The University of Manitoba

REGULATION OF FOOD INTAKE IN THE STEER

by

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ABSTRACT

The main purposes of this experiment were to determine the effects of frequency of feeding and of form of diet on the voluntary consumption of food by steers and to study the mechanisms involved in regulation of appetite. The diet was formulated to contain 60 per cent barley, 38 per cent alfalfa and 2 per cent trace mineralized salt. It was offered with the hay chopped and mixed with rolled barley (C) or with the entire diet ground and pelleted (P). The two types of diets were fed either continuously (CC, CP) or twice each day (TC, TP) to four steers according to a 4 x 4 Latin Square Design.

The average quantities of food voluntarily consumed were 9.20, 9.00, 7.93 and 7.02 kg per day for treatments CC, CP, TC and TP, respectively. The average gains in body weight were 0.81, 0.75, 0.61 and 0.55 kg per day, respectively. These values were significantly higher when the steers were fed continuously than when they were fed twice each day. The coefficients of apparent digestibility for dry matter, organic matter, and energy of the diet were also significantly higher when the steers were fed continuously and the values for chopped food were significantly greater than those for pelleted food. These results for digestibility applied

to the forestomachs and to the entire gut.

There was little change with time of day in the magnitudes of all measurements made when the steers were fed continuously. However, the effect of time of sampling was significant when the steers were fed twice each day. The assumption has been made in this thesis that the critical values of physical and chemical factors that induce satiety were close to those maintained when the steers were fed continuously. Satiety, at the end of a meal in the steers fed twice each day, may therefore have arisen from distension of the reticulorumen, the relatively large flow rates of abomasal digesta or elevated concentrations of VFA in rumen fluid and in the plasma. The volumes of rumen contents and flow rates of abomasal content were smaller at the end of a meal for treatment TP than for TC. Therefore, these physical factors may not have been as influential in regulating the intake of food in treatment TP as they were in treatment TC. The pH of rumen fluid may have controlled the size of a meal indirectly by inhibiting the motility of the forestomachs and thus limiting the rate of outflow of contents from these organs. The osmolality of rumen fluid and of plasma and the packed cell volume of blood, did not seem to be involved in the control of eating behavior because results for these measurements increased to maximum values at 0.5 hr from the start of a meal and yet the steers continued to eat for about one hour.

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INTRODUCTION

A better understanding of the mechanisms controlling appetite and satiety in ruminants is necessary since production and hence profit margins may be increased if animals can in some way be induced to eat more food. Mature animals usually maintain relatively constant body weights indicating that a regulatory mechanism exists, ensuring that the amount of food consumed daily is sufficient to offset the energy utilized to maintain normal body function. A number of mechanisms have been described for the control of voluntary food intake, and most of them involve the transmission of neural activity to the central nervous system, in particular to centers in the hypothalamus. It is believed that the voluntary intake of poor to medium quality roughages by ruminants is limited by the amount of digesta in the reticulorumen. Differences in the intake of a variety of roughages can therefore be related to the rate at which they disappear from the reticulorumen. There is, accordingly, a positive correlation between the voluntary intake of roughages and their digestibility. However, the variations in the rate of passage of digesta do not appear to be the only factors responsible for regulation of food

intake in ruminants. The voluntary intake of concentrate diets by ruminants is limited by a chemostatic mechanism which appears to set an upper limit to caloric intake.

The rates of weight gain by growing animals, such as sheep and steers, and of milk production by dairy cows has been increased by feeding the animals more frequently than once a day. Also, the medium quality roughages, when ground and pelleted, are consumed to a greater extent, resulting in increased production and increased profits to the agricultural producer.

The present experiment was designed with the hope of obtaining more information about the physical and chemical mechanisms involved in the regulation of food intake in ruminants. A diet, consisting of 38 per cent alfalfa hay and 60 per cent barley, which could be used to fatten ruminants in feedlots was offered to steers in two physical forms and at two frequencies of feeding. Their voluntary food intakes per day and rates of weight gain were measured because these are of economic importance. In addition, the physical factors involved in the regulation of food intake, such as the volume and weight of rumen contents, the rate of flow of digesta into the small intestine and the apparent digestibility of the food were studied. Chemical factors, such as pH and the concentrations of volatile fatty acids in rumen and abomasal fluid and the osmolality of rumen fluid and of plasma were also investigated.

LITERATURE REVIEW

Regulation of Food Intake in the Ruminant

1. The role of the central nervous system in the regulation of food intake

Feeding behavior in the mammal is thought to be regulated according to the firing rates or levels of neural activity in single cells in the "satiety center" of the ventromedial hypothalamus and in the "appetite center" of the lateral hypothalamus (Anand, 1967). Anand (1961) working with rats showed that high levels of neural activity in the satiety center were associated with low levels in the appetite center and vice versa. The satiety state is characterized by a high firing rate of neurones in the satiety center and that of hunger by a high rate in the lateral hypothalamus. These characteristics are thought to reflect the changes in the body resulting from feeding behavior. Brobeck (1955) pointed out that the satiety center, by suppressing the activity of appetite center, brings about the cessation of food intake or the state of satiety. Subsequently, when the food eaten is disposed of through conversion to heat, work, or some form of stored energy, the firing rates of single cells in the satiety center decrease and the appetite center become more active; this leads to the state of hunger.

Wyrwicka and Dobrizecka (1960) showed that the electrical stimulation of the ventromedial hypothalamus of goats depressed food intake. However, a lesion created in this area in rats and mice resulted in obesity as a result

of hyperphagia (Hetherington and Ranson, 1942; Mayer, French, Zighera and Barnett, 1955; Brobeck, 1946). Stimulation of the lateral hypothalamus in satiated sheep, goats and rats caused these animals to start eating (Larsson 1954; Morgane, 1961). Teitelbaum and Stellar (1954) and Baile, Mahoney and Mayer (1967) found that rats and goats with lesions in the lateral hypothalamus refused to eat or drink.

Anliker and Mayer (1957) reported that satiety is regulated rather than hunger since destruction of the satiety center causes hyperphagia and obesity. However, Anand (1967) stated that there are problems in determining what animals are regulating with reference to food intake, and in determining what changes, taking place in the body, act as signals for the regulating system. Mayer (1967) concluded that a type of regulation operating over long periods of time may correct the errors of the day to day or short term regulating system and hence cause mature animals to maintain a relatively constant body weight.

2. General review of factors affecting food intake

The following discussion indicates briefly the effects of energy balance, glucose utilization, body temperature, fatness and pregnancy on the voluntary consumption of food by animals.

a. Energy balance

Conrad (1971) pointed out that animals transfer all the energy obtained from food to heat, work, or the production of fat, growing tissue, milk and so on and that these "output" responses may influence the amount of food voluntarily consumed. Campling (1966) showed that with *ad libitum* feeding of hay to lactating dairy cows, the food intakes were increased to 29 per cent more than for dry animals. The mechanisms regulating food intake appear to be the same as those that control energy balance. For example, cattle given diets differing in caloric density consumed quantities of food that provided similar intakes of digestible energy (Montgomery and Baumgardt, 1965).

b. Glucose utilization

The rate of glucose utilization has been considered by Mayer (1955) as a component in a feedback system controlling food intake in monogastric animals. Anand (1967) recorded electroencephalographic activity in the unanesthetized rat through electrodes chronically implanted in the satiety and feeding centers and found that hyperglycemia, produced by intravenous glucose infusion, increased the frequency of electrical activity in the satiety center, with a concommitant drop in voltage and frequency of the electrical activity in the appetite center. Conversely hypoglycemia, produced by an intravenous injection

of insulin, decreased the electrical activity in the satiety center. Oomura, Ono, Ooyama and Wagner (1969) found single neurones sensitive to glucose concentration in the hypothalamus of the rat. However, the concentration of glucose in blood is probably not important for controlling food intake in ruminants. Glucose and insulin concentrations in blood vary little with time after feeding (Manns and Boda, 1967). Furthermore, the amount of food consumed by the goat was not affected following injections of glucose intra-ruminally, intraperitoneally, into the jugular or ruminal veins, into the carotid artery or into the cerebral ventricle (Baile and Mayer, 1970). In ruminants, there is no evidence that receptors exist in the hypothalamus which are sensitive to glucose concentration or utilization rate.

c. Production of lactic acid

Baile and Mayer (1970) showed that lactate, injected into the rumen or jugular vein, decreased food intake in goats. The effects often lasted for several days. They also suggested that this response may account for the depression of food intake in some disorders of digestion such as grain engorgement (Ryan, 1964).

d. Temperature

Single neurones sensitive to temperature have

been found in the hypothalamus of monogastric animals (Nakayama, Hammel, Hardy and Eisenman, 1963). Apparently, neurones sensitive to temperature can affect feeding behavior in the goat. Andersson and Larsson (1961) cooled the ventromedial hypothalamus of a satiated goat from 39°C to 29°C and caused the animal to start eating. They heated the same area from 39°C to 49°C caused a hungry goat to stop eating. Baile and Mayer (1968) found little evidence that hypothalamic temperature changes or the rate of surface heat loss served as sources of feedback signals to control food intake. This has recently been confirmed by Dinius, Kavanaugh and Baumgardt (1970).

e. Fatness and pregnancy

Fatness, as well as pregnancy may restrict the space for the digestive tract and its contents in the abdominal cavity. Taylor (1959) obtained an inverse relationship between the amount of fat in the abdomen of sheep and the quantity of food voluntarily consumed. He concluded that fatness could affect the voluntary intake of forages. The intake of food by ewes was reduced during the last week of gestation, and the effect was greater in the animals carrying twins than in those carrying singles (Reid, 1968).

Recently, Forbes (1969) showed that the concentration of estrogens in the blood may be significant in decreasing the food intake in pregnant ewes. Muir (1970) found that

estrogen, fed to cattle at the level of 219 mg per day per 600 kg body weight, decreased the consumption of food. There was a significant interaction between the effects of estrogen and progesterone. The cows receiving both hormones ate more than those treated with estrogen but they ate less than the control animals.

Forbes and Rook (1970) infused oestradiol into lactating goats and found that the resultant depression in food intake was greatest when the animals were in oestrus. Food intakes in cattle and sheep were consistently depressed by infusions containing quantities of oestrogen similar to those secreted in late pregnancy. This provides a possible explanation for the observed decline in concentrate intake by cattle and sheep in late pregnancy (Forbes, 1971).

3. Possible origins of satiety signals

A. Physical factors in the control of hunger and satiety

a. Oropharyngeal metering of food intake

Balch (1958) concluded that oropharyngeal metering mechanisms did not limit food intake in the ruminant. Campling and Balch (1961) fed cows a diet of hay and for three hours they collected boluses of food at the cardia and removed these from the rumen. The length of the period of eating was almost doubled, and the cows ate 177 per cent of their pretrial intakes. The cows did slow down in their rate of eating at the end of the extended period. It was

concluded that satiety in cows was not determined by exhaustion of the salivary glands or of the musculature in the jaw or reticulorumen.

b. Effects of distension of the stomachs and duodenum

Paintal (1953) claimed that gastric stretch receptors were important in the peripheral mechanism of hunger and thirst in cats. Distension of the stomach in the dog decreased the intake of food during a meal (Grossman, 1960). Iggo (1957) found distension sensitive nerve endings in the esophagus, stomach and small intestine of the cat which produced afferent electrical activity in the cervical vagus. Anand and Pillai (1967) measured single neurone activity in several areas of the hypothalamus in cats and concluded that distension of the stomach brought about satiation through vagal afferents affecting the hypothalamic satiety mechanism.

Iggo and Leek (1967) reported the presence of receptors sensitive to distension in the reticulum of sheep. These probably act as detectors of fill in the reticulo-rumen and hence may be important in limiting the intake of poor to medium quality roughages by ruminants. Harding and Leek (1972) have found receptors in the abomasum and proximal duodenum which are sensitive to distension and tactile stimulation.

c. Digestibility of food and gut fill

Crampton (1957) showed a positive relationship between the voluntary intake and the digestibilities of roughages by sheep. Crampton, Donefer, and Lloyd (1960) suggested that recurring hunger in ruminants was caused by a reduction of rumen load. Blaxter *et al* (1961) measured the voluntary intake by sheep of hays with digestibilities ranging from 44.7 to 74.2 per cent and found that intake increased with increasing digestibility and with faster rates of passage in such a way that the amount of dry matter contained by the gut at the end of a meal was the same, whatever the quality of the hay eaten. Similar results were obtained with steers (Blaxter and Wilson, 1962). Blaxter, Wainman and Wilson (1961) claimed that, within the limits of the qualities of forage used, the amount of food that was consumed by sheep was determined by the capacity of their digestive tracts rather than by physiological factors.

Campling, Freer and Balch (1962) observed that cows voluntarily consumed more hay than straw and that the rate of passage of digesta through the alimentary tract was faster with the hay diet. With these foods there were similar amounts of digesta in the rumen just before a meal (Freer and Campling, 1963). However, in another experiment a more digestible diet, dried grass containing 66 per cent digestible dry matter, was fed and the amount of digesta in

the reticulo-rumen immediately after feeding was about the same as with hay but was much less just before the next meal. They concluded that for diets of straw, hay and dried grass, there were limiting values for fill in the reticulo-rumen both before and after feeding.

Freer and Campling (1963) observed that rumen-fill was small and not limiting the voluntary intake of concentrate diets. Conrad, Pratt and Hibbs (1964) suggested that the voluntary intake by dairy cows of roughages with digestibilities between 55 and 67 per cent is limited by physical factors. Beyond 67 per cent the weight of food consumed daily decreased as the digestibility increased and physiological factors appeared to trigger satiety. Montgomery and Baumgardt (1965) suggested that food intake with these types of diets may be regulated to keep energy intake constant. Cowsert and Montgomery (1969) and Vidal, Hogue, Elliot and Walker (1969) who also worked with ruminants have obtained similar results. When physical factors are operating and limiting intake, the signal controlling the cessation of eating presumably arises from distension of the reticulo-rumen. Mayer (1967) pointed out that distension of the reticulorumen is probably responsible for the short-term or day-to-day regulation of voluntary roughage intake by ruminants. However, it is also possible to integrate physical control with the long-term regulation of energy balance that adult animals exhibit. For example, the

changes in the physiological status of an animal due to lactation, may modify and increase the capacity of the reticulorumen and thus provide conditions for the increased intake of food associated with lactation.

McCullough (1969) showed that the rate of weight gain in steers decreased as the proportion of hay in the roughage-concentrate diets increased. This indicates that physical factors associated with the feeding of roughage limited the energy intake of the animals and hence reduced their growth rates.

d. Rate of passage

Campling, Freer and Balch (1962) showed that the addition of dilute solutions of urea to the reticulo-rumen of cows offered oat-straw *ad libitum* improved its digestibility, reduced the time of retention of food residues in the reticulo-rumen and increased the voluntary intake of the food by 39 per cent. They suggested that the increased rate of disappearance of digesta probably resulted from improved cellulolytic activity of the rumen microflora which in turn increased the rate of breakdown of food to particle sizes small enough to pass through the omasum. The size of particle does have a marked effect on the rate of passage of food residues from the reticulo-rumen (Rodrigue and Allen, 1960; Campling *et al.*, 1962).

e. Rate of flow

The capacity of the abomasum and intestines to contain digesta may limit food intake by setting upper limits to the rate of flow of digesta through the digestive tract. Distension of the abomasum reflexly inhibits the primary cycle of reticulo-rumen motility and hence the rate of flow of digesta from the reticulum to the abomasum (Kay, 1965).

The flow of digesta from the abomasum is regulated by the quantity of digesta in the small intestine. Phillipson (1952) reported that the flow from the abomasum was reduced or temporarily abolished by distension of the duodenum with a balloon or by the rapid introduction of digesta into the duodenum. Conversely, the flow rates of digesta through the abomasum were abnormally high when abomasal or duodenal digesta were collected and not returned into the duodenum (Phillipson, 1952; Hogan and Phillipson, 1960, Phillipson and Ash, 1965).

Phillipson and Ash (1965) found that the volumes of contents in the rumen and the abomasum affect the flow of digesta through the omasum.

Titchen, Reid and Vleig (1966) decreased the voluntary intakes of hay by sheep by infusing fat into the duodenum. They suggested that distension of the duodenum and delayed abomasal emptying were responsible for the depression in food intake.

Goodall and Kay (1965) found that distension of the ileum of sheep, due to a blockage of cannulae, caused sheep to stop eating. When the blockage was removed digesta were released and the sheep recovered their appetites within a few hours.

Campling, Freer and Balch (1963) and Campling and Freer (1966) have suggested that in cattle, distension of the hindgut with digesta may limit the rate of disappearance of digesta from the reticulo-rumen and hence act as an indirect means of limiting the voluntary intake of roughage. This may be caused by the rumino-colic and gastro-colic reflexes (Ruckebush, 1970).

B. Chemical factors in the control of hunger and satiety

a. Osmolality of rumen fluid

The voluntary intake of lucerne hay by sheep decreased when the osmolality of the ruminal liquor was increased either by the additon of NaCl to the food (Wilson, 1966) or by the infusion of volatile fatty acids or their salts into the rumen (Rook, Balch, Campling and Fisher, 1963; Montgomery Schultz and Baumgardt, 1963).

Ternouth and Beattie (1971) added solutions of NaCl, KCl, or salts of acetic, propionic and butyric acid to the rumen of sheep at the beginning of a feeding period and decreased the intakes of a roughage diet. They added similar volumes differing in osmolality to the rumen and

as the osmolality of electrolytes was linearly increased, the food intake was linearly decreased. This decrease was mainly due to a reduced food intake during the first hour of the feeding period. Ternouth and Beattie (1971) also fed the sheep a diet of highly digestible lucerne chaff containing 1% NaCl. Whenever water was added to the rumen, food intake increased, but the increase was significant only when the water was given within one hour of, or immediately before, feeding. They concluded that the rumen becomes hyperosmolar from the ingestion of the salt and the fermentation of food and that the addition of water to the rumen decreased the osmolality of the rumen fluid and consequently increased food intake. Warner and Stacy (1965) reported that rumen osmolality in sheep rose to about 400 mosmol/kg one hour after feeding. Thus the osmolality of rumen fluid may be an important part of the system regulating feeding behaviour in ruminants.

b. Osmolality of plasma

The volume of extracellular fluid in sheep decreased by 10 per cent shortly after they were fed 350 g lucerne chaff (Ternouth, 1968). The volume returned to the prefeeding level 3 hours later. The osmolality and the concentration of sodium and chloride in the plasma began to increase one hour after the start of feeding and reached maximal values 3 to 5 hours later. Christopherson and Webster (1972)

found that the plasma volume of sheep decreased quickly by about 300 ml at the beginning of the meal and then increased slowly after a meal had ended. Hematocrit values increased and then decreased accordingly. The extracellular fluid volume was significantly decreased by about 1000 to 1500 ml during the meal. While ruminants eat, a large volume of fluid leaves the plasma and extracellular fluid space and enters the digestive tract in the form of saliva (Bailey, 1961, Stacy and Warner, 1966) and possibly by direct movement across the rumen wall (Ternouth, 1968). Ternouth and Beattie (1971) found that saline infused into the peritoneal cavity of sheep beginning 30 minutes after the start of feeding, increased food intake by 17% over that recorded in a preinfusion control period. They suggested that a reduction of extracellular fluid volume, rather than an increased plasma osmolality, causes satiety in the ruminant.

