbit.

Gastric acidity. While hydrochloric acid is not a digestive enzyme, it will be discussed because its production in sufficient quantities is required for optimum pepsin activity.

Grutzner (1875) was unable to find acid in the fetal stomachs of cattle, sheep, pigs and dogs. The stage of fetal development was not reported. In contrast, Moriggia (1875) reported that the stomach contents of the bovine fetus were always slightly acid after the third month.

Hydrochloric acid has been found to be present in the human fetal stomach as early as the nineteenth week of development (Keene and Hewer, 1929).

Gmelin (1902, 1904) studied the effect of increasing age on the hydrochloric acid production of the dog. In both studies it was found that the level of acid was low at birth and increased rapidly after 18 days of age. In the second study Pavlov fistulas were installed in order to eliminate any direct effect of the food on the stomach acidity.

Stomach contents of premature infants have been reported to be lower in acid content than the contents of full term infants (Miller, 1941). In general, the acid content was relatively high at birth. It dropped to a low at 10 days of age and then increased.

Kvasnitskii and Bakeeva (1940) found that hydrochloric acid did not appear in significant quantities in the pig until 20 to 30 days of age. Lewis et al. (1955a) recorded pH values of 4.2, 3.5, 3.9, 3.6, 4.0 and 4.2 on 6-hour fasted baby pigs stomachs at 1, 7, 14, 21, 28 and 35 days of age.

A pH of 4.4 for the combined contents of the omasum and abomasum of calves at birth has been reported by Parrish and Fountaine (1952). For the rumen and reticulum a pH of 6.4 was recorded. Since the omasum does not secrete

hydrochloric acid, a somewhat lower pH would be expected if the abomasum had been considered alone.

Trypsin. Langendorf (1879) was able to demonstrate the presence of trypsin in the human fetus as early as the fifth month of development. These findings have been confirmed by Ibrahim (1909), Keene and Hewer (1929) and Schmidt (1914). Zweifel (1874) and Schmidt (1914) reported that trypsin was present in the newborn infant but the level was not high. In quantitative studies of the duodenal juice of infants, Vazquez (1951) found that trypsin was present at low levels at birth. The levels increased with age and at 3 weeks the level was approximately the same as the level at 3 months of age. Werner (1948) compared the tryptic activity of premature infants (2.5 kg.) with that of full term infants (3.0 kg.). The premature infants showed little or no activity while the full term infants showed about 85% of the activity of the adult human.

The only data for species other than the human was reported by Langendorf (1879). Trypsin was usually found in the pig fetus after the 100 mm. stage of development and in the bovine fetus after the 250 mm. stage. In rats, both the fetus and the newborn exhibited trypsin but the activity was low. Trypsin was present in the new-born rabbit.

Other proteolytic enzymes. Ibrahim (1909) found that enterokinase appears in the human fetus at about the same time as trypsin. The presence of erepsin (a mixture of exopeptidases in the intestinal juice) has been demonstrated in the newborn human (Cohnhiem, 1909), premature infants (Jaiggy, 1907), and as early as the fifth month of fetal development (Jaiggy, 1907; Keene and Hewer, 1929; Langstein and Soldin, 1908).

Pancreatic Amylase. Much of the information concerning pancreatic amylase is conflicting. This conflict is probably due to the variety of techniques and to the small numbers used in many of the studies. Langendorf (1879) was not able to detect pancreatic amylase at the 6-months stage in the human fetus. However, Keene and Hewer (1929) found it as early as the twenty-second week of fetal development, but it did not appear in all individuals as late as full term. Zweifel (1874) has reported that pancreatic amylase was absent in the newborn and did not appear in significant quantities until the end of the second month of age. In contrast, considerable diastatic activity of the intestinal contents of the newborn has been reported by Schmidt (1914). In quantitative studies of the duodenal juice from 1 to 21 days of age, Vazquez (1951) found the amylolytic activity was either low or absent during this period.

There is a paucity of data concerning species other than the human. Langendorf (1879) found pancreatic amylase to be present in the 100 mm. pig embryo and the 250 mm. bovine embryo. Both the rat fetus and the newborn rat exhibited amylolytic activity and the level was high at 3 to 4 days of age. Pancreatic amylase was not detected in the newborn rabbit.

A quantitative study using pigs has been conducted by Kitts et al. (1956). The pancreatic amylase activity was approximately 100 units per kilogram body weight at birth, increased to 1500 units at the sixth day of age and remained at this level through the twenty-second day of age. It had increased to 4300 units at 37 days of age.

Lactase. Lactase is one of the last of the digestive enzymes to appear in the development of the fetus. Ibrahim (1910) and Ibrahim and Kaumheimer (1910) reported that it was not present until the seventh or eighth month in the fetal development of the human. In some premature infants it was absent in the stool on the first day after birth but it appeared soon thereafter. Keene and Hewer (1929) were not able to detect lactase in the human fetus except at full term. Plimmer (1906) did not find lactase in the rat fetus 2 days prepartum but did find it 12 hours prepartum. Lactase was reported to be present in the pig embryo by Mendel (1906) but the stage of development was not given.

From his studies of a large number of species, Plimmer (1906) came to the conclusion that lactase is present in high concentrations at birth in all mammalian species and then decreases with increasing age. He also found it to be present in adult omnivores and carnivores but not in adult herbivores. However, Mendel (1906) was not able to demonstrate lactase in the adult pig. In recent studies with the pig, Bailey et al. (1956) found lactase to be present at a high level at birth and it remained at this level until about 20 days of age. The level then began to decrease rapidly and by 50 days of age the level of lactase was negligable. Similar results have been found with rabbits and cattle (Heilskov, 1951). In the bovine the lactase activity decreased about 50% during the period from 3 to 30 days of age.

Maltase. In the human fetus, maltase has been found as early as the fifth month by Ibrahim (1910) and the sixth month by Keene and Hewer (1929). However, it may not be present in all individuals at full term (Keene and Hewer, 1929).

Mendel (1906) has qualitatively shown the presence of maltase in the suckling pig. In quantitative studies with pigs, Bailey et al. (1956) found that the level of maltase was negligible at birth. It increased to a maximum

at 25 days of age and remained at this level until the experiment was terminated at 50 days.

Sucrase. The behavior of sucrase is very similar to that of maltase with the exception that it appears at an earlier stage of development in the fetus (Bailey et al., 1956; Ibrahim, 1910; Keene and Hewer, 1929; Mendel, 1906).

