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Rumen By-Pass of Protein
Through Esophageal Groove Closure
in Lactating Cows

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Frank E. Standaert

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M.S. degree in Dairy Science

J. T. Huber
Major professor

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RUMEN BY-PASS OF PROTEIN THROUGH ESOPHAGEAL GROOVE CLOSURE IN LACTATING COWS

By

Frank E. Standaert

A THESIS

Submitted to
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in partial fulfillment of the requirement
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ABSTRACT

RUMEN BY-PASS OF PROTEIN
THROUGH ESOPHAGEAL GROOVE CLOSURE
IN LACTATING COWS

By

Frank E. Standaert

Nineteen Holstein heifers, (22-23 mo), were trained in two trials to suckle fluids from a nipple pail. In both trials, animals were forced fed fluids daily through a nipple pail after a 10-12 h withdrawal from water. As animals began actively suckling, water was gradually re-introduced until free choice. Training periods lasted 2-3 weeks after which most animals would suckle. Several single crossover design trials with two 1-day periods were used to determine rumen by-pass. Blood glucose tolerance curves were compared for animals that suckled a 500g glucose solution to those which received 500g glucose directly in the rumen. In all trials, mean serum glucose levels at 1 and 2 hours after glucose load was greater ($P<.05$) for the suckled animals, though a few heifers showed no rise in blood glucose after suckling. Six animals which maintained the suckling habit were used in a single cross over experiment to determine the effect of suckling milk on milk and milk protein production. The suckled group was offered sufficient whole milk to furnish 500g milk protein daily but mean intake was

only 320g. Control animals received 500g casein daily in the concentrate. Periods lasted 3 weeks. All animals were fed a corn silage-haylage mix ad libitum and 1 kg of 12% protein concentrate for every 3 kg milk produced. Milk yields, milk protein percent and protein yields for the suckled and control groups were: 22.2 kg, 21.2 kg; 3.64%, 3.51% ($P<.1$); 808g, 735g ($P<.05$). The suckled and control groups consumed 92% and 103.8% of NRC requirements for protein.

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The author also expresses deepest thanks to his fellow graduate students who may have helped in a great variety of ways. Help with training, feeding and daily care of the animals is sincerely appreciated. Also, the stimulating discussions with fellow graduate students was a very valuable portion of this author's graduate study.

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INTRODUCTION

Efficiency of use of nutrients by animals is determined by two basic factors. The digestive system of the animal and the composition and quality of the feedstuffs containing the nutrients. For ruminant nutritionists, the presence of the rumen presents the first major obstacle in the efficient use of quality feeds. High quality proteins are almost completely degraded in the rumen to yield lower quality microbial protein in lesser quantities than the protein originally added. Because the nonruminants do not have a pre-gastric microbial fermentation, the full benefit of a high qualtiy protein source can be appreciated. At the same time, the presence of the rumen enables the ruminant to utilize a more diverse selection of lower quality and high fiber feeds. The ruminant can convert low quality proteins, NPN and roughages into higher quality microbial protein for productive function. To increase productivity then, either the capacity to produce microbial protein must be increased or methods by which high quality protein can pass through the rumen undergraded must be employed. Esophageal groove closure is the natural means in young ruminants by which milk "by-passes" the undeveloped rumen and enters the abomasum for efficient digestion and absorption. This reflex closure of the groove, if operable in the adult

cow, may present one way in which nutrients could escape the inefficient fermentation in the rumen and be utilized to a greater extent by the animal.

The object of this investigation is to determine whether esophageal groove closure cows and if it can be used to feed the dairy cow a portion of the nutrients required for lactation.

LITERATURE REVIEW

Rumen By-Pass

The beneficial effects of by-passing the rumen with protein have been observed for some time. Many of these effects were seen in experiments comparing the protein quality of different feed sources for ruminants. Chalmers and Synge (1954) correlated the greater value of herring meal supplements over casein supplements for sheep with the lower evolution of ammonia in the rumen from the herring meal. A greater proportion of the herring meal supplement passed through the rumen undegraded and resulted in better wool growth and increased nitrogen retention. Earlier, Cuthbertson and Chalmers (1950) had shown that casein had little value as a protein supplement for sheep unless the rumen was by-passed. The rapid deamination of this high quality protein has a detrimental effect on its utilization. These workers showed an increase in nitrogen retention when casein was administered into the duodenum of sheep. They also showed that this increase diminished as the basal intake of nitrogen increased from 6g to 25g per day. No difference in nitrogen retention was obtained at the high level of nitrogen intake (Chalmers et al. 1954).

The solubility of different protein sources in rumen liquor was found to be a major factor influencing the degree

of by-pass. McDonald (1952) showed that dietary proteins soluble in the rumen fluid were rapidly fermented. Fontaine et al. (1944) reported better utilization of the nitrogen in ground nut meals when the percentage of salt-peptizability or solubility of the protein is low. Solubility, however, is not the only important factor involved in nitrogen utilization. El-Shazly (1958) showed that casein gave better nitrogen retention than meat meal and beans, both of which are less soluble and generate lower rumen ammonia concentrations. Chalmers (1961) pointed out that the nitrogen digestibility of these protein sources has a controlling effect on the nitrogen retention observed. She reported that the digestibility of nitrogen for meat meal, beans and casein were 47, 59 and 70 percent and nitrogen balance decreased as nitrogen digestibility decreased, concluding the ideal ration for maximum use of nitrogen should contain protein of good digestibility with a low solubility in rumen fluid. When casein was heated or denatured into hard lumps and then ground, the solubility in rumen fluid decreased and the nitrogen balance increased (Chalmer et al. 1954).

The results of these and other studies were a major impetus for extensive research in two areas of protein nutrition over the last 15 years. One line of work investigated the various methods by which protein could be protected from degradation in the rumen and still be well-digested in the intestine, the other studied more extensively the overall effects of rumen by-pass of nutrients utilizing the abomasal

and duodenal cannulae.

Abomasal Infusion Studies in Lactating Cows

Because casein is the major milk protein and should provide an ideal mixture of amino acids for milk protein synthesis, it has been the protein source used most often in postruminal infusion studies. Also, there is some difficulty in getting plant and other animal proteins into solution or a suitable suspension. Glucose and amino acids have also been used widely for infusion studies.

Clark (1975) recently published an excellent review of the postruminal infusion studies in lactating cows. He reported an increase in milk yield of 1 to 4 kg per cow per day when casein was supplemented postruminally. The response in milk yield was accompanied by a 10-15% increase in milk protein yield. Various factors affect these responses although they are not all clearly understood. Level of production exerts some effect. Greatest increases in milk yield after postruminal protein infusion is from high producing cows. Cows producing over 20 kg milk per day had increase greater than 1 kg of milk per cow per day when casein was infused postruminally. When production was less than 20 kg, increases greater than 1 kg per cow per day were seldom seen. Clark (1975) believes that cows producing in excess of 30 kg milk per day fail to produce to their genetic potential because of the lack of some key nutrient or nutrients. Estimates of rumen microbial protein yields in high producing cows support this concept (Hogan and Weston 1970; Burroughs et al. 1975; Hume et al. 1970; Ibrahim and Ingalls, 1972).

Hale and Jacobsen (1972) infused casein, gelatin, partially delactosed whey and zein into the abomasum and found no response in milk yield, however, the cows in this study averaged only 6 kg milk per day. Vik-Mo, Emery and Hubes (1974) observed negative responses in milk yield with cows producing less than 20 kg milk per day. Fluctuations in feed intake may have contributed to the negative response although energy and protein intake were usually above standard allowances. Broderick et al. (1970) reported a significant depression in concentrate intake when 800g of methionine-supplemented casein was infused into the abomasum. Most workers (Clark, Spires and Derrig, 1973; Derrig, Clark and Davis, 1974; Hale and Jacobsen, 1972; Hale, Jacobsen and Hemken, 1972; Vik-Mo, Emery and Huber, 1974) observed no effect on feed intake when 300-600g of casein was infused into the abomasum. Others (Ørskove, Fraser and Pirie, 1973; Papas, Hatfield and Owens, 1974; Spechter, 1972) have reported an increase in feed intake when protein was fed to by-pass the rumen.

Clark (1975) suggested the optimum quantity of infused casein for increasing milk production appears to be from 300-500g per day. Infusion of greater quantities has not further increased milk yield. Even for cows fed above NRC standards for energy and protein, infusion of 300-500g casein into the abomasum usually resulted in maximal increases in milk and milk protein yield (Broderick, Kowalczyk and Satter, 1970; Derrig, Clark and Davis, 1974; and Vik-Mo, Emery and Huber, 1974).