Gutman and Krausz (1969) have been able to increase or decrease the food intake of rats by increasing and decreasing their extracellar fluid volumes. Lepkovsky, Lyman, Fleming, Nagumo and Dimick (1957) reported that rats mobilized their tissue fluids to maintain their gastric contents at a water content of 49%. They suggested that the intake of food was limited by the quantity of tissue fluid available to maintain the stomach contents in a fluid state.

c. pH of fluid digesta

Receptors for pH have been found in the mucosa of the stomach of the sheep (Iggo, 1957), and in the abomasum and proximal duodenum of the sheep (Harding and Leek, 1972). These receptors in the sheep also acted as mechanoreceptors as they could be excited by stroking the mucosa.

Harding and Leek (1972) found that receptors in the reticulo-rumen responded to chemicals as did abomasal receptors. The receptors in the reticulo-rumen responded to a variety of acids such as HCl, volatile fatty acid, lactic acid and alkalies such as NaOH. They suggested that these receptors may be involved in the inhibition of forestomach mobility that occurs during intra-ruminal infusion of acid (Ash, 1959) and in decreased food intake by acetate administration (Baile and Mayer, 1969).

Hunt and Knox (1969) found that acid, included in a meal, slowed gastric emptying in humans. They suggested that receptors in the duodenum,sensitive to hydrogen ion concentration of pH less than 6,activated a mechanism which decreased the rate of emptying of contents from the stomach (enterogastrone and enterogastric reflex). The rate of gastric emptying can thus be indirectly influenced by secretions from the pancreas and duodenum which neutralize the acid digesta leaving the stomach.

Scarisbrick (1954) and Jones (1957) reported that reticuloruminal motility was suppressed following a decrease

in the pH of rumen fluid. Montgomery, Schultz and Baumgardt (1963) reduced the *ad libitum* hay intake of cattle by infusing a solution of acetic acid, with a pH of less than 5.0, into the rumen. However, a similar acid solution titrated to pH 6.0 did not affect the quantity of food consumed. Bhattacharya and Warner (1967) infused acid solutions into the rumen of steers to lower and maintain the rumen pH at about 6.0 The daily intakes of hay were decreased significantly from 12.3 kg in the control animals receiving water, to 6.0, 6.3 and 8.0 kg in the animals receiving phosphoric acid, lactic acid, and citric acid solutions, respectively. In control animals, rumen pH fell gradually through the hours of feeding from 7.03 before infusion to 6.58 six hours later. They concluded that the pH of rumen fluid influenced voluntary food intake in steers and that this characteristic could be of physiological importance with some diets.

d. Volatile fatty acid concentrations in rumen fluid and blood

Volatile fatty acids are produced in the forestomachs of ruminants by microbial fermentation of food. These products are an important energy source for the ruminant and are mostly absorbed into the blood stream through the wall of the reticulorumen. Their concentrations in rumen fluid and their rates of production and absorption increase shortly after feeding has begun. Simkins *et al.* (1965)

described experiments with lactating and non-lactating holstein cows and suggested that acetic, propionic, and butyric acid concentrations in rumen fluid may induce satiety. They proposed that chemoreceptors for these acids were located in the rumen wall, in the portal system and in the liver. The concentrations of the butyric and propionic acids in peripheral circulation are low because they are utilized rapidly in the rumen epithelium and the liver.

Volatile fatty acids, injected into the rumen, have decreased the intake of food by cattle (Montgomery, Schultz and Baumgardt, 1963; Weston, 1966; Baile and Mayer, 1969) and goats (Baile and Mayer, 1967). Baile and Mayer (1970) showed that the most sensitive receptors for acetate or the greatest number of these receptors, or both, are located on the lumen side of the ruminoreticulum especially in the dorsal sac. Baile (1971) showed that the decrease in food intake by sheep, associated with additions of acetic acid to the rumen, could be partially eliminated by adding a local anesthetic to the solution infused. A local anesthetic (xylocaine), injected near nerves innervating the dorsal rumen, increased food intake slightly and also partially eliminated the depression of food intake caused by injecting sodium salt of acetic or propionic acids into the rumen.

Evidence from the experiments discussed indicates that the amount of acetic acid produced daily in the rumen, its concentration in rumen fluid, and its rate of metabolism

in tissue may be of major importance in the control of feeding behaviour in ruminants.

4. Effects of grinding and pelleting on the intake of food by ruminants

Blaxter, Graham and Wainman (1956) fed sheep dried grass that had been either chaffed or ground and cubed. The finely ground and cubed roughage had a faster rate of passage through the digestive tract and a lower digestibility. Wilkins, Lonsdale, Tetlow and Forrest (1972) fed cattle and sheep diets of ground and dried grass in the wafer form which differed only in fineness of grind. With increasing fineness there was an increase in intake but a decrease in digestibility. The diet having the largest particles passed at the slowest rate through the digestive tract. Similar results were obtained with lactating cows by Rodrigue and Allen (1960).

Hogan and Weston (1967) reported substantial increases in consumptions of lucerne and wheaten hays due to grinding and pelleting of the diets. They assumed that the enhanced intakes were due to a faster rate of removal of organic matter from the rumen. The lower digestibility of both foods in the pelleted form was accounted for by the decreased digestibility of the cell wall constituents.

The rate of secretion of saliva is lower for ground and pelleted than for long roughage diets (Wilson and Tribe, 1963; Weston and Hogan, 1967). Also, cattle and sheep spent

less time eating and relatively little time ruminating diets that were ground and pelleted (Freer and Campling, 1963; Weston and Hogan, 1967).

Campling, Freer and Balch (1962), working with cattle fed rye grass, found that there was 20 per cent less water present in the rumen when the food was given in the ground and pelleted form than when it was long. A similar decrease of 13 per cent was observed by Weston and Hogan (1967) in sheep fed wheaten hay.

King, O'Dell and Brannon (1962) compared the digestibilities of the baled, ground, and ground and pelleted coastal Bermuda grass hay in dairy cows.

Digestibility (%)

	T.D.N.	D.M.	Protein	Fiber
Baled Hay	52.1	54.0	67.4	59.1
Ground Hay	49.2	51.3	64.3	54.9
Pelleted Hay	43.6	45.3	59.8	42.0

5. Effect of frequency of feeding

It has long been the practice of good stockmen to feed their animals a little at a time but often. Dawson and Kopland (1949) observed that dairy cows, when fed twice daily, produced 6 per cent more milk and consumed 10 per cent more hay than when they were fed once daily. Growing

cheviot ewes fed a mixed diet in 8 meals per day gained 260 per cent more weight than those fed the same diet once daily (Gordon and Tribe, 1952). Rakes, Hardison and Albert (1957) found that the rates of body weight gain of dairy heifers given a mixed diet of concentrate and forage in 4 meals per day were 60 and 52 per cent greater than those of heifers given the same amount of food but in one and two meals per day, respectively. Also, heifers fed an all-hay diet in 10 meals per day gained 92 per cent more body weight than others receiving the same amount of food in two meals. Rakes *et al.* (1961) gave young and adult sheep restricted quantities of food in one or 8 meals per day. The higher frequency of feeding increased weight gains by 65 per cent in the young animals (6 months old) but did not affect the rate of gain in the sheep 2.5 years of age. The young animals produced less heat when fed more frequently. The average pH of the ruminal ingesta were not influenced by feeding frequency. However, it fluctuated more in the animals fed once daily. Mochrie, Thomas and Lucas (1956) observed no difference in the yield of 4 per cent fat-corrected milk or in the body weight changes of first lactation cows fed the same amounts of a mixed diet in 2 or 8 meals per day.

Moir and Somers (1957) found that the digestibility of dry matter was 63.9 and 67.1 per cent in sheep fed once and 4 times per day, respectively. There were lower concen-

trations of bacteria in the rumen fluid of the sheep fed once a day. Campbell and Marilan (1961) fed lactating Gurnsey cows two, four, or seven times daily. Food intakes were positively related to the frequency of feeding and the digestibilities of the dry food were 51.59, 52.52 and 55.10 per cent, respectively.

Purser and Moir (1959) found a marked diurnal fluctuation in the numbers of ciliate protozoa in rumen fluid. The numbers decreased after a meal to about one-third of the prefeeding levels, probably because the ability of the protozoa to multiply is strongly inhibited by conditions of low pH.

Ibrahim, Ingalls and Stanger (1970) found that the low pH of rumen contents, associated with a high level of volatile fatty acid production after feeding, reduced the protozoal population in rumen fluid. A stable and active protozoal population was obtained by maintaining the pH of rumen fluid near neutrality in dairy cows (Ibrahim *et al.*, 1970).

The volume, composition and onward flow of rumen contents fluctuate much less when sheep are fed continuously rather than twice daily (Kay, 1963). Increasing the frequency of feeding, while maintaining the food intake constant, also decreased the extent of daily fluctuations of fermentation rate as exhibited by ammonia and volatile fatty acid concentrations in rumen fluid (Ibrahim, Ingalls and Phillips, 1969).

MATERIALS AND METHODS

Animals and Surgery

Four steers of about one and one half years of age were used in this work. Number 18 was of the Holstein breed and numbers 16, 15 and 55 were Herefords. Their body weights at the beginning of the first period were 385, 408, 396 and 390 kg, respectively. They were kept in single stalls in a barn throughout the experiment. Each steer was fitted with two cannulae, one in the dorsal sac of the rumen[1] and the other in the pyloric region of the abomasum[2]. The operations to insert the cannulae were performed at least three months prior to the start of the experiment (see position of cannulae in Figures 1 and 2).

Diet and Method of Feeding

The diet consisted of 38 per cent alfalfa hay, 60 per cent barley, and 2 per cent trace mineralized salt (Table 1). This diet was formulated in accordance with the National Research Council recommendations for growing steers (1963). Some of the chemical constituents of the

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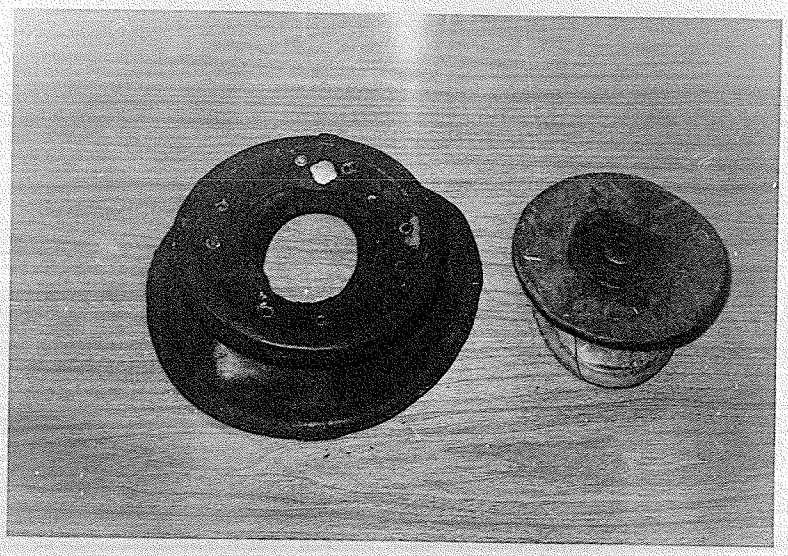
[1] Avon Rubber Co. Ltd., Melksham, Wiltshire, England.
[2] Made in a mold from polyvinyl chloride, Norton International Inc., Akron, Ohio.

PART A



PART B

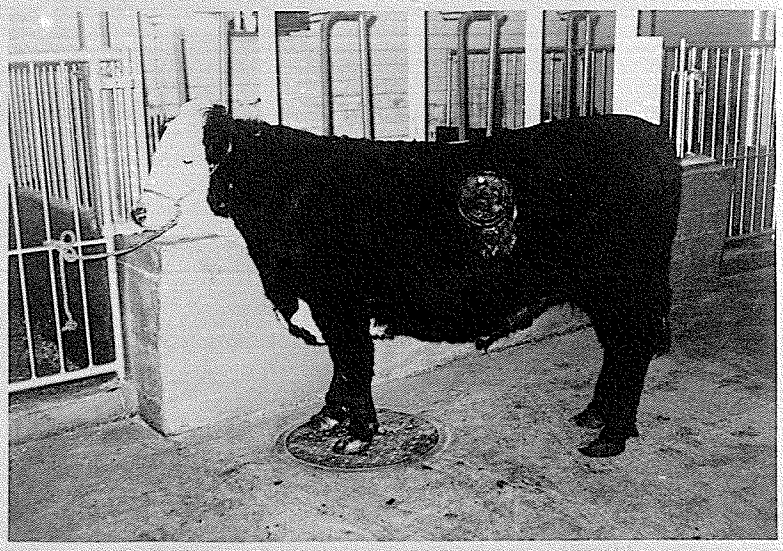
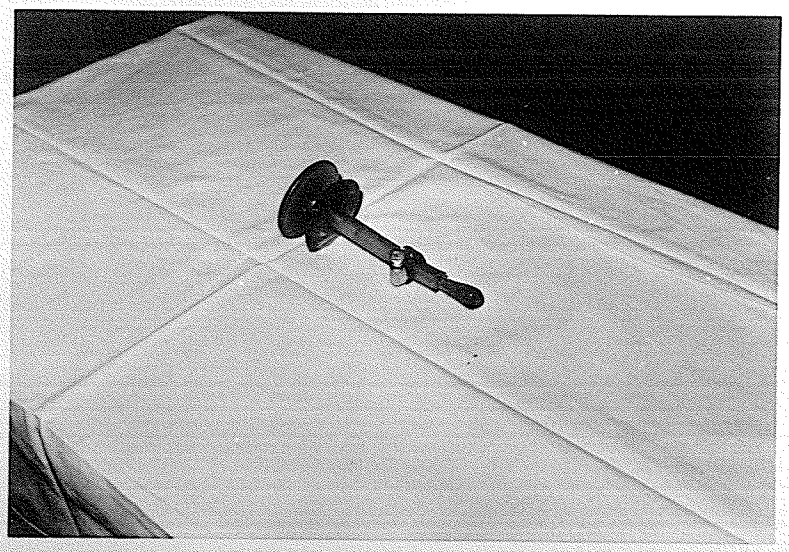


FIGURE 1. Rumen cannula used Part A and the steer fitted with this device.

PART A



PART B

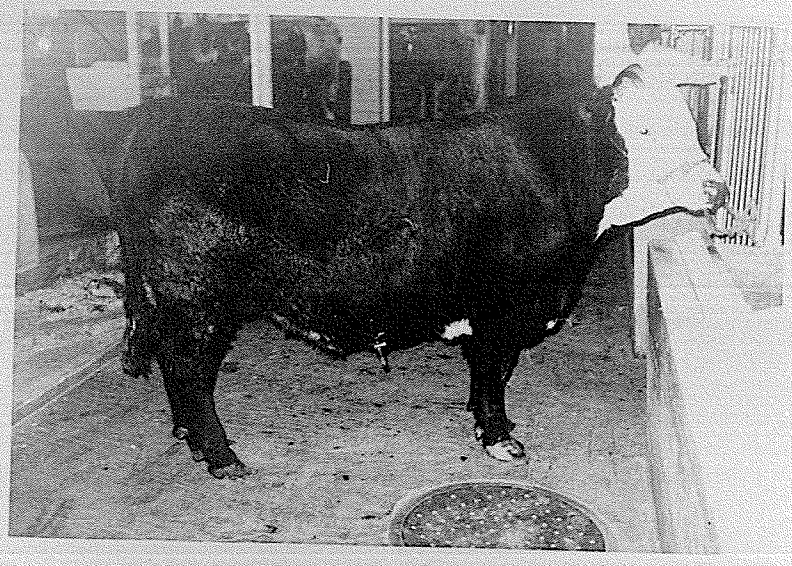


FIGURE 2. Abomasal cannula used Part A and the steer fitted with this device.

TABLE 1. Composition of the diets fed

Item		Per cent by Weight
Barley		60
Alfalfa Hay		38
Trace mineral salt		2
- Sodium chloride	96.5 %	
- Iodine	0.010%	
- Cobalt	0.004%	
- Iron	0.160%	
- Copper	0.330%	
- Manganese	0.120%	
- Zinc	0.400%	
Vitamin A		2200 IU/kg of food
Vitamin D		275 IU/kg of food

food are described in Table 2. Half of the complete diet was ground and pelleted. For the remaining portion, the alfalfa was chopped and the barley was rolled.

An automatic apparatus was used to feed half of the cattle at two minute intervals throughout the experiment (Figure 3). This is referred to in the text as continuous feeding. The remaining animals were fed twice a day by hand.

Treatments and Experimental Design

The treatments used in the experiment were as follows:

A. Chopped alfalfa hay was mixed with rolled barley and fed continuously throughout a period in the experiment (CC).

B. The entire diet was ground and pelleted and fed continuously (CP).

C. The form of the food was as in A, but the animals were fed at 09:00 AM and 09:00 PM each day (TC).

D. The entire diet was as in B, but the animals were fed at 09:00 AM and 09:00 PM each day (TP).

The four treatments A,B,C, and D were allocated to the four steers in four periods of time according to a 4 x 4 Latin square design, as follows:

TABLE 2. Chemical composition of the diet and ingredients

	Dry Matter Content (%)	Composition of Dry Matter by Weight		
		Energy Cal/gm	Crude Protein %	Ash %
Pelleted diet	89.60	4221	12.68	8.33
Barley	88.13	4341	10.78	3.26
Alfalfa Hay	87.83	4226	13.17	10.69

	Steer Number			
	18	16	15	55
Period	Treatments			
1	A	B	C	D
2	B	A	D	C
3	D	C	B	A
4	C	D	A	B

The periods were subdivided on the average into a ration adjustment period of 13 days, and an experimental period of 16 days.

