

FACTORS AFFECTING APPETITE IN DAIRY CALVES

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## INTRODUCTION

Appetite may be defined simply as the desire for food (45). At this station (14), appreciable variation among calves with respect to feed intake for a given ration has been observed. It was determined that approximately 50% of the variation among early weaned calves in weight gains to 8 weeks of age was associated with differences in dry feed intake. After removing the effects of sex, season, and related factors by blocking, about 15% of the total variation in weight gains to 8 weeks of age was accounted for by differences in birth weight, leaving approximately 35% of the variation in weight gains accounted for by differences in feed intake alone. Since the performance of young calves reared using an early weaning plan is so closely related to the amount of dry feed voluntarily consumed, it is important to identify the basic factors responsible for differences in intake.

The basic belief concerning the feeding of ruminant animals, as recently stated by Lassiter (43), is that maximum feed intake, appetite, and maximum animal performance are usually highly correlated. An increase in feed intake presumably represents a certain increment of increase of the energy intake of the animal. Consequently, the energy or appetite field may have the potential for increasing animal performance more than any other aspect of nutrition. In order to attain the maximum feed intake potential of the calf, and thus obtain more growth



and maximum animal performance, information concerning the essential factors controlling appetite is needed.

It is well known (4) that animals consuming only concentrate diets never fill their reticulo-rumens. On the basis of this fact, a thermostatic or chemostatic mechanism in regulation feed intake may be applicable to ruminants, though the gastro-intestinal tract fill appears to control the intake of roughages. Accordingly, and due to little information concerning the factors affecting concentrate consumption by ruminant animals, this research was conducted to study: (a) the relationship, if any, between the levels of certain metabolites, acetate and glucose, in the blood and the intake of a concentrate diet by young dairy calves, and (b) the threshold level, if any, of blood acetate with respect to feed intake.

## REVIEW OF LITERATURE

This review is limited as far as possible to the most pertinent and recent studies involving blood levels of certain metabolites, the presence of material in the digestive tract, frequency of feeding, hormones, and other factors influencing feed intake.

### Blood Levels of Certain Metabolites:

Anand (1) indicated that food intake is regulated through the interaction of a "satiety center" in the ventromedial hypothalamus and an "appetite center" in the lateral hypothalamus of the brain of the animal. In particular, he stated that the level and utilization of blood glucose have an important role in the regulation of the activity of hypothalamic centers. The glucoreceptor mechanisms are believed to be located in the satiety centers, since alterations in their electrical activity are more pronounced than in the feeding centers when blood glucose content is changed. He also stated that the activity of satiety centers may indirectly or directly influence the feeding centers.

Larsson (42) working with goats, showed that electrical stimulation of the extreme parts of the lateral hypothalamic nuclei produced an immediate eating response, often with licking and exaggerated masticatory movements. However, no significant changes in the blood sugar values were observed in relation to hyperphagia and rumination.

Glucose plays a relatively minor part in the energy metabolism in ruminant animals, since a major portion of ingested carbohydrates

are degraded to volatile fatty acids, which are absorbed into the blood stream via the rumen wall (5).

Martin et al. (48) indicated a definite volatile fatty acid absorption through the ruminal wall in 3-week-old calves fed concentrate mixtures, with or without volatile fatty acid salts added in addition to milk. More recently, Head et al. (37) studied glucose metabolism and the relative effect of intravenous infusion of acetate in three nonpregnant, nonlactating Holstein dairy cows. They demonstrated the qualitative importance of glucose in the overall metabolic economy of the ruminant, though the glucose was noted to be of less quantitative importance than acetate. These authors suggested the preferential utilization of acetate by the peripheral tissues during intervals of increased acetate availability.

It has been observed that the level of blood glucose decreases with age until it attains the blood glucose level of the mature cow (49). Reid (62, 63) reported blood glucose values in mature fed sheep from 20 to 50 mg/100 ml and little fluctuation in glucose levels associated with feeding. Arterio-venous differences (carotid-jugular) were always less than 2 mg/100 ml, indicating that little glucose was utilized by the peripheral tissues. In a different study (65), it was observed that the rate of glucose disappearance was slow in sheep fed on roughage diets, but as high as those normally observed in man on high intakes of a diet containing 50 per cent of cracked maize. Warner et al. (73) reported that the whole blood glucose of male Holstein calves declined uniformly for the first five weeks of age. The level of feed intake appeared to be of more importance in their results than the type of feed. Ratcliff et al. (61) found that whole blood reducing-sugar values declined

for the first 8 weeks of age and that limiting some of the Holstein calves (males) to a liquid diet fed by nipple did not alter appreciably the nature of these trends. Hibbs and co-workers (38) observed higher blood sugar values in calves continued on whole milk beyond 7 weeks of age than in calves of the same age which were fed only hay and dry concentrates. Martin et al. (48) reported that the postfeeding glucose level in venous blood increased sharply when young calves were fed whole milk only, but decreased slightly when fed either a control diet or a diet containing salts of volatile fatty acids. Davis and Brown (26) suggested that differences in blood glucose concentrations of 3 crossbred young calves (ranging in age from 10 to 14 days) and 3 crossbred steers (ranging in age from five to seven months) reflected differences in pool size of glucose. These data were interpreted as evidence that glucose utilization was dependent upon the amount available to the tissue.

In studying the effects of intravenous infusion of glucose on feed intake of adult ewes, Manning et al. (47) demonstrated that infusion of from 1.67 to 8.3 g of glucose per kilogram of body weight over a 2-hour period did not affect the feed consumption of the ewes or their feeding behavior. The authors indicated that the observations cast some doubt upon the possible role of glucose as the regulating mechanism in short-term appetite control in ruminants. They concluded that a chemoreceptor mechanism of regulation of feed intake in ruminants may depend on any of several blood metabolites. In the light of this exploratory work, Dowden and Jacobson (27) conducted a series of trials, using identical twin cows, to study the effects of several major intermediate metabolites on appetite. The authors reported that raised levels of blood

glucose of the cows infused with glucose (4 or 25% of body digestible energy, D.E., maintenance requirement) had little or no effect on the appetite of dairy cattle. However, administration of sodium acetate, acetic acid or propionic acid (12.5% of D.E. maintenance requirement) resulted in significant reductions in feed intake of the treated cows during the 8-hour infusion and the following 16-hour period. They concluded that one of the possible mechanisms of regulation of feed intake involves the absorption of acetic acid and propionic acid or, as indicated by the experiment using sodium acetate, the acetate and propionate ions into the blood stream, which at threshold-levels would elicit a chemoreceptor response inhibiting feed intake. In determining the effect of intra-ruminal infusion of certain rumen metabolites on voluntary hay consumption by dairy cows, Montgomery et al. (54) found that infusion of acetic acid over a 4-hour period significantly decreased daily hay consumption. When the acetic acid was supplied as the sodium salt or when partially neutralized to pH 5.0 with sodium hydroxide, there was only a small reduction in hay intake. However, infusion of propionic, butyric or lactic acid did not result in significant decrease in hay consumption, although propionic acid infusion resulted in a moderate reduction in hay intake. The amount of acid infused was equal to one-half of the 24-hour production figures. These amounts, expressed on the basis of a 1500-lb. animal, were: acetic acid, 870 g; propionic acid, 280 g; butyric acid, 260 g; lactic acid, 340 g.

In studying the relationship between acetic and propionic acid content of blood and feed intake in young dairy animals, Little and Hawkins (46) conducted an experiment using twelve dairy steers weighing from 200 to 610 lb. Two experimental rations consisting of alfalfa hay

and coastal Bermuda grass hay, respectively, were used. Total feed intake per eating interval of the animals was significantly correlated with: (a) blood content of acetic and propionic acids at initiation and termination of eating intervals, and (b) change in blood levels of these acids during the interval. Further, these workers observed no consistent threshold blood levels of acetic or propionic acid at which feed intake was initiated and terminated. Baumgardt et al. (7) reported a significant correlation ( $r = 0.63$ ) between total feed intake by Holstein heifers and concentration of acetate in the venous blood. Blood sugar responses were variable but tended to decrease after feeding.