Pancreatic lipase. Keene and Hewer (1929) found that pancreatic lipase was usually present after the thirty-second week of fetal development in the human. The lipase levels have been found to be high at birth in the human (Zweifel, 1874; Schmidt, 1914). Kitts et al. (1956) have shown that the level of lipase is high at birth in the pig and it remained high throughout the 50-day period of the study.

Supplementation of Diets with Enzymes and Hydrolysates

This section will deal with two aspects of the use of supplemental enzymes and hydrolysates in the diet. These are the studies of experimentally produced enzyme deficiencies and the use of enzymes and hydrolysates to improve the utilization of foods by young animals. While a great number of studies have been conducted using hydrolysates with older animals, these studies will not be covered here because it is felt that they do not apply directly to the problem being considered.

Studies of enzyme deficiencies. Cruickshank (1915) was one of the first investigators to use substitution therapy in a study with depancreatized dogs.[1] When a meat diet was fed to these dogs the coefficients of digestibility for protein and fat were 78 and 33%, respectively. Feeding raw pancreas increased these values to 92 and 79%, respectively. Similar findings have been reported by Coffey et al. (1940a, 1940b), but the effects were somewhat inconsistant in the case of fat. They also reported that the loss of carbohydrate in the feces was reduced considerably when fresh pancreas was fed.

Several studies have been reported in which pancreatin was administered to depancreatized dogs. Selle (1937) found that 3 g. of pancreatin per 100 g. of a meat diet reduced the nitrogen loss in the feces by 60% but failed to check the loss of fat. Larger amounts of pancreatin further reduced the nitrogen loss but were not more effective in checking the fat loss. Enteric-coating of the pancreatin to prevent destruction in the stomach gave no improvement. Schmidt et al. (1937) obtained similar reductions in nitrogen losses when using 25 g. of pancreatin per day with a beef diet. In addition, fecel fat loss was reduced

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[1]For convenience, the term "depancreatized" will be used whenever all of the external secretion of the pancreas was excluded from the intestines, whether by depancreatization, ligation of the pancreatic ducts, etc.

by a similar amount. These reductions occurred regardless of the level of food intake. Coffey et al. (1940b) reported that 2 g. of pancreatin per day was not effective in reducing fecal nitrogen and fat losses but was effective in reducing fecal carbohydrate losses. In four cases of achylia pancreatica in humans, Beazell et al. (1941) found that oral administration of pancreatin resulted in 60% reductions in fecal fat and nitrogen losses.

In depancreatized dogs fed a 62% starch diet, Beazell et al. (1937) found that supplementing the diet with various amylases decreased the fecal starch loss by 30 to 50%. In general amylases of vegetable origin which are inactivated at a lower pH were more effective than pancreatic amylase. Enteric-coating of the pancreatic amylase improved its effectiveness. Even with very high levels of amylases it was not possible to reduce the fecal starch loss to its pre-operative level.

Ivy et al. (1937) have shown that as much as 20% of the starch in the diet may be digested in the stomach when supplemental amylases were used.

Use of enzymes and hydrolysates in feeding young animals. The use of enzymes in feeding young animals is not a new idea. Kellner (1924) credited Liebig with the formulation of a milk substitute for calves which contained starch that had been saccharified with malt. Noller (1955)

has reveiwed several studies on the use of malt-treated starch in calf feeding. While satisfactory results were obtained, it is difficult to interpret these studies because of the large amount of lactose which was available from the skim milk in the diets.

The results of the few studies on the use of supplemental enzymes are somewhat conflicting. Johnson (1949) found that sucrase added to the diet did not prevent the deleterious effects from feeding sucrose to 2-day old pigs. However, since Becker et al. (1954b) obtained similar effects from feeding fructose, this failure may not have been due to the lack of activity of sucrase.

Williams and Knodt (1951) found that supplementation of a milk replacer with papain or pancreatin resulted in decreased growth and feed consumption by calves. The milk replacer used was of the skim milk type.

Studies involving the use of supplemental proteolytic enzymes with soybean-type diets for pigs have been reported by Lewis et al. (1955a, 1955b). The rate of gain was increased as much as 29% from 1 through 4 weeks of age. Pepsin provided a greater response than proteolytic enzymes of plant or fungus origin.

Jorpes et al. (1946) reported increased weight gains in premature infants when a pancreatic digest of casein was added to the diet. This response was not obtained when

undigested casein was used. Feeding an enzymatic digest of bovine plasma to infants from 1.5 to 9 months of age produced growth rates and nitrogen retention as great as evaporated milk (Albanese et al., 1951).

Summary of the Literature Review

The most important points of the literature review as they apply to the current problem may be briefly summarized as follows. Calves fed a corn-soybean flour milk replacer do not begin to grow at the normal rate until they are about 30 days of age. Results of digestion trials indicate that this lack of growth is due to the young calf's inability to digest these milk replacers. It would appear that young animals are not able to utilize nutrients which are not present in milk and which require enzymatic degradation before absorption. The apparent deficiencies of a number of digestive enzymes in the young animal would tend to support this conclusion. There is some evidence that supplemental enzymes will improve food utilization in cases of enzyme deficiencies. However, the results with the use of supplemental enzymes have not been uniformly successful.

EXPERIMENTAL PROCEDURE

The first of the two experiments was carried out to determine if predigesting a soybean flour-corn milk replacer with malt diastase and/or papain would improve its utilization by calves. A 2 x 2 factorial design using 24 Holstein calves was employed in this experiment. The control group received Milk Replacer 1 (Table 1). The chemical analysis of this replacer is presented in Table 2. The remaining groups received Milk Replacer 1 that had been predigested with the following enzymes; 1.0% malt diastase,[1] 0.2papain,[2] and a combination of these treatments.

The selection of treatment levels was based on the strength of the enzyme preparations and the calculated amount of substrate present in the milk replacer. The replacer to be predigested was mixed with four parts of water and the digestion was carried out at 40° C. for 12 hours. The digestion was carried out daily in amounts sufficient to meet the needs of the following day.

The calves used in the experiment were obtained from the University herd and from local dairymen. They were

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[1]Dry malt syrup (60° L), produced by Standard Brands, Inc., New York.

[2]No. 1 powder, produced by Paul-Lewis Laboratories, Inc., Milwaukee.