Ørskov, Grubb and Kay (1977) studied postruminal infusion of casein and glucose with early lactating cows in negative energy balance. These workers observed an increase in milk yield and amount of nitrogen in milk when either casein or glucose was infused postruminally. Vik-Mo et al. (1974) also showed a response in milk yield to glucose infusion, but less than that of casein, which suggests that energy may be limiting at the mammary gland. In a second experiment, Ørskov et al. (1977) observed a significant linear and quadratic increase in milk yield and yield of nitrogen when levels of casein infused increased from 0 to 750g per day. Casein infusion also doubled the negative energy balance for the cows in this trial as calculated using the equation of Gaines and Overman (1938). These results indicate that cows in early lactation and negative energy balance can be limited by an inadequacy of both energy and amino acids for milk protein synthesis.

It can be concluded, therefore, that the response in milk yield to protein supplemented postruminally is of a magnitude which might have practical interest. Chalmers (1961), Allison (1970), Hogan and Weston (1970), Smith (1975), and Ørskov et al (1977) were all in agreement when they called for the development of a protein rich supplement which escapes ruminal degradation by micro-organisms to a substantial extent and yet remains digestible in the small intestine.

Methods Used to Facilitate Rumen By-Pass of Protein

Much of the early work on the protection of proteins from ruminal degradation was done at the Prospect Laboratory

in Australia and the Rowett Research Institute in Scotland. Reis and Schinkel (1964) demonstrated a biological response that could be used to test the effectiveness of various techniques of protection when they noted a marked increase in wool growth of sheep after abomasal infusion of casein or sulfur amino acids. This wool growth response has since been used to evaluate the degree of natural protection of feedstuffs and test various processing techniques (Reis, 1967, 1970; Reis and Tunks, 1969). Work in this area includes heat treatment of protein, chemical modification of proteins, inhibition of proteolytic activity of rumen microbes with antibiotics and reducing the mean residence time of ingesta in the rumen.

Heat Treatment of Proteins

Application of heat to grains or forages during processing can decrease ruminal degradation of protein (Hale, 1973; Schoeman, deWet and Burger, 1972). The reduced rate of microbial fermentation has been attributed to the reduced solubility of the protein (Tagari, Ascarelli and Bondi, 1962). However, Chalmer et al. (1954) indicated that grinding of the coarse lumps of insoluble protein may be necessary to enhance rapid passage of the material out of the rumen. Heat processing to obtain protection is at a disadvantage because decreases in digestibility and biological value may also result. The Maillard reaction between sugar aldehyde groups and the free amino groups of protein is responsible for much of the decrease in digestibility (Ferguson, 1975; Chalupa, 1975). This heat damage can also occur in the absence of sugar or oxidizing fat (Bjarnason and Carpenter 1969, 1970).

Goering and Waldo (1974) attributed decreased protein digestibility and animal performance to heat damage in forages. Some work has been done evaluating the effects of heating time, temperature and moisture on the degree of damage in forages (Goering and VanSoest, 1967). Chalupa (1975) indicated that if the Maillard reaction can be controlled to decrease protein solubility in the rumen and yet maintain intestinal protein digestibility, animal performance could be increased. This is indeed the case when evaluated by nitrogen retention, weight gain or feed efficiency (Goering and Waldo, 1974; Hudson, Glimp, Little and Woolfolk, 1970; Sherrod and Tillman, 1962, 1964).

Chemical Treatment of Protein

Chemical agents which form reversible cross-linkages with amino and amide groups have been studied as a means of protecting proteins. These linkages decrease the solubility of protein at rumen pH, but are subsequently destroyed in the acidic abomasum making the protein available for digestion by the host animal. Formaldehyde has been studied most extensively for its protective ability. The reaction of formaldehyde with protein has been widely used industrially (Walker, 1964) and has been described in detail (Fraenkel-Conrat and Olcott, 1946, 1948). Formaldehyde treatment of casein has resulted in increased nitrogen retention, wool growth and muscle growth (Barry, 1972, 1973; Faichney, 1971; Faichney and Weston 1971; Hemsley, Reis and Downes 1973; Reis and Tunks, 1969; and Wright, 1971). Treatment of plant proteins

feed efficiency have been improved (Chalupa, 1975). Formaldehyde treatment of forages at ensiling also promotes increases in animal performance (Barry, Fennessy and Duncan, 1972; Brown and Valentine, 1972; Waldo, Keys and Gordon, 1973). Weston (1971) and McRae et al. (1972) showed that formaldehyde treatment of casein decreased total nitrogen digestibility but the amount of non-ammonia nitrogen entering and absorbed in the small intestine was significantly increased. However, work by Schmidt et al. (1973) and Wachira (1973) indicates formaldehyde protection of plant proteins will probably affect rumen microbial metabolism, microbial protein production and intestinal digestibility.

The use of tannins to protect dietary protein has also been considered. McLeod (1974) suggested that tannins found in seeds and forages may be responsible for some of the natural protection of proteins that is observed. Tannins have been chemically classified as either hydrolysable or condensed. Zelter et al. (1970) reported that tannin-protein complexes formed by condensed tannins are not likely to be hydrolysed to yield amino acids in the abomasum. Hydrolysable tannins, on the other hand, form reversible cross-linkages with proteins, presumably by hydrogen bonding. These bonds are weakened or reinforced by ionic forces (Ferguson, 1975). Little attention has been given to controlled experiments using tannins as a means of protection. It has been shown that bird-resistant sorghum grain having a high tannin content is less readily fermented in the rumen than normal sorghum (Saba, Hale and Theurer, 1972). However, Manson

et al. (1973) showed that this sorghum also has a lower net energy and apparent crude protein digestibility than normal sorghum.

Other chemical agents have been investigated but will not be discussed in detail. These include phosphonitrilic halides, polymerized unsaturated carboxylic acids and halo triazines, (Miller 1972), sulfonyl halides and acrolein acetals (Miller 1973), hexamethylenetetramine (Schmidt et al. 1973) and acetylenic esters (Wildi and Miller, 1973).

Antibiotics

Hogan and Weston (1969) studied several antibiotics in attempting to control rumen protease and deaminase activity. The results were not encouraging and problems with feed intake were encountered. Schelling et al. (1972) found that 1g per day of oxytetracycline had no overall effect on rumen metabolism in sheep. When oxytetracycline was given in combination with lysine and methionine, these amino acids were increased in the abomasum and plasma. This effect was attributed to the presence of oxytetrocycline. However, these workers also experienced problems with depressed feed intake and digestive disturbances.

Esophageal Groove Closure

Several researchers have given consideration to the esophageal groove as a means to obtain rumen by-pass. The closure of the eosphageal groove involves a series of co-ordinated reactions of the caudal thoracic esophagus, the reticular groove and the reticulo-omasal orifice (Titchen and New-

hook, 1975). These reactions, which are normal functions in young ruminants, facilitate the passage of suckled liquid from the esophagus through the reticular groove and omasal canal into the abomasum. This direct passage of suckled liquid into the abomasum is associated with the contraction of the reticular groove. Stimulation of the efferent fibers of the vagus nerve produces groove contraction and vagal nerve section eliminates it (Comline and Titchen, 1951; Duncan, 1953). Factors believed to influence groove closure are age, temperature of the liquid, posture of the animal while suckling and composition of the suckled liquid (Ørskov, 1972).

Reik (1954) reported that the presence of sodium salts in the mouth activated groove contraction in calves. Watson and Jarret (1941) also observed groove contraction in sheep using copper salts while Mönning and Quin (1935) used salts of silver and zinc to produce the same effect. Glucose solutions also evoked groove closure in cattle, (Titchen, 1968).

Wester (1926) stated that the esophageal groove mechanism regressed with age due to a failure of the groove to develop proportionately with the rumen and reticulum. Its vagal innervation, he stated, also regressed with age. Two years later, Schalk and Amadon (1928) sucessfully revived the mechanism in an adult cow by re-establishing a liking for drinking milk. After studying groove activity in 15 normally fed cows with rumen fistulae, Schalk and Amadon, (1928) concluded that the groove functions only upon rare occasions in the mature animal.

There exists considerable disagreement in the literature concerning the mechanisms of groove closure. Ørskov and Benzie (1969) found that the nature of the fluids tested had no influence on the function of the groove. Earlier workers had shown that milk (Wise and Anderson, 1939) and solutions of sodium salts (Wester, 1930; Trautmann and Schmitt, 1933) or copper sulphate (Watson and Jarrett, 1941) would promote closure of the groove. Ørskov et al. (1969) and Watson (1944) agree that if lambs are trained to suck from a bottle at weaning and do so voluntarily and eagerly, milk would continue to pass to the abomasum for months or years. Wise and Anderson (1939) concluded that with the dairy calf, suckling from a from a nipple pail promoted groove closure while drinking from an open pail failed to enhance the passage of milk to the abomasum. In a subsequent study, they stated that elevation of the head while suckling had no influence on groove function (Wise et al. 1942). Ørskov et al. (1970) on the other hand, showed that fluids entered the abomasum regardless of whether they were suckled or drunk normally as long as the liquid was consumed as a result of a "pleasurable anticipation" as opposed to a quenching of thirst. After numerous studies, Ørskov et al. (1970) believe that the groove mechanism is a conditional reflex depending on the mood of the animal. They showed that groove contraction occurs even before suckling as a result of anticipation for receiving a suckled meal.