#### Presence of Material in Digestive Tract:

Balch (3) and Blaxter et al. (10) suggested that the reason for differences in appetite of sheep for dried grass given as pellets or in long form was due to the more rapid passage of the former through the digestive tract, and that it was possible that the sheep ate to constant fill, that being the amount of dry matter present in the intestinal tract at the end of a meal. Campling and associates (17, 18, 19, 32) concluded that the amount (in weight) of the reticulo-ruminal contents has a direct effect on the voluntary intake of roughage. In a study of feed intake with three mature nonlactating cows, Freer and Campling (32) found that the voluntary intake of concentrates was not related to the digestibility of the feed or its mean time of retention in the alimentary tract in the same way as the voluntary intake of roughages. It was suggested that the concentration of products of digestion of the concentrates might have limited their intake. McCullough (51) found, from feeding trials with 34 silages fed to dairy cows as the only roughage, that dry matter intake decreased as crude protein, and



crude fiber increased. On the other hand, dry matter intake increased as dry matter digestibility and total digestible nutrients required by the cows increased.

Blaxter and his associates (10) suggested that, within the limits of fodder quality studied, the amount of feed sheep eat is determined by the capacity of their digestive tracts and that physical factors rather than physiological factors regulate appetite. From feeding trials, involving thirty male Holstein calves fed a commercial milk replacer in different reconstitutions (5, 10, 15, 20 or 25% dry matter), Pettyjohn et al. (56) concluded that at the 5% dry matter concentration the physical capacity of the calves limited the amount of feed eaten. And, at the higher nutrient concentrations, the calf, generally, limited its level of nutrient intake. Presumably, total intake (weight) of the respective diets was largely dependent upon their energy content. Veltman and Thomas (71) suggested that cows do not normally consume feed to the maximum capacity of the digestive tract for that particular feed. They also indicated that rumen retention time decreases as rate of feed intake increases. Freer et al. (33) suggested that the limit to the amount of each of several different roughage diets eaten is the result of the time required for the breakdown of particles by chewing and digestion to a size at which they can be transferred through the reticulo-omasal orifice, although the mean frequencies of the biphasic contraction of the reticulum in cows, during eating and rumination, were relatively constant with the different diets and at all levels of intake. Conrad et al. (23) concluded that physical and physiological factors regulating feed intake by lactating dairy cows change in importance with increasing digestibility. At low digestibilities the

factors were: Body weight (reflecting roughage capacity), undigested residue per unit body weight per day (reflecting rate of passage), and dry matter digestibility. At higher digestibilities intake appeared to be dependent on metabolic size, production, and dry matter digestibility.

On the basis of gastro-intestinal tract fill values calculated using rate of passage curves, Baumgardt et al. (7) suggested that distention of digestive tract did not limit the intake by eight Holstein heifers of pelleted rations consisting of different ratios of alfalfa meal to corn.

Pope et al. (57) and Hughes et al. (40) indicated that the caloric content of the diet is a more important factor controlling feed intake of fattening cattle than bulk or density of the ration. However, Brown and Lassiter (12) reported that the influence of the protein-to-energy ratio on growth rates did not appear to be expressed through the consumption of feed (in weight), since only slight differences in feed consumption were observed among nine groups of young calves receiving three protein levels, 14, 16 and 18%, each of which contained three protein-to-energy ratios, 1:46, 1:48 and 1:50, up to 86 days of age. However, as the protein-to-energy ratio became wider, the growth rates decreased, particularly, after milk feeding was discontinued.

Wayman et al. (74) reported that lactating cows reduced their feed intake, whenever possible, under high-temperature stress. During this study, when total intake was maintained constant by force feeding through ruminal fistulae, but less than the digestive tract fill, the marked decrease in voluntary feed intake provides evidence confirming the existence of factors, other than rumen capacity alone, controlling



appetite. Eng (28) reported a consistent decrease in the feed intake of sheep as the environmental temperature was raised from 55 to 100°F.

#### Frequency of Feeding:

Rakes et al. (59) presented data suggesting that a response from frequent feeding can be attained with young growing animals, but not with older mature animals. Mohrman et al. (53) found that feed consumption and daily gain were improved by feeding beef cattle six times over a 24-hour period day as compared to feeding twice daily. Campbell and Marilan (16) reported a decrease approaching significance ( $P = 0.10$ ) in feed consumption of 21 lactating dairy cows fed only two times as compared to 4 or 7 times daily. Rhodes and Woods (66) have shown that feed consumption was improved ( $P < .01$ ) by self-feeding as compared to feeding fattening lambs an equalized daily intake of feed at different intervals. In comparing the performance of steer calves fed ad libitum vs. twice daily in individual stalls, Church and Ralston (20) found that ad libitum feeding resulted in greater feed intake than did individual feeding. In contrast, Clark and Keener (21) reported that, in four successive trials, no significant differences in feed consumption of growing Holstein heifers were observed as a result of feeding 24X or 10X vs. 2X daily. In relating rumination to the effect of frequent feeding on efficiency of feed utilization, Gordon (35) found that the rate of regurgitation of boli was more rapid in sheep frequently fed, and it was postulated that this may reflect a general increase of motility, secretion and digestion in the rumen.

The proportion of volatile fatty acids was not affected by frequency of feeding according to Leffel and Komarek (44), but Mochrie (52) reported less diurnal variation in volatile fatty acid concentrations

when animals were frequently fed. Knox and Ward (41) observed higher volatile fatty acid concentrations and a lower acetic/propionic ratio when animals were fed frequently. Putnam et al. (58) have shown that the average rumen volatile fatty acid concentrations of Angus heifer calves were somewhat greater when fed ten times daily than when fed two times daily. However, within pair comparisons were inconsistent and the difference did not approach statistical significance. The authors also indicated that the frequently fed animals appeared to have greater appetites. Thus, more frequent feeding has been reported to result in less variation in the concentration of total VFA (41, 52) and a lower molar percentage of acetic acid (41) in the rumen. Consequently, lower peak concentrations of acetic and/or propionic acids in the blood resulting from less variation in rumen VFA may increase the appetite of the ruminant animal.

#### Hormones and Other Factors Influencing Feed Intake:

Balch and Campling (4) stated that treatment of animals with hormones likely will influence the energy requirement, and thus indirectly alter the long-term regulation of food intake; however, there is no experimental proof of the direct influence of hormones on the mechanisms regulating intake. These authors referred to Ferguson who showed, in at least one experiment, that injection of mono-sodium-L-thyroxine stimulated feed intake in sheep. Godfrey and Tribe (34) reported that implantation of sheep with a single dose of three 30 milligram tablets of L-thyroxine did not increase their intake of hay. But, the authors attributed the lack of effect to the poor quality of the hay. From results of many experiments with dairy cows, Blaxter et al. (8) concluded that thyroxine has a stimulating effect on the intake of mixed

feeds of hay and concentrates. But, Singh and Donker (68) found that feeding thyroprotein to one of each pair of identical twin dairy heifers did not increase voluntary intake of hay.

Balch and Campling (4) noted that artificial estrogens appear to have inhibited intake of feed by goats and sheep. They further indicated that in a recent experiment variable effects on feed intake by lambs were obtained with stilbestrol given in the feed, or progesterone with estradiol given by injection. However, more recently, Wickersham and Schultz (75) showed that during a 280-day period, from 6 to 15 months of age, feeding diethylstilbestrol (DES) stimulated a highly significant ( $P < 0.01$ ) increase in the average daily roughage dry matter consumption (0.97 lb per animal) of seven treated Holstein heifers, although the treatment resulted a slight but nonsignificant reduction (-5.8%) in feed efficiency. However, the DES treatment had no significant effect on feed intake of the dairy heifers during the period from 15 to 24 months.

Okamoto et al. (55) found no relationship between the free histamine concentration in silage and the silage dry matter intake of dairy heifers.

## EXPERIMENTAL PROCEDURE

Two experiments were conducted to study (a) the relationship between the levels of certain metabolites, acetate and glucose, in the blood and the intake of a concentrate diet by young dairy calves, and (b) the threshold level of blood acetate with respect to feed intake,

### Experiment I:

Management and Feeding: Twenty Holstein male calves were placed on experiment at three days of age as they became available. To minimize, to some extent, the variation attributable to differences in initial weight, only calves weighing between 70 and 110 pounds were used. The calves were raised in individual pens with wood shavings being used for bedding. Each calf received colostrum from its dam during the first two days following birth and then whole milk via nipple pail until 28 days of age. Whole milk was fed at the daily rate of 10% of initial body weight during the first 12 days in the experiment, and at the rate of 8% of initial weight for an additional 14 days. Starting at a few days of age, the calves were encouraged to eat calf starter. The same calf starter ration was fed to all calves free-choice throughout the seventy-day experiment and constituted the sole diet after abrupt weaning at 28 days of age.