Table 1. Composition of the experimental milk replacers

Ingredient	Milk Replacer		
	1	2	3
	(%)	(%)	(%)
Soybean flour	33.4	41.9	-
Dried skim milk	-	-	57.9
Finely ground corn	48.0	-	-
Cerelose	-	39.5	23.5
Corn distillers dried solubles	10.0	10.0	10.0
Dried whey	5.0	5.0	5.0
Aurofac D[1]	1.0	1.0	1.0
Dicalcium phosphate	2.0	2.0	2.0
Salt	0.5	0.5	0.5
Vitamin and mineral mixture[2]	0.1	0.1	0.1

[1]Contained 5.0 gm. Aureomycin and appreciable Vitamin B_{12} per lb.

[2]Mixture contained:

Vitamin A concentrate 20,000 U.S.P. units/gm.	20 gm.
Irradiated yeast, 9,000 I.U. Vitamin D/gm.	5 gm.
Cobalt Chloride	1.5 gm.
Cupric sulfate	1.25 gm.
Ferrous sulfate	6 gm.

Table 2. Chemical composition of the feeds used in the two experiments[1]

Feed	Dry matter	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract	Ash
	(%)	(%)	(%)	(%)	(%)	(%)
Replacer 1	84.9	27.6	1.5	2.1	47.3	6.4
Replacer 2	90.7	26.8	3.9	1.3	52.6	6.1
Replacer 3	94.1	24.8	1.3	0.4	59.2	8.4
Starter	88.0	18.4	3.5	4.7	56.1	5.3
Alfalfa hay	86.8	16.0	1.4	29.8	32.5	7.1

[1]The feeds were analyzed using accepted A.O.A.C. (1950) procedures.

placed on experiment as they became available throughout the year with the exception that no calves were on experiment during the summer months. The calves were randomly assigned to the various experimental groups. Any animal that was apparently sick or abnormal during the first days of the experiment was removed and replaced by the next animal of the same sex and relative weight.

The calves were left with their dams for the first 48 hours. They were then weighed and placed into individual pens bedded with wood shavings. The milk replacers were fed as a gruel using the amounts of milk and/or water

indicated in the feeding schedule (Table 3). The treated milk replacers were fed through 40 days of age in order

Table 3. Daily feeding schedule

Age (days)	Whole milk (lb.)	Milk replacer (lb.)	Water (lb.)
0-2	with dam	-	-
3-5	6	0.2	-
6-9	4	0.5	4
10-20	2	0.8	8
21-40	-	1.0	12
41-60	-	-	ad libitum

to be certain that all calves were past the critical period when milk replacer feeding was terminated. The calves were kept on experiment an additional 20 days to determine if there were any carry-over effects from the treatments. Milk Replacer 1 in dry form was fed ad libitum to all calves after 7 days of age and was the only feed used during the last 20 days of the experiment.

Body weights were obtained at the beginning of the experiment, at 40 and 60 days of age, and at weekly intervals throughout the experiment. The weights presented in the results for 20 days of age were obtained by interpolation. The amounts of feed fed to the individual calves

were recorded daily. Refusals of milk and milk replacer were recorded at the time of feeding. Unconsumed dry feed was weighed and discarded at the same times that body weights were taken.

The feed consumption and growth data for the various 20-day periods, the period from 3 to 40 days, and the period from 3 to 60 days were subjected to the usual methods of analysis of variance (Snedecor, 1956). In the few cases in which a calf died before the end of the experiment, a missing value was calculated following the method outlined by Snedecor (1956). In the event that significant differences were found, the individual differences between means were tested by means of a multiple range test (Duncan, 1955).

Because of the inconclusive (in the case of malt diastase) and unsatisfactory (in the case of papain) results of the first experiment, a second experiment was carried out in order to further evaluate the use of supplemental enzymes with milk replacers. Three milk replacers (Table 1) were fed with and without 0.5% pepsin.[1] Pepsin was chosen because (1) it normally occurs in the digestive tract and, therefore, would not be toxic and (2)

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[1] 1:3000 N. F., produced by Cudahy Laboratories, Omaha, Neb.

it was the enzyme that showed the most promise in the pig studies (Lewis et al., 1955a). The level of pepsin used was the one which had been recommended for use with pigs (Lewis et al., 1955a). The milk replacers were not predigested in order to eliminate another possible cause of the unsatisfactory results obtained from papain.

If malt diastase was effective in the first experiment one would expect as great or greater effect from the use of the end-product of carbohydrate digestion. Therefore, Milk Replacer 2 (Table 1) was formulated in which the corn was replaced by cerelose. Milk Replacer 3 utilized skim milk as the primary source of protein. Its purpose was to serve as a positive control.

A starter[2] and second-cutting alfalfa hay were fed ad libitum to all calves after 7 days of age. This change from the first experiment was made in order to conform more closely with the usual methods of feeding calves.

The body weights were taken at five day intervals throughout this experiment.

With the above exceptions the second experiment was conducted in the same manner as the first experiment.

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[2]Composed of 42.8% ground corn, 31.3% crimped oats, 13.3% soybean oil meal, 10.0% distillers solubles, 1.0% dicalcium phosphate, 1.0% salt, 0.5% Aurofac 2A, and 0.1% vitamin A and D mix.

RESULTS

The results of the two experiments are presented in Tables 4 and 5, respectively. Although data had been collected at 5-day intervals, it was not practical to use these detailed data because of relatively large errors inherent in the measurement of body weight. The use of 20-day intervals for the presentation of the data was arbitrarily adopted because the relative error in body weight measurement was reduced to a reasonable size and because these intervals coincided with the most radical changes in the feeding regime. It is recognized that this arbitrary treatment of the data may tend to cover up some of the trends that were present in the more detailed data. Therefore these detailed data will be discussed in those cases where it is felt that it would make important contributions to the interpretation of the data.

Milk and Milk Replacer Consumption

The consumption of whole milk and milk replacer was similar for all groups in both experiments (Tables 4 and 5). This would be expected because of the controlled feeding regime that was used. The small variations were caused by occasional feed refusals by a few calves. In general,