Methods by which successful groove closure has been assessed include radiographic observation (Ørskov et al. al.
1970), visual inspection or palpation through rumen fistulae

(Wise and Anderson, 1939), plasma urea nitrogen and feeding of glucose or strontium via nipple bottle and their subsequent determination in the blood and rumen respectively (Robinson et al. 1977; Heddie and Ward, 1973; Reik, 1954).

Several workers have shown improvement in growth rate and feed efficiency when nutrients by-passed the rumen via closure of the esophageal groove (Ørskov, 1972). Guilhermet et al. (1977) observed improvements in growth and feed efficiency with liquid feeding of casein and soya flour to 8 week old calves. However, Robinson et al. (1976) observed no significant differences in performance of calves fed supplemental protein via nipple pail compared to calves fed the same protein included in the basal ration. They also observed a trend towards reduced weight gains and dry matter intake with nipple fed calves.

If groove closure can be re-initiated in adult ruminants to allow substantial high quality protein to enters the abomasum, post-ruminal infusion studies suggest that there would be marked improvement in weight gain, feed efficiency, nitrogen balance or milk and milk protein production, depending on the animal's productive function.

Research Proposal: The objectives of this study were:

1. Train lactating cows to suckle fluids from a nipple pail.
2. Determine whether rumen by-pass of the suckled fluid occurs.
3. Determine the effects of suckling a high quality protein as a portion of the ration on milk and milk protein yield.

MATERIALS AND METHODS

Animals and Management

A total of 19 Holstein-Friesian heifers were used in this study. The animals were approximately 22 months of age at the beginning of the training trials and were due to freshen at about 25 months of age. All heifers were born and raised in the Michigan State University dairy herd. They were housed in a stanchion barn equipped for individual feeding throughout the trial. After freshening, the animals were milked at 0400 and 1500 hours daily. On most days, the cows were turned into a dry lot for 2-3 hours after the morning milking before returning to the stanchion barn.

TRAINING TRIAL I: Feed was withheld from 6 heifers in an attempt to initiate a desire for suckling. A nipple bottle containing whole milk was introduced into the animal's mouth the morning and evening of each day during the training period. This "force feeding" procedure required two workers. One would restrain the animal's head and the other worker would direct the nipple into the mouth of the animal. Milk was forced out of the nipple by one worker and the mouth was held tightly shut around the nipple to force swallowing. Approximately 10-20 minutes were spent with each animal during each attempt. After two days of trying this method, no

progress had been made towards initiating a desire to suckle. On the third day, dry hay was fed ad-libitum and water was witheld for 12-14 hours. The animals were not allowed access to water until about 1 hour after being forced to suckle whole milk. After several days of continued training the animals began to show less anxiety for the nipple and more active suckling. Thereafter, the majority of the animals needed only to be introduced to the nipple bottle and suckling was completed with few problems (see Plate I, Appendix). The animals were allowed to suckle approximately 4 liters of whole milk followed by as much water as they would voluntarily consume through the nipple, which ranged from 2-10 after each feeding. After two weeks of training, free choice water gradually re-introduced to animals. Beginning with the third week of training, heifers were fed by nipple only in the mornings so as to maintain the suckling habit.

Rumen By-pass Determination

Before the heifers freshened, three trials were conducted to determine if rumen by-pass was occurring. A two-period changeover design with two treatments was employed. Four liters of whole milk containing approximately 500g of D-glucose was either suckled or placed directly into the rumen according to the schedule in Table I. For trials II and III, a centrifugal pump was used to force the solution into the rumen of controls because of delays encountered in voluntary drinking in Trial I. Blood samples were drawn prior to and at timed intervals after ingestion of the milk. The following day treatments were switched for each animal. Specific informa-

tion on controls used and time interval between sampling are also shown in Table I. Water was removed from the animals 12 h before each trial to insure rapid suckling of test solutions.

TRAINING TRIAL II: Thirteen heifers were trained using a slightly different procedure. The animals suckled water instead of whole milk. Training was only during mornings. Water was restricted for about 12 hours before each morning feeding. The animals received dry hay ad-libitum and 1 kg of a grain mix per day. After 8 days of training, water was gradually re-introduced to animals that suckled willingly. By the tenth day only 2 animals required water deprivation to encourage suckling.

Rumen By-pass Determination

The basic protocol was the same as for other by-pass trials but water containing 500g D-glucose was used instead of milk. Control animals received the same amount of glucose in 1 kg grain. Blood samples were drawn through jugular cannulae prior to and .5, 1, 2 and 4 hours after ingestion of glucose. On the second day, treatments were reversed. Water was again witheld overnight before each trial day to insure rapid suckling of the glucose solution.

Production Trial

Eight animals trained to suckle were selected on the basis of willingness to suckle and increase in blood glucose during rumen by-pass trials. They were used in a production trial to test the effect of suckling on milk yield and composition. A two-period changeover design was used with periods lasting three weeks.

Table 1.--Ruben By-Pass Determination Trials, Solutions Used, Method of control, Time and method of Sampling.

Trial	Solution	Treatment 1	Treatment 2 (Control)	Blood Sample	Blood Sampling Times
I	4 liters whole milk + 500g glucose	suckled	Drank Normally	Tail Vein	Before, 1, 2 and 4 hours after feeding
II	same as I	suckled	Stomach tube in rumen	Tail Vein	Before, 1, 2, 4 and 6 hours after feeding
III	same as I	suckled	Stomach tube into rumen	Jugular Cannulae	Before, 1/2, 1, 2, 4 and 6 hours after feeding

Animals were randomly assigned to either a suckled group or a control group. The suckled group was allowed to suckle up to 15 liters of whole milk daily which provided approximately 500g of milk protein. The control group received daily 500g of casein in the concentrate. All animals were fed ad-libitum a mix of 50 percent corn silage and 50 percent haylage and concentrate at 1.0 kg per 2.5 kg milk. The concentrate allowance was adjusted weekly. The silage mix was fed at approximately 0900 hours and grain at 1400 hours. Feed left in the manger at 0700 hours was removed and weighed. Ingredient composition of the concentrate is shown in Table 2.

Metal holders for nipple pails were placed just above the feed manger of each animal, so that cows had unobstructed access to feed but could also reach the nipple pail (see Plate 2, Appendix). The pails were filled twice daily with whole milk for the suckled group and water for controls. Consumption of suckled milk, feed weighbacks and milk yields were recorded daily. Body weights were determined weekly.

The whole milk used for the supplement was taken daily from the bulk tank at the MSU Dairy Cattle Center. Whole milk, silage mix concentrate were sampled weekly. Milk samples of two consecutive milkings were composited from each cow twice weekly, usually on Monday P.M., Tuesday, A.M. and Thursday, P.M., Friday, A.M. Tail vein blood samples were collected on Monday and Thursday of each week. After three weeks, treatments were switched.

Table 2.--Ingredient composition of concentrate used throughout the experiment.

Ingredient	%
Ground Shell Corn	54.0
Rolled Oats	26.5
Soybean Meal (50%)	11.5
Molasses	5.0
Trace Mineral Salt[a]	1.0
Dicalcium Phosphate	1.5
Limestone	.5
Vitamin A	4400 lu/kg
Vitamin D	400 lu/kg

[a]Trace mineral salt contains a guaranteed minimum of: .35% Zn, .12% Fe, .15% Mg, .03% Cu, .005%Co, .007% I, and 96.00% NaCl.

Near the beginning and end of each treatment period, a glucose tolerance test was conducted on each animal receiving the suckled whole milk. Approximately 500g of glucose was added to the milk on the morning of the test. Blood samples were drawn from the tail vein prior to and at 1 and 2 hours after ingestion of suckled supplement.

Final Rumen By-pass Trial

After the production trial, 5 cows that had previously been trained, and were still suckling satisfactorily, were used in a final trial to determine rumen by-pass. The objective was to compare suckling to normal drinking and observe the efficacy of rumen by-pass for the two delivery methods. The trial was held on 5 consecutive days and the schedule of treatments for each animal is in Table 3. Blood samples were drawn from the tail vein prior to and at 1 and 2 hours after treatments.

Handling and Processing of Samples

Feed: Silage mix, concentrate and casein samples were composited for each period. Concentrate was ground through a 50 mesh screen and silage in a model 84142 Hobbart Silage Chopper.[1] Dry matter, total nitrogen, acid detergent fiber (ADF), and acid detergent nitrogen (ADN) were determined on each composite sample.

Dry matter was determined by drying in a forced air oven at 100 C for 48 hours. Total nitrogen was determined by

[1]Hobbart Manufacturing Company, Troy, Ohio.

Table 3.--Schedule of treatments for final by-pass trial[a].