Introduction to Methods

A general summary of experimental plan for each period is given in Table 3. The amount of food voluntarily consumed by each animal was determined daily. To study the physical factors that may limit food intake, the weights and volumes of digesta in the rumen and rates of flow of digesta into the intestines were determined. To investigate the chemical factors involved, samples of rumen and abomasal contents were obtained to determine the osmolality and the concentrations of volatile fatty acids, NH_3 and H^+ in the fluid fraction. Samples of blood were analyzed for osmolality and the total concentration of volatile fatty acids.

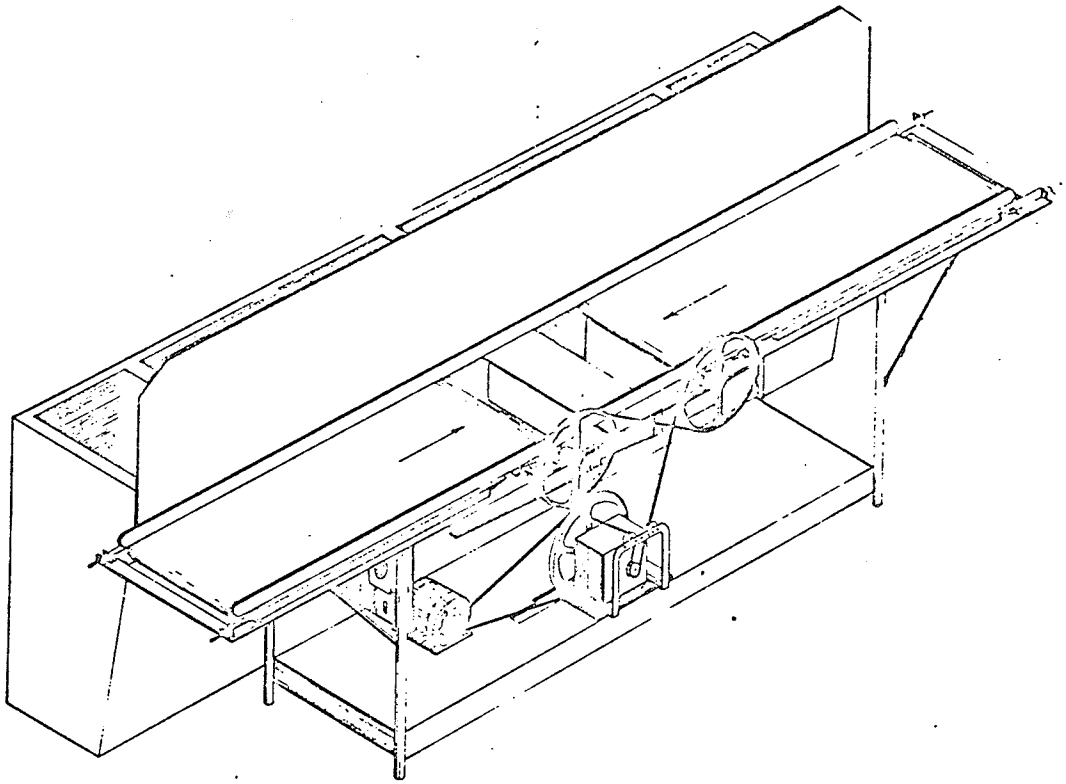


Figure 3. The automatic feeder.

TABLE 3. Experimental protocol during each period

Day of the Period	Item
1 to 13	Preliminary feeding period
14 to 28	Measured voluntary food consumption daily and weighed the animals
17 to 18	Collected blood, rumen and abomasal samples for analyses of volatile fatty acid, NH_3, pH, and osmolality
19 to 27	Injected a solution of PEG into the rumen of each steer, then infused the marker solution into the rumen continuously
20 to 28	Collected feces to determine the digestibility of the dry matter, organic matter, protein, and energy contents of the food
25 to 27	Collected abomasal samples to determine the rate of flow of digesta into the intestine and to calculate the extent of the digestion of food in the forestomach.
28 to 29	Measured the weight and the volume of the rumen contents

Methods Used to Study Physical Factors Controlling Food Consumption

Determination of voluntary intake of food

The animals were given 3 kg of food on the first day of each period. Their rations were then increased by 10 per cent on successive days until the weight of weighbacks collected daily were approximately 10 per cent of the weight of the food given. Maximum levels of food intake were usually attained by day 10 of each period. The *ad libitum* intakes of food were determined daily between days 14 and 28 (Table 3). The weighbacks for the animals fed twice daily were collected approximately 2 hr after the start of each meal. By this time the animals had stopped eating. The weighbacks for animals fed continuously were collected every day at 09.00 A.M. The weighbacks obtained daily for each steer between days 14 and 28 of each period were frozen. A representative sample for each steer was analyzed for its dry matter, organic matter, protein and energy contents.

Weight and volume of rumen contents

The weight and volume of rumen contents in each steer were measured on two consecutive days by manually removing digesta through the fistula. For the animals fed continuously, the rumens were emptied at 11.00 P.M.

on day 28 and at 11.00 A.M. on day 29. For the steers fed twice daily, the rumens were emptied at 11:00 PM on day 28 and at 8:30 AM, 11:00 AM and 8:30 PM on day 29.

Rate of flow of digesta fluid and dry matter through the abomasum

Polyethylene glycol with a molecular weight of about 4,000 (PEG) was used as a marker of fluid digesta (Hyden, 1955). On day 19 of each period, 100 gms of PEG in an aqueous solution was injected into the rumen as a priming dose. The continuous infusion was begun immediately after the priming dose with the concentration of 20.83 mg PEG/ml. The marker solution was pumped into the rumen through Jayon tubing (O.D. 1/4", I.D. 1/8") at a rate of 2,400 ml per day for 8 days using Buechler micropumps (Buechler instruments Inc., Fort Lee, New York). Samples of abomasal digesta were collected at the times given in Table 4 on days 25, 26, and 27 of each period. The recovery of PEG in the feces at these times was, on the average, 100 per cent.

The samples were frozen and later thawed to determine the concentration of PEG in the fluid phase.

The rate of flow of fluid digesta through the abomasum was determined using equation 1 (Phillips and

TABLE 4. Times in each period at which abomasal samples were collected to determine the rate of flow of digesta into the intestine.

Day of Period	Twice a Day Feeding	Continuous Feeding
25	2:00 p.m.	
	4:00 p.m.	4:00 p.m.
	6:00 p.m.	
	8:00 p.m.	8:00 p.m.
	10:00 p.m.	
	12:00 p.m.	12:00 p.m.
26	2:00 a.m.	
	4:00 a.m.	4:00 a.m.
	6:00 a.m.	
	8:00 a.m.	8:00 a.m.
	10:00 a.m.	
	12:00 a.m.	12:00 a.m.
	2:00 p.m.	
	4:00 p.m.	4:00 p.m.
	6:00 p.m.	
	8:00 p.m.	8:00 p.m.
	10:00 p.m.	
	12:00 p.m.	12.00 p.m.
27	2:00 a.m.	
	4:00 a.m.	4:00 a.m.
	6:00 a.m.	
	8:00 a.m.	8:00 a.m.
	10:00 a.m.	
	12:00 a.m.	12:00 a.m.

Dyck, 1964).

$$\text{Flow rate (ml/hr)} = \frac{\text{PEG infused into rumen (mg/hr)}}{\text{Conc. of PEG in abomasal fluid (mg/ml)}} \quad (1)$$

Separate samples of abomasal digesta were collected to determine the rate of flow of digesta dry matter through the abomasum. These were taken at intervals of 4 hr from 02.00 P.M. on day 25 to 12.00 A.M. of day 27 of each period. The outflow from the abomasal cannula of each steer was mixed well and 100 ml was transferred to a plastic bag (Whirl-pak) and frozen immediately. These samples were dried in a Virtis freeze drier (Virtis Co., Gardiner, New York) and weighed individually. The amount of dry digesta (D.M.) flowing through the abomasum per day was calculated as shown in equation 2.

$$\text{Dry matter (g/d)} = \frac{\text{Weight of D.M. (g)}}{\text{1 ml abomasal fluid}} \times \text{Flow rate of abomasal fluid (ml/d)} \quad (2)$$

The dried samples for each steer were pooled, ground and kept in the freezer for later analyses of their protein, energy and ash contents.

Fecal Collections

Feces were collected for 7 to 8 days between days

20 to 28, as shown in Table 3, in each period. The daily collections from each steer were weighed and mixed, and a sample (10 per cent) was frozen. At the end of each collection period the samples for each animal were thawed and mixed. About 10 per cent of the composite samples were refrozen and their dry matter, protein, ash and energy contents were determined at the end of the experiment.

Digestibility

The apparent digestibilities of the dry matter, organic matter, energy, crude protein of the food in the alimentary tract were calculated as illustrated in equation 3 for dry matter.

$$\text{D.M. digestibility (\%)} = \frac{\text{Mean D.M. intake/d} - \text{Mean output of D.M. in feces/d}}{\text{Mean D.M. intake/d}} \times 100 \quad \ldots (3)$$

The digestibilities of dry matter, organic matter, energy and protein in the forestomach were calculated as shown in equation 4 for dry matter.

$$\text{D.M. digested in the rumen (\%)} = \frac{\text{Mean D.M. intake/d} - \text{D.M. flow through the abomasal/d}}{\text{Mean D.M. intake/d}} \times 100 \quad \ldots (4)$$

Methods Used to Study Chemical Factors Controlling Food Consumption

Sampling fluid from rumen and abomasal contents

Digesta was removed from the ventral sac of the rumen and from the abomasum of each steer on days 17 and 18 of each period at the times given in Table 5. Each sample was filtered through gauze into a polypropylene bottle and the pH of the fluid was measured immediately. Two drops of $HgCl_2$ solution (25 mg-%, w/v) were mixed with each sample to inhibit bacterial activity. The samples were then frozen. At the end of the experiment, they were thawed to determine the concentrations of volatile fatty acids and NH_3. The rumen fluid was also analyzed for osmolality.

Blood sampling

The techniques used to cannulate the jugular vein and to obtain blood samples were described by Phillips (1968). Thirty ml samples of blood were withdrawn from the jugular vein of each steer on days 17 and 18 of each period at the times given in Table 5. The blood was heparinized and a portion was centrifuged at 2,500 rpm for ten minutes. The plasma samples were decanted and stored in vials at -5°C for later analyses of the concentration of volatile fatty acid and osmolality.

TABLE 5. Timetable for collecting rumen, abomasal and blood samples to study chemical factors limiting food intake.

Day	Twice a Day Feeding	Continuous Feeding
	(Time of Sampling)	
17	3:00 p.m.	3:00 p.m.
	6:00 p.m.	6:00 p.m.
	9:00 p.m.	9:00 p.m.
	9:30 p.m.	9:30 p.m.
	10:00 p.m.	10:00 p.m.
	12:00 p.m.	12:00 p.m.
18	3:00 a.m.	3:00 a.m.
	6:00 a.m.	6:00 a.m.
	9:00 a.m.	9:00 a.m.
	9:30 a.m.	12:00 a.m.
	10:00 a.m.	
	12:00 a.m.	

The hematocrit of the remaining blood was measured using a capillary tube 70 mm long. The tubes were filled with blood, sealed at one end and centrifuged for 7 min in an "International" microhematocrit centrifuge at about 12,000 rpm. The depth of packed cells in the tube was determined in an "International" capillary tube reader.

Chemical analyses

The energy contents of the samples were determined using a Parr Bomb Calorimeter with automatic water temperature controls.

The nitrogen content of dry sample material was determined by a macrokejeldahl technique (AOAC, 1959).

The ammonia concentration in fluid digesta was determined in Conway units (Conway, 1957).

The concentrations of volatile fatty acids in fluid digesta were determined by the method of Erwin, Marco, and Emery (1961). Six microliters of solution were analyzed using a Burrell Corporation gas chromatograph.

The concentrations of volatile fatty acids in plasma were determined using a steam distillation method (Annison, 1954). Ten microliters of a concentrated solution of VFA were analyzed using a Burrell Corporation gas chromatograph.

The osmolalities of fluid digesta and plasma were measured with an osmometer (Model 31, Advanced Instruments

Inc., Massachusetts). Two ml samples were used for each determination. Samples of dry matter were ashed in a furnace at 600°C for 24 hr.

Samples of wet abomasal digesta were freeze dried. Wet feces, food and weighbacks were dried at 75°C in a forced air oven for 4 days.

Statistical Methods

The results were tested for treatment, period and animal effects by an analysis of variance for the Latin Square Design. The significance of effects of frequency of feeding, form of food and the interaction of frequency and form were tested with individual degrees of freedom. Also, the significance of differences among treatment means were analyzed using Duncan's Multiple Range Test (Steel and Torrie, 1960).

The statistical model was:

Source	Degrees of Freedom
Animal	3
Period	3
Treatments	3
Frequency	1
Form	1
Interaction	1
Error	6

RESULTS

Voluntary Food Intake

The average quantities of food voluntarily consumed were 9.20, 9.00, 7.93 and 7.02 kg per day for dietary treatments CC, CP, TC and TP, respectively. The treatment effect in the Latin Square analysis of variance was not significant (Table 6). Further analysis indicated that the animals fed continuously consumed significantly more food than those fed twice each day ($p < 0.05$) but the form of the food, that is chopped or pelleted, had no effect on intake. The mean voluntary food intakes for the treatments CC and CP did not differ significantly from that for TC but were significantly greater than that for TP (Table 6). The average time of eating a meal was 1.5 hr for the steers fed twice each day.

Body Weight Gain

The average daily weight gains of the steers while on the treatments CC, CP, TC and TP were 0.81, 0.75, 0.61, 0.55 kg per day, respectively.

There were significant differences among the treatment means (Table 6). The cattle fed continuously gained signifi-

Table 6. Analysis of variance for voluntary food intake, body weight gain, and the coefficients of digestion for steers.

	Mean Square in the Analysis of Variance								Means of Treatments†			
	Steer	Period	Treatment	Frequency	Form	Interaction	Error	Residual Standard Deviation	CC	CP	TC	TP
Food Intake (kg/day)	0.6238	1.7333	4.1118	10.5950*	1.2430	0.4970	1.2036	1.0970	9.20^{A}	9.00^{A}	7.93^{AB}	7.02^{B}
Body Weight Gain (kg/day)	0.0026	0.0148	0.0586*	0.1620**	0.0140	0.0006	0.0109	0.1040	0.81^{A}	0.75^{AB}	0.61^{BC}	0.55^{C}
Total Digestion (%)												
Dry Matter	0.1633	0.1701	8.1583**	20.2100**	4.0200**	0.2500	0.2475	0.4970	80.50^{A}	79.25^{B}	78.01^{C}	77.25^{C}
Organic Matter	0.1876	0.1163	8.4943**	18.2115**	7.0600**	0.2090	0.1649	0.4060	81.28^{A}	79.72^{B}	78.91^{C}	77.81^{D}
Energy	0.0645	0.4147	19.1179**	48.1980**	7.5490*	1.6070	1.1142	1.0560	79.87^{A}	77.86^{B}	75.76^{C}	75.02^{C}
Protein	1.0807	0.6690	37.3063*	73.1450*	29.2410	9.5330	5.9973	2.4490	77.70^{A}	76.53^{A}	74.96^{AB}	70.72^{B}
Digestion in Forestomach (%)												
Dry Matter	3.2726	4.3377	186.8230**	495.0600**	63.3620	2.0450	14.4517	3.8010	$61.12^{A\#}$	57.85^{A}	50.71^{B}	46.01^{B}
Organic Matter	4.1243	4.5493	184.3060**	438.2700**	114.4900**	0.0003	10.7711	3.2820	64.41^{A}	59.05^{AB}	53.93^{BC}	48.59^{C}
Energy	7.4174	29.7502	198.0877**	453.2600**	140.780*	0.2210	13.0693	3.6153	63.17^{A}	57.48^{AB}	52.76^{BC}	46.60^{C}
Protein	3.8280	41.5936	86.9964**	151.1050**	83.1280*	26.7550	10.8912	3.2890	-20.91^{A}	-13.76^{B}	-12.17^{B}	-10.20^{B}

* significant at $p < 0.05$
** significant at $p < 0.01$
† means with different superscripts are significantly different at $p < 0.05$
\# 61.12 as a percentage of 100 not 61.12 units out of a total of 80.50.