Table 4. The results of the first experiment

	Control	Malt diastase	Papain	Combination	Malt diastase		Papain	
					With	Without	With	Without
	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)
Weight								
Average Initial	93.7	95.0	93.3	90.8	92.9	93.5	92.2	94.4
Average Gain								
3 to 20 days[b]	0.8	2.8	0.0	1.2	4.0	0.8	1.2	3.7
21 to 40 days[b]	13.5	15.2	3.8	12.2	13.7[a]	8.7[a]	8.0[a]	14.3
41 to 60 days[c]	23.3	39.1	26.5[a]	27.8[a]	34.0[a]	24.6[a]	27.3[a]	31.3
3 to 40 days[b]	14.3	18.0	3.8	13.3	15.7	9.1	8.6	16.2
3 to 60 days[d]	37.6	57.1	34.5[a]	42.2[a]	50.4[a]	36.4[a]	38.7[a]	47.4
Average total feed consumption								
Whole milk	54.3	53.0	54.3	52.9	53.0	54.3	53.6	53.7
Treated milk replacer	30.3	29.9	30.2	29.7	29.8	30.3	30.0	30.1
Untreated milk replacer								
3 to 20 days[b]	6.5	6.3	4.2	4.8	5.6	5.3	4.5	6.4
21 to 40 days[e]	27.5	25.2	13.8	14.2	19.7[a]	20.7[a]	14.0[a]	26.3
41 to 60 days[f]	57.2	71.3	45.8[a]	64.2[a]	69.0[a]	52.6[a]	56.0[a]	64.3
3 to 40 days[g]	34.0	31.5	18.0	19.0	25.3	26.0[a]	18.5	32.8
3 to 60 days[h]	91.1	102.8	68.5[a]	85.8[a]	95.0[a]	82.1[a]	78.1[a]	97.0

[a] These values are for the surviving calves.
[b] No significant differences.
[c] With malt diastase > without malt diastase. ($P < 0.05$).
[d] With malt diastase > without malt diastase. ($P < 0.05$).
[e] Without papain > with papain. ($P < 0.01$).
[f] With malt diastase > without malt diastase. ($P < 0.01$).
[g] Without papain > with papain. ($P < 0.01$).
[h] Without papain > with papain. ($P < 0.05$).

Table 5. The results of the second experiment

	Replacer 1		Replacer 2		Replacer 3		Replacer			Pepsin	
	Control	Pepsin	Control	Pepsin	Control	Pepsin	1	2	3	With	Without
	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)
Weight											
Av. initial	88.7	95.8	89.7	89.2	87.8	90.8	92.3	89.5	89.3	91.9	88.7
Av. gain											
3 to 20 days[b]	6.8	3.2	6.8	8.5	13.3	13.5	5.0	7.7	13.4	9.0	8.4
21 to 40 days[c]	16.2	23.8	9.8[a]	16.0	23.3	25.0	20.0	12.9[a]	24.2	21.6	16.4[a]
41 to 60 days[d]	29.2	24.8	22.0[a]	23.7	24.8	27.2	27.0	23.0[a]	26.0	25.2	25.6[a]
3 to 40 days[e]	23.0	27.0	16.7[a]	24.5	36.7	38.5	25.0	20.6[a]	37.6	30.0	25.4[a]
3 to 60 days[f]	52.2	52.2	45.8[a]	48.2	61.5	65.7	52.2	47.2[a]	63.6	55.3	54.1[a]
Av. total feed consumption											
Whole milk	60.0	56.5	57.4	55.2	56.0	55.8	58.3	56.3	55.9	55.8	57.8
Milk replacer	30.3	30.6	30.6	30.4	30.6	30.5	30.5	30.5	30.6	30.5	30.5
Starter											
3 to 20 days[d]	2.5	2.4	1.5	1.0	2.0	2.2	2.5	1.2	2.1	1.8	2.0
21 to 40 days[g]	20.7	29.8	11.2[a]	11.0	20.2	18.3	25.3	11.1[a]	19.3	19.7	17.4
41 to 60 days[d]	48.6	55.0	41.6[a]	44.6	56.7	48.4	51.8	43.4[a]	52.5	49.3	49.9[a]
3 to 40 days[h]	23.3	32.2	12.7[a]	12.0	22.2	20.5	27.7	12.3[a]	21.3	21.5	19.4[a]
3 to 60 days[d]	71.9	87.5	57.4[a]	56.6	78.9	69.1	79.7	56.9[a]	74.0	71.1	70.9[a]
Hay											
3 to 20 days[d]	0.9	0.9	0.6	0.8	0.5	1.0	0.9	0.7	0.8	0.9	0.6
21 to 40 days[d]	10.2	9.1	6.9[a]	12.0	8.0	14.4	9.6	9.5[a]	11.2	11.8	8.4[a]
41 to 60 days[d]	16.4	18.6	19.5[a]	20.4	14.0	24.2	17.5	20.0[a]	19.1	21.0	16.3[a]

Table 5. Continued

	Replacer 1		Replacer 2		Replacer 3		Replacer			Pepsin	
	Control	Pepsin	Control	Pepsin	Control	Pepsin	1	2	3	With	Without
	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)
Hay (continued)											
3 to 40 days[d]	11.0	9.9	7.5[a]	12.8	8.4	15.4	10.5	10.2[a]	11.9	12.7	9.0[a]
3 to 60 days[d]	27.4	28.5	30.5[a]	33.3	22.3	39.7	28.0	32.2[a]	31.0	33.8	26.3[a]

[a] These values are for the surviving calves.
[b] Replacer 3 > Replacers 1 and 2, ($P < 0.05$).
[c] Replacers 1 and 3 > Replacer 2, ($P < 0.01$); With pepsin > without pepsin, ($P < 0.05$).
[d] No significant differences.
[e] Replacer 3 > Replacers 1 and 2, ($P < 0.01$).
[f] Replacer 3 > Replacer 2, ($P < 0.05$).
[g] Replacer 1 > Replacer 2, ($P < 0.05$).
[h] Replacer 1 > Replacer 2, ($P < 0.05$).

acceptability of the milk replacers was not a problem in these experiments.

Starter Consumption[1]

In both experiments the level of starter consumption was low for all calves through 20 days of age (Tables 4 and 5). The differences among the various groups were not statistically significant in either experiment.

In the first experiment the use of papain decreased starter consumption to a considerable degree. This decrease was statistically significant during the period 21 through 40 days and for the total consumption through 40 days. Following 40 days the consumption of starter followed a trend similar to that of the preceding period. However, the difference was of smaller magnitude and was not significant. For the entire experiment the calves receiving papain consumed significantly less starter than the other calves.

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[1]For convenience when referring to the first experiment, the term "milk replacer" refers to the treated milk replacer that was fed as a gruel and the term "starter" refers to the untreated milk replacer that was fed in the dry form. From a functional standpoint, the untreated milk replacer was used as a starter in this experiment. The composition of Milk Replacer 1 was the same as the starter used in the second experiment with the exception of the use of finely ground corn and soybean flour in place of coarsely ground corn, crimped oats, and soybean oil meal.

Malt diastase had no effect on starter consumption during the periods in which it was used (through 40 days of age). However, during the period from 41 through 60 days, the calves that had received malt diastase consumed significantly more starter than the other calves. The increased starter consumption by these calves in the complete experiment was completely accounted for by the increase in the last period and was not significant.