The starter consisted of dehydrated alfalfa crumbles 20%, crimped corn 24%, crimped oats 24.3%, wheat bran 8.2%, corn distillers solubles 5.5%, dried molasses 4%, soybean meal 11.5%, trace mineral salt 1%,

dicalcium phosphate 1%, and antibiotic-vitamin premix 0.5% (to give chlortetracycline, 25 mg.; vitamin A, 2250 I.U.; vitamin D, 280 I.U. per pound of ration, respectively).

Health Observations: Observations were made on the health of the calves throughout the experiment. Fecal consistency was rated and recorded twice daily on an arbitrary scale of one through four denoting: 1 - normal, 2 - very soft, 3 - semi-fluid, and 4 - extremely fluid feces. The thriftiness of each calf was rated upon termination of the experiment using the following scale: very thrifty, thrifty and unthrifty.

Experimental Measurements: The relationship between the levels of the metabolites, acetate and glucose, in the venous blood and appetite of young calves was evaluated by measuring (a) the amount of feed consumed, and (b) the levels of blood glucose and acetate at the initiation and at the termination of specific eating intervals. An eating interval was defined as the period from start of eating until a calf stopped eating for at least 5 minutes. Samples of venous blood obtained from the jugular vein were collected at the initiation and termination of the eating interval following a 12-14 hour fasting period at 3, 5, 7, and 9 weeks of age ( $\pm$  3 days).

The amount of dry feed consumed by each calf was recorded daily (at 11:00 A.M.). In addition, the amount consumed during each eating interval at the ages mentioned above was recorded. Body weights of the calves were determined at the initiation of the experiment and at weekly intervals thereafter.

Acetate Tolerance Test: At the end of the 10-week period, an acetate tolerance test on each calf was carried out in the morning following an 18-20 hour fasting period. An acetate solution, containing

20.98 per cent (weight/volume) acetic acid neutralized to pH 7.4 with sodium hydroxide, was injected into a jugular vein of the first seven calves on the experiment. The attempt was made to inject 100 ml of the acetate solution in each calf, but none of the animals received the complete quantity due to some losses during injection. Six calves received amounts ranging from 82 to 97 ml of acetate, over a period of 2 to 4 minutes. One calf was given only 56 ml of the acetate solution due to discomfort evidenced by coughing and other symptoms of shock.

Due to the signs of discomfort shown by several of the first seven calves during the injections, the concentration of acetate solution was decreased from 28.68 per cent to 20.50 per cent. Therefore, the remaining 13 calves received amounts ranging from 80 to 97 ml of the less concentrated acetate solution (Table I) in the same manner as described above. During each test, blood samples were taken from the jugular vein of each calf immediately before and at 0, 30, 60, 90 and 120 minutes following acetate injection.

#### Experiment II:

Management and Feeding: Four Holstein male calves, weighing 94.5, 86.0, 95.0, and 100 pounds, respectively, were placed on experiment when three days of age. They were raised in individual pens with wood shavings used for bedding. Each calf received colostrum from its dam during the first two days following birth and then whole milk via nipple pail until 28 days of age. Whole milk was fed at the daily rate of 10% of initial body weight. The same calf starter ration used in Experiment I, was fed to all calves free-choice throughout the experiment and constituted the sole diet after abrupt weaning at 28 days of age.

TABLE I

AMOUNTS OF ACETATE INJECTED FOR TOLERANCE TEST ON 20 CALVES AT APPROXIMATELY 10 WEEKS OF AGE

Calf no.	Body weight, (W)  (1b)	Na acetate solution injected		Injected acetate calculated as acetic acid		
		Volume (ml)	Concentration (w/v %)	(g/calf)	(g/100 lb W)	(g/100 W <sub>kg</sub> <sup>.75</sup> )
200	142.5	88	28.68	18.5	12.9	80.9
3	170.0	97	"	20.4	12.0	78.1
63	126.0	88	"	18.5	14.6	88.7
75	126.4	92	"	19.3	15.3	92.5
79	125.2	96	"	20.1	16.1	97.2
39	144.6	82	"	17.2	11.9	74.5
30	129.9	56	"	11.7	9.0	55.2
4	142.0	97	20.5	14.5	10.2	63.9
38	173.7	97	"	14.5	8.4	54.9
67	169.8	95	"	14.2	8.4	54.7
181	161.8	96	"	14.4	8.9	57.3
182	150.3	84	"	12.6	8.4	53.0
183	134.8	91	"	13.6	10.1	62.3
185	126.0	97	"	14.5	11.5	69.9
186	164.6	95	"	14.5	8.7	56.0
204	150.0	97	"	14.5	9.7	61.3
6	186.0	86	"	12.9	6.7	46.3
13	135.0	92	"	13.8	10.2	62.9
53	135.0	90	"	13.5	10.0	61.6
20	138.5	87	"	13.0	9.4	58.4

Health Observations: Observations were made on the health of the calves throughout the experiment. The same systems used in Experiment I were followed in the evaluation of fecal consistency and thriftiness of each calf.

Acetate Infusion: Exteriorization of one carotid artery of each experimental calf at 1 to 3 weeks of age was made using the technique for relocation of the carotid artery described by Butler (15). Infusion studies were carried out on each calf at different ages within the period from 42 to 151 days of age (Table VI). An 18- to 20-hour fasting period preceded each infusion study. Calves 88, 4, and 92 received acetate solutions of various concentrations ranging from 1.37 to 20.55% (w/v) at a rate of 0.97 ml/minute (Table VI). Quantities ranging from 6 to 55 ml of the acetate solutions were infused. To determine the effects of infusion, calf 211 received 20 ml of saline (0.85%) solution at a rate of 0.194 ml per minute when it was 46 days of age.

Calves 88, 4, and 92 received autoclaved acetate solutions made of acetic acid neutralized to pH 7.4 with sodium hydroxide in saline at 46, 71, and 42 days of age, respectively. Calf number 88 received acetate solutions composed of anhydrous sodium acetate dissolved in distilled water when it was 67, 88, 100, and 107 days of age. Calf number 4 received acetate solutions composed of anhydrous sodium acetate dissolved in saline solution when it was 88 and 151 days of age; and calf number 88 received an acetate solution made in the same manner when it was 121 days of age.

The infusion was made into the exteriorized carotid artery through a small sterile intramedic polyethylene tubing inserted previously through an 18 gauge thin wall or 17 gauge needle (Figure 1). The





Fig. 1. Acetate infusion technique. View illustrating the insertion of a small polyethylene tubing into the exteriorized carotid artery prior to infusion.

polyethylene tubing was held on the neck of the calf with adhesive tape in such a way that it remained in place throughout each infusion period, and allowed the animal to move its head freely. The rate of infusion was controlled by using a 600-930 Quadruple-syringe infusion-withdrawal pump (Figure 2) The effect of raised level of blood acetate and its threshold level with respect to appetite were determined by the observations made on the general performance, the heart rate, the respiration rate, the amount and frequency of urination and defecation, the body temperature, the amount of water consumed, the amount of dry feed consumed, and the time the animal stopped eating during each test period.

Experimental Measurements: Samples of venous blood were obtained from the jugular vein of each calf immediately before start of infusion and after the animal stopped eating at each test and used to ascertain the threshold level of acetate in terms of feed intake. The amount of dry feed consumed during each infusion period was recorded. Body weights of the calves were determined at the initiation of the experiment, at each infusion and weekly throughout the experiment.

Determination of Blood Metabolites:

Blood samples from calves in both experiments were collected in prepared collection tubes and kept cooled in an ice bath after the blood was collected. The collection tubes were prepared by evaporating 0.2 ml of a potassium oxalate solution (0.1 g/ml) in each tube prior to blood sampling. Protein-free filtrates of the blood were prepared according to the method of Folin and Wu (31) and stored in a deep freeze until analyzed.