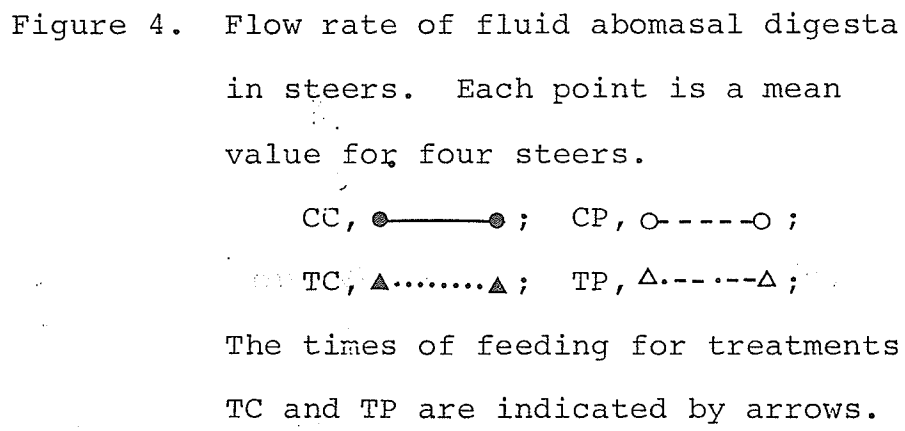
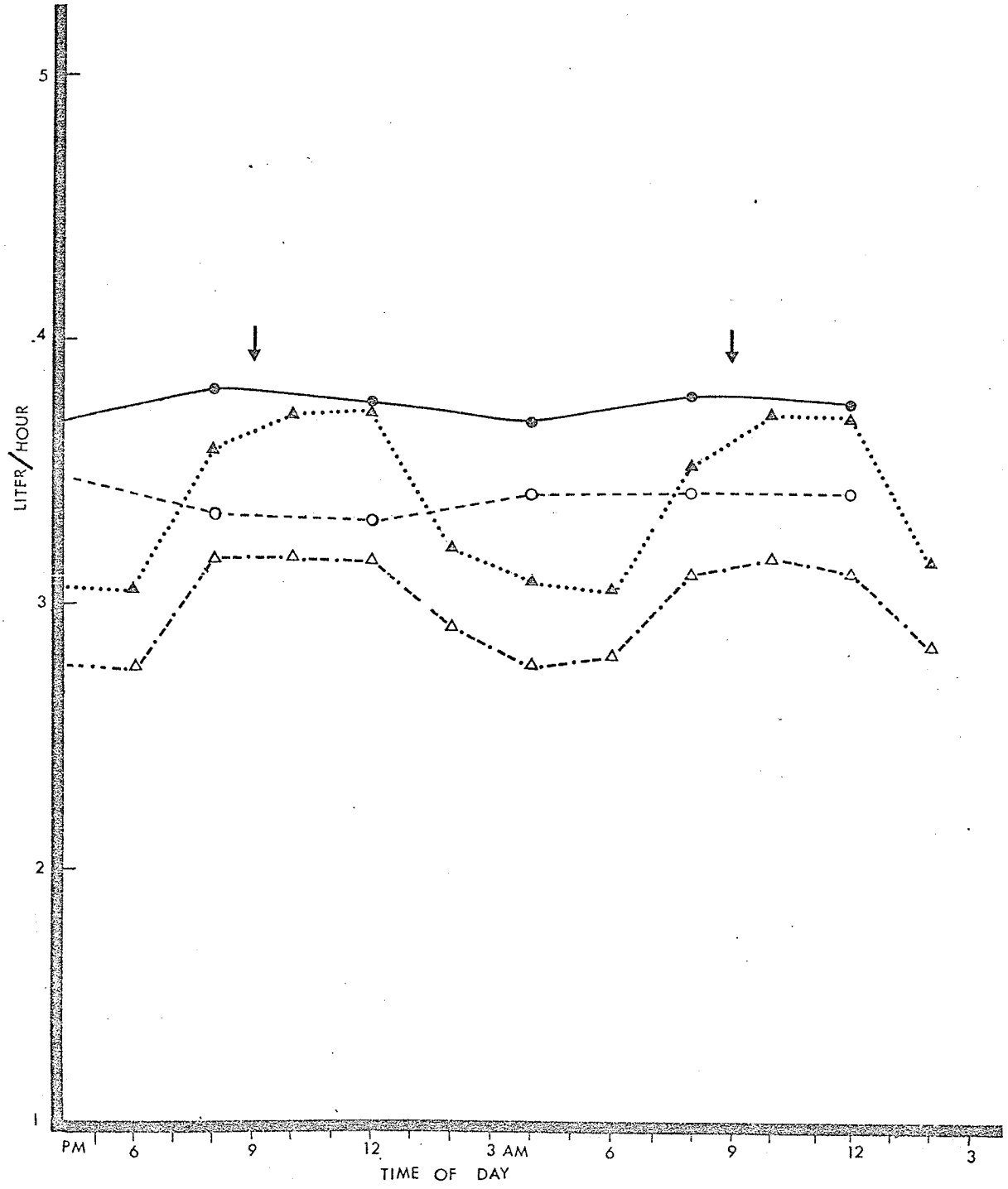


Figure 4. Flow rate of fluid abomasal digesta in steers. Each point is a mean value for four steers.

CC, ●——●; CP, ○- - - -○;

TC, ▲.....▲; TP, △- - - -△;

The times of feeding for treatments TC and TP are indicated by arrows.


5
4
3
2
1
LITER/HOUR
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6
9
12
3 AM
6
9
12
3
TIME OF DAY

cantly faster than those fed twice per day ($p < 0.05$).

Flow Rate of Fluid Digesta Through the Abomasum

There was a marked diurnal fluctuation in the flow rate of abomasal fluid in the animals fed twice a day but this was virtually absent in those fed continuously. The effect of time of sampling on the rate of flow was significant when the animals were fed twice each day ($p < 0.01$) but was not when they were fed continuously (Appendix 2).

The flow rate began to increase in the animals fed twice a day at least one hour before the time of feeding (Figure 4). The largest average flow rates, 3.7 and 3.2 l/hr for treatments TC and TP, respectively, were attained within an hour after the start of a meal and were maintained at this high level for another two hours. The rates then declined gradually, reaching the lowest values of 3.1 and 2.8 l/hr, respectively between six to nine hours after the beginning of the meal. The flow rates for treatments CC and CP were maintained on the average at about 3.7 and 3.4 l/hr, respectively.

The flow rates of abomasal fluid for steers fed twice a day were significantly smaller than those for steers fed continuously at 4:00 and 8:00 PM and at 4:00 and 8:00 AM ($p < 0.05$) but the differences were not significant at 12:00 noon or at 12:00 midnight (Table 7). These differences in rates of flow at other times of the day were not tested.

Table 7. Flow rate of abomasal digesta of steers.

Time of Day	Mean Square in the Analysis of Variance								Means of Treatments (l/hr)†			
	Animal	Period	Treatment	Frequency	Form	Inter-action	Error	Residual Standard Deviation	CC	CP	TC	TP
4:00 PM	0.0911	0.2456**	0.6105**	1.4702**	0.3570**	0.0046	0.0212	0.146	3.660^{A}	3.390^{B}	3.060^{C}	2.762^{D}
8:00 "	0.1356	0.1942*	0.3182*	0.3364*	0.6162**	0.0020	0.0359	0.189	3.785^{A}	3.415^{BC}	3.584^{AB}	3.152^{C}
12:00 "	0.1004	0.3682**	0.3364**	0.1369	0.8100**	0.0625	0.0332	0.182	3.739^{A}	3.415^{BC}	3.723^{AB}	3.149^{C}
4:00 AM	0.0821	0.1906**	0.6775**	1.7623**	0.2627**	0.0077	0.0186	0.136	3.682^{A}	3.469^{A}	3.086^{B}	2.752^{C}
8:00 "	0.1423	0.2409*	0.3255**	0.1702**	0.8055**	0.0011	0.0320	0.179	3.809^{A}	3.341^{BC}	3.520^{AB}	3.107^{C}
12:00 "	0.1067	0.3009*	0.3596**	0.0033	1.0252**	0.0203	0.0357	0.189	3.742^{A}	3.308^{B}	3.679^{A}	3.105^{B}

* significant at $p < 0.05$
** significant at $p < 0.01$
† means with different superscripts are significantly different at $p < 0.05$

The flow rates at all times of the day listed in Table 7 were significantly larger when the animals were fed chopped hay than when they were fed pelleted food ($p < 0.01$) (Figure 4).

The interactions in the analysis of variance between the effects of frequency of feeding and forms of the food were not significant (Table 7).

Digestion of the Food and its Components

The apparent coefficients of digestibility for the dry matter, organic matter, energy and protein contents of the food were significantly greater when the animals were fed continuously rather than twice a day ($p < 0.01$) (Table 6). The coefficients for all components except protein were greater when the food was given in the chopped form than in the form of pellets ($p < 0.01$). The digestion coefficients were greatest for the treatment CC, and least for the treatment TP, the means being 80.50 and 77.25, respectively.

The Apparent Digestion of Food in the Forestomachs

The coefficients of digestion of dry matter, organic matter, energy, and protein were significantly greater when the animals were fed continuously than when they were fed twice each day. The highest and lowest means, for CC and TP, were 61.12 and 46.01 per cent respectively for dry matter

(Table 6). The steers fed chopped food digested significantly more of the organic matter and energy of food in the reticulorumen than did those given pelleted food. The amount of protein leaving the forestomachs was greater than that ingested for each treatment. The apparent increase was greater when food was given continuously than when it was given twice each day. The amounts of protein were also greater for food in the chopped form than in pelleted form (Table 6).

Weight, Volume and Density of the Reticulorumen Contents

There was a marked diurnal fluctuation in both weight and volume of the contents of the reticulorumen in the steers fed twice each day (Figures 5 and 6). However, there was little change with time of day in these values when the animals were fed continuously.

The mean volume and weight of contents of the reticulorumen were significantly greater when the steers were fed continuously than at 0.5 hr before the start of a meal (8:30) when they were fed twice each day ($p < 0.01$) (Table 8). At two hours after the start of a meal there was no significant effect of frequency of feeding on the total weight and volume of the reticulorumen contents.

The mean volumes and weights of contents were always greater for chopped food than for pelleted food. However, the effect of form of food was significant only for weight of contents at two hours after the start of a meal in the

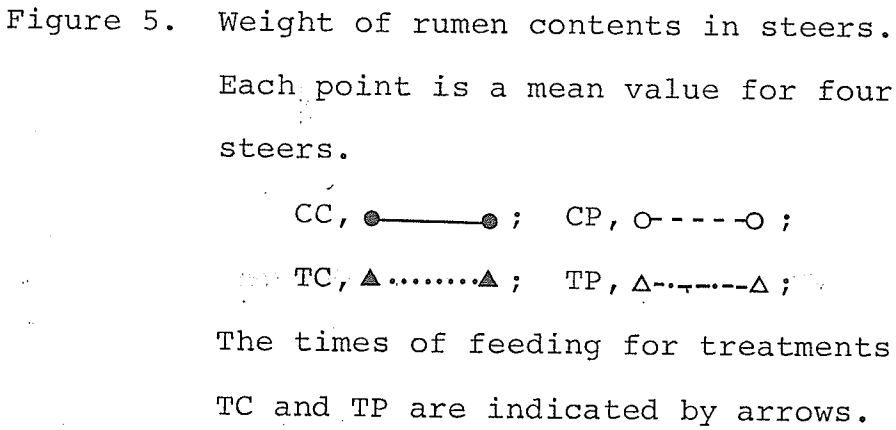
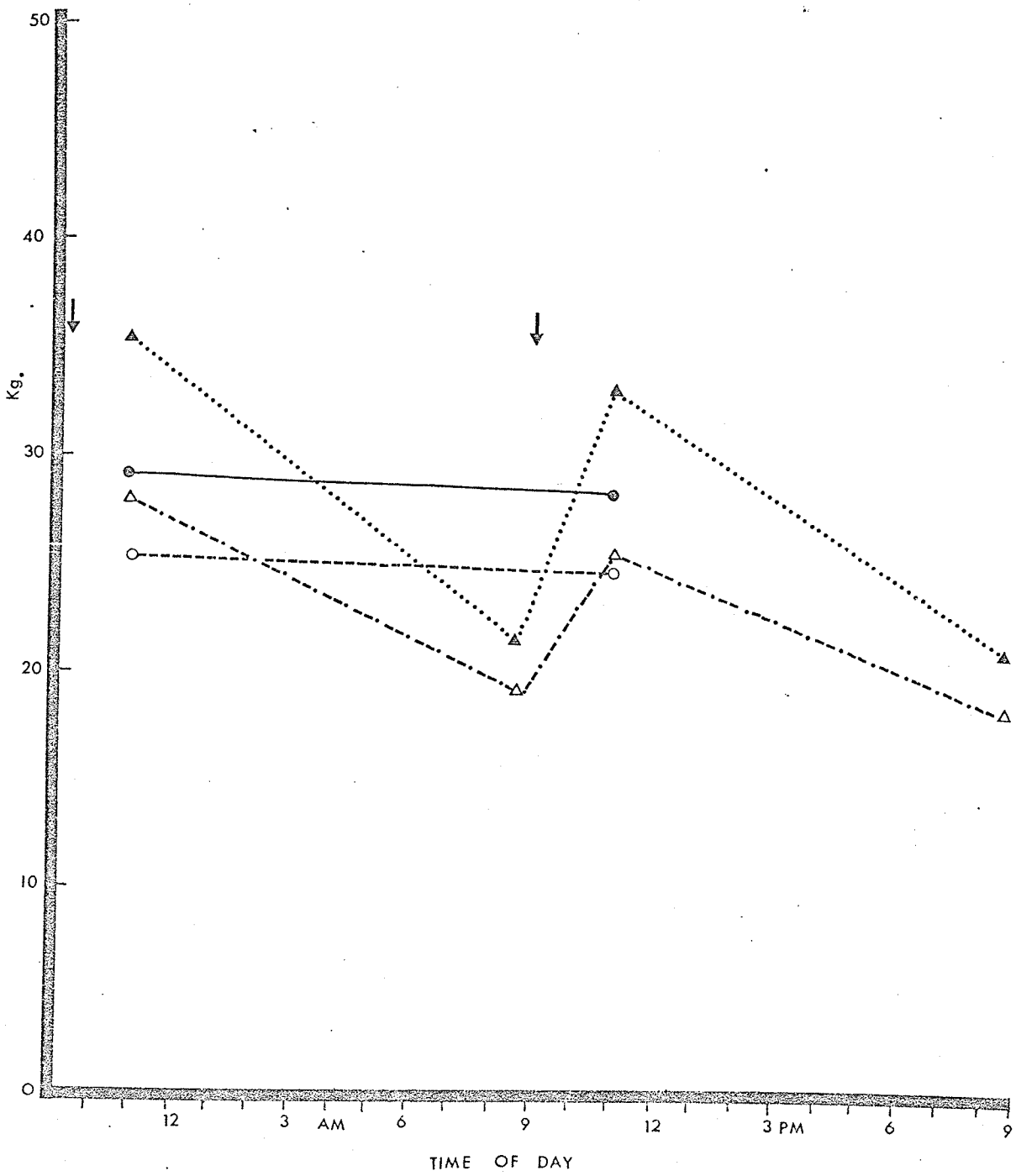


Figure 5. Weight of rumen contents in steers. Each point is a mean value for four steers.

The times of feeding for treatments TC and TP are indicated by arrows.


50
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12
3 PM
6
9
TIME OF DAY

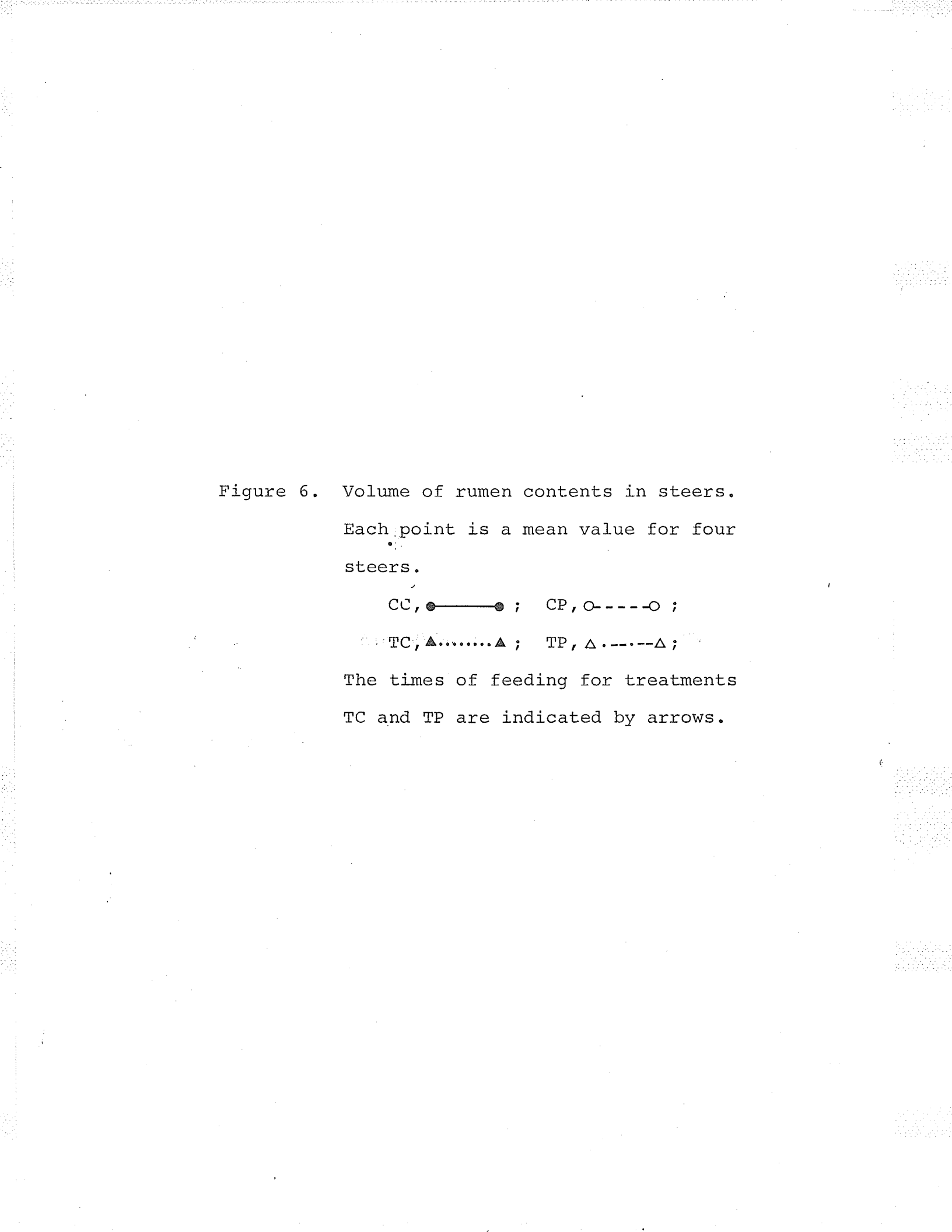


Figure 6. Volume of rumen contents in steers. Each point is a mean value for four steers.

The times of feeding for treatments TC and TP are indicated by arrows.