In the second experiment the calves receiving pepsin consumed approximately the same amount of starter as the control calves. The small differences were not significant in any of the periods considered.

The rate of starter consumption from 21 through 40 days of age appeared to be inversely related to the level of cerelose in the milk replacer. The results of the statistical analysis revealed that only the difference between the calves receiving Milk Replacer 1 and the calves receiving Milk Replacer 2 was significant. Similar results were obtained when the total consumption through 40 days was considered. From 41 through 60 days the rate of starter consumption by the calves that had received Milk Replacers 1 and 3 was approximately the same and was somewhat lower for the calves that had received Milk Replacer 2. However, this difference was not significant. Similar relationships were obtained when the starter consumption for the entire

experiment was considered and these differences were likewise not significant.

Hay Consumption

Hay consumption by the individual calves of the second experiment varied from 0 to 55 lbs. and did not appear to be related to starter consumption or weight gain (Table 5). The differences among the various groups were not significant in any of the periods.

Weight Gain

In general, the rate of gain by the various groups in the first experiment tended to follow trends similar to the rates of starter consumption (Table 4). In all periods the calves that had received papain exhibited a lower rate of gain than the calves that had not received papain and the calves that had received malt diastase exhibited a higher rate of gain than the calves that had not received malt diastase. These differences were not statistically significant with the exceptions of the differences due to malt diastase from 41 to 60 days and for the complete experiment.

In the second experiment the use of pepsin produced no significant effect on the rate of gain during the first and last periods of the experiment. However, from 21

through 40 days the rate of gain for the calves receiving pepsin was significantly greater than the rate for the control calves. A large part of this difference was accounted for by two calves in the control group that died shortly after the end of this period. The differences for the total gain through 40 days and through 60 days were not significant.

The calves that had received skim milk (Replacer 3) exhibited a rate of gain approximately equal to the normal growth rate for Holstein calves (Matthews and Fohrman, 1954). The calves receiving the two vegetable replacers exhibited the typical critical period. From 3 to 20 days the calves receiving Replacer 3 gained significantly more weight than the other replacer groups. From 21 to 40 days the difference between Replacers 1 and 3 was not so pronounced and was not significant. The rate of gain by the Replacer 2 group was significantly lower than the rates for the other groups in this period. When the data for these periods was combined it was found that the rate of gain for the Replacer 3 group was significantly greater than the rates for the other groups.

The rates of gain for the three milk replacer groups did not vary greatly in the last period and the differences among the groups were not significant. When the entire

experiment was considered the only significant difference among the groups was the difference between Replacers 2 and 3.

Mortality

In the first experiment two calves from the group that had received papain alone and one calf from the group that had received the combination of enzymes died after 40 days of age. The history of all cases was similar. These calves consumed little or no starter up to 40 days of age. After the feeding of milk replacer was terminated at 40 days, the calves still refused to consume significant amounts of starter and all efforts to get them to do so failed. The calves became extremely emaciated and died within 3 to 10 days. At necropsy the adrenal cortex of all three calves showed hemorrhage and/or necrosis. Mild hyperemia and some hemorrhages were noted in the digestive tract. At the present time these lesions should be considered to be of a non-specific nature. They were probably caused by the long period of reduced nutrient intake preceding death (Follis, 1948).

In the second experiment two calves fed Milk Replacer 2 without pepsin died under circumstances similar to those in the first experiment. However, at necropsy no gross lesions were found.

DISCUSSION

Most of the increased gain by the calves that had received malt diastase occured after 40 days of age when the calves no longer received the enzymatically treated milk replacer. This period of increased gain coincided with a period of significantly increased starter intake. While it is possible that the increased starter consumption and weight gain was due to some indirect effect of the use of malt diastase, this possibility should be discounted until there is more evidence to support it. This is particularly true in view of the studies which have been reported since the completion of this experiment which indicate that malt diastase could not be expected to be effective under conditions of this experiment.

Ratcliff *et al.* (1957) found no growth response from feeding animal diastase to calves. However, in this study the enzyme was not used until the calves were 28 days of age at which time they should have been past the critical period. In studies more comparable to the present one, Dollar and Porter (1957) found that predigestion of oat flour and flaked corn with alpha- and beta-amylase did not improve these feeds for calves through three weeks of age. Chromatographic examination of the digestion mixture showed

that the primary end-product was maltose. From studies using the blood sugar response method, Dollar and Porter (1957) concluded that glucose and lactose are the only carbohydrates that can be utilized by the young calf. The ability to utilize lactose decreased with age. Ingestion of sucrose, maltose, dextrin and soluble starch gave no blood sugar response. They also examined the amylolytic activity of the pancreas and the maltase, lactase and sucrase activities of the intestinal mucosa. The results gave good correlation with the blood sugar findings: lactase activity was high in the very young calf, but that of amylase and maltase was very low; the activity of amylase and maltase increased with age while that of lactase decreased; no sucrase activity was detected. Larson et al. (1956) found that maltose is readily hydrolysed and absorbed when introduced posterior to the rumen of a nine-months-old bovine, but they were unable to demonstrate the digestion of starch. It may be possible that the amylolytic enzyme systems of the bovine never become well developed because with a normally functioning rumen very little starch is presented to the lower portions of the digestive tract.

In studies on the carbohydrate utilization in the young pig using the blood sugar response method, Dollar et al. (1957) obtained results identical to those obtained in

calves. In contrast, Cunningham and Brisson (1957a, 1957c) reported that starch and maltose digestion in the two-day-old pig was practically complete. However, as Dollar and Porter (1957) have pointed out, "(digestibility studies) are clearly misleading as they ignore the contribution of the bacterial flora of the large gut to the degradation of carbohydrate--a process that does not necessarily contribute to the nutrition of the host."

It follows from the above findings that predigestion of cereal starches by amylases must be complemented by the action of maltase to complete the degradation to glucose before they can be utilized by the young calf. Therefore, it would appear that the improved gain obtained from the calves that had received malt diastase was not due to the action of the enzyme.

Since cerelose is composed largely of glucose, its use in the second experiment should have overcome the deficiency of a utilizable carbohydrate in the diet. The lack of significant improvement in gain through 20 days of age indicates that the lack of an available energy source is not the sole deficiency in the corn-soybean flour milk replacer. If a milk replacer composed of an animal protein (i.e., casein) and corn had been used, it would have been expected to provide some information on whether available protein is the sole deficiency. However, Dollar and

Porter (1957) have employed this type of diet with unsatisfactory results which indicate that both protein and energy are lacking in the corn-soybean flour milk replacer.