Animal	Days				
	1	2	3	4	5
1470	T	S	N	S	N
1482	N	T	S	N	S
1486	N	S	T	S	N
1503	S	N	N	T	S
1509	S	N	S	N	T

[a]500g glucose in 4 liters water was:

T = tubed into rumen
S = suckled
N = drunk normally

Kjeldahl (AOAC 1965) as modified by Wall and Gehrke (1975). Acid detergent fiber and ADN were by the Van Soest method (Goering and Van Soest, 1970).

Milk: All milk samples, including those of the whole milk supplement were analyzed within 48 hours after sampling. Milk fat was determined by the Babcock method; milk protein by the Udy dye method (Udy, 1971); and total solids by drying 3 ml at 90 C for 3 hours in a forced air oven.

Blood: Except for those samples taken by jugular cannulae in some by-pass trials, all blood was drawn from the tail vein using single draw needles[2] with 15 ml vacutainer tubes.[3] Blood tubes were placed in the cooler at 8-10°C immediately after collection for 5-6 hours and then centrifuged for serum separation at 6500xg for 20 minutes. Serum was drawn off with a pipette and placed in 7 dram plastic vials[4] for storage at -20 C until analyzed.

Glucose Tolerance Tests: Those samples collected to determine rumen by-pass of sugar were analyzed for glucose using the coupled system of glucose oxidase and peroxidase.[5] The values obtained were plotted against time of sampling and analyzed statistically.

Production Trial: Serum samples collected during the production trial were analyzed for glucose as previously

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[2]Single Draw Vacutainer Needle (silicone coated, 20 G). Becton-Dickinson, Rutherford, N.J. 07070

[3]Vacutainer, non-sterile, 15 ml. Becton-Dickinson, Rutherford, N.J. 07070.

[4]Fisher Scientific Company, Pittsburg, PN.

[5]Worthington Biochemical Corporation, Freehold N.J.

described and serum urea-N according to Okuda (1965) and Kulasek (1972, 1976).

Statistics

Glucose tolerance tests: Paired t tests were used to compare mean serum glucose levels at 1 and 2 hours after feeding (Steel and Torrie, 1960).

Production Trial: The analysis of variance for each of the parameters in this trial was a balanced single crossover experiment (Gill, 1978) according to the model in (1):

(1) $Yijk = u + Di + Pj + Tk + E(ijk)$

Where Yijk is the observed value in the ith subject, jth period and kth treatment;

u is the overall mean;

Di is the effect of the ith subject; i = 1, 2..6;

Pj is the effect of the jth period; j = 1,2;

Tk is the effect of the kth treatment; k = 1,2;

E(ijk) is the residual error.

Table 4 shows the degrees of freedom for each source of variation.

Table 4.--Sources of Variation and degrees of freedom for model used in production trial.

Sources of Variation	d.f.[a]
Cows	5
Periods	1
Treatments	1
Error	4
TOTAL	11

a. degrees of freedom

RESULTS AND DISCUSSION

Training Trials

Four of the 6 animals were suckling successfully 10 months after the beginning of the first training trial (Table 5), and only 2 at 10 months of the second training trial (Table 5). Animals were considered to be suckling successfully when an animal would readily suckle 4-8 liters of solution (water or milk). The results of this study indicate that 2 year old heifers can be forced to re-initiate the suckling habit (See Plate 1 and 2). Inducing thirst in the animal is first necessary to stimulate a desire to suckle. In the first training procedure, feed withdrawal did not stimulate a desire to suckle. During the first 3 days of this trial, the force-feeding procedure apparently upset the animals, making them nervous and difficult to work with. Overnight withdrawal from water, however, caused thirst in heifers which they would satisfy through suckling. In both training trials, all heifers showed interest in the nipple after withdrawal of water. This behavior was a learned response. As training continued, less time was needed to force each animal to suckle. At the end of training, most animals would willingly reach for the nipple and no restraint was necessary. Although time required for consumption of the desired amount of fluid was not recorded, it was reduced as the training period

Table 5.--Results of Training Trials. Number of animals suckling successfully at several times after beginning of training.

Time after beginning of training	Number of animals suckling 1st trial[a]	2nd trial[b]
3 days	0[c]	13
1 week	5	13
1 month	4	8
2 months	4	4
10 months	4	2

[a]Six heifers used in Trial 1.

[b]Thirteen heifers used in Trial 2. Water was restricted from beginning of training and returned after 2 weeks.

[c]Water was restricted after this point and returned after 2 weeks.

progressed. Approximately 10-15 minutes were required for the animals to consume 4-5 liters of fluid at the beginning of training, but this was reduced to less than 5 minutes by the end of training.

Persistency of the suckling habit after training was greater in the first trial (Table 5). It is difficult to assess the exact reason for this difference, but several possibilities exist: 1.) Suckling twice daily in trial 1 as opposed to once daily in trial 2, even though after 2 weeks the animals in trial 1 were allowed to suckle only in the mornings. Whether this 2 weeks of twice-daily suckling caused a greater desire to suckle 10 months later is not known; 2.) Whole milk was used to train animals in trial 1 and water in trial 2. Hence, the animals might have remembered the more palatable taste of milk. However, after the training periods of both trials, water was used to maintain suckling; 3.) A third possibility is that fewer animals were trained in trial 1 and more time was devoted in training each animal.

Cows suckling on one another is an undesirable trait in the dairy industry. One recommendation for cows exhibiting this behavior is to cull them. The cows trained in this study were released from stanchions with the remainder of the herd for 2-3 hours per day, but were never observed sucking other cows, nor did they show any desire to do so. Apparently, these heifers, after being trained to suckle, made no association between milk from the nipple and that which might

be obtained from another cow's udder.

Rumen By-pass Determination

The method used to determine rumen by-pass occurrence has previously been used by other workers (Huber et al. 1967; Robinson et al. 1976). The rapid rise in serum glucose after suckling showed that suckled glucose by-passed the rumen. Table 6 lists the mean serum glucose levels for each treatment from all the animals in the four by-pass trials. An average increase of 20 mg/100 ml was observed for heifers which suckled glucose with the peak at 2 hours post-feeding. There was little change in blood glucose when glucose was placed directly in the rumen. Figure 1 plots the values shown in Table 6 against time of sampling. Figures 2 and 3 indicate the difference in responses for two individual heifers after suckling the glucose solution; one increased greatly in serum glucose (Fig. 2) the other showed showing no increase (Fig. 3). While both of these heifers suckled actively, the animal represented in figure 3 showed a failure of closure of the esophageal opening into the rumen.

Only 4 of the 19 animals studied showed no rise in serum glucose after suckling. Three of these were not active sucklers. Thus, all but one of the heifers that suckled actively actively training showed a rapid rise in serum glucose. This increase was usually in excess of 30 mg/100 ml. The peak level for the suckled group shown in Figure 1 was less than 30 mg/100 ml above controls because animals that did not obtain by-pass were included in the mean. Figure 4, which includes only values from those animals that obtained rumen by-pass, shows increases are

Table 6.--Mean serum glucose levels (mg/100ml) for all animals in the 4 by-pass trials.

Time	Control	Suckled
0[a]	47.44	48.51
.5[b]	44.14	56.70
1[a]	50.44	62.92
2[a]	52.78	68.08*
4[a]	54.64	61.38
6[c]	64.59	64.26

a Means of 37 values.

b Means of 25 values.

c Means of 15 values.

* Significantly different ($P<.1$).