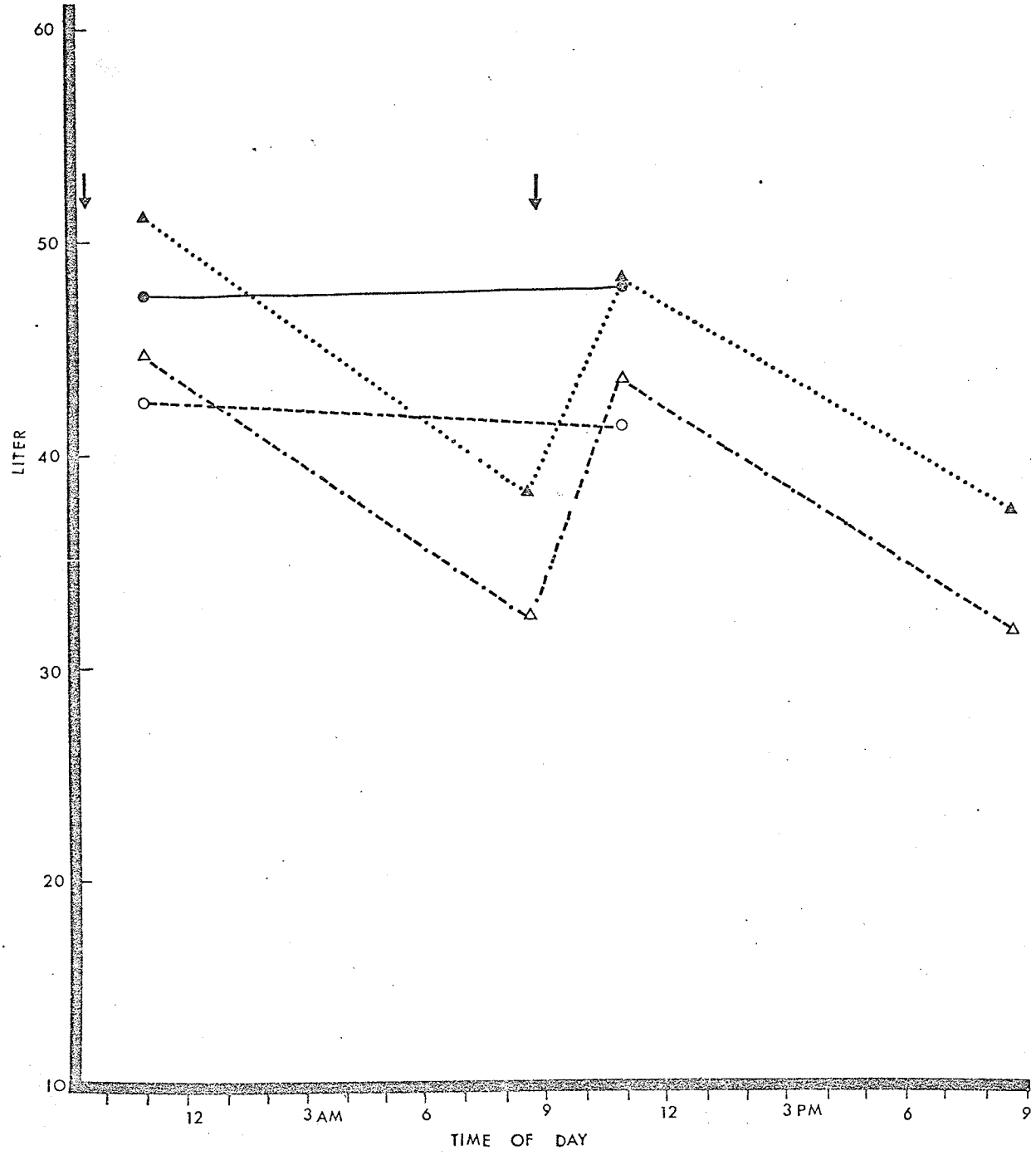

60
50
40
30
20
10
LITER
12
3 AM
6
9
12
3 PM
6
9
TIME OF DAY

Table 8. Analysis of variance of the weight, volume and density and the dry matter and water contents of digesta in the reticulo-rumen of steers.

	Mean Square in the Analysis of Variance								Mean of Treatment[#]			
	Animals	Period	Treatment	Frequency	Form	Inter-action	Error	Residual Standard Deviation	CC[±]	CP[±]	TC[†]	TP[†]
Total Weight (kg)												
8:30	121.1059**	109.4755**	77.9312*	189.6120**	41.990	2.1904	9.5552	3.0910	38.90^{A}	34.92^{AB}	31.28^{BC}	28.78^{C}
11:00	200.2774*	110.4062**	64.3360	49.9495	130.930*	12.1278	13.9431	3.7340	38.90^{AB}	34.92^{A}	44.18^{B}	36.71^{A}
Total Volume (l)												
8:30	337.2291**	18.0208	180.7708*	410.060**	132.250	0	27.2291	5.2180	47.63^{A}	41.88^{AB}	37.50^{BC}	31.75^{C}
11:00	497.1914**	65.1914	49.2330	19.6914	127.972	0.0352	28.1080	5.3020	47.63	41.88	49.75	44.19
Density (kg/l)												
8:30	0.0378	0.1406**	0.0258	0.0541	0.0150	0.0086	0.0100	0.1000	0.81	0.83	0.88	0.99
11:00	0.0186*	0.1184**	0.0114	0.0333*	0.0002	0.0008	0.0028	0.0530	0.81^{A}	0.83^{AB}	0.92^{B}	0.91^{AB}
Total D.M. (kg)												
8:30	2.4405**	0.7466*	3.5371**	9.4400**	0.6440	0.1541	0.1308	0.3620	5.40^{A}	5.26^{A}	4.06^{B}	3.46^{B}
11:00	4.3467**	1.1445	4.1592**	8.9550*	2.1240	1.3983	0.4099	0.6400	5.40^{A}	5.26^{A}	7.48^{B}	6.16^{A}
Total Water (l)												
8:30	87.0519**	89.3285**	44.3388	101.6560*	28.5960	2.7640	8.2115	2.8660	33.09^{A}	29.59^{AB}	27.22^{B}	25.38^{B}
11:00	128.7373**	69.0740**	21.6674	6.4009	58.2169*	0.3906	6.8312	2.6136	33.09^{AB}	29.59^{A}	34.67^{B}	30.54^{AB}
Dry Matter (%)												
8:30	3.6988	2.3692	10.4684*	29.0520*	0.7656	1.5880	1.2243	1.1065	14.99^{A}	15.18^{A}	12.92^{B}	11.86^{B}
11:00	5.4114	1.2955	6.5408*	18.5330*	0.2700	0.8190	1.3170	1.1476	14.99^{A}	15.18^{A}	17.59^{B}	16.88^{AB}

* significant at $p < 0.05$
** significant at $p < 0.01$
\# means with different superscripts are significantly different at $p < 0.05$
± the results for CC and CP at 11:00 AM and PM were used in the analyses at 8:30 and at 11:00 assuming that the values for the different measurements varied little with time of day when the steers were fed continuously
† the values given for 8:30 and 11:00 are means for results obtained at 8:30 AM and 8:30 PM (0.5 hr before the start of a meal) and at 11:00 AM and 11:00 PM respectively.

steers fed twice each day.

The mean values for density of digesta were always less than 1.0 kg/l (Table 8). The values when the steers were fed continuously were smaller than those when the animals were fed twice each day, the effect being significant only at two hours after the start of a meal ($p < 0.05$; Table 8). The effect of form of food on the density of rumen contents was not significant.

Weight of Dry Matter, Volume of Water and the Proportion of Dry Matter in Digesta from the Reticulorumen

The total volumes of water, weights of dry matter, and the per cent dry matter in digesta increased after feeding in the treatments TC and TP. These values for treatments CC and CP were similar at 11:00 PM and 11:00 AM (Appendix 2).

The mean weights of dry matter and the per cent dry matter in the total reticulorumen contents were significantly smaller for treatments TC and TP than for treatments CC and CP at 0.5 hr before the times of feeding ($p < 0.05$; Table 8), but were significantly greater ($p < 0.05$) at two hours after the start of feeding. There were no significant effects of form of food on these measurements at either of the times given.

The average rates of removal of dry matter from the reticulorumen in the treatment TC were 299 and 529 g/hr between and during meals, respectively. These values for the treatment TP were 277 and 684 g/hr, respectively.

The mean volumes of water in the reticulorumen were significantly smaller for treatments TC and TP than for treatments CC and CP at 0.5 hr before the times of feeding ($p < 0.05$). The effect of form of food was not significant but the means for treatments involving chopped food were greater than the means for treatments involving pellets. At two hours after feeding, the effect of frequency of feeding was not significant but the effect of form of food was, the means for food in the chopped form being significantly greater than those for pelleted food.

The mean values for per cent dry matter of digesta from the reticulorumen were significantly smaller for treatments TC and TP than for treatments CC and CP at 0.5 hr before the time of feeding ($p < 0.05$), but were greater ($p < 0.05$) at two hours after the start of a meal. The effects of form of food were not significant at either time.

Osmolality of Rumen Fluid and Plasma

There was a marked diurnal variation in the osmolality of rumen fluid when the steers were fed twice each day (Figure 7). The osmolalities decreased to minimum values of 515 and 511 mosmol/l for the treatments TC and TP,

respectively just prior to feeding. The maximum values of 675 and 760 mosmol/l were attained within one and one-half hours after the start of a meal, respectively. The osmolalities were maintained at relatively high values for the next three hours but they decreased markedly during the three hours before the next time of feeding. There was little variation throughout the day in the osmolalities of rumen fluid when the steers were fed continuously. The average levels for the treatments CC and CP were 591 and 658 mosmol/l, respectively (Table 9, Figure 7). The effects of time of sampling on osmolality of rumen fluid were significant, by analysis of variance, for steers fed twice each day ($p < 0.01$), but were not significant for steers fed continuously (Appendix 2).

The osmolality of rumen fluid just before the start of a meal at 9:00 PM, from the steers fed twice each day, was significantly smaller than that taken at the same time from the animals fed continuously. But at 0.5 hr after feeding had commenced, the osmolality was significantly greater for the steers fed twice each day (Table 9). The osmolality of rumen fluid was generally larger when the steers were fed pellets than when they were fed chopped hay. This result was found for both frequencies of feeding but the effect was significant at only 9:30 PM and at 3:00 and 12:00 AM (Table 9).

The osmolality of plasma for treatments TC and TP

Table 9. Analysis of variance of osmolality of rumen fluid from steers.

Time of Day	Mean Square in the Analysis of Variance								Means of Treatments† (mosmol/l)			
	Animals	Period	Treatment	Frequency	Form	Interaction	Error	Residual Standard Deviation	CC	CP	TC	TP
3:00 PM	1595.229	13172.563	6685.229	3451.560	16576.560	27.560	6469.812	80.435	579	646	611	673
6:00 "	2816.063	10657.229	3835.396	3.063	11502.560	0.563	2473.979	49.739	591	645	593	646
9:00 "	673.730	9765.730	17504.060*	43995.06**	4128.060	4389.060	2951.060	54.324	588^{AB}	653^{A}	516^{B}	515^{B}
9:30 "	356.230	8789.560	17862.560*	25520.06*	27972.560*	95.060	3462.470	58.843	596^{A}	675^{AB}	671^{AB}	760^{B}
10:00 "	1655.229	9213.063	7438.729	15190.56*	5738.060	1387.560	2070.729	45.505	594^{A}	651^{AB}	675^{AB}	694^{B}
12:00 "	1005.229	17817.729*	7105.396	6847.560	14220.560	248.060	2833.563	53.231	603	655	637	704
3:00 AM	3481.729	20363.396**	10423.729*	138.063	30363.060**	77.006	1896.729	43.551	600^{AB}	673^{BC}	592^{A}	693^{C}
6:00 "	2283.729	14237.229*	3729.063	280.563	10455.060	451.563	2032.729	45.086	587	649	606	646
9:00 "	258.560	13640.730*	17467.890*	37733.060**	1040.060	13630.560*	2017.640	44.918	588^{AB}	663^{A}	549^{BC}	507^{C}
12:00 "	1081.229	10780.063*	10734.896*	12488.060*	17889.060*	1827.560	1874.229	43.292	584^{A}	672^{B}	661^{B}	706^{B}

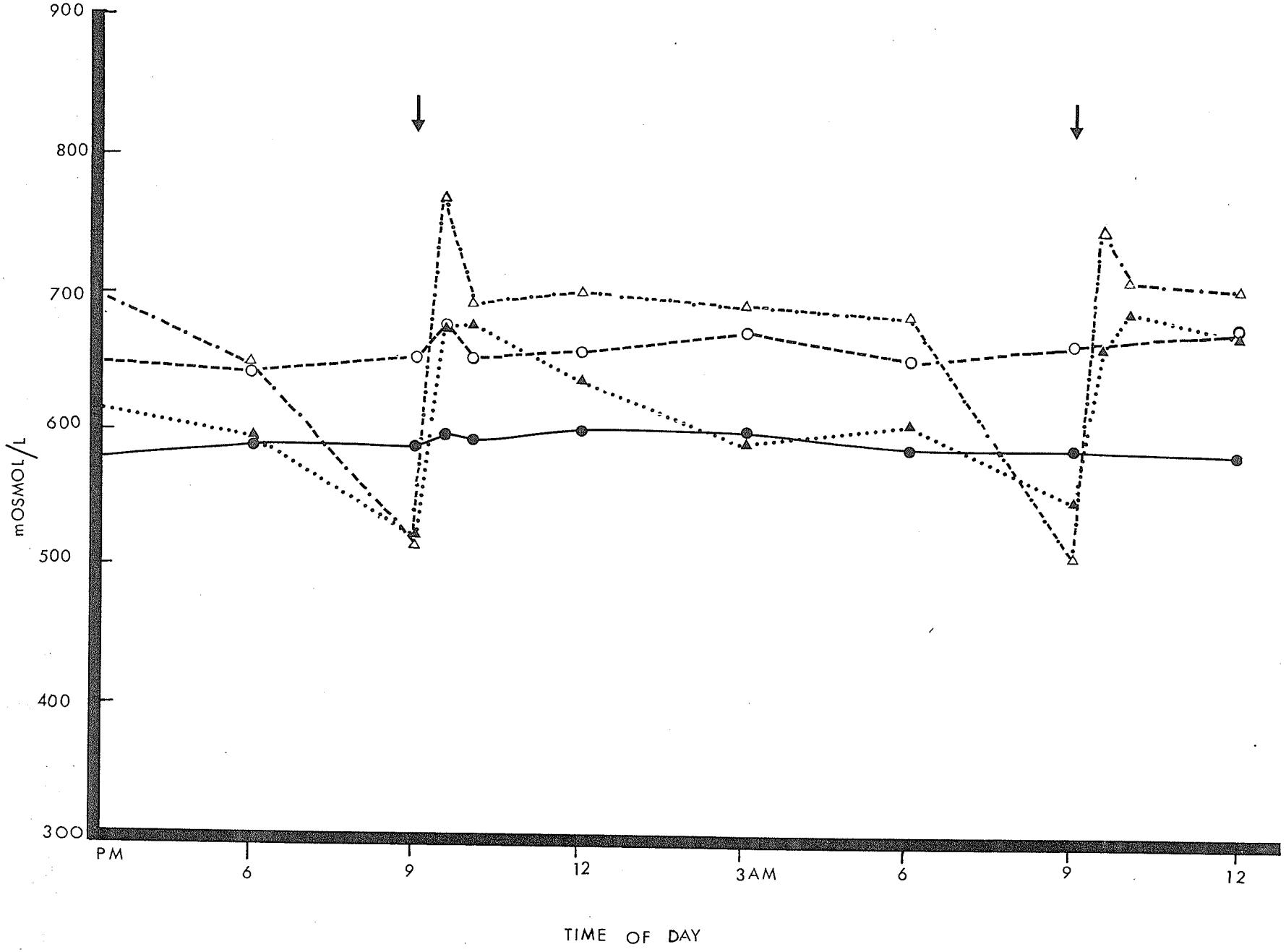
* significant at $p < 0.05$
** significant at $p < 0.01$
† means with different superscripts are significantly different at $p < 0.05$.

Figure 7. Osmolality of fluid digesta from the rumen of steers. Each point is a mean value for four steers.

CC, ●——● ; CP, ○----○ ;

TC, ▲.....▲ ; TP, △-.--.--△ ;

The times of feeding for treatments TC and TP are indicated by arrows.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TIME OF DAY

increased from values of 270 and 265 mosmol/l just before the start of a meal at 9:00 PM to maximum values of 291 and 294 mosmol/l, respectively at 9:30 PM (Table 10). The osmolality then decreased gradually to the new prefeeding levels of 271 and 274 mosmol/l, respectively. This pattern of changes in osmolality did not occur in the animals fed continuously. The average osmolalities were 280 and 281 mosmol/l for the treatments CC and CP, respectively. There was a significant effect of time of sampling on the osmolality values when the steers were fed twice each day ($p < 0.01$). This was not found when the steers were fed continuously. The osmolality of plasma from steers fed twice each day was significantly greater than that for steers fed continuously at 0.5 hr after the start of the meal at 9:00 PM ($p < 0.05$; Table 10).

Packed Cell Volume in Blood

The packed cell volume, obtained when the steers were fed twice each day was 30 per cent for six hours prior to feeding at 9:00 AM and increased to 34 and 33 per cent for treatments TC and TP at 9:30 AM, respectively. By 10:00 AM, the values had returned to 30 per cent. The packed cell volume for the steers when fed continuously were maintained at 30 per cent except that at 9:30 AM they were 29 and 30 per cent for the treatments CC and CP, respectively. The time of sampling had a significant effect on values for packed cell volume when the animals were fed

Table 10. Analysis of variance of the osmolality of plasma.

Time of Day	Mean Square in the Analysis of Variance								Means of Treatments# (mosmol/l)			
	Animals	Period	Treatment	Frequency	Form	Inter-action	Error	Residual Standard Deviation	CC	CP	TC	TP
3:00 PM	14.5625	98.5625	40.2291	105.063	10.563	5.063	98.3958	9.919	280	280	276	273
6:00 "	30.2291	226.2291	124.2291	248.063	39.063	85.562	117.8125	10.854	278	280	275	267
9:00 "	72.0833	178.4166	216.0833	576.00	16.00	56.250	129.6666	11.387	279	280	270	265
9:30 "	41.4166	30.7500	222.9166*	650.250**	6.250	12.250	35.0833	5.9231	280^{A}	280^{A}	291^{B}	294^{B}
10:00 "	78.0625	70.0625	125.0625	280.562	76.562	18.063	57.6458	7.592	279	281	285	292
12:00 "	36.9166	78.4166	73.0833	9.00	110.250	100.00	53.4166	7.309	280	281	276	287
3:00 "	29.7291	36.2291	14.3958	10.563	27.563	5.063	25.3958	5.039	279	280	276	280
6:00 "	0.4166	60.9166	34.7500	49.00	49.00	6.250	67.5833	8.221	279	281	274	279
9:00 "	17.4166	35.7500	81.0833	225.00	16.00	2.250	73.1666	8.554	280	281	271	274
12:00 "	23.5625	22.7291	9.2291	3.063	10.562	14.063	14.3958	3.794	281	281	283	280

* significant at $p < 0.05$
** significant at $p < 0.01$
\# means with different superscripts are significantly different at $p < 0.05$.

twice each day ($p < 0.001$). The effect was not significant when the animals were fed continuously (Appendix 2).