The results from 21 to 40 days of age indicate that the use of high levels of cerelose with plant protein is unsatisfactory. The large depression of weight gain by the Replacer 2 can be largely accounted for by the depressed starter intake compared to the Replacer 1 group. While the consumption of starter by the calves receiving Replacer 3 was somewhat depressed when compared to the calves receiving Replacer 1, this depression was not important because of the superior ingredients contained in the milk replacer. The recovery of appetite by the calves that had received the high level of cerelose after the termination of milk replacer feeding lends further support to the conclusion that high levels of cerelose depress appetite.

The glucostatic theory of the regulation of food intake (Mayer, 1953) provides a means of explaining this appetite depression. The glucose in Replacer 2 would be readily absorbed and would cause elevated blood sugar levels for some time after feeding. In Replacer 3 a considerable portion of the carbohydrate was lactose which does not give as great an increase in blood sugar as glucose in calves of this age. Most of the carbohydrate in Replacer 1 was starch which should give no increase in blood sugar

level.[1] It follows that one would expect the greatest starter consumption by the calves that received Replacer 1, an intermediate consumption by the calves that received Replacer 3, and the least consumption by the calves that received Replacer 2. This is in agreement with the results of the experiment.

The use of papain in the first experiment appears to have produced deleterious effects. This is indicated by the depressed growth rate, the significantly depressed starter consumption, and the higher mortality rate. Further support for this conclusion is provided by the recovery of appetite and the relative improvement in rate of gain by the surviving calves after the termination of papain use. These results are in agreement with those of Williams and Knodt (1952).

The cause of these deleterious effects cannot be determined from the results of this experiment or from the literature. Lewis et al. (1955a) used papain at the rate of 1% of the diet of young pigs without the production of adverse effects. No toxic effects were found in laboratory animals when it was fed at much higher rates than were used in this study (Weiner, 1955). It may be possible that the deleterious effects are dependent upon the specie

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[1]Results of blood glucose determinations which are not included in this thesis are in agreement with this.

used. Another possibility is that these effects were in some way associated with the predigestion process. In this regard the results of the study by Cunningham and Brisson (1957b) on the use of pepsin in baby pig diets is of some interest. They found that soybean protein predigested with pepsin resulted in severe diarrhea and death of 2-day-old pigs. When the diet was not predigested there was no adverse effect from the use of pepsin.

The effect of pepsin in the second experiment cannot be evaluated with certainty on the basis of the available data. Although the rate of gain was increased significantly from 21 to 40 days, most of this increase can be attributed to the low rate of gain of the two calves in the control group which died shortly after the end of this period. The rate of gain of these calves was the lowest of all calves during this period. In addition, starter consumption by these calves was very low which would indicate that they were not normal. At the present time it is not possible to attribute these deaths to the diet or to suggest a mechanism by which pepsin could have prevented the deaths. In addition to these considerations, if pepsin were effective in improving weight gains, one would have expected as much or more improvement in the period preceding 20 days of age. This improvement was not obtained. Therefore, the most reasonable conclusion at the present

time is that pepsin was not effective in improving the rate of gain.

This conclusion is in agreement with the more recently reported work with calves (Ratcliff et al., 1956) and pigs (Alsmeyer et al., 1957; Conrad and Beeson, 1957; Cunningham and Brisson, 1957b; Hanson, 1957).[1] It is not in agreement with the earlier pig work reported by the Iowa group (Baker et al., 1956; Lewis et al., 1955a; Lewis et al., 1955b).

The low production of hydrochloric acid and the specificity of the various proteolytic enzymes provide two possible explanations for the ineffectiveness of pepsin in most studies. In the case of the pig it has been reported that the pH of the stomach contents was never lower than 3.5 and it was usually nearer 4.0 (Lewis et al., 1955a). Since the pH optimum for pepsin activity is from 1.5 to 2.0 and since pepsin is relatively inactive at pH 4 (Fruton and Simmonds, 1953), it would not appear likely that pepsin could improve food utilization in the young pig through its proteolytic activity.

There is no information available concerning the hydrochloric acid production by the abomasum of the young

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[1] In addition to the negative results cited, a number of other studies yielding essentially the same results have come to the author's attention, either by personal communication or by the popular press.

calf. However, since a number of other species have yielded essentially the same results as the pig it is possible that a similar situation exists in the calf.

A second explanation is analogous to that discussed in regard to the ineffectiveness of supplemental amylases. From the specificity of the various proteolytic enzymes (Fruton and Simmonds, 1953), it follows that the end-products of digestion by an individual enzyme would be a mixture of peptides. These peptides would not be utilized unless the animal possessed the other enzymes necessary to complete the degradation of the peptides to a utilizable form. Therefore, the use of supplemental pepsin could be expected to be effective only if it or an enzyme of similar specificity was the only enzyme deficient.

Evidence supporting this possibility is lacking because the studies concerning enzyme production in the young have not dealt with specific enzymes and because little is known about the amino acid sequence of different proteins. Since the proteins of milk are almost completely digested by the young animal, it must follow that the young animal has a sufficient quantity of all enzymes required for the complete degradation of milk proteins. It is possible that some of the specific enzymes required for complete degradation of plant proteins are different than those required for milk proteins and that these enzymes

are deficient in the suckling animal. In this regard it has been shown that pancreatic and peptic digests of plant proteins yield end-products which differ considerably from those obtained from animal proteins (Birk and Bondi, 1956; Bondi and Birk, 1956). In the plant protein digests a considerable quantity of peptides were precipitated by trichloroacetic acid. These peptides were not obtained from animal protein digests.

In conclusion, it would appear from a considerable amount of direct and circumstantial evidence that animals at birth are deficient in many if not all of the digestive enzymes except those required for the complete degradation of the various components of milk. Therefore, any attempt to develop a feeding regime excluding milk would necessitate the use of ingredients which can be completely degraded by the enzymes present or the provision of all of the additional enzymes required for the degradation of the given ingredient together with the necessary conditions for the action of these enzymes (pH, etc.). It would appear that most studies including the present ones have not met these conditions.

SUMMARY

Two experiments were carried out to determine the effect of supplementing milk replacers with enzymes. In the first experiment a corn-soybean flour milk replacer was predigested with malt diastase, papain, and a combination of these enzymes. Fifty-five pounds of milk were fed through 20 days of age and 30.5 lb. of the treated milk replacer were fed through 40 days of age. The experiment was terminated at 60 days. Decreased starter consumption and growth from 20 to 40 days and an increased mortality rate indicated that papain produced deleterious effects. Malt diastase produced no improvement in gain during the periods it was used.