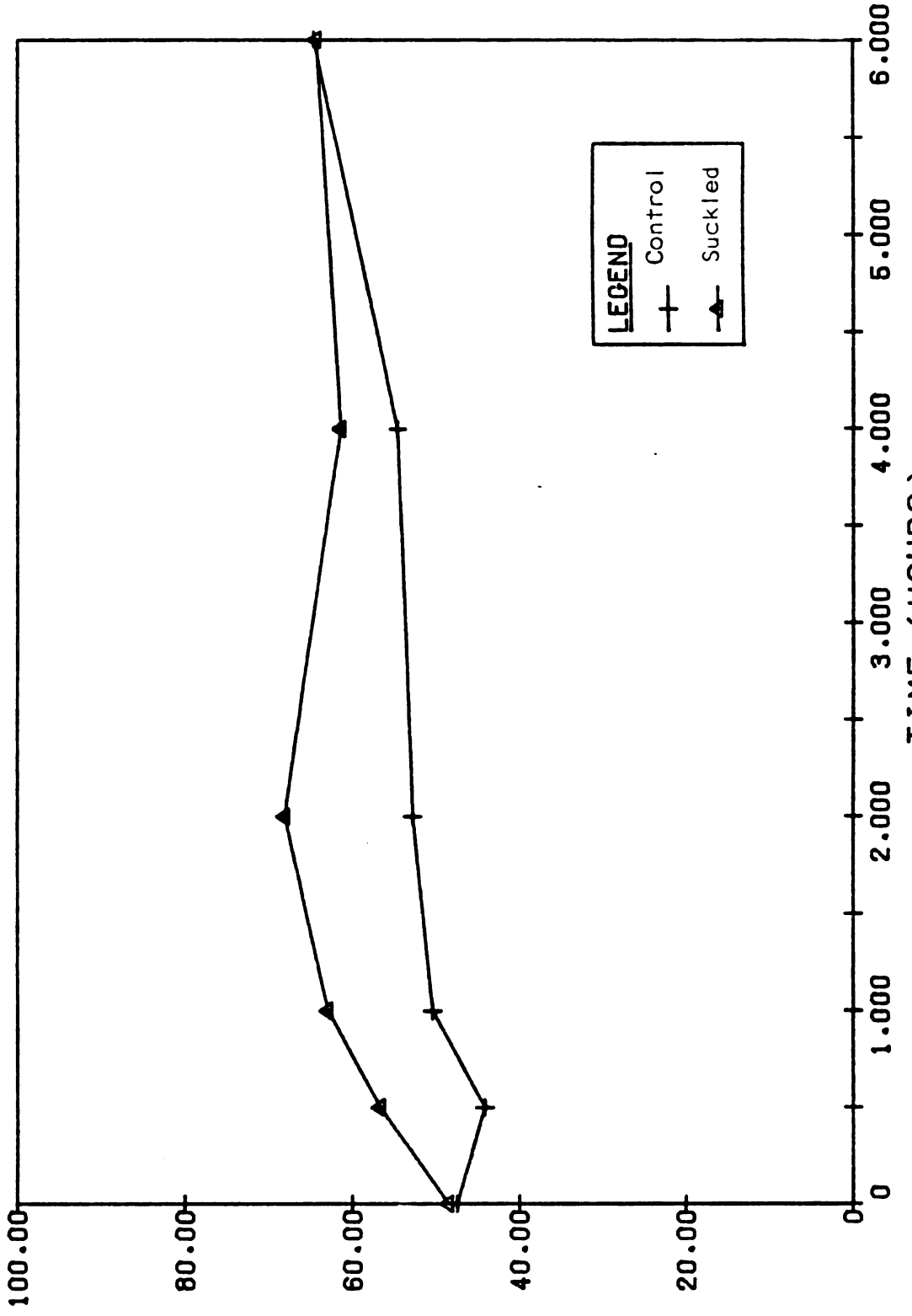


Figure 1-- Serum glucose response to glucose either suckled or placed directly into the rumen for all animals in by-pass trials.

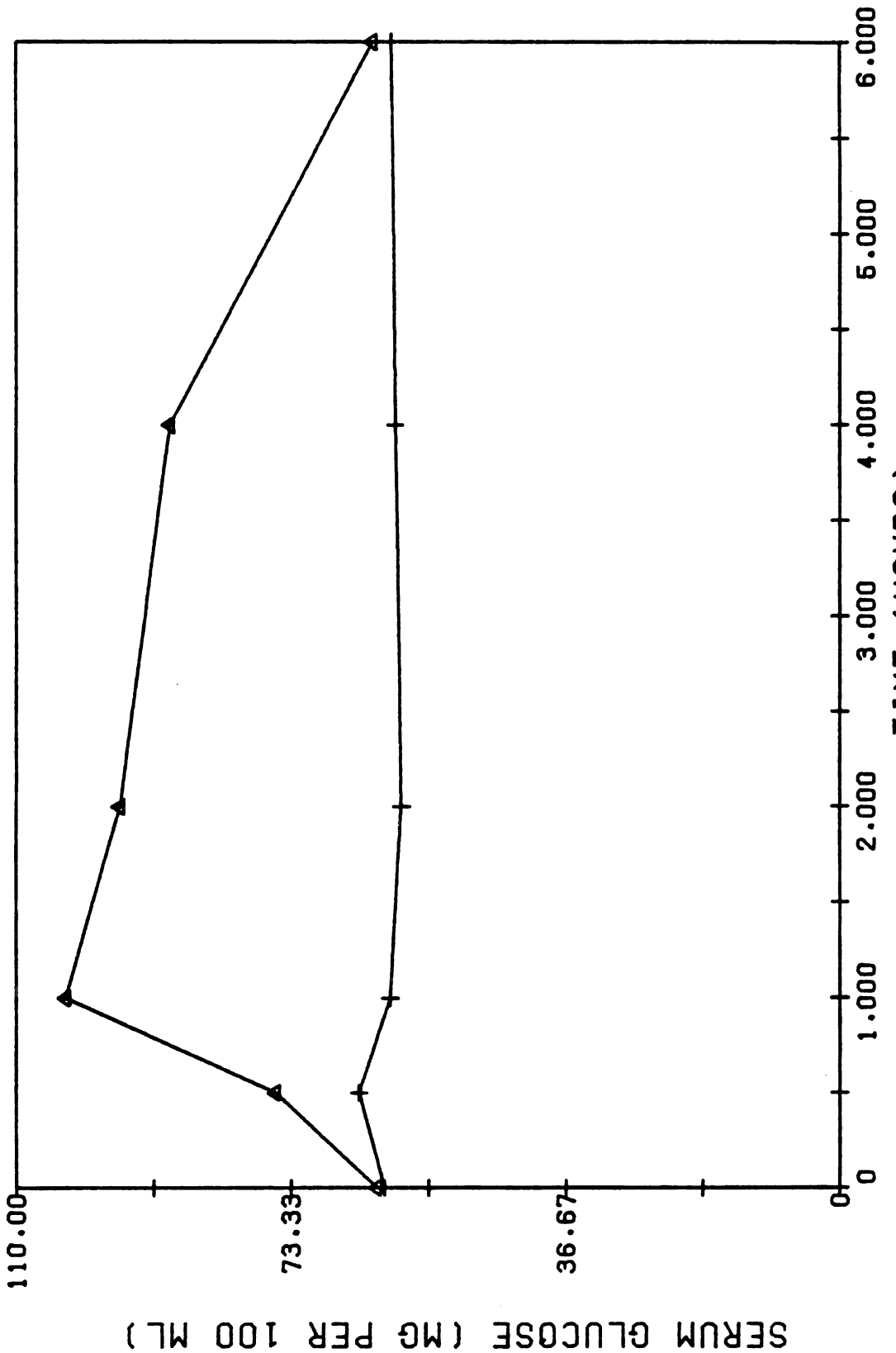


Figure 2-- Serum glucose response to glucose for animal obtaining rumen by-pass.