Determination of Blood Reducing Sugar: Eight standard solutions of glucose, containing 10, 20, 40, 60, 75, 80, 90 and 100 ug glucose/ml,

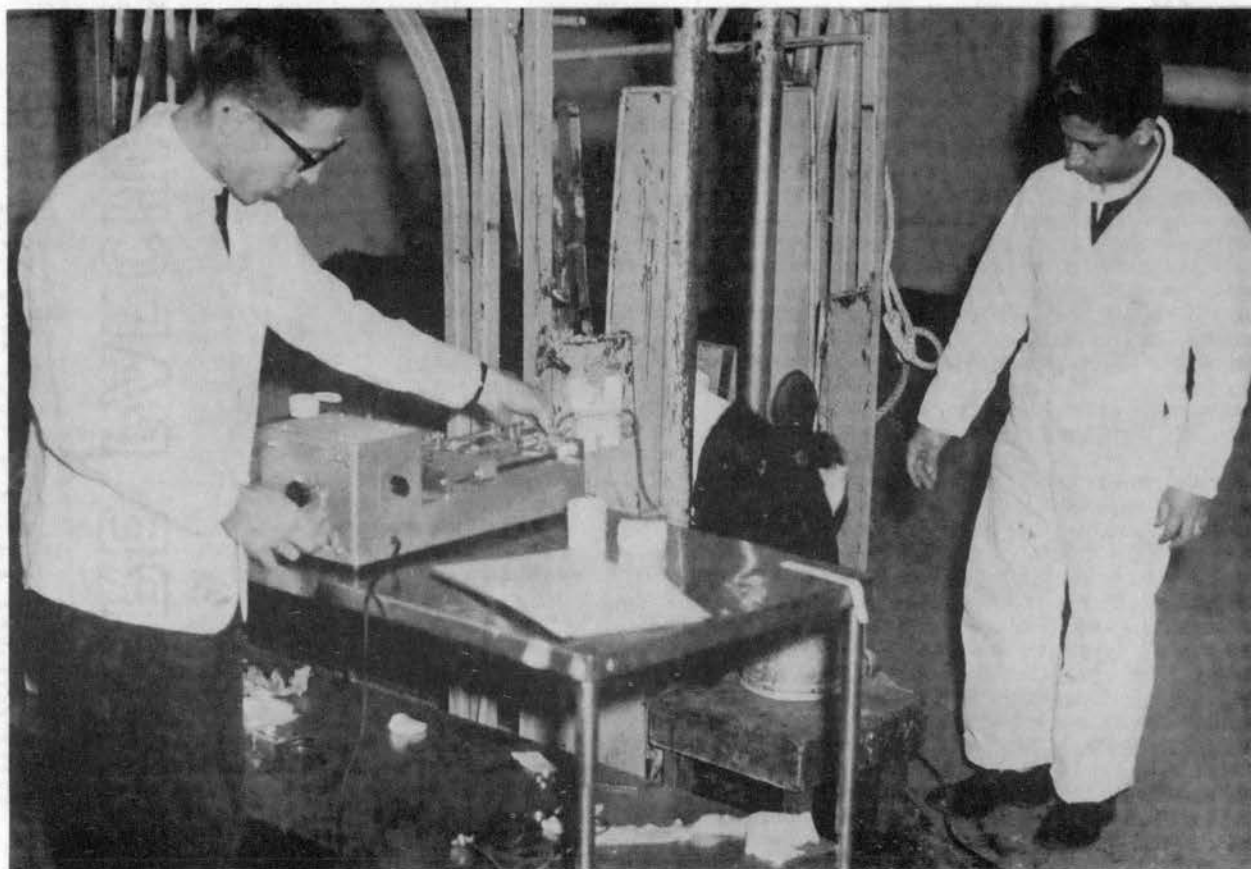


Fig. 2. Acetate infusion technique. View illustrating the connection of the tubing to an infusion-withdrawal pump with which the rate of infusion was controlled.

respectively, were prepared according to Roe (67) to establish a glucose standard curve used for determining the reducing sugar values of the blood samples. A one-milliliter portion of each sample filtrate was analyzed for blood glucose by the anthrone reagent technique as described by Colvin et al. (22), except that a Klett-Summerson colorimeter was used instead of spectrophotometer for determining the amount of reducing sugar.

Determination of Blood Acetic Acid: A 50 or 100 ml portion of each filtrate was used for determining the blood acetic acid using a modification of the methods reported by Erwin et al. (29) and Baumgardt (6).

A standard solution of VFA containing acetic, propionic and butyric acids was prepared to use in determining the unknown quantity of the individual fatty acids in the blood samples. The quantities of the VFA used in the standard corresponded to values representing five times the concentration of previously reported average amounts (8.4, 1.21 and 1.49 mg/100 ml of acetic, propionic and butyric acids, respectively) present in the blood of young calves (39, 49, 50, 60, 62, 69). To prepare a liter of standard solution, appropriate amounts (in volume) of the acids were measured into a 10 ml stoppered volumetric flask containing distilled water, and weighed individually. The contents were then emptied into a one-liter volumetric flask. After rinsing the 10 ml flask several times and emptying into the one-liter flask the solution was made up to one liter, shaken, and placed in the cooler.

Sample Preparation for Chromatography: To form the sodium salts of the fatty acids 1 or 2 ml of 3N NaOH solution was added to a 50 or 100 ml portion of protein free blood filtrate, respectively. The resulting solution was evaporated to dryness in an oven set on a

constant temperature (approximately 90°C).

To the dry residue in each flask 1 ml of 3.2N H<sub>2</sub>SO<sub>4</sub> or 6.4N H<sub>2</sub>SO<sub>4</sub> was added to the 50 or 100 ml of filtrate, respectively, and the contents carefully mixed. The liquid was transferred to a one-gram glass vial and stored in a deep freeze ready for direct gas-liquid chromatographic analysis.

Chromatographic Method: The instrument used was an Aerograph Model A-600-C, Hy Fi, (Wilkins Instrument and Research, Inc., Walnut Creek, California). This instrument was equipped with a hydrogen-flame ionization detector which, being insensitive to water, permits the use of aqueous solutions, and an 8-step precision attenuator that reduced the signal by half with each step from 1 to 128. A range switch (10 to 1000 for the hydrogen flame or 1 to 10 for electron capture, E.C.) further reduced the signal by 100 times.

The Aerograph hydrogen generator Model A-650 was used to supply filtered hydrogen necessary for the operation of the flame ionization detector. Cylinder laboratory grade oxygen was used instead of air.

Cylinder laboratory grade nitrogen was used as the carrier gas and a conventional two-stage pressure regulator was employed.

A Sargent Model SRV (S-72180-20) recorder with a 1 mv range, 0 to 100 scale chart paper, and  $\frac{1}{2}$ , 1 and 2 inches per minute chart speeds, was used for recording the eluted fatty acid peaks.

A column material consisting of 20% neopentylglycol succinate (NPGS) and 2% H<sub>3</sub>PO<sub>4</sub> on 60-80 mesh firebrick, was packed in a 1/8" x 5' stainless steel column. This column was then heated at a constant temperature of approximately 220°C for at least 48 hours before operation.

The following operating conditions were found to be the most satisfactory for the above mentioned column in separating the blood VFA:

- 1 - oven temperature, 135-150°C.
- 2 - injector temperature, approximately 160°C.
- 3 - carrier gas (nitrogen) flow rate at detector head,  
28 ml per minute.
- 4 - hydrogen flow rate to detector, 20 ml per minute.
- 5 - oxygen flow rate to detector, approximately 60 ml per minute.
- 6 - set range switch on 10 "flame."
- 7 - sample size: 1 microliter of reconstituted blood filtrate  
per injection.
- 8 - record speed, 1 inch per minute.
- 9 - attenuations of 8X and 64X used for detecting the lowest  
and highest acetic acid contents of blood samples, respectively.

Calculation of Blood Acetic Acid: The blood acetic acid was calculated from the peak obtained following the injection of 1.0 ul of reconstituted blood filtrate in the gas chromatography instrument. The area of the respective peaks eluted following the injection of the standard solution (Table II) was assumed to represent the computed micrograms per 1.0 ul. The amount of the acetic acid in the whole blood (mg/100 ml) was then computed using the following formula:

$$\text{Concentration of blood acetic acid (mg/100 ml)} = \frac{(\text{sample peak area}) (\text{ug/1.0 ul of standard})}{(\text{standard peak area}) (\text{concentration factor})} \times 100$$

TABLE II  
STANDARD VFA SOLUTION

Acids	ul/liter	g/liter	ug/ul
Acetic	400.38	0.4200	0.4200
Propionic	61.05	0.0605	0.0605
Butyric	77.71	0.0745	0.0745

## RESULTS AND DISCUSSION

All calves appeared to be thrifty and healthy during the major portion of the respective experiments. Relatively severe diarrhea was evident in a few cases, but it did not last more than 2 or 3 days at the most. The growth rate of the calves in Experiment I was found to be less than the optimum rate previously reported at this station (76). However, all calves consumed a substantial amount of the starter ration throughout the respective experiments.

### Experiment I:

Relationship Between Acetate and Glucose Content of Blood and Feed Intake: The average blood levels of acetic acid at the start and termination of the eating intervals were nearly the same at all ages studied in this experiment (Table III). The acetic acid levels were comparable to those reported by other workers (46, 48, 49); however, the lack of change in concentration of blood acetate with age was in contrast to the results of McCarthy et al. (49), who found the level of blood VFA (calculated as acetic acid) to increase from 1.78 to 6.53 mg/100 ml between the ages of 1 to 15 weeks.