The packed cell volume for steers fed twice each day was greater than that for steers fed continuously at 0.5 hr after feeding ($p < 0.01$). There was no significant effect due to form of the diet or interaction.

pH of Fluid from Rumen and Abomasal Digesta

There was a diurnal fluctuation in the pH of rumen liquor when the steers were fed twice each day (Figure 8). The pH was highest before the start of the meals at 9:00 PM and 9:00 AM, the mean values for treatments TC and TP being 6.42 and 6.57, respectively. The pH decreased slightly 0.5 hr later and reached the minimum values of about 5.6 and 5.7, respectively from one to three hours after the start of a meal. The pH of the rumen liquor samples then increased slowly to new high values before the next time of feeding. When the steers were fed continuously, there was substantially less variation in the pH of rumen liquor throughout the day (Figure 8), average values for the treatments CC and CP being 6.29 and 5.86, respectively. The effect of time of sampling on pH values was significant for treatments TC and TP ($p < 0.01$) but not for treatments CC and CP.

The pH values for samples of rumen fluid from steers fed twice each day were significantly greater than those for steers fed continuously at 9:00 and 9:30 PM but were signifi-

Table 11. Analysis of variance for pH of rumen fluid of steers.

Time of Day	Mean Square in the Analysis of Variance								Means of Treatments[#]			
	Animal	Period	Treatment	Frequency	Form	Interaction	Error	Residual Standard Deviation	CC	CP	TC	TP
3:00 PM	0.0714	0.0885	0.3326*	0.0189	0.9752**	0.0039	0.0607	0.246	6.21^{A}	5.75^{B}	6.18^{A}	5.65^{B}
6:00 "	0.0660	0.0710	0.1939	0.0031	0.2756	0.2756	0.0835	0.289	6.34	5.81	6.16	6.16
9:00 "	0.0856	0.1968	0.4993**	1.1556**	0.0010	0.3025*	0.0439	0.210	6.20^{A}	5.83^{B}	6.46^{BC}	6.64^{C}
9:30 "	0.0805	0.0980	0.2964*	0.5439*	0.1314	0.2139	0.0585	0.242	6.26^{AB}	5.85^{A}	6.40^{B}	6.45^{B}
10:00 "	0.0800	0.0300	0.3900*	0.0689	0.0452	1.0764**	0.0500	0.224	6.28^{A}	5.86^{BC}	5.62^{C}	6.25^{AB}
12:00 "	0.1100	0.0300	0.3100**	0.5439**	0.1914*	0.1914*	0.0300	0.173	6.29^{A}	5.85^{B}	5.70^{BC}	5.70^{C}
3:00 AM	0.0185	0.0714	0.2943**	0.1314*	0.7439	0.0076	0.0216	0.147	6.34^{A}	5.95^{B}	6.20^{A}	5.73^{B}
6:00 "	0.0159	0.0634	0.1276	0.1502	0.0083	0.1502	0.0449	0.212	6.26	5.93	6.26	6.31
9:00 "	0.0108	0.0575	0.2316	0.3025	0.0090	0.3025	0.0629	0.251	6.39	5.96	6.39	6.51
12:00 "	0.1672	0.4039	0.3176	0.0026	0.9506	0.0016	0.1114	0.334	6.30	5.83	5.77	5.74

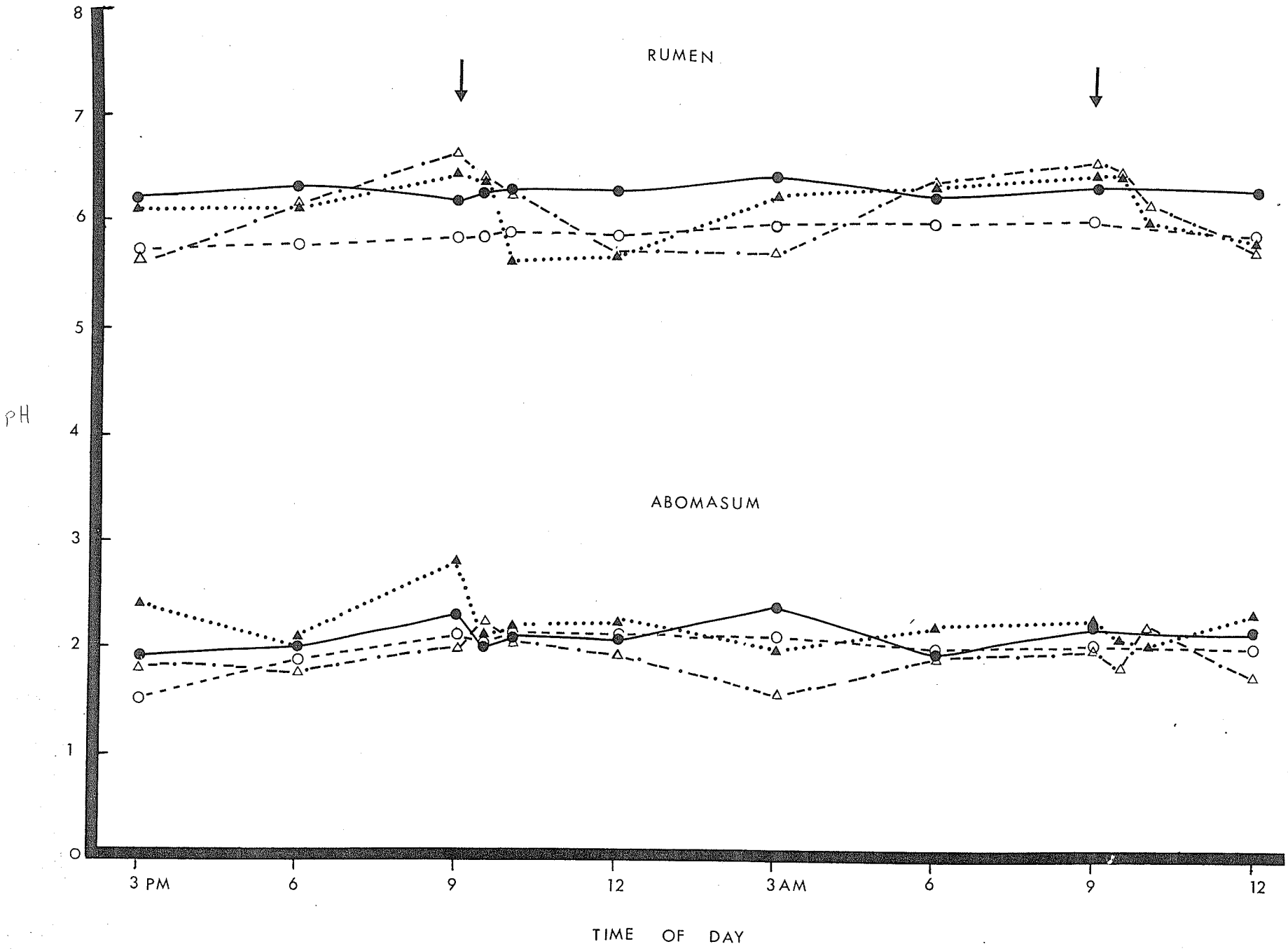
* significant at $p < 0.05$
** significant at $p < 0.01$
\# means with different superscripts are significantly different at $p < 0.05$.

Figure 8. pH of fluid from rumen and abomasal digesta of steers. Each point is a mean value for four steers.

CC, ●——●; CP, ○- - - -○;

TC, ▲.....▲; TP, △-.-.-.-△;

The times of feeding for treatments TC and TP are indicated by arrows.


RUMEN
ABOMASUM
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TIME OF DAY

cantly less at 12:00 midnight and at 3:00 AM. The pH values for treatment CC were always greater than those for treatment CP (Figure 8 and Table 11), but the means for TC and TP showed no consistent ranking of magnitude.

The pH of abomasal liquor ranged between 1.58 and 2.83 (Figure 8). There was no significant differences among the mean values for the four treatments.

Total Concentrations of Volatile Fatty Acids in Fluid Digesta from the Rumen and Abomasum, and in Plasma

The total concentrations of volatile fatty acids (VFA) in rumen fluid from steers fed twice each day increased to maximum values at three hours from the start of a meal, the means being 16.53 and 16.93 mmol/100 ml for the treatments TC and TP, respectively. The concentrations decreased gradually to the minimum values of 10.00 and 9.17 mmol/100 ml, respectively before the start of a meal (Figure 9). The total concentrations of VFA in rumen fluid from steers fed continuously were relatively less variable than those for steers fed twice each day. The values for treatments CC and CP were maintained at about 13.04 and 14.53 mmol/100 ml, respectively, throughout the day. The effect of time of sampling was not significant for animals fed continuously but was for those fed twice each day ($p < 0.01$; Appendix 2).

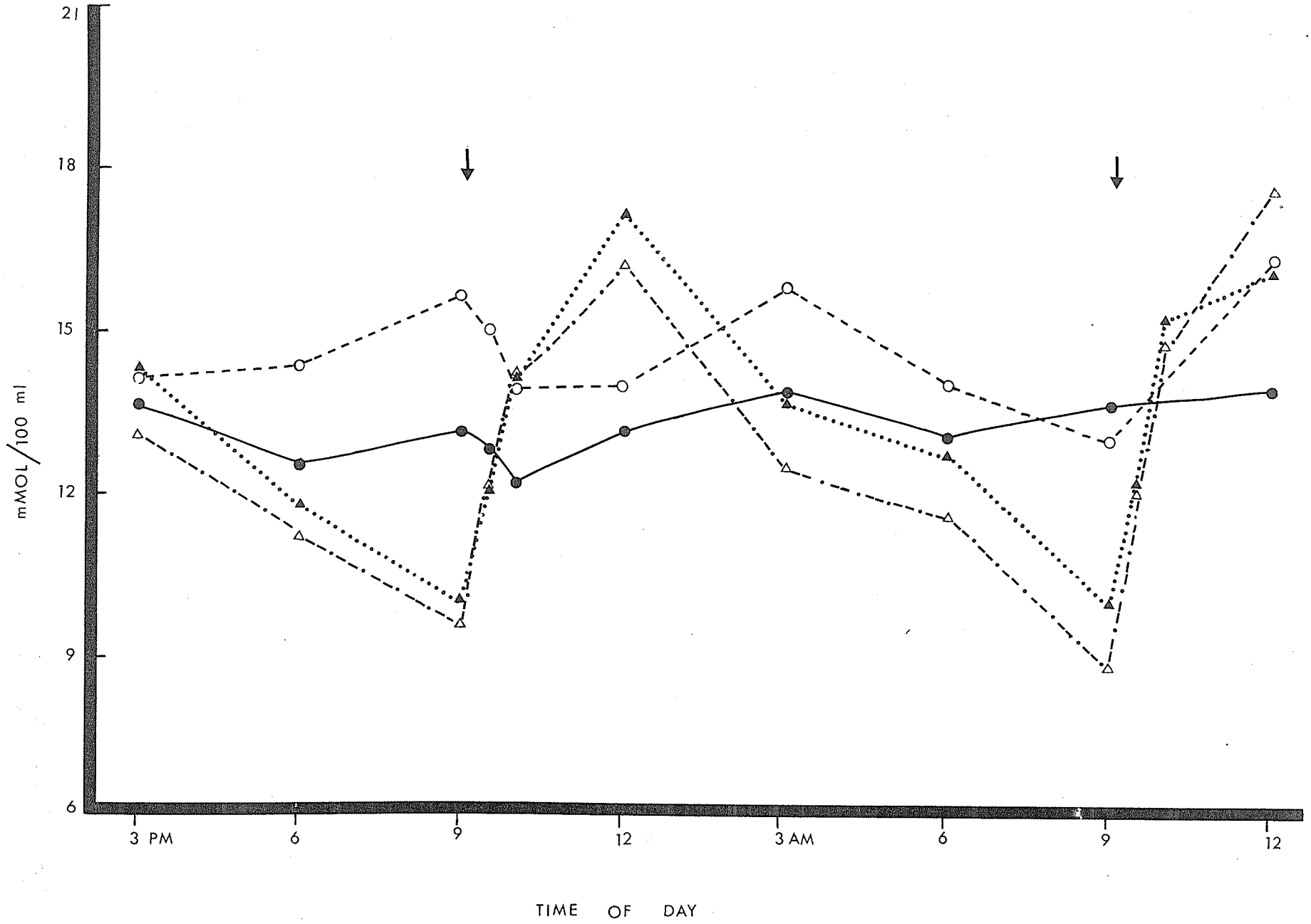
The mean VFA concentrations for treatments TC and TP were significantly lower than those for treatments CC and CP

Figure 9. The total concentration of volatile fatty acid in fluid digesta from the rumen. Each point is a mean value for four steers.

CC, ●——● ; CP, o- - - -o ;

TC, ▲.....▲ ; TP, △.--.--△ ;

The times of feeding for treatments TC and TP are indicated by arrows.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3 AM
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TIME OF DAY

Table 12. Analysis of variance for concentrations of volatile fatty acids in fluid digesta from the rumen of steers.

Time of Day	Mean Square in the Analysis of Variance							Residual Standard Deviation	Means of Treatments[#] (mmol/100ml)			
	Animal	Period	Treatment	Frequency	Form	Inter-action	Error		CC	CP	TC	TP
3:00 PM	9.3885	3.2232	1.1489	0.0944	0.3449	3.0076	4.9006	2.214	13.55	14.13	14.27	13.10
6:00 "	6.9969	0.1068	7.5023	14.4952	1.7882	6.2238	7.5326	2.745	12.42	14.34	11.77	11.19
9:00 "	4.4281	2.3749	33.2017	85.0960*	4.6731	9.8360	8.8319	2.972	13.06^{AB}	15.71^{A}	10.01^{B}	9.53^{B}
9:30 "	2.8653	4.0456	7.5056	13.7715*	5.2487	3.4969	2.6666	1.633	12.88^{AB}	14.95^{A}	11.95^{B}	12.16^{AB}
10:00 "	9.6488	0.2358	3.8958	5.9915	2.5019	3.1943	3.8419	1.960	12.16	13.85	14.28	14.18
12:00 "	5.0070	4.7543	13.7150	37.9179*	0.0044	3.2229	5.2138	2.283	13.13	14.07	17.11	16.25
3:00 AM	7.7405	3.3656	7.9982*	12.8397*	0.6753	10.4798*	1.3996	1.183	13.83^{AB}	15.86^{A}	13.66^{B}	12.45^{B}
6:00 "	2.1540	1.5479	3.8336	5.4080*	0.0070	6.0861*	1.0033	1.002	12.89^{AB}	13.96^{A}	12.76^{AB}	11.57^{B}
9:00 "	7.1793	2.2291	21.6711*	61.4264**	3.2490	0.3381	4.0677	2.017	13.62^{A}	13.01^{A}	10.00^{B}	8.80^{B}
12:00 "	5.5140	7.6407	15.3857	27.5100	17.7915	0.8556	5.7266	2.393	12.87	15.44	15.95	17.60

* significant at $p < 0.05$
** significant at $p < 0.01$
\# means with different superscripts are significantly different at $p < 0.05$.

at 9:00 and 9:30 AM and at 9:00 PM ($p < 0.05$; Table 12). The effect of frequency of feeding was not significant at 10:00 PM, but at 12 midnight, the concentrations for steers fed twice each day were significantly greater than those for steers fed continuously. At 3:00 and 6:00 AM, the concentrations of VFA in steers fed twice each day, were significantly lower than those for animals fed continuously.

There were no significant effects of form of food on the concentration of the VFA in rumen fluid.

The total concentrations of volatile fatty acids in abomasal liquor were small, ranging from 0.76 to 1.33 mmol/100 ml. The analysis of variance showed that there were no significant effects of frequency of feeding or form of food at any of the times of sampling.

The total concentrations of VFA in the plasma were low ranging from 0.44 to 1.56 mmol/l (Figure 10; Table 13). The concentrations of VFA, when the steers were fed twice each day, were relatively low before the start of a meal being on the average 0.69 and 0.56 mmol/l for treatments TC and TP, respectively. The concentrations increased quickly for one hour after a meal and reached maximum values of 1.34 and 1.51 for treatment TC and TP, respectively at three hours after the start of a meal. They then declined slowly to new low levels before the start of the next meal (Figure 10). The mean concentration for the treatments CC and CP throughout the times of sampling were 0.92 and 1.17 mmol/l, respectively.

Table 13. Total concentration of VFA in the plasma of steers.