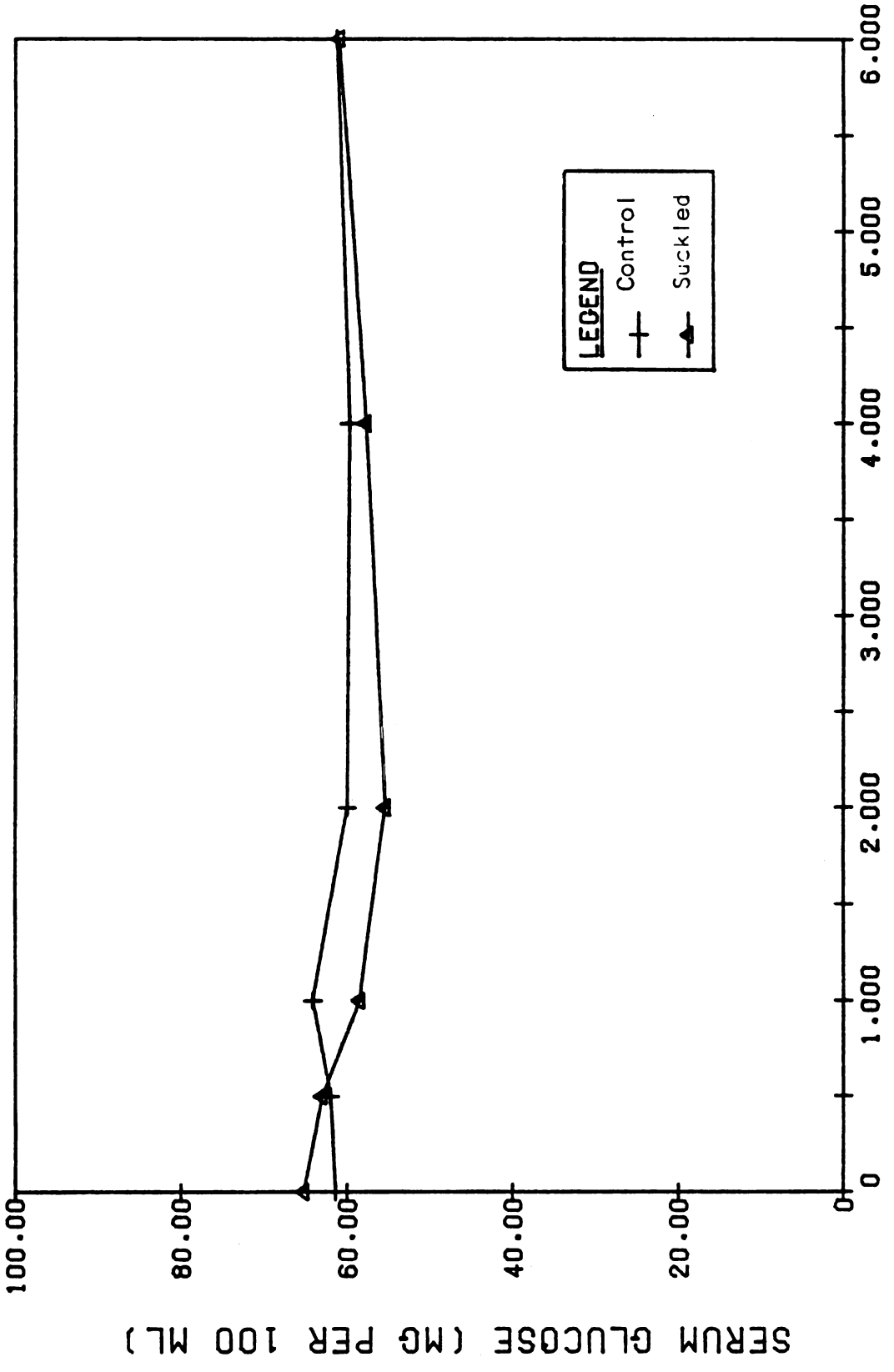


Figure 3-- Serum glucose response to glucose for animal not obtaining rumen by-pass.

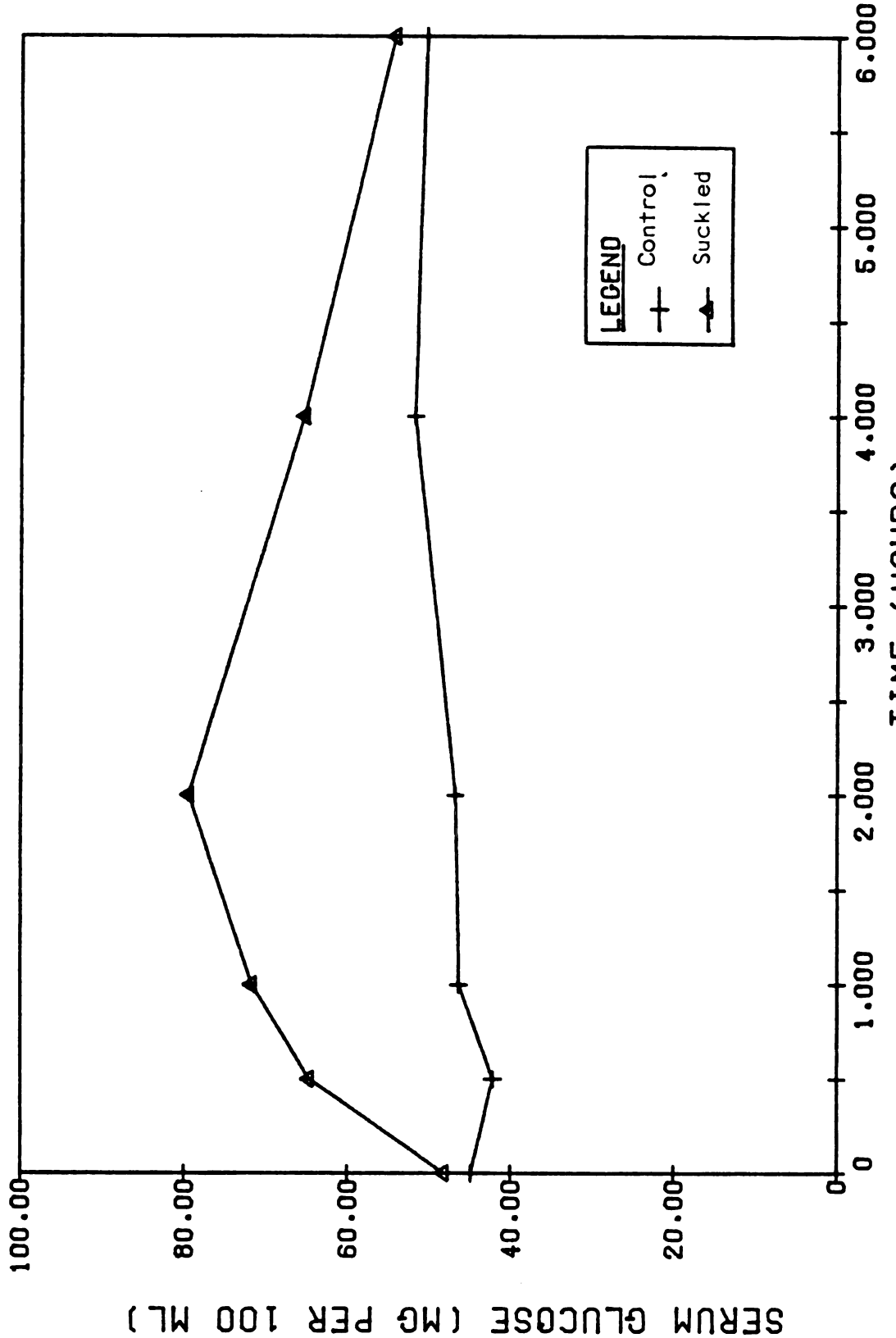


Figure 4-- Serum glucose response to glucose either suckled or placed directly into the rumen for only animals obtaining by-pass.

greater for animals in which by-pass was confirmed. Table 7 lists the mean values plotted in Figure 4.

The final by-pass trial was conducted to determine if animals previously trained to suckle would obtain by-pass when drinking normally. The results of the first trial, where normal drinking was used as a control, suggested such a response in 4 of the 6 heifers. Normal drinking was compared to tubing directly into the rumen and suckling. Table 8 lists the differences in mean serum glucose level between the 0 and 2 hour sample for the 5 animals used. Only one animal, 1503, showed a large initial rise in serum glucose after normal drinking. The following day, when normal drinking was again tested on the same heifer, no rise was observed. Number 1503 did show a significant increase when the glucose solution was suckled. On the occasion when the increase after normal drinking was observed, the entire test solution was consumed within 1 minute after initiation of drinking. This may have caused a portion of the solution to flow down the esophageal groove and escape rumen fermentation.

The animals in the production trial were chosen on the basis of successful suckling activity and positive by-pass. Table 9 lists the mean serum glucose levels for the suckled animals in the production trial and Figure 5 plots these values against time of sampling. The average level at 2 hours was 37 mg/100 ml above the 0 hour level. As Figure 5 indicates, these animals continued to obtain rumen by-pass throughout the trial.

Table 7.--Mean serum glucose levels (mg/100 ml) for only animals obtaining rumen by-pass.

Time	Control	Suckled
0[a]	44.84	48.19
.5[b]	42.21	64.72
1[a]	46.39	71.75*
2[a]	46.85	79.51*
4[a]	51.83	65.35
6[c]	50.40	54.28

a Means of 31 values.

b Means of 19 values.

c Means of 15 values.

* Significantly different ($P<.05$).

Table

Table 8.--Difference in mean serum glucose (mg/100 ml) between 0 and 2 hours after administration of treatment[a].

Animal	Day				
	1	2	3	4	5
1470	-2.0 T	25.6 S	.8 N	14.8 S	-5.0 N
1482	-1.6 N	-3.0 T	28.2 S	-3.8 N	27.5 S
1486	-5.0 N	-1.2 S	-1.9 T	-7.0 S	2.9 N
1503	21.1 S	19.7 N	-4.0 N	-6.0 T	25.1 S
1509	-7.1 S	-4.0 N	-3.8 S	0.0 N	-2.6 T