The average blood levels of glucose at the start and termination of the eating intervals were slightly more variable from one age to another than those of acetate; however, the values were comparable to those reported by other workers (2, 48, 49, 76). The lack of decrease in concentration of blood glucose with age was in



TABLE III

FEED INTAKE AND BLOOD LEVELS OF ACETIC ACID AND GLUCOSE AT FOUR DIFFERENT AGES

	Age (wk)	Feed intake per eating interval (lb)	Acetic acid level			Glucose level		
			initial	terminal	change in level	initial	terminal	change in level
			(mg/100 ml)			(mg/100 ml)		
Means	3 <sup>a</sup>	0.24	7.8	6.3	-1.5	107	88	-19
	5	0.52	7.3	7.1	-0.2	87	84	-3
	7	0.99	7.1	7.5	0.4	87	90	3
	9	1.50	7.5	7.8	0.3	92	94	2
Standard deviation	3 <sup>a</sup>	0.14	1.5	3.2	3.3	17.3	27.9	25.5
	5	0.25	4.5	2.1	4.1	24.3	22.1	18.5
	7	0.33	3.2	1.7	3.8	15.1	14.0	10.8
	9	0.35	2.8	2.9	4.5	15.3	14.9	18.3
Coefficient of variation	3 <sup>a</sup>	58.3	19.2	50.8	--	16.2	31.7	--
	5	48.1	61.6	29.6	--	27.9	26.3	--
	7	33.3	43.8	23.3	--	17.4	15.5	--
	9	23.3	37.3	37.2	--	16.6	15.8	--

<sup>a</sup>At 3 weeks of age, all values are calculated from observations on 11 calves, while at other ages, observations on 20 calves were used.

contrast to the results previously obtained at this station (76) and those of other workers, including Attebery and Colvin (2) who found that inclusion of grain and hay in the diet increased the rate of decline in the fasting blood glucose levels in dairy calves from 1 to 13 weeks of age.

The average daily dry feed intake of the calves during the first 10 weeks of this experiment was 2.02 lb. which was similar to that previously found in this station (76). The average weight gain, 0.77 lb/day, was comparatively less than that previously reported (76). Nevertheless, the growth rate of the calves was considered satisfactory for the purpose of this experiment since the calves were thrifty and healthy as noted above. The average dry feed intake during the specified eating intervals increased with age, particularly from 3 to 5 weeks of age during which time the calves were weaned (Table III).

There was a considerable amount of variation among the calves in concentration values for both blood glucose and acetic acid (Tables III and VII). The wide variation among blood levels of glucose and acetate obtained in this study could be attributed to at least several factors involved in feeding of young calves. For example, the exact age at which rumination started, the time and amount of natural inoculation, the difference in size of the reticulo-rumen, and the difference in rate of rumination could have contributed to the variation in acetate and glucose concentrations. Since the starter ration fed in this study contained 20% dehydrated alfalfa crumbles, a few calves occasionally consumed the grain portion of the ration and left most of the alfalfa crumbles. This mode of eating could have been another source

of variation in acetate content of the blood in young calves, since changing the grain to hay ratio has been observed to change the molar proportion of acetate in the rumen, regardless of the form of hay in the diet (13, 36).

The coefficient of variation in concentration of acetic acid in the venous blood of the calves was greater than it was for glucose at each age at which samples were taken (Table III). However, on the basis of the correlation coefficients presented in Tables IV and V, no consistent linear relationship appeared to exist between blood concentrations of acetate or glucose and feed intake of the young calves. The incidence of a few relatively large correlation coefficients was likely due to chance only, since some of the other correlation coefficients ( $r = 0.47$  and  $0.54$ , Table V;  $r = -0.896$ , Table IV) did not fit with either the chemostatic or the thermostatic theories, and the remainder ( $r = -0.61$ , Table IV;  $-0.48$ ,  $0.62$ ,  $0.60$ ,  $0.59$ ,  $0.53$ ,  $0.59$ ,  $0.897$  and  $0.467$ , Table V) were inconsistent with respect to eating interval and age of the animal.

The chemostatic theory is that some metabolite found in the blood circulation may stimulate the hypothalamus to halt or initiate eating and that the absolute blood levels as well as arteriovenous differences of glucose affect voluntary feed intake (1, 4). The thermostatic theory is that "animals eat to keep warm and stop eating to prevent hyperthermia," and that the extra heat liberated as a result of the specific dynamic action of feed consumption could well be the signal to the hypothalamus for the animal to cease eating (1, 4).

In contrast to the results obtained in the present study, Little and Hawkins (46) found the initial and final levels of blood acetic

TABLE IV  
CORRELATION COEFFICIENTS BETWEEN FEED INTAKE DURING EATING INTERVALS  
AND BLOOD ACETIC ACID OF YOUNG CALVES

Age	Feed intake variable	Blood level of acetic acid		
		initial	terminal	change
3 weeks	Total amt/interval	0.02	0.28	-0.131
	Amt/interval/100 lb.	0.02	0.30	0.195
	Amt/interval/100 W <sub>kg</sub> <sup>0.75</sup>	0.07	0.28	0.224
5 weeks	Total amt/interval	-0.13	0.20	0.148
	Amt/interval/100 lb.	-0.19	0.23	0.203
	Amt/interval/100 W <sub>kg</sub> <sup>0.75</sup>	-0.18	0.19	0.193
7 weeks	Total amt/interval	0.03	-0.26	-0.128
	Amt/interval/100 lb.	-0.03	-0.33	-0.061
	Amt/interval/100 W <sub>kg</sub> <sup>0.75</sup>	-0.61*	-0.896*	-0.103
9 weeks	Total amt/interval	-0.11	-0.35	0.292
	Amt/interval/100 lb.	-0.08	0.22	0.188
	Amt/interval/100 W <sub>kg</sub> <sup>0.75</sup>	-0.12	0.21	0.220

\*Statistically significant ( $P < 0.05$ )

TABLE V  
CORRELATION COEFFICIENTS BETWEEN FEED INTAKE DURING EATING  
INTERVALS AND BLOOD GLUCOSE OF YOUNG CALVES

Age	Feed intake variable	Blood level of glucose		
		initial	terminal	change
3 weeks	Total amt/interval	0.20	0.557	-0.474
	Amt/interval/100 lb.	0.38	0.62*	0.897*
	Amt/interval/100 W <sub>kg</sub> <sup>75</sup>	0.27	0.60*	0.483
5 weeks	Total amt/interval	-0.051	-0.0004	0.342
	Amt/interval/100 lb.	-0.04	0.11	0.114
	Amt/interval/100 W <sub>kg</sub> <sup>75</sup>	-0.48*	0.02	0.467*
7 weeks	Total amt/interval	0.47*	0.417	-0.358
	Amt/interval/100 lb.	0.43	0.39	-0.039
	Amt/interval/100 W <sub>kg</sub> <sup>75</sup>	0.35	0.37	-0.086
9 weeks	Total amt/interval	0.54*	0.59*	0.246
	Amt/interval/100 lb.	0.27	0.53*	0.214
	Amt/interval/100 W <sub>kg</sub> <sup>75</sup>	0.36	0.59*	0.202

\*Statistically significant ( $P < 0.05$ )

and propionic acids and change in levels of these acids significantly correlated with feed intake during specific eating intervals. The different results obtained by these authors could have been due to the differences in diet, age, and amount of variation in body weight of the animals used.

If a consistent correlation were observed between blood concentrations of acetate and glucose and feed intake, the importance of the blood content of glucose should decrease and that of acetate increase quantitatively in relation to feed intake during the transition phase (the period of transition from monogastric into ruminant) of growth of young ruminant animals. In reference to the transition phase, other workers (48, 49) have observed that the level of blood glucose decreases and that of acetate increases with the age of young calves. Degradation of ingested carbohydrates to volatile fatty acids (5) increases as a result of simultaneous development of the rumen and the establishment of a population of ruminal microorganisms (5, 30, 70, 72).

The lack of statistically significant correlations between the levels of acetic acid and glucose in the blood and feed intake does not necessarily provide evidence that these metabolites are not related, in one way or another, to feed intake of ruminant animals. Hyperglycemia induced by intravenous injection of glucose has been observed to depress and intravenous injection of VFA to slow the rate of contraction of the reticulo-rumen (4). However, it is suggested that neither acetate nor glucose per se is an important metabolite regulating feed intake in the blood of young dairy calves, particularly those of the ages used in this study. As far as blood glucose

is concerned, this finding confirms that of Larsson (42) who found no significant changes in the blood sugar values in relation to hyperphagia when electrical stimulation of the extreme parts of the lateral hypothalamic nuclei produced an immediate eating response in goats. There is no information available at the present time in regard to the effect of acetate circulating in the blood on bilateral parts of the hypothalamus in relation to feed intake of the ruminant animals.