Treatment	Time of Day											
	PM						AM					
	3:00	6:00	9:00	9:30	10:00	12:00	3:00	6:00	9:00	9:30	10:00	12:00
	Concentration (mmol/l)											
TC	0.6013	0.6149	0.6076	1.0331	1.1477	1.4214	1.0738	1.0482	0.7717	1.0364	1.2121	1.2554
TP	0.5520	0.6590	0.4560	1.2390	1.2940	1.5640	0.7140	0.4375	0.6670	1.1650	1.2370	1.4580
CC	0.8790	0.9080	0.8050	1.0150	0.9240	1.0790	1.0590	0.9000	0.9180	-	-	0.7360
CP	1.1660	1.2070	1.1720	1.0460	1.2060	1.5490	1.0310	1.1340	1.1470	-	-	1.0330

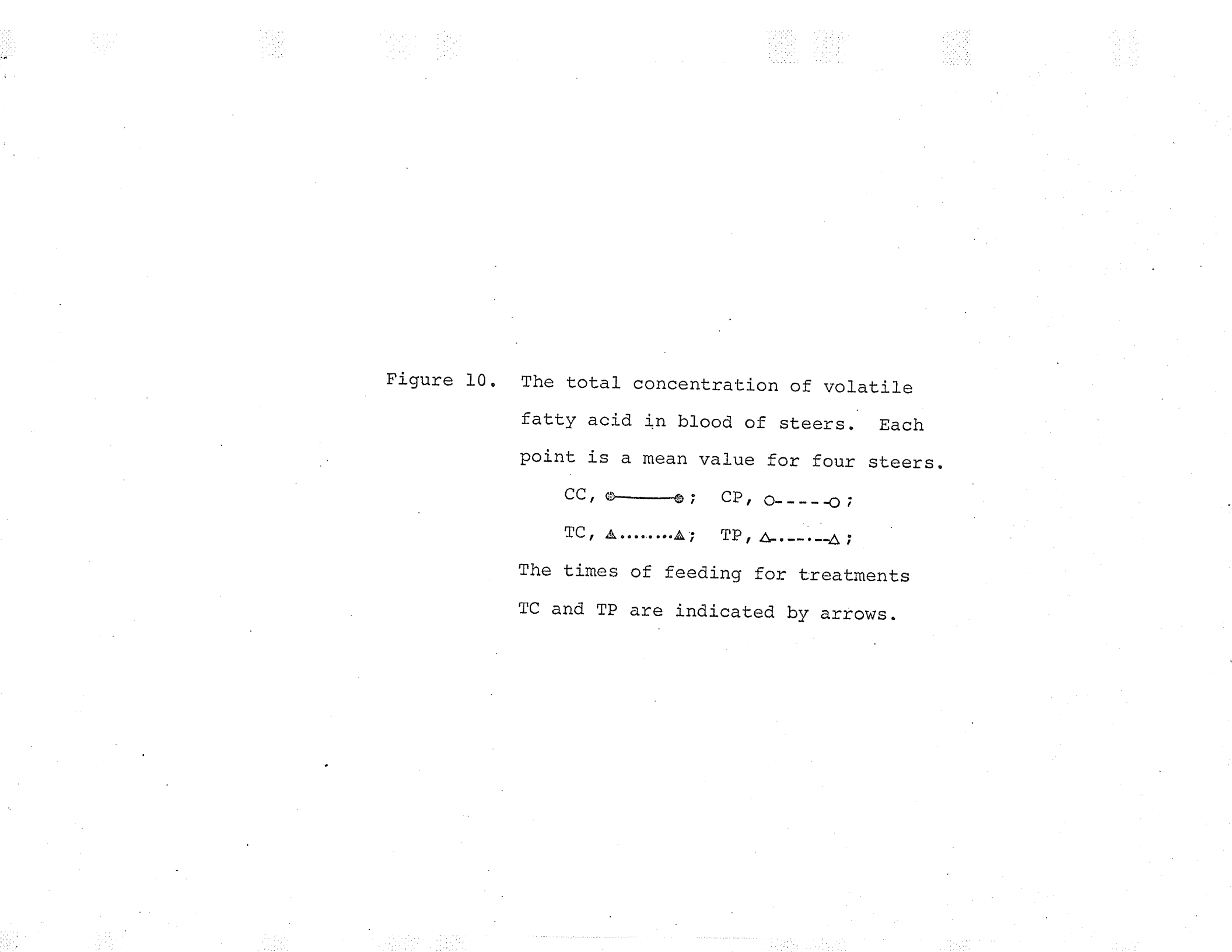
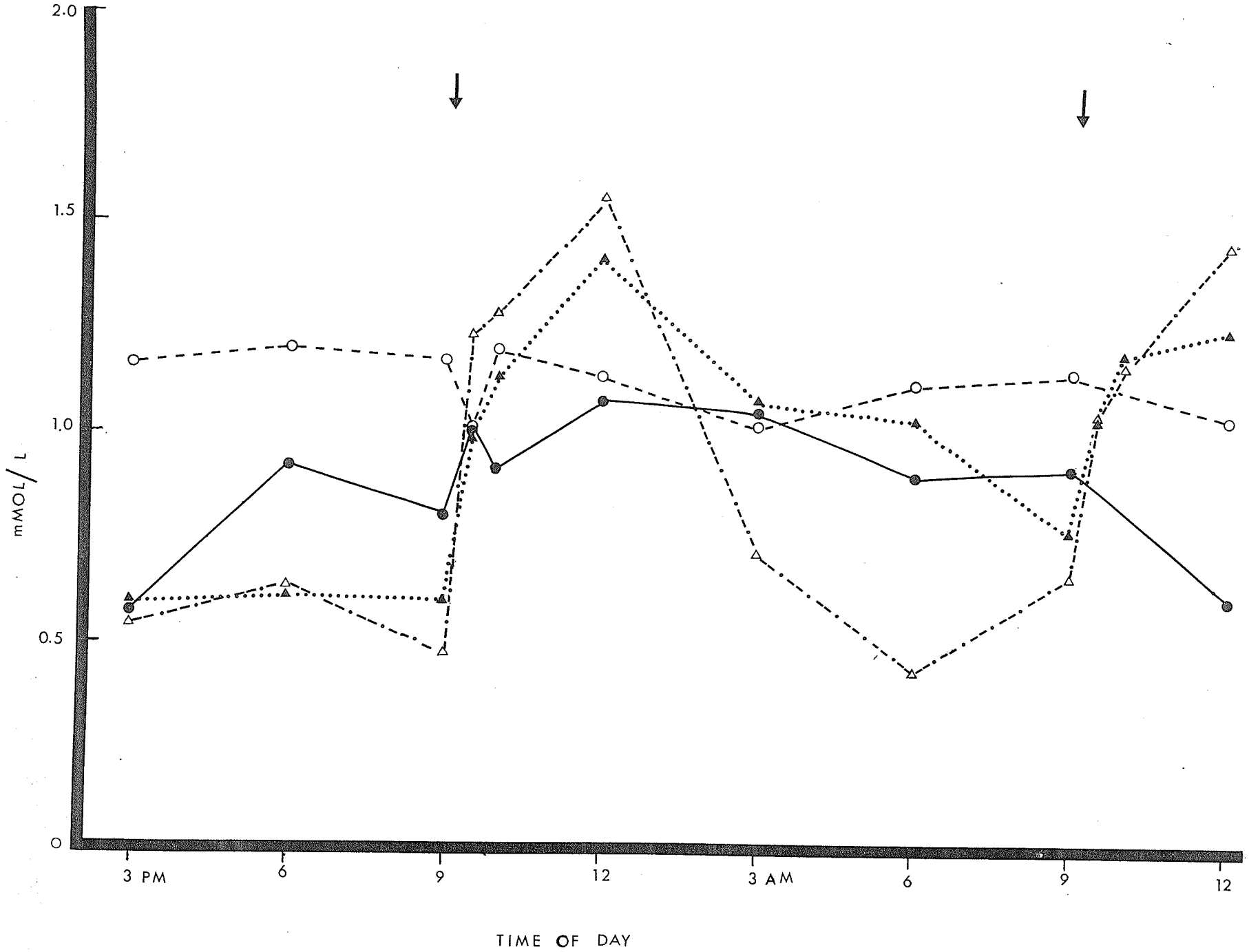


Figure 10. The total concentration of volatile fatty acid in blood of steers. Each point is a mean value for four steers.

CC, ●——●; CP, ○-----○;

TC, ▲.....▲; TP, △-.-.-.-△;

The times of feeding for treatments TC and TP are indicated by arrows.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TIME OF DAY

The Concentrations of Ammonia in Fluid Digesta from the Rumen and Abomasum

The fluctuations in concentrations of ammonia in rumen liquor were small when steers were fed continuously but large peaks in concentration were found within three hours after the start of a meal when the animals were fed twice each day (Figure 11). The effect of time of sampling on ammonia concentrations in rumen liquor was significant in an analysis of variance for the animals fed twice each day ($p < 0.01$) but was not significant for the animals fed continuously (Appendix 2).

The mean concentrations of ammonia in the rumen liquor, from the steers fed twice each day, were significantly higher at 0.5 and 1.0 hr after feeding commenced than the values obtained for the steers fed continuously ($p < 0.05$; Table 14). The concentrations for treatments TC and TP were lower than those for treatments CC and CP from midnight until 9:00 AM (Figure 11) but the differences were not significant (Table 14).

The average concentrations of ammonia in abomasal liquor were 5.11, 5.24, 4.81 and 4.99 mg/100 ml for the treatments CC, CP, TC and TP, respectively. There were no significant effects of frequency of feeding or form of food on these concentrations. There were no significant effects of time of sampling in any of the results.

Figure 11. The concentrations of ammonia in rumen fluid of steers. Each point is a mean value for four steers.

CC, ●———● ; CP, ○- - -○ ;

TC, ▲·····▲ ; TP, △-.--.--△ ;

The times of feeding for treatments TC and TP are indicated by arrows.

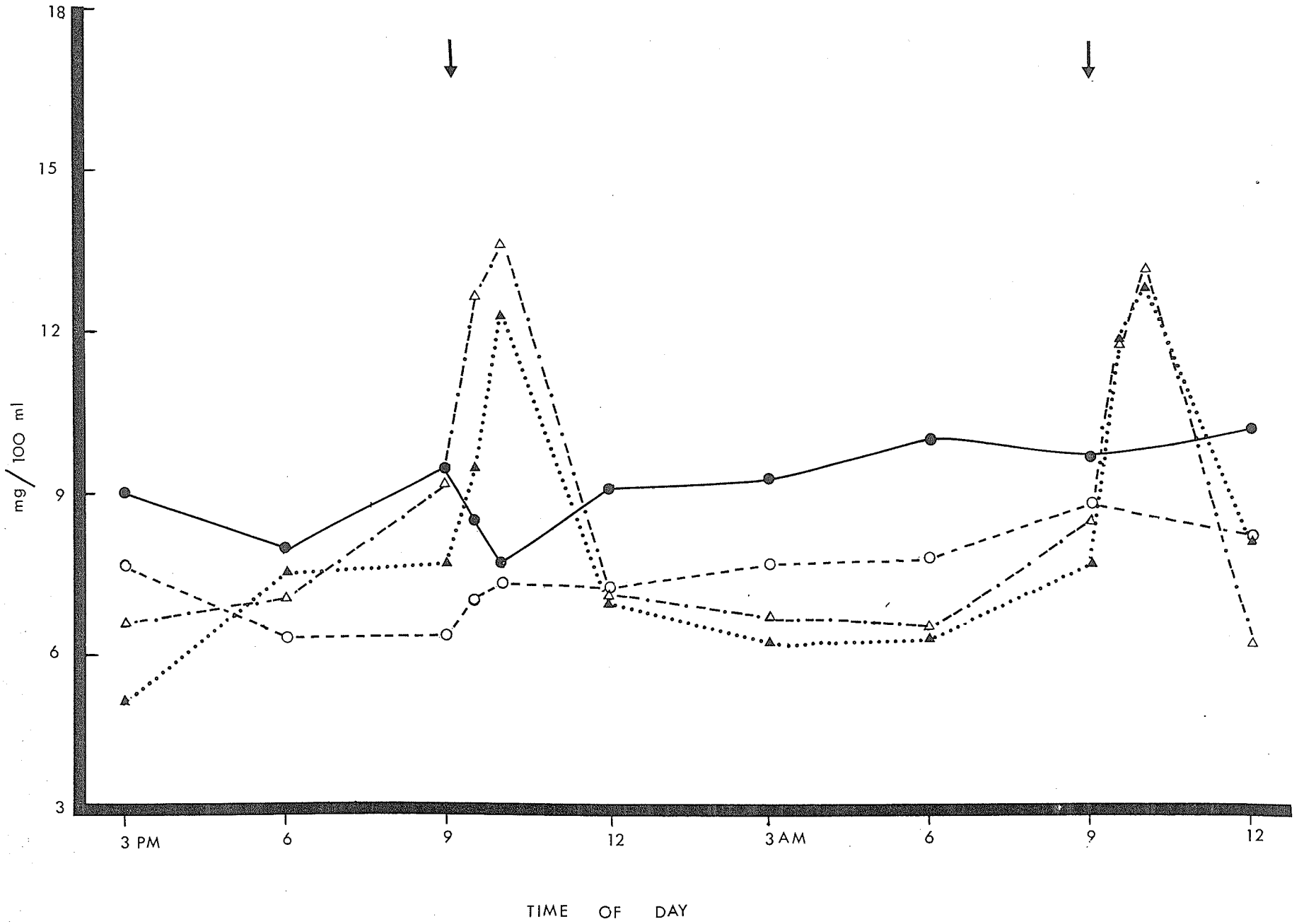
18
15
12
9
6
3
mg / 100 ml
3 PM
6
9
12
3 AM
6
9
12
TIME OF DAY

Table 14. Analysis of variance of the concentration of ammonia in fluid digesta from the rumen of steers.

Time of Day	Mean Square in the Analysis of Variance								Means of Treatments[#] (mg/100 ml)			
	Animal	Period	Treatment	Frequency	Form	Inter-action	Error	Residual Standard Deviation	CC	CP	TC	TP
3:00 PM	1.5179	3.0654	10.0120*	22.3256**	0.0100	7.7006	1.7385	1.3190	9.03^{A}	7.63^{AB}	5.21^{B}	6.65^{B}
6:00 "	5.7426	1.2355	2.1780	0.0689	4.6764	1.7889	2.2728	1.5080	8.00	6.25	7.46	7.05
9:00 "	7.9835	3.1668	8.5022	0.8556	2.3256	22.3256	4.2485	2.0610	9.43	6.31	7.54	9.14
9:30 "	14.1729	4.4879	23.1604*	43.5600*	2.6406	23.2806*	3.6239	1.9040	8.51^{A}	6.91^{A}	9.40^{AB}	12.63^{B}
10:00 "	4.9455	13.3905	40.9130*	118.5377**	1.1827	3.0189	4.4618	2.1120	7.65^{A}	7.30^{A}	12.20^{B}	13.61^{B}
12:00 "	2.7193	6.9597	3.8355	5.4639	2.8477	3.1952	7.4399	2.7280	8.94	7.20	6.88	6.93
3:00 AM	0.3377	1.9368	7.2210	16.0000	1.5625	4.1006	4.6954	2.1670	9.18	7.54	6.16	6.55
6:00 "	3.4254	1.0989	12.3018	25.8064	4.4944	6.6049	9.3103	3.0510	10.04	7.69	6.21	6.44
9:00 "	2.6444	2.0105	2.8251	4.6440	0.1260	3.7830	4.8840	2.2100	9.59	8.55	7.54	8.40
12:00 "	5.8851	10.1239	9.9647	14.9189	14.9189	0.0564	16.3337	4.0410	10.23	7.98	7.98	6.16

* significant at $p < 0.05$
** significant at $p < 0.01$
\# means with different superscripts are significantly different at $p < 0.05$.

DISCUSSION

Food Intake, Body Weight Gain and Digestion of Food

The steers fed continuously consumed significantly more food than those fed twice each day. The average values for the two frequencies were 9.10 and 7.47 kg/day, respectively. The diet fed was of medium quality, having a crude protein content of 12.68 per cent, but grinding and pelleting this food did not significantly increase its daily consumption by the steers. This finding is not in agreement with the conclusion of Ben-Saud (1970).

The steers also gained significantly more weight when fed continuously as opposed to twice each day. This result has been shown previously in cattle (Gordon *et al.*, 1952; Hardison *et al.*, 1957; Rakes *et al.*, 1957) and in sheep (Rakes *et al.*, 1961). Rakes *et al.* (1961) reported that young sheep given eight meals each day gained body weight faster than those fed once each day but a similar response was not obtained in mature sheep.

The coefficients of apparent digestion of food dry matter in the entire gut were higher than the expected value of about 70 per cent. The coefficients for organic matter and energy were also higher than expected. The long

period of exposure of the feces to air before collection, and a loss of some feces during collection might have caused these apparent errors of determination. Assuming this discrepancy to be similar for all treatments, the digestibility of the dry matter, organic matter and energy in the fore-stomachs and in the entire gut were significantly higher when the steers were fed continuously than when they were fed twice each day. This is in agreement with the results of Moir and Somers (1957). Campbell and Merilon (1961) also found that more frequent feeding increased the digestibility of the food, which in turn resulted in increased intakes and increased daily gains of body weight.

The lower digestibility of the food in the steers fed twice each day may have been caused by a decreased microbial count in rumen fluid after feeding. Purser and Moir (1959) found marked diurnal fluctuation in the number of micro-organisms in rumen fluid, the number decreasing after a meal to about one-third of the value observed before the start of a meal. Purser and Moir (1959) reported that the maximum value of the pH of the rumen fluid before feeding was 7.13. The minimum pH of 5.26 for rumen fluid after feeding decreased the numbers of protozon in the rumen. In the experiment now reported, the pH of rumen contents from steers fed twice each day varied between about 6.50 and 5.70 before and after a meal, respectively. The small variation of the pH of the rumen fluid in this experiment might not

have been significant in affecting the numbers of protozoa.

The rates of flow of contents from the forestomachs of steers fed continuously were maintained relatively constant with no evidence of a diurnal pattern such as was found in the steers fed twice each day. The flow rates of dry digesta from the reticulorumen during meals and between meals were 529 and 299 g/hour, respectively for the steers receiving the treatment TC. The values were 684 and 277 g/hour, respectively,for the treatment TP. The high flow rate of dry matter during a meal corresponded with the high rate of flow of digesta through the abomasum and this may have caused a larger amount of food to escape digestion in the rumen compared with that for steers fed continuously.

The amount of protein leaving the forestomachs was greater than that ingested for every treatment presumably due to the synthesis of microbial protein in the rumen (Allison, 1970).

The apparent digestibilities of the dry matter, organic matter, and energy of pelleted food in the forestomachs were lower than those for food in the chopped form. This may have been caused by shorter retention times of the pelleted food in the reticulorumen. Small particles of food are retained in the reticulorumen for a shorter time than long particles (Blaxter *et al.*, 1956; Rodrigue *et al.*, 1960; Hogan *et al.*, 1967; Wilkins *et al.*, 1972).

The differences between the coefficients of dry matter

digestion in the treatments CC and TP were smaller for the entire gut than those for the forestomachs. This indicated that the proportion of digestion occurring in the intestines was greater for the pelleted food than for the food in chopped form. Grinding and pelleting depressed dry matter digestion before the small intestine but more dry matter was digested in the caecum and colon of steers given this diet, perhaps because the particles of the pelleted food were fermented by the microbial enzymes in the caecum and colon faster than the food in chopped form.