a. T = tubed into rumen

S = suckled

N = drunk normally

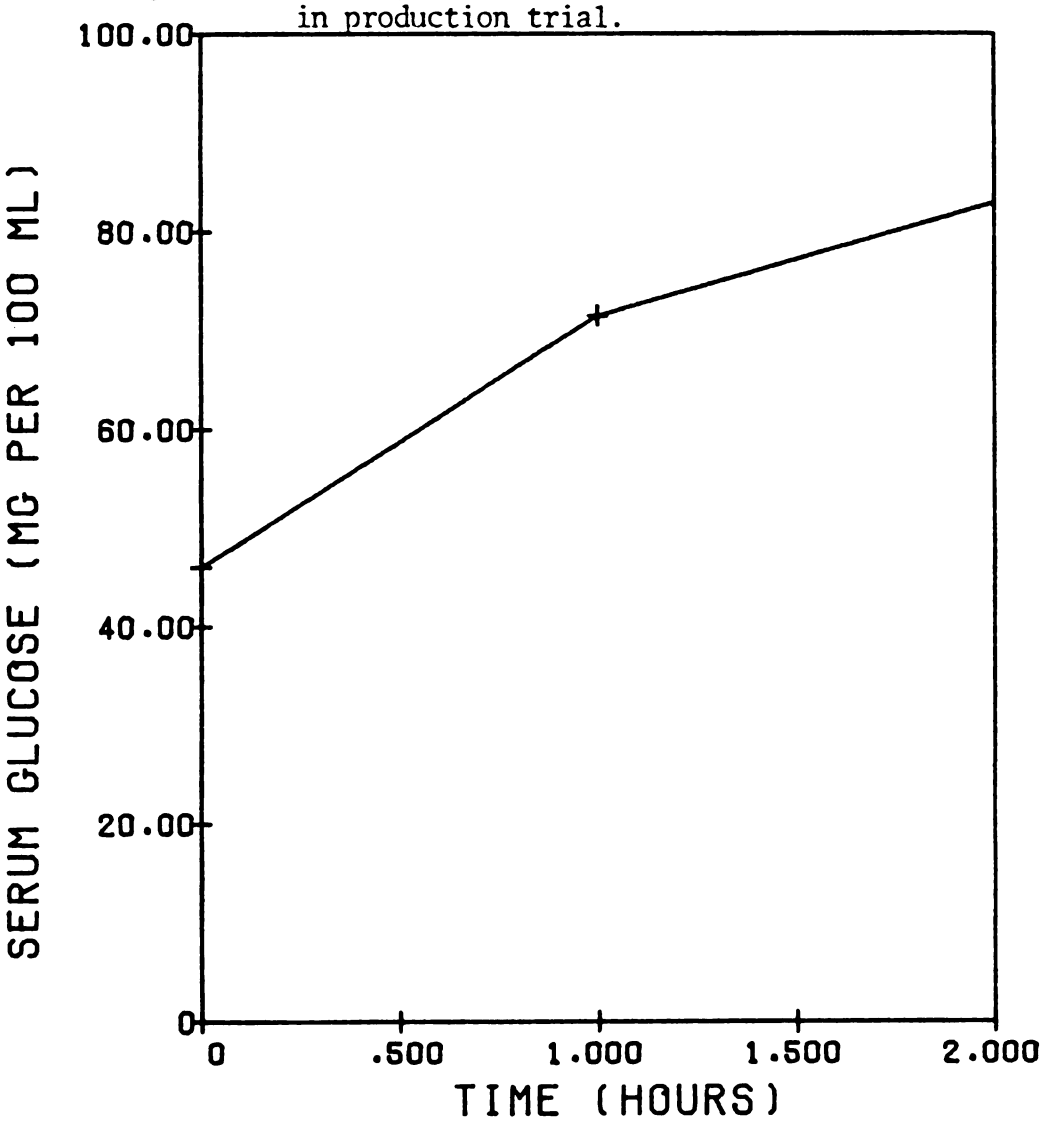
Table 9.--Mean serum glucose levels (mg/100 ml) two hours after ingestion of 500g glucose for animals in production trial.

Time	Serum Glucose (mg/100 ml)[a]
0	45.98
1	71.48*
2	82.88**

[a]Includes 12 values in each mean

*significantly different from 0 hour ($P<.05$)
**significantly different from 0 hour ($P<.01$)

Figure 5-- Serum glucose response to glucose for animals in production trial.



Production Trial

Two of the eight animals used in this trial were not included in the statistical analysis. One of these had developed ketosis and was also operated on to correct a displaced abomasum. As a result of this, the heifer stopped suckling. The other heifer also lost interest in suckling and consumed less than 30g milk protein per day. Thus, the data reported are means of values obtained from 6 cows.

Feed Analysis: Ingredient composition of the concentrate used has already been listed in Table 2. Analysis of the concentrate, silage mix, casein and milk supplement are shown in Table 10. The percent protein in the total ration was 12.3 for the suckled group and 13.5 for controls.

Feed Intake: Table 11 lists intake of nutrients for each group. Consumption of whole milk through nipple pails did not affect total dry matter, silage and concentrate intake. The control group had 130g/day greater supplement intake than the suckled group. The control group received a constant amount (500g) of supplemental casein in the concentrate daily. The concentrate was usually completely consumed, therefore 450g of casein entered the rumen daily in controls. The supplement intake for the suckled cows, on the other hand, was quite variable. Table 12 lists the protein intake from whole milk for each of the 6 cows that completed the production trial. These ranged from 200-454g with a mean of 320.5g. Only one animal, 1481, approached the goal of 500g suckled protein. The actual

Table 10.--Dry matter content and concentration of nutrients in the dry matter of feedstuffs used in the production trial.

Feed	DM[a]	Total N	ADN[b]	CP[c]	ADF[d]	NEL[e]
Concentrate	89.8	1.92	.20	12.00	6.15	1.81
s.e	.47	.07	.02	.32	.39	--
Forage mix	44.12	2.54	.23	15.87	30.65	1.43
s.e.	.79	.04	.01	.21	.54	--
Casein	92.30	14.54	--	90.90	--	--
s.e.	.64	.13	--	.54	--	--
Whole milk	12.2	4.69	--	29.31	--	--
s.e.	.32	.09	--	.38	--	--

a. dry matter

b. acid detergent nitrogen

c. crude protein = total N x 6.25

d. acid detergent fiber

e. estimated net energy for lactation

f. standard error

Table 11.--Effect of Suckled Whole Milk on Dm Intake, Supplement Intake, Total Protein Intake and NE_1 Intake

	Treatment		s.e.[a]	Overall mean	Period	
	Suckled	Control			1	2
Dry Matter Intake, kg/day	16.7 (3.25)[b]	16.6 (3.22)	± .13	16.63 (3.23)	16.3 (3.20)	16.9 (3.28)
Supplement Intake, g/day (milk protein - dry basis)	320.5	450**	±28.3	385.2	400.9	369.5
Total Protein Intake,kg/day	2.05	2.24*	± .027	2.14	2.12	2.16
NE_1 Intake, Mcal/day	24.5	25.6*	± .122	25.0	24.4	25.6

* Significantly different ($P<.01$).

**Significantly different ($P<.05$).

[a]Standard error.

[b]Values in parentheses are DM intake as a percent of body weight.

amount of milk protein reaching the abomasum was probably somewhat lower than the values listed in Table 12. Work by Wise and Anderson (1939) suggests that there may be spillover of liquid as it is directed down the esophageal groove in older animals. The fact that the groove fails to develop proportionately with the rumen (Wester, 1926) also suppports this hypothesis. Therefore, the values listed in Table 12 are considered to be the maximum amount of supplemental milk protein by-passing the rumen. Total protein intake (Table 11) therefore, was greater for controls, primarily due to the consumption of greater amount of supplemental protein.

The total protein intake as a percent of NRC requirements was 103.8 for controls and 92 for the suckled group. NE_1 intake, as a percent of NRC, was 106.7 and 99.5 for respective groups. Thus, the nutrient intake was not so high as to mask a response from protein supplemented to the abomasum (Clark, 1975; Ørskov et al. 1977). There were no effects of periods for any of the intake parameters.

Production Effects: Suckled supplemental milk protein increased milk protein yield ($P<.05$) 70g over control animals (Table 13). Milk fat yield was also increased by approximately the same amount, although not significantly so. Milk yield was increased by almost 1 kg/day this also was not statistically significant ($P<.20$). This 5% increase in milk yield, however, coupled with the 10% increase in protein yield may have considerable economic significance. According to Smith and Johnstone (1978) and Johnson (1971), reasonable estimates

Table 12.--Whole milk protein consumption through nipple pails for each cow on production trial.

Cow Number	Consumption (g/day)
1481	454.0
1482	199.7
1483	238.3
1490	295.1
1503	372.3
1470	363.2

Table 13.--Effect of Suckled Whole Milk on Yield of Milk, Milk Fat and Milk Protein, Milk Fat Percent and Milk Protein Percent.

	Treatment		s.e.[a]	Overall mean	Period	
	Suckled	Control			1	2
Milk yield, kg	22.20	21.22	±.49	21.8	22.1	21.4
Milk fat, %	3.12	2.93	±.13	3.02	2.88	3.17
Milk protein, %	3.64	3.51**	±.03	3.58	3.58	3.58
Milk fat yield, g	692.0	621.7	± 27	650.7	634.2	667.1
Milk protein yield, g	808.1	735.5*	± 18	771.0	781.8	760.2

* Significantly different ($P<.05$).

**Significantly different ($P<.1$).

[a]Standard error.

[b]Six Cows per treatment.

for protein differentials range from 3-6 cents per tenth of a pound of protein. Then for every tenth of a point the protein percent is raised, the dairyman could expect a increased income of 3-6 cents per hundredweight. If one assumes the price of milk to be $10.00 per cwt., with a 6 cent differential on protein test and a 9 cent differential on fat test, then for the cows in the study:

1.) The suckled cows lose 36 cents on fat (3.5-3.1 = .4 pct. points and x.09 = .36) but gain 6 cents on protein (3.6-3.5 = .1 pct. points.), making the price per cwt. $9.70.