Acetate Tolerance Test: Very low concentrations of acetic acid were observed in the blood samples taken immediately after acetate injection in five calves as compared to the remaining 15 calves in this study (Table VIII). One possible explanation for this occurrence is that Calves 67, 204, 6, 53, and 20 (Table VIII) did not receive the correct amount of acetate solution in their blood due to inadvertent difficulties encountered during the acetate injection. It is possible that after proper insertion of the needle, it passed through the jugular vein or was pulled out of it owing to the excitement of the animal during injection. In any event, the data on these five calves were considered invalid, and only the data from 15 calves were used for the calculation and interpretation of the results (Tables VIII and IX).

The intravenous injection of sodium acetate solution (20.5 or 28.68%) in the young calves fasted for 18-20 hours did not appear to have any influence on blood level of glucose, during the 2-hour period of the tolerance test (Figure 3).

The correlation coefficients between the exponential rate of acetate utilization of the calves and average feed intake (for 10 days prior to the tolerance test), the average feed intake per 100 kg body weight, or the average feed intake per 100 units of metabolic size,

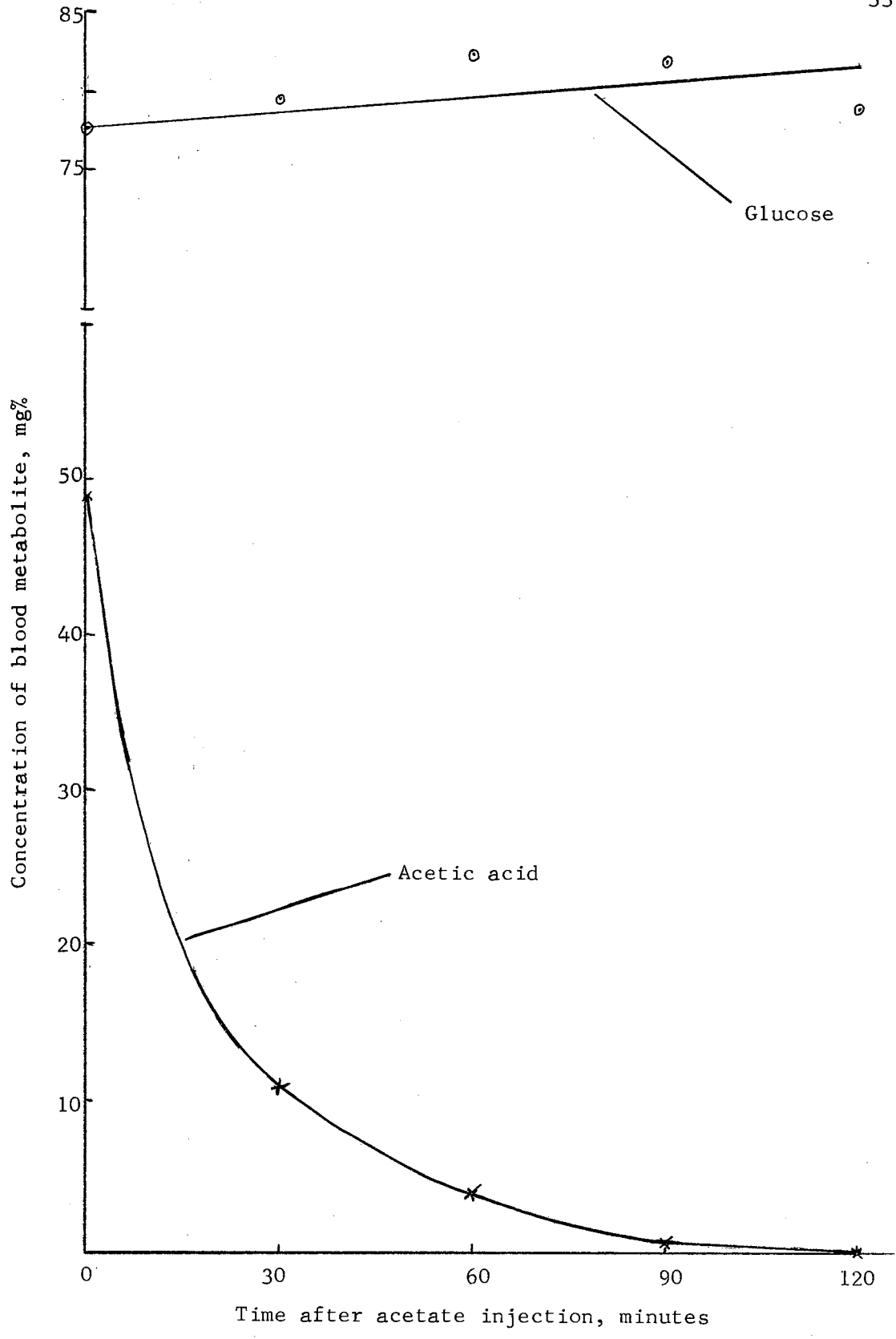


Figure 3. Acetate tolerance test conducted on 15 calves at approximately 10 weeks of age (concentration of acetic acid = total observed conc.-Pre-injection conc.).



were calculated to be -0.43, -0.23, and -0.32, respectively. These values were found to be statistically not significant ( $P > 0.10$ ). Thus, there was no definite indication of relationship between utilization of blood acetate by body tissue and feed intake, although acetate has been considered by other workers (11) to be the major volatile fatty acid utilized for energy in the peripheral blood of ruminants. The acetate may have been inefficiently used for energy during the tolerance test as it has been suggested (11) that acetic acid is not readily oxidized unless carbohydrate or a carbohydrate precursor is given to the animal at the same time.

#### Experiment II:

Acetate Infusion: Infusion of 20 ml of saline solution (0.85%) into the arterial blood at a rate of 0.194 ml per minute did not have any adverse effect on Calf 211. No other infusion studies were made on this calf at later ages due to difficulties encountered in placing the needle into the carotid artery.

Various volumes (6 to 55 ml) of acetate solutions having concentrations of 1.37, 4.38, 8.49, 9.59 to 20.55 g/100 ml were infused at an arbitrary rate of 0.97 ml per minute into the arterial blood of Calves 88, 4, and 92 (Table VI).

At 46 days of age, 12 ml of 1.37% and 5 ml of 9.59% acetate solution (in physiological saline) were infused into the arterial blood of Calf 88 during the first and last portions of a 25-minute infusion period, respectively. The amount of acetate infused during the first portion of the infusion period was calculated to be equivalent to 10 per cent of the blood acetate flow rate in the carotid artery of a calf.

TABLE VI

## DATE PERTAINING TO THE ACETATE INFUSION STUDIES

Calf no.	Age at infusion date	Body weight	Na acetate solution infused into arterial blood of calves				Volume infused per calf	Feed intake during infusion	Venous blood level of acetic acid	
			Concentration as acetic acid	Concentration of Na acetate	Rate of infusion	Rate of infusion in terms of acetic			initial	terminal
	(days)	(lb.)	(g/100 ml)	(g/100 ml)	(ml/min)	(mg/min)	(ml.)	(lb.)	(mg/100 ml)	
211	46	113	saline	saline	0.194		20	0.7	8.1	7.2
92	42	118	7	9.59	0.97	67.9	18	0.7	2.9	7.1
88	46	125	1	1.37	"	9.7	12	1.0	5.1	7.7
	67	143	7	9.59	"	67.9	5			
	88	182	6.2	8.49	"	60.1	55	1.6	9.2	8.6
	88	182	7	9.59	"	67.9	33	2.5	7.6	6.1
	100	201	15	20.55	"	145.5	42	2.0	9.2	7.8
	107	192	7	9.59	"	67.9	44	2.0	7.6	10.0
	121	221	15	20.55	"	145.5	40	3.0	4.9	9.0
4	71	a	7	9.59	"	67.9	6	0.6	5.0	6.0
	88	a	3.2	4.38	"	31.04	30	2.2	6.4	8.4
	151	246	15	20.55	"	145.5	38	3.5	8.8	4.3

<sup>a</sup>Missing data

Since it was desired that any possible response to the infused acetate occur before the calf had sufficient time to satisfy its appetite by eating, the concentration of the acetate solution was arbitrarily changed from 1.37 to 9.59%. After 5 ml of the more concentrated solution had been infused, the calf stopped eating and showed discomfort; therefore, the infusion was stopped.