In the second experiment, soybean flour-corn, soybean flour-cerelose, and skimmilk-cerelose milk replacers were supplemented with pepsin but were not predigested. All other aspects of the experiment were similar to the first experiment. It was concluded that pepsin produced no improvement in growth with any of the replacers. The skimmilk-cerelose replacer produced significantly greater weight gains than the other replacers during the first 20-day period. In the second 20-day period the soybean flour-cerelose replacer produced significantly smaller gains than

the other replacers. Starter consumption by this group was also significantly depressed.

From considerations of the literature and the results of the present experiments it appears that young animals possess only those enzymes which are necessary for the degradation of the components of milk. Future use of enzymes in diets replacing milk will require a greater consideration of enzyme specificity and optimum conditions for enzyme activity than has been used in previous experiments.

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APPENDIX

Appendix Table 1. Summary of analysis of variance: The first experiment

Source of variation	Degrees of freedom	0-20 days		21-40 days		41-60 days		0-40 days		0-60 days	
		MS[a]	F	MS	F	MS	F	MS	F	MS	F
		Weight gain									
Total	23[b]										
Malt diastase	1	15.0	0.08	150	1.53	523	4.47[c]	260	2.24	1520	5.87[c]
Papain	1	9.4	0.05	240	2.45	171	1.46	345	2.97	1001	3.86
Malt diastase x papain	1	1.0	0.01	67	0.68	253	2.16	52	0.45	77	0.30
Residual	20[b]	177.3		98		117		116		259	
		Untreated milk replacer									
Total	23[b]										
Malt diastase	1	0.4	0.04	6	0.06	1700	8.50[d]	4	0.03	1552	3.34
Papain	1	22.1	2.15	912	10.02[d]	748	3.74	1219	8.96[d]	3876	8.36[c]
Malt diastase x papain	1	1.0	0.10	11	0.12	43	0.22	18	0.13	117	0.25
Residual	20[b]	10.3		91		200		136		465	

[a]Mean square.

[b]These degrees of freedom are reduced by three for 41 to 60 days and 0 to 60 days because of the missing values that were calculated for the three calves that died.

[c]Significant, ($P < 0.05$).

[d]Significant, ($P < 0.01$).

Appendix Table 2. Summary of analysis of variance: The second experiment

Source of variation	Degrees of freedom	0-20 days		21-40 days		41-60 days		0-40 days		0-60 days	
		MS[a]	F	MS	F	MS	F	MS	F	MS	F
		Weight gain									
Total	35[b]										
Milk replacer	2	222	5.29[c]	388	6.81[d]	124	0.70	934	9.83[d]	1468	3.52[c]
Pepsin	1	4	0.10	240	4.21[c]	11	0.06	187	1.97	307	0.74
Milk replacer x pepsin	2	23	0.55	30	0.53	74	0.42	28	0.29	140	0.34
Residual	30[b]	42		57		177		95		417	
		Starter consumption									
Total	35[b]										
Milk replacer	2	4.62	1.78	607	4.60[c]	392	0.93	717	4.78[c]	2073	2.16
Pepsin	1	0.25	0.25	49	0.37	6	0.01	43	0.29	95	0.10
Milk replacer x pepsin	2	0.41	0.16	104	0.79	198	0.47	102	0.68	487	0.51
Residual	30[b]	2.59		132		420		150		961	
		Hay consumption									
Total	35[b]										
Milk replacer	2	0.08	0.12	11	0.25	15	0.16	11	0.22	46	0.17
Pepsin	1	0.51	0.76	108	2.55	359	3.86	123	2.41	916	3.39
Milk replacer x pepsin	2	0.26	0.39	48	1.12	49	0.53	54	1.06	176	0.65
Residual	30[b]	0.67		43		93		51		270	

[a]Mean square.

[b]These degrees of freedom are reduced by two for 41 to 60 days and 0 to 60 days because of the missing values that were calculated for the two calves that died.

[c]Significant, ($P < 0.05$).

[d]Significant, ($P < 0.05$).

Appendix Table 3. Data for the individual calves: The first experiment.

	Calf number											
	1	4	5	15	24	33	2	3	13	19	22	31
	Control						Malt diastase					
Sex	M	M	M	M	F	M	M	M	M	F	M	M
Weight												
Initial	111	84	89	93	87	98	100	100	92	85	105	88
Gain												
0 to 20 days	-7	6	5	0	2	-1	-4	3	7	0	5	6
21 to 40 days	16	5	26	9	22	3	2	13	28	11	28	9
41 to 60 days	40	12	32	10	42	4	50	39	33	24	33	44
0 to 40 days	9	11	31	9	24	2	-2	16	35	11	33	15
0 to 60 days	49	23	63	19	66	6	48	55	78	35	66	61
Total feed consumption												
Whole milk	56.0	56.0	58.0	58.0	44.0	54.0	56.0	52.0	48.0	43.0	64.0	55.0
Treated milk replacer	30.5	30.5	30.2	30.2	29.5	30.7	30.5	29.0	30.3	29.7	29.2	30.5
Untreated milk replacer												
0 to 20 days	5.0	7.0	5.0	8.0	10.0	4.0	4.0	15.5	3.0	3.5	9.0	4.5
21 to 40 days	25.0	26.0	31.0	25.0	40.0	18.0	14.0	34.5	42.0	10.5	32.0	19.5
41 to 60 days	79.0	35.5	66.5	44.5	79.0	36.5	74.5	70.0	69.0	59.5	72.0	82.0
0 to 40 days	30.0	33.0	36.0	33.0	50.0	22.0	18.0	45.0	45.0	14.0	43.0	24.0
0 to 60 days	109.0	68.5	102.5	77.5	129.0	58.5	92.5	115.0	114.0	73.5	115.0	106.0

Appendix Table 3. Continued.