2.) The control cows likewsie lose 54 cents on fat and gain nothing on protein so the price per cwt is $9.46.

Thus the value of the milk produced in one day by the suckled cows was $4.73 and that for the controls was $4.41. Even when both groups are not penalized for the low fat content and just credited for increased protein, the value of the average day's milk for the suckled cows is 25 cents greater than controls as calcuted in (3) and (4).

3.) 10.00 + .06 = 10.06/cwt. or .1006/lb

.1006 x 48.84 lbs = $4.91 for suckled cows.

4.) 10.00/cwt or .1/lb

.1 x 46.64 lbs = $4.66 for control cows.

One must also consider the cost of producing the extra milk. In this case 320 g of casein, at 72 cents[6].

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[6]Milk Specialties Inc., Chicago, Ill.

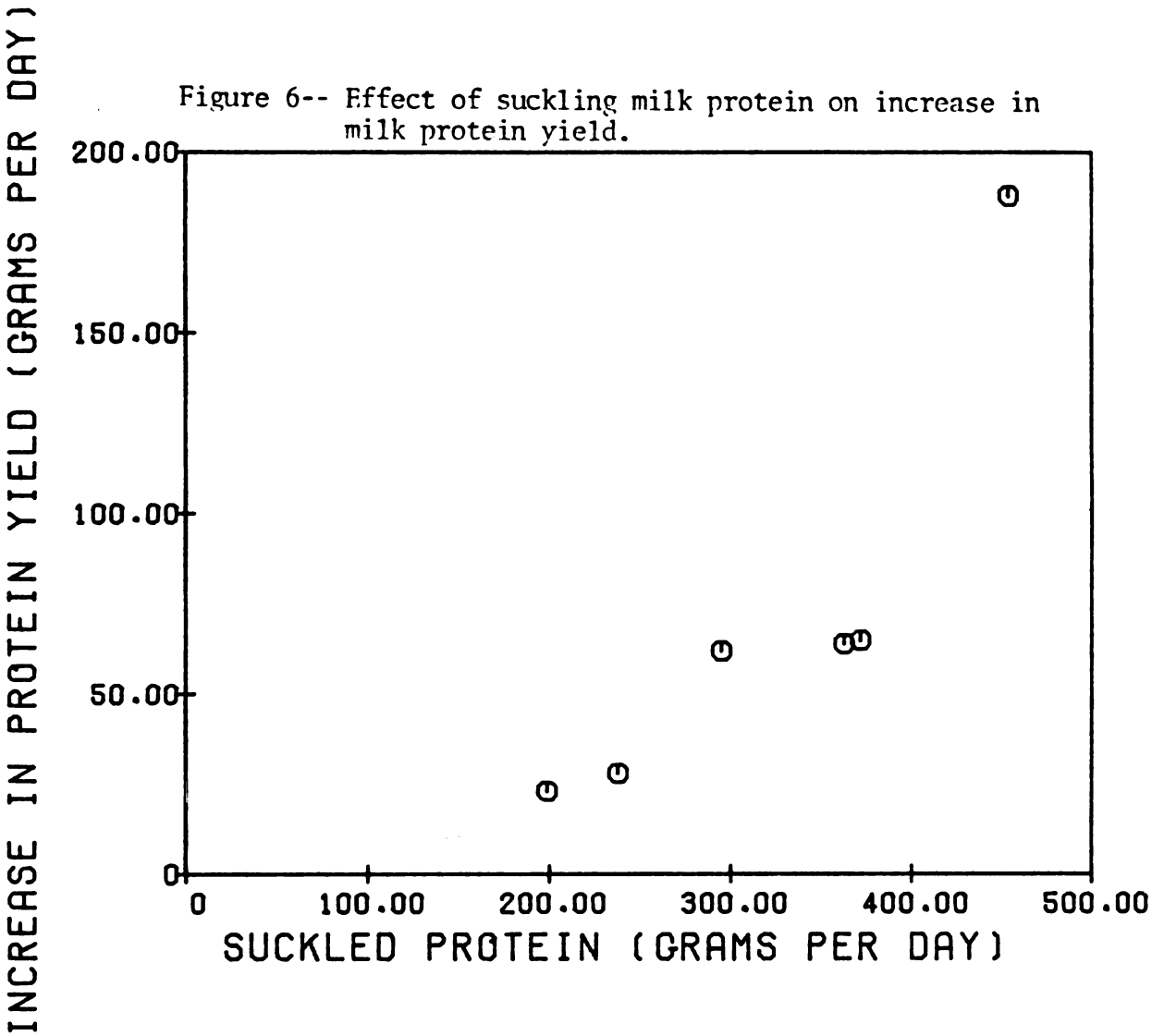
per lb, the daily cost of the casein supplement was 51 cents giving a 26 cent daily profit decrease. If, however, a soyflour solution were used and the same results obtained, the daily profit increase would be 15.5 cents, assuming a cost of 13.25¢/lb[6] per lb soyflour. Unless milk yield and protein content could be increased even further, however, the increased labor involved in training the animals to suckle and maintaining the suckling habit would make this scheme of supplementation unfeasable to the average dairyman.

Since whole milk was used as the supplement, it is difficult in this study to attribute the effects only to protein. Milk fat was consumed as well as protein. The slight increase in fat yield could have resulted from the highes fat intake (Bitman et al, 1973; Mattos and Palmquist, 1974; Wright et al. 1974). For discussion purposes, it will be assumed that the effects on protein yield were influenced by suckled protein only. In gross terms, efficiency of protein utilization (total milk protein out total protein in) was 39.3% and 32.4% for the suckled and control groups, respectively.

Figure 6 plots the increase in milk protein against the intake of suckled protein for each individual animal. This relationship is linear and has a regression coefficient of .87. A similar response was noted by Ørskov et al. (1977).

A more desirable way to look at this, however, might be to

Figure 6-- Effect of suckling milk protein on increase in milk protein yield.



estimate the amount of protein reaching the abomasum. Bucholtz and Bergen (1973) suggested 16.5g microbial protein production per 100g organic matter when rumen turnover of protein was 26%. Protein at the abomasum in this study was calculated as in (4) and (5):

$$(4)\quad DOM = \sum_{i=1}^{3} (IT \times DM \times OM \times TDN)$$

$$(5)\quad ABPR = DOM - (TPXBP) \times .165 + (TPXBP)$$

Where DOM is intake of digestible organic matter, kg; i is 1 for silage mix; 2 for concentrate; and 3 for casein supplement in rumen.

IT is intake of feed on as is basis, kg;

DM is dry matter in feed, %;

OM is dry matter minus ash, %;

TDN is total digestible nutrients, %;

ABPR is total protein at abomasum, kg;

TP is true protein, kg;

BP is plant protein by-passing the rumen, %;

.165 is kg microbial protein per kg DOM.

Estimates of total protein available at the abomasum at several rates of by-pass are listed in Table 14. If 40% of the protein entering the rumen were by-passed in to the abomasum, both groups of cows would be received approximately the same amount of protein at the abomasal level. The increased milk protein yield for the suckled group then might be explained by the better utilization of the milk protein entering the abomasum. On the other hand, if one assumes that there is no by-pass except for that protein that is suckled, then the

Table 14.--Total protein (kg/day) available at the abomasal level for cows in this study.

True Protein bypassing the rumen, % of intake	Treatment	
	Suckled	Control
0	1.57	1.43
10	1.72	1.62
20	1.86	1.81
30	2.01	1.99
40	2.15	2.18

efficiency of conversion of abomasal protein to milk protein is the same for both groups, as calculated in (6) and (7).

(6) suckled: 100 (.808/1.57) = 51%.

(7) control: 100 (.735/1.43) = 51%.

The production results obtained support the findings of abomasal infusion studies. If the amount of suckled protein could be increased, the production response may even be greater.

Other Effects: Mean levels of urea-nitrogen and glucose in the serum of cows on production trial were not different between treatments (Table 15). Body weights of the animals in each treatment were also not different and were 513.2 kg and 514.3 kg for the suckled and control groups respectively.

Conclusions

Two year old heifers can be trained to re-initiate suckling. The suckling habit continued for up to 10 months in some animals. More work is needed on maintaining the suckling habit for longer periods.

The majority of animals that suckled actively obtained rumen by-pass of the suckled fluid.

When whole milk was suckled and by-passed the rumen, milk yield was increased by 5% and protein yield by 10%.

This method of rumen by-pass certainly has merit from a research standpoint, but may not provide a great enough response to be commercially feasable at the present time.

Table 15.--Effect of suckling whole milk on mean serum levels of urea N and glucose.[a]

	Suckled	Control	s.e.[b]
Urea-nitrogen	8.32	8.98	± .87
Glucose	48.51	47.44	±2.32

a. 48 values per mean.

b. standard error

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BIBLIOGRAPHY

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APPENDIX

Plate 1: Typical view of feeding procedure with a trained animal.

Plate 2: Metal holders were used in the production trial to give animals access to milk throughout the day.

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