Upon receiving 9 ml of the 9.59% acetate solution 15 minutes after start of an infusion, Calf 92 stopped eating, and 2 minutes later it urinated and started to act restless by shaking its head and licking its nostrils. The calf continued to act in the same manner until it began to show discomfort for a few minutes and, after infusion of a total of 18 ml of 9.59% acetate solution, it died. Immediately after death, a blood sample from the heart of the calf was obtained for the determination of level of acid. No evidence of any kind of infection was found in the body of the animal. The blood level of acetic acid for the initial sample (obtained 12 minutes before infusion) and that for the terminal sample (obtained 14 minutes prior to the death of the animal) were determined to be 2.9 and 7.1 mg % (Table VI), respectively; whereas, the blood level of acetic acid for the sample obtained from the heart was 12.1 mg %.

To study a possible change in the blood pH, 6 ml of the same acetate solution (9.59%) was infused in Calf 4 at 71 days of age. The infusion was terminated when the calf stopped eating. Its blood pH was determined 10 minutes after the termination of the test and found to be 7.6.

In an attempt to determine the reason for the death of Calf 92 and the restlessness and abnormality of Calves 88 and 4 during the

acetate infusions, acetate solutions of different concentrations, prepared in different ways, were used in subsequent infusions.

At 67 days of age, Calf 88 ate grain during the entire 62-minute period of infusing 55 ml of 8.49% acetate solution, except while drinking water. It seemed to be getting restless at the end, but no definite reactions developed. But, at 88 days of age, this calf stopped eating after a 37-minute period of infusing 33 ml of 9.59% acetate solution. It did not show any sign of abnormality during the acetate infusion period. However, after 42 ml of 20.55% acetate solution was infused at 100 days of age, the calf stopped eating and started to act restless. Its heart rate (120 per minute) and respiration rates appeared to be accelerated by this time. When 3 minutes later, resumption of infusion was attempted, the animal did not at first eat, but then consumed a small amount of feed. At 107 days of age, this calf showed discomfort and stopped eating after 38 minutes of infusion; eating was afterwards resumed, but stopped again at the end of 52 minutes, by which time 44 ml of 9.59% acetate had been infused. Its heart rates were found to be 90, 94 and 120 beats per minute immediately before, 26 and 35 minutes after starting the infusion, respectively. At 121 days of age, this calf did not stop eating and showed no restlessness during a 44-minute period of infusing 40 ml of 20.55% acetate solution. However, its heart rate was found to be 112 beats per minute at the end of the infusion. In general, this calf showed a tendency to tolerate the higher amount of acetate solution as it became older. The levels of acetic acid in the venous blood of this calf did not appear to be affected appreciably by infusion of various amounts of acetate (Table VI). No indication of threshold level of acetate in regard to feed intake was observed.

At 88 days of age, Calf 4 stopped eating after a 35-minute period of infusing 30 ml of 4.38% acetate solution, but did not show any abnormal reaction. It urinated once and drank a half gallon of water during the infusion period. But, when 38 ml of 20.55% acetate solution was infused over a 42-minute period, at 151 days of age, the calf stopped eating 32 minutes after infusion started and its heart rate was found to be 112 beats per minute. The animal started to show discomfort and its heart rate increased up to 116 beats per minute by the time the infusion was terminated. Since saline was used in the preparation of the acetate solutions and since generally less quantities were required for this calf to stop eating, as compared to those required by Calf 88, it was thought that saline in the acetate solution could have resulted in an adverse physiological effect. No appreciable change in levels of acetic acid in venous blood of the calf was observed during the infusion periods mentioned above, and no indication of threshold level of acetate in terms of feed intake were observed.

The cause of death of Calf 92 could be attributed to the combination of autoclavation and saline content of the acetate solution infused. This can be reasonably believed to be the true cause of the death of the animal since Calves 88 and 4 showed relatively more restlessness and abnormality upon infusion with the same 9.59% acetate solution, as used for Calf 92, at 46 and 71 days of age, respectively, than when infused with other solutions (Table VI). Moreover, it has been suggested that infusion of acetate solution made in saline may result blood alkalosis.

The rather arbitrary, progressive changes in the rate of infusion, acetate concentration, and the composition and treatment of the acetate

solution were made to achieve certain conditions providing better indication of the effect, if any, of acetate infusion on voluntary feed intake of young calves. No evidence of a threshold level of blood acetate with respect to feed intake could be inferred from the results of this experiment. This conclusion is in agreement with that of Little and Hawkins (46) who likewise found no threshold level of blood acetic or propionic acid with respect to feed intake. However, it is recognized that different results might have been obtained in the present study if the acetate solution had been infused into the superior hypophyseal artery supplying the hypothalamus.

Dowden and Jacobson (27) observed the roughage intake of identical twin cows to be depressed during an 8-hour infusion with either acetate or propionic acid and also during the 16-hour period following the infusion. These observations were interpreted as evidence for a chemostatic mechanism in the regulation of feed intake by ruminants, although the levels of metabolites they infused may have been unphysiologic (4). Furthermore, Montgomery et al. (54) reported that intraruminal infusion of acetic acid significantly decreased daily hay intake by dairy cows. This effect could have been attributed to the stress condition which resulted from their attempt to infuse large amounts of acetic acid into the rumens of the animals.

Further research is needed to study the following aspects of appetite control in dairy cattle:

- (a) The influence of simultaneous intra-ruminal infusion of acetate and lactate on the voluntary feed intake.
- (b) The threshold blood level of lactate with respect to feed intake.

- (c) The influence of simultaneous intravenous infusion of glucose and acetate on the voluntary feed intake.
- (d) The utilization rate of either acetate or lactate by the hypothalamus of the brain of the ruminant.

## SUMMARY AND CONCLUSIONS

One experiment involving 20 male Holstein calves was conducted to study the relationship between the blood level of the metabolites, glucose and acetate, and feed intake. At the end of the experiment, an acetate tolerance test on each calf was conducted, at ten weeks of age ( $\pm 3$  days), to study the relationship between disappearance rate of blood acetate and feed intake. In a second experiment, an acetate infusion study was conducted on each of four male Holstein calves to study the blood threshold level of acetate in terms of dry feed intake. A constant infusion, for less than 2-hour periods, of acetate solution was made into an exteriorized carotid artery of each calf at various ages.

Calves in Experiment I were on experiment from three days to 10 weeks of age. Calves in Experiment II were on experiment between the ages of 3-151 days. The same kind of calf starter ration was fed to all calves free-choice throughout both experiments.

Determinations of glucose and/or acetic acid were made on venous blood samples obtained in both experiments. Determinations were made of the amounts of dry feed voluntarily consumed by each calf during each eating interval (Experiment I), each infusion period (Experiment II) and daily during the course of this study.

In Experiment I, no consistent linear relationship was found between the blood metabolites, glucose and acetate, and feed intake



by young dairy calves. In the acetate tolerance test, no significant correlation ( $P > 0.10$ ) was found between the exponential rate of disappearance of blood acetate and dry feed intake. In Experiment II, no evidence of a blood threshold level for acetate with respect to feed intake was observed.

It was concluded that neither acetate nor glucose per se is an important blood metabolite regulating feed intake of young dairy calves. A complex series of blood metabolites are likely involved as feed intake regulators.

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APPENDIXES



TABLE VII

INITIAL AND TERMINAL LEVELS OF ACETIC ACID AND GLUCOSE IN THE VENOUS BLOOD OF YOUNG CALVES AND THEIR DRY FEED INTAKE PER EATING INTERVAL AT FOUR DIFFERENT AGES

Calf no.	Blood sample	3 wk of age			5 wk of age			7 wk of age			9 wk of age		
		Acetic acid (mg/100 ml)	Glucose (mg/100 ml)	Feed intake (lb.)	Acetic acid (mg/100 ml)	Glucose (mg/100 ml)	Feed intake (lb.)	Acetic acid (mg/100 ml)	Glucose (mg/100 ml)	Feed intake (lb.)	Acetic acid (mg/100 ml)	Glucose (mg/100 ml)	Feed intake (lb.)
200	initial	6.93	57		15.00	62		14.52	81		13.05	103	
	terminal	— <sup>a</sup>	—	0	6.89	35	0.4	6.83	81	1.25	2.12	79	1.0
3	initial	9.18	85		8.35	82		8.23	92		11.45	96	
	terminal	—	—	0	5.58	42	0.6	7.71	96	1.00	6.75	66	1.28
63	initial	13.04	139		7.62	72		6.80	71		6.24	74	
	terminal	—	—	0	5.40	91	0	8.38	74	0.90	4.79	88	1.29
75	initial	6.87	97		7.86	78		11.63	75		8.83	93	
	terminal	5.46	66	0.15	6.05	78	1.0	7.95	92	1.11	7.84	97	1.29
79	initial	7.82	137		6.43	74		6.80	86		6.39	91	
	terminal	2.50	102	0.20	7.61	75	0.45	9.33	87	0.70	9.13	89	1.32
39	initial	9.70	110		7.16	74		5.75	84		8.56	91	
	terminal	4.44	72	0.30	8.89	98	1.15	6.61	76	0.85	3.12	105	1.65
30	initial	7.72	109		2.18	71		10.02	78		11.36	103	
	terminal	3.44	84	0.20	8.62	78	0.5	11.29	100	0.29	8.88	98	1.66
4	initial	6.31	70		7.19	76		5.07	78		5.36	59	
	terminal	4.58	50	0.10	5.05	84	0.4	6.51	97	1.26	4.97	98	1.18

<sup>a</sup>No blood sample was taken because no dry feed was consumed by the animal.