	Calf number											
	6	7[a]	11	14[b]	25	26	8	9	10[c]	21	23	32
	Papain						Combination					
Sex	M	M	F	M	M	M	M	F	M	M	M	F
Weight												
Initial	92	78	98	97	87	108	98	81	78	90	122	76
Gain												
0 to 20 days	3	0	-2	1	-4	2	3	0	8	-7	0	3
21 to 40 days	5	-3	10	-7	-3	21	20	10	0	24	14	5
41 to 60 days	26	-	30	-	26	24	23	30	-	35	27	24
0 to 40 days	8	-3	8	-6	-7	23	23	10	8	17	14	8
0 to 60 days	34	-	38	-	19	47	46	40	-	52	41	32
Total feed consumption												
Whole milk	56.0	52.0	54.0	56.0	52.0	56.0	55.0	56.0	56.0	43.5	53.0	54.0
Treated milk replacer	30.5	30.0	29.8	30.5	29.9	30.5	29.8	30.5	30.5	27.9	30.1	29.4
Untreated milk replacer												
0 to 20 days	6.5	3.5	6.0	0.0	3.0	7.0	6.5	2.5	4.5	3.0	9.0	5.0
21 to 40 days	16.5	11.5	14.0	2.0	11.0	27.0	24.5	5.5	2.5	13.0	26.0	13.0
41 to 60 days	58.5	-	39.5	-	50.0	33.5	65.0	62.5	-	73.5	73.5	45.0
0 to 40 days	23.0	15.0	20.0	2.0	14.0	34.0	31.0	8.0	6.0	16.0	35.0	18.0
0 to 60 days	81.5	-	59.5	-	64.0	67.5	96.0	70.5	-	89.5	108.5	63.0

[a]Died at 51 days of age.
[b]Died at 43 days of age.
[c]Died at 46 days of age.

Appendix Table 4. Data for the individual calves: The second experiment.

	Milk Replacer 1											
	11	26	32	34	43	69	3	27	30	39	50	52
	Control						Pepsin					
Sex	M	M	F	M	M	M	M	M	M	F	F	M
Weight												
Initial	92	119	75	81	80	85	75	94	109	104	87	106
Gain												
0 to 20 days	11	9	4	6	1	10	9	5	2	-12	7	9
21 to 40 days	14	18	20	16	12	17	3	23	40	34	18	25
41 to 60 days	38	28	32	30	19	28	-4	41	40	25	11	38
Total feed consumption												
Whole milk	57.0	57.0	55.0	77.0	57.0	57.0	57.0	53.0	57.0	58.0	57.0	57.0
Milk replacer	30.7	30.7	30.5	28.7	30.7	30.7	30.7	30.2	30.7	30.5	30.7	30.7
Starter												
0 to 20 days	0.5	2.5	0.5	1.0	3.0	7.0	2.5	5.5	2.0	0.0	1.0	3.0
21 to 40 days	10.5	28.0	15.0	19.0	25.0	27.5	6.0	43.5	49.0	33.5	7.0	42.5
41 to 60 days	64.0	46.0	43.5	48.0	49.0	41.5	13.5	73.5	93.5	63.5	20.0	67.0
Alfalfa hay												
0 to 20 days	0.5	0.5	2.5	0.0	0.0	1.0	0.5	1.0	0.0	0.5	2.0	0.5
21 to 40 days	10.5	15.5	22.5	3.0	2.0	8.0	4.5	6.0	2.5	13.5	22.5	6.0
41 to 60 days	14.0	26.0	31.5	10.0	3.0	13.0	20.5	10.5	21.5	14.0	31.0	15.5

Appendix Table 4. Continued.

	Milk Replacer 2											
	4[a]	25	29	40	47	53[b]	6	22	24	31	51	56
	Control						Pepsin					
Sex	M	M	F	M	F	M	M	F	M	M	F	M
Weight												
Initial	82	91	90	95	82	98	110	84	86	90	82	83
Gain												
0 to 20 days	3	11	23	7	2	-5	11	7	13	6	10	4
21 to 40 days	1	8	16	20	8	5	31	13	7	23	14	8
41 to 60 days	-	13	18	34	23	-	40	26	22	32	19	3
Total feed consumption												
Whole milk	57.5	57.0	59.0	57.0	57.0	57.0	57.0	51.0	56.0	55.0	57.0	57.0
Milk replacer	30.2	30.7	30.4	30.7	30.7	30.5	30.7	29.9	29.9	30.5	30.7	30.7
Starter												
0 to 20 days	3.0	0.0	2.5	1.0	0.5	2.0	3.5	1.0	0.0	0.5	0.5	0.5
21 to 40 days	1.0	8.5	30.0	19.5	1.0	7.0	22.5	12.5	3.0	14.0	10.0	4.0
41 to 60 days	-	23.5	59.0	50.0	34.0	-	79.5	36.5	34.5	63.0	31.0	22.5
Alfalfa hay												
0 to 20 days	0.5	0.0	0.0	2.0	1.0	0.0	0.5	1.0	2.5	0.0	0.5	0.0
21 to 40 days	2.5	6.0	0.0	15.0	20.0	0.0	7.0	15.0	18.5	11.0	14.5	6.0
41 to 60 days	-	22.5	0.5	23.0	32.5	-	19.0	24.5	28.0	13.0	26.0	13.0

[a]Died at 50 days of age.
[b]Died at 59 days of age.

Appendix Table 4. Continued.

	Milk Replacer 3											
	7	8	20	21	44	59	12	18	23	28	45	55
	Control						Pepsin					
Sex	M	M	F	M	M	M	M	M	M	F	M	M
Weight												
Initial	106	99	80	64	84	94	76	119	99	81	106	64
Gain												
0 to 20 days	17	10	16	16	13	8	19	7	21	15	2	17
21 to 40 days	23	26	21	18	24	28	32	26	27	19	20	26
41 to 60 days	21	33	26	16	43	10	25	44	36	3	32	23
Total feed consumption												
Whole milk	57.0	55.0	53.0	57.0	57.0	57.0	57.0	52.0	57.0	55.0	57.0	57.0
Milk replacer	30.7	30.5	30.3	30.7	30.7	30.7	30.7	30.4	30.7	29.9	30.7	30.7
Starter												
0 to 20 days	4.5	1.0	1.0	1.5	1.0	2.5	1.5	3.0	3.0	1.5	0.5	3.0
21 to 40 days	19.0	20.0	12.5	14.5	20.0	35.5	7.0	28.0	35.0	7.0	11.5	22.0
41 to 60 days	70.5	61.5	47.5	39.5	74.5	47.0	48.0	70.0	72.5	15.5	39.0	46.5
Alfalfa hay												
0 to 20 days	0.5	0.0	0.0	0.5	1.5	0.0	1.5	2.5	1.5	0.5	0.5	0.5
21 to 40 days	9.5	10.5	7.0	5.0	16.5	0.0	16.0	15.0	16.0	19.5	13.5	6.0
41 to 60 days	16.0	24.5	18.5	5.0	19.0	0.0	27.5	30.0	23.0	28.5	24.0	12.5