TABLE VII (Continued)

Calf no.	Blood sample	3 wk of age			5 wk of age			7 wk of age			9 wk of age		
		Acetic acid	Glucose	Feed intake	Acetic acid	Glucose	Feed intake	Acetic acid	Glucose	Feed intake	Acetic acid	Glucose	Feed intake
		(mg/100 ml)		(lb.)	(mg/100 ml)		(lb.)	(mg/100 ml)		(lb.)	(mg/100 ml)		(lb.)
38	initial	6.89	103		5.93	106		7.41	112		12.58	103	
	terminal	7.13	70	0.05	7.70	101	0.5	6.51	98	1.16	8.32	79	1.49
67	initial	3.67	58		5.31	91		3.64	111		2.56	97	
	terminal	—	—	0	5.78	91	0.65	11.39	98	1.25	10.24	129	1.73
181	initial	7.98	95		9.78	80		7.93	86		6.79	108	
	terminal	—	—	0	7.46	79	0.22	9.12	93	0.30	14.83	99	1.12
182	initial	8.25	41		6.23	62		2.68	93		6.23	112	
	terminal	—	—	0	13.48	81	0.66	9.33	92	1.22	9.53	111	1.99
183	initial	6.46	93		3.82	56		5.59	78		7.53	93	
	terminal	—	—	0	7.01	72	0.32	6.41	68	0.85	7.27	102	1.81
185	initial	8.39	104		5.57	79		7.88	116		7.66	104	
	terminal	—	—	0	6.99	83	0.46	5.65	109	1.29	9.63	107	2.16
186	initial	7.99	111		9.68	84		13.68	94		9.36	98	
	terminal	9.27	95	0.1	6.07	83	0.60	5.43	104	1.29	9.68	109	1.90
204	initial	8.88	163		5.81	84		5.82	111		5.74	85	
	terminal	—	—	0	7.59	88	0.63	8.41	116	1.51	10.90	91	1.70
6	initial	4.26	98		9.52	117		6.09	82		6.31	118	
	terminal	7.28	100	0.52	9.75	111	0.40	8.22	87	1.0	8.82	89	2.00

TABLE VII (Continued)

Calf no.	Blood sample	3 wk of age			5 wk of age			7 wk of age			9 wk of age		
		Acetic acid	Glucose	Feed intake	Acetic acid	Glucose	Feed intake	Acetic acid	Glucose	Feed intake	Acetic acid	Glucose	Feed intake
		(mg/100 ml)		(lb.)	(mg/100 ml)		(lb.)	(mg/100 ml)		(lb.)	(mg/100 ml)		(lb.)
13	initial	10.30	123		10.41	153		3.22	69		6.70	74	
	terminal	7.96	69	0.30	6.22	143	0.40	4.25	62	0.5	7.13	84	1.00
53	initial	9.41	119		4.79	131		5.70	72		5.6	66	
	terminal	13.40	149	0.40	3.71	84	0.50	6.15	91	1.1	6.12	76	1.20
20	initial	9.05	97		7.41	103		4.28	69		4.17	82	
	terminal	3.81	116	0.30	5.43	87	0.60	5.42	72	1.1	5.67	77	1.30

TABLE VIII

BLOOD LEVELS OF ACETIC ACID AND GLUCOSE DURING AN ACETATE TOLERANCE TEST CONDUCTED  
ON DAIRY CALVES AT APPROXIMATELY TEN WEEKS OF AGE

Calf no.	Pre-injection		Time after injection, minutes									
	Acetic	Glucose	0		30		60		90		120	
			Acetic	Glucose	Acetic	Glucose	Acetic	Glucose	Acetic	Glucose	Acetic	Glucose
(mg/100 ml)												
200	5.4	94	70.43	86	16.14	104	8.55	88	6.15	93	7.10	60
3	6.3	88	71.08	109	19.59	94	12.88	80	8.96	89	6.59	87
63	5.7	65	52.06	54	28.13	48	10.90	61	7.10	77	7.11	67
75	4.8	31	82.92	79	26.83	86	26.95	43	5.82	62	6.04	59
79	6.3	75	59.89	66	34.64	74	19.95	101	9.87	83	7.53	83
39	6.09	67	76.51	55	18.07	97	8.64	89	7.73	97	6.32	74
30	4.78	94	37.26	78	11.39	82	6.54	100	7.36	71	4.77	101
4	9.48	58	35.90	71	9.74	53	6.63	71	6.06	56	7.26	52
38	9.98	98	48.79	29	16.16	74	4.32	53	7.84	88	7.57	82
67 <sup>b</sup>	4.5	80	6.92	73	9.85	82	10.59	73	9.18	80	4.03	82
181	8.8	76	49.71	97	16.28	109	11.86	115	4.99	117	8.83	121
182	8.5	34	55.9	108	23.85	24	14.90	107	6.48	78	7.73	108
183	5.93	97	51.16	98	14.73	89	6.30	96	6.70	96	6.18	69
185	9.16	79	49.42	66	12.35	74	9.90	80	10.57	79	4.54	79
186	4.15	82	39.36	83	7.14	72	6.48	76	9.47	69	6.65	67
204 <sup>b</sup>	8.55	99	7.57	98	7.43	101	5.74	99	6.89	98	7.64	97
6 <sup>b</sup>	5.31	94	13.04	89	6.78	55	6.43	61	11.29	114	6.25	51
13	<sup>a</sup>	<sup>a</sup>	53.92	88	13.67	113	6.88	76	4.81	73	6.72	73
53 <sup>b</sup>	7.46	99	6.34	98	10.3	95	9.36	99	9.2	98	8.52	99
20 <sup>b</sup>	6.09	73	5.05	79	5.16	84	9.33	74	10.01	74	8.92	96
Avg.	6.81	74.14	55.62	77.80	17.91	79.53	10.78	82.40	7.33	81.87	6.79	78.80

<sup>a</sup>Missing data.

<sup>b</sup>Values for these calves were not included in calculating the averages.

TABLE IX  
 VARIOUS EXPERIMENTAL MEASUREMENTS IN RELATION TO AN  
 ACETATE TOLERANCE TEST ON 15 CALVES

Calf no.	Exponential rate of acetate utilization, <sup>a</sup> X 10 <sup>-3</sup>	Avg. daily feed intake <sup>b</sup>	Avg. body weight <sup>b</sup>	Avg. daily feed intake per 100 W <sub>kg</sub>	Avg. daily feed intake per 100 W <sub>kg</sub> <sup>75</sup>
				(lb.)	
200	8.00	3.8	139	6.1	17.2
3	8.00	4.4	166	5.9	17.3
63	7.75	3.1	120	5.7	15.6
75	9.80	3.0	119	5.5	15.1
79	7.80	3.3	114	6.4	17.1
39	8.44	4.5	136	7.3	20.6
30	6.58	4.0	115	7.6	20.5
4	5.30	4.2	128	7.3	20.1
38	6.44	4.8	162	6.5	19.0
181	6.70	3.8	153	5.5	15.8
182	7.60	4.3	146	6.5	18.5
183	7.26	3.7	126	6.4	17.6
185	7.20	4.2	123	7.5	20.5
186	4.73	4.2	157	5.8	16.9
13	7.54	3.5	131	5.9	16.3

<sup>a</sup>Disappearance rate of blood acetate, expressed as logarithmic value of change in acetic acid concentration (mg/100 ml) per minute.

<sup>b</sup>Feed intake and body weight for the 10 days prior to the day preceding that of acetate injection.

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