The Factors Controlling Hunger and Satiety in the Animals Fed Continuously

The amount of food consumed when the steers were fed continuously was significantly greater than that when they were fed twice each day. The continuous feeding method eliminated variations with time of day in the measurements studied in this experiment. The average time between the triggering of satiety and the start of a meal was much shorter when the steers were fed continuously than when they were fed twice each day. A slight decrease in the volume of rumen contents or the concentration of VFA in the rumen fluid for example may have caused the steers fed continuously to start to eat. The rumen volumes or VFA concentrations may then have increased slightly and brought

about the triggering of satiety. As a result, fairly constant values were maintained throughout the day for all the measurements studied. The largest values for some measurements might be thought of as the critical levels for the triggering the satiety in the steers fed continuously or in those fed twice each day.

Physical Factors in the Control of Hunger and Satiety

Amount of digesta in the reticulorumen

The weights and volumes of reticulorumen contents were maintained at a relatively constant level throughout the day when the steers were fed continuously. The rumen volumes of the steers fed twice each day were significantly smaller before feeding than those of animals fed continuously but at the end of the meal the volumes were not significantly different. The steers given the treatment TC may therefore have stopped eating at the end of a meal because the reticulorumen was filled to a critical volume. The volumes of contents were smaller for pelleted food than for food in the chopped form. Thus this argument to explain satiety may not be true for pelleted food. Distension of the reticulorumen possibly acts as one of the feedback mechanisms in the regulation of hunger and satiety (Campling, 1970). The steers fed twice each day probably had a desire to eat well before the times of feeding but

food was not available for them to eat. As a result, the steers consumed more food when fed continuously than when fed twice a day.

Flow rate of abomasal contents

Continuous feeding resulted in a regular movement of digesta through the abomasum into the duodenum. However, for the dietary treatments TC and TP, comparatively large amounts of digesta flowed into the duodenum for three hours from the start of the meal. The steers fed twice each day stopped eating at about 1.5 hours after the start of a meal, which for treatment TC corresponded to the time of maximum flow rate of the digesta through the abomasum. The values for TC were significantly greater than those for TP. The high rates for treatment CC and for TC after a meal, might have stimulated the mechanoreceptors located in the wall of abomasum (Harding *et al.*, 1971) to limit appetite directly, or output from these receptors may have inhibited the primary cycle of reticulorumen motility and hence reduced the rate of flow of the digesta from the reticulorumen. The same reasoning may not hold when the steers were fed pelleted food because of the relatively low flow rates obtained. Coombe and Kay (1965) suggested that the capacity of the abomasum and intestines might limit food intake, either directly or indirectly, by limiting the rate of flow of digesta through the digestive tract.

Campling *et al.* (1963) suggested that the voluntary intakes of diets of ground and pelleted hay and of dried grass by cows may have been limited by the large amount of digesta in the abomasum and intestines by an inhibition of the flow of digesta from the reticulorumen.

The flow rate of abomasal digesta increased before the start of feeding. This result has been found by Harris and Phillipson (1962), Phillips and Dyck (1963) and may be associated with a cephalic phase of gastric (abomasal) secretion (McLeay and Titchen, 1970).

Chemical Factors in the Control of Hunger and Satiety

Osmolality of the rumen fluid

The values obtained in this experiment were higher than expected. The samples of rumen fluid were not free of particulate matter and this may have caused an error in the determination of osmolality.

The production of volatile fatty acids, ammonia, and other metabolites by microbial fermentation would have contributed to the increase in osmolality after the start of a meal in the steers fed twice each day. The osmolalities of rumen fluid for treatments TC and TP increased above those for treatments CC and CP, respectively, within 0.5 hr after the start of a meal and were maintained in this manner for about three hours after the start of a meal. The steers

fed twice each day continued to eat for about 1.5 hr indicating that the relatively high levels of rumen osmolalities were not effective in the triggering of satiety. Bergen (1972) also reported that rumen osmolality was not an important factor in the control of food intake in sheep. However, Ternouth and Beattie (1971) added electrolyte solutions of differing osmolality to the rumen contents of sheep and found that the intake of food was linearly decreased as the osmolality of the solution infused was linearly increased. They concluded that the osmolality of rumen fluid may be associated with the regulation of food intake in sheep.

Packed cell volume

The packed cell volume for the steers fed twice each day increased about 15 per cent within 30 minutes of the start of a meal and returned to prefeeding levels within one hour. A similar trend was not observed in the steers fed continuously. The increase for the animals fed twice a day correponded to the initial increase in the osmotic pressure of rumen fluid. Water from plasma may have diffused into the rumen at this time. However, the packed cell volume returned to prefeeding values within one hour after the start of a meal even though the osmolalities of rumen fluid remained at high values. Ternouth (1968) found that the osmotic pressure of rumen fluid in sheep was

about 20 per cent higher than that of plasma shortly after the start of a meal. Under similar circumstances water from the blood diffused through the wall of the rumen into the contents of the rumen (Engelhardt, 1970). Gutman and Krausz (1969) increased and decreased the food intake of the rat by increasing and decreasing their extracellular fluid volumes. Their theory of tissue dehydration limiting food intake may not be applicable to the regulation of food intake in ruminants because the steers fed twice each day stopped eating at 10:30 AM and 10:30 PM, these times being 1.5 hr after the start their meals.

Osmolality of plasma

The osmolality of plasma for treatments TC and TP increased from 270 and 265 mosmol/l before the start of a meal to maximum values of 291 and 294 mosmol/l 0.5 hr later, respectively. The values then decreased slowly until the next time of feeding. These results suggest that the amount of food consumed in a single meal may not be related to the osmolality of plasma since the steers spent about 1.5 hr eating.

The increase in plasma osmolality after feeding, in the steers fed twice each day, probably resulted from the absorption of end products of microbial fermentation in the rumen or from the transfer of water from plasma across the ruminal wall into the rumen contents.

pH of the rumen liquor and abomasal fluid

The pH of rumen liquor was maintained relatively constant when the steers were fed continuously. The pH's for treatments TC and TP declined gradually during a meal and after about 2.5 hr from the start of a meal they were below the values for steers fed continuously. These relatively low pH's may have affected the voluntary food intake in the animals fed twice each day. Bhattacharya and Warner (1967) reported that daily hay intake by steers dropped from 12.3 kg in the control animals (water infused) to 6.0, 6.3 and 8.0 kg in the steers receiving phosphoric acid, lactic acid, and citric acid, respectively. In the animals infused with acid the pH of the rumen fluid dropped from 7.08 to about 6.00. Results of this study indicated that a drop in rumen pH influenced voluntary food intake in steers. Harding and Leek (1972) reported the presence of receptors for pH in the wall of the reticulorumen which caused reflex inhibition of the motility of the reticulorumen.

The pH of abomasal fluid was low and was not affected by feeding in treatments TC and TP. There were no significant differences among the treatments. It is most unlikely that the pH of abomasal fluid is involved in the control of food intake in the steer.

Volatile fatty acid concentrations in fluid digesta from the rumen and abomasum and in plasma

The total concentrations of VFA in the rumen fluid of steers fed twice each day increased above those for steers fed continuously at about 1.5 hr after the start of a meal. The concentrations of acetic, propionic, and butyric acids in the rumen fluid were shown to be involved in the control of voluntary food intake in ruminants (Baile *et al.*, 1969). This report also suggested that the concentration of VFA in rumen fluid may act as one of the factors controlling voluntary food intake in steers fed twice each day, as the steers stopped eating 1.5 hr after the start of a meal. By this time the concentrations of VFA in the rumen fluid of steers fed two times each day were similar to those of steers fed continuously. These concentrations may have been effective on receptors for VFA in the rumen wall. The impulses on arrival at the satiety center in the ventromedial hypothalamus may have caused satiety.

Only small concentration of VFA were found in the abomasal fluid. Statistical analyses did not reveal any significant differences among the treatments. Presumably, the VFA in the abomasal fluid are not involved in the control of food intake in steers.

The VFA produced in the rumen are absorbed rapidly

through the epithelial wall of the reticulorumen. The concentrations in the peripheral circulation are low because they are diluted by tissue fluid and plasma and they are utilized rapidly in various parts of the body such as the rumen epithelium, the liver , and in the body tissue. The concentrations of VFA in the plasma increased after the start of a meal in the steers fed twice each day. This is related to the increase in concentration of VFA in the rumen fluid. At 1.5 hr after the start of a meal, the total concentrations of VFA in the plasma of the steers fed twice each day were greater than those for steers fed continuously. These high concentrations could have stimulated VFA receptors located in the walls of blood vessels in the portal system and caused satiety in the steers fed twice each day. Baile and Mayer (1970) proposed that the most sensitive chemoreceptors for acetic acid were located on the lumen side of the reticulorumen and especially in the dorsal rumen. High concentrations of propionic acid, which were also effective in reducing food intake, may be sensed by similar receptors. Both goats and sheep have receptors sensitive to propionic acid located in the ruminal vein and portal system. Baile and Mayer (1970) suggested that the initiation of a meal was probably affected by a combination of a decrease in neural activity and inhibitory action of the ventromedial hypothalamus with the concomitant increase in neural activity in the

hypothalamus. These changes were thought to be caused by low concentrations of volatile fatty acids in the rumen fluid and in the blood.

Concentrations of ammonia in rumen and abomasal digesta

The concentrations of ammonia in rumen and abomasal fluid were measured to study the patterns of production in steers fed at different frequencies. The concentrations of ammonia in body fluids have not been involved directly in the regulation of food intake. However, it is possible that the concentration of ammonia in rumen fluid is indirectly involved in limiting the intakes of roughage diets low in nitrogen. The addition of urea to these diets increases their intakes by ruminants and also increases the rate of breakdown of cotton thread in the rumen. Bryant and Robinson (1963) suggested that ammonia is the preferred source of nitrogen for most bacteria in the rumen and that it is essential for the growth of several important species. However, the point at which the concentration of ammonia becomes limiting for growth of these bacteria has not been clearly defined. Microbial fermentation in the reticulorumen contributes to the degradation of particles of food to sizes that will pass easily through the omasum.

SUMMARY AND CONCLUSIONS

Under the conditions of this experiment the following conclusions were drawn.

A. The steers fed continuously ate more food, gained body weight at a faster rate and digested more dry matter, organic matter, and energy of the food in the forestomachs or in the whole gut than they did when fed two times per day. The weights of protein flowing from the forestomachs were larger than those consumed. The values were greater, as a proportion of food intake, when the steers were fed continuously than when they were fed twice each day.

B. The steers fed continuously had relatively constant levels throughout the day for values of all measurements made. For this method of feeding, the times between meals were short and the volume of contents in the rumen or the concentrations of VFA in rumen fluid or plasma may have been maintained near critical levels which induce satiety. The steers may have started to eat when these values decreased slightly and stopped eating when they increased to, or slightly above, the critical levels.

C. The steers fed twice each day spent about 1.5 hr eating each meal. In these steers there was a diurnal pattern for changes in the volume of rumen contents, the rate of flow of abomasal digesta, the concentration of VFA

in rumen fluid and plasma, the osmolality of rumen fluid and plasma and the packed cell volume of blood. The values for these measurements were lower than those for the steers fed continuously before the start of a meal but were significantly higher shortly after that. The pH of the rumen fluid was at a maximum just before the time of feeding but declined gradually after eating commenced. The values at about two hours after the start of a meal were lower than those obtained when the steers were fed continuously.

The mechanoreceptors which are located in the walls of the reticulorumen and abomasum may have been stimulated by the large volumes of rumen contents and by the relatively large rates of flow of digesta through the abomasum at the end of a meal. Also, the increases in concentrations of VFA in rumen fluid and plasma after the start of a meal may have stimulated receptors located in the wall of the reticulorumen and in the portal system. The combined effects of these physical and chemical factors may have increased the neural activity in the ventromedial hypothalamus at the end of a meal and brought about satiety. The fall in the pH toward the end of a meal may have stimulated the receptors of pH in the wall of the reticulorumen and indirectly affected appetite by inhibiting slightly the primary cycle of reticulorumen motility and hence the rate of flow of digesta from the reticulorumen.

The steers fed twice each day were thus satiated in

a short time compared with the time between meals. Since the steers only had access to food during a meal, they were probably hungry well before the established meal times. As a result, the animals consumed less when they were fed twice each day than when they were fed continuously, having food always present before them.

D. The observed values for packed cell volume in blood, for osmolality of rumen fluid and plasma, for the concentration of VFA in abomasal fluid and for pH of abomasal fluid were such that these parameters may not be involved in the control of food intake in steers.

E. It is concluded that the amount of food consumed by steers was regulated by physical and chemical factors acting together. These factors were not necessarily acting independently because all stimuli are thought to be transformed by receptors into neural activity which then influences the appetite and satiety centers in the central nervous system. Also, it seems unlikely that any one factor will be universally responsible for regulation of food intake in steers.

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Appendix 1

Method for Measurement of Polyethylene Glycol in Fluid Digesta

The techniques used were similar to that described by Dyck (1963). The procedure used for measuring the concentration of Polyethylene Glycol dissolved in liquid was as follows:

A. Five millilitres of fluid were placed in a 40 ml centrifuge tube and the following reagents were added in order with thorough mixing after each addition.

a. 2 ml 0.3 N $Ba(OH)_2$

b. 2 ml 5% (W/V) $ZnSO_4 \cdot 7 H_2O$

c. 1 ml 5% (W/V) $BaCl_2 \cdot 2 H_2O$

The first two reagents are used to precipitate the protein and other solids present in the liquid. It is essential that the $Ba(OH)_2$ and $ZnSO_4$ be neutral as measured with phenolphthalein. BaCl is added to precipitate any excess sulfate.

B. The resulting suspension was then centrifuged at 581 x g for 10 min to precipitate the solids.

C. The supernatant was filtered through Whatman #40 filter paper.

D. To 5 ml of the clear liquid, an equal volume

(5 mls) of trichloracetic acid (TCA), 30% (W/V) TCA; 5.9% (W/V) $BaCl \cdot 2H_2O$; reagent was added, with thorough mixing. Addition of the TCA reagent to a solution containing PEG produces turbidity, the density of which is directly proportional to the PEG concentration.

E. Five minutes after the addition of the TCA reagent the percentage light transmittance was measured on a colorimeter using a blank filter.

For accurate measurement of PEG a concentration of 0.05 to 0.50 mg per 5 ml of clear filtrate was required.

The samples of abomasal contents required at least a 10-fold dilution with water to reduce the concentration of those pigments which interferred with the colorimeter reading. The concentration of PEG infused into the rumen was calculated so that at least a 10-fold dilution of the abomasal samples was required.

The concentration of PEG in the clear filtrate was determined from a standard curve of percent transmittance versus concentration of PEG in mg per ml. The standard curve was prepared using concentrations of 0.00 - 0.20 mg/ml of PEG standard solution using the previously described analytical procedure.

To insure that fecal recovery of PEG was close to 100 per cent a priming dose of PEG was injected into the rumen, followed by continuous infusion of 20.83 mg/ml PEG solution for six to seven days. The recovery of PEG in the

feces after six to seven days of infusion was very close to 100 per cent.

The abomasal collection was carried out on the seventh, eighth and ninth days. Total feces was also collected. The continuously fed animals had abomasal contents collected every four hours whereas those fed twice a day were sampled every two hours.

The expected PEG concentration was calculated for each sample and on appropriate dilution was made to give a PEG concentration of 0.05 to 0.50 mg for 5 mls of clear filtrate. The PEG concentration of the clear filtrate determined from the standard curve was multiplied by two to give the PEG concentration of the diluted samples.

To calculate abomasal outflow per hour, the concentration of PEG in the diluted samples was multiplied by the dilution factor to give the true PEG concentration in abomasal contents. The concentration of PEG infused was divided by the PEG concentration in the abomasal contents and then multiplied by the weight of PEG infused per hour to give the abomasal outflow per hour.

The fecal PEG concentration in the diluted samples was multiplied by the dilution factor to give the total concentration of PEG in the feces. The PEG recovered was then calculated from the concentration per unit feces times the total quantity of feces from which the sample was initially prepared and then divided by the total PEG infused.

The abomasal samples were diluted 10-fold (V/V) with water. Ten per cent of every daily fecal collection was thoroughly mixed and pooled for the duration of the experiment. Ten per cent of the total pooled feces were diluted to 10-fold with water and the resulting suspension further diluted to 20-fold, thus resulting in a 200-fold (W/V) dilution.

Appendix 1.

Table 1. Percent recovery of PEG in the feces.

	Feeding Method			
	Continuous		2 times/day	
Steer No.	Chopped Hay	Pellet	Chopped Hay	Pellet
18	112.50	108.30	101.57	102.95
16	107.50	105.33	112.51	103.70
15	99.73	100.20	108.50	104.20
55	102.40	99.00	104.16	99.10
Average	105.53	103.21	106.69	102.49

Appendix 1.

Table 2. Percent dry matter of abomasal content.

	Feeding Method			
	Continuous		2 times/day	
Steer No.	Chopped Hay	Pellet	Chopped Hay	Pellet
18	4.31	4.84	4.97	5.38
16	3.88	5.79	4.21	4.39
15	4.58	5.56	4.82	5.82
55	4.84	4.43	5.82	6.12
Average	4.40	5.16	4.96	5.43

Appendix 2. Summary of analyses of variance (mean squares) to determine the effect of time of sampling on measurements made on steers.

Measurement	Feeding Continuously						Feeding Twice Each Day					
	CC			CP			TC			TP		
	Time†	Animal†	Error†	Time	Animal	Error	Time	Animal	Error	Time	Animal	Error
Abomasal flow rate	0.013	0.890**	0.023	0.013	0.490	0.022	0.357**	0.490**	0.010	0.142**	1.470**	0.008
Osmolality of rumen fluid	218.820	56169.0**	711.460	357.013	52535.290**	188.640	11311.430**	22871.010**	1180.110	25666.820**	47226.470**	1394.300
Osmolality of plasma	2.130	446.360**	3.840	1.740	92.020**	2.900	207.960**	347.690**	44.490	370.470**	155.800	86.990
Packed cell volume	0.090	4.120**	0.130	0.100	1.480**	0.090	6.790**	15.720**	0.580	5.510**	2.950**	0.320
pH of rumen fluid	0.010	0.100**	0.011	0.010	0.140**	0.029	0.350**	0.170**	0.045	0.490**	1.060**	0.084
pH of abomasal fluid	0.090	0.190	0.120	0.040	1.740**	9.034	0.210	0.190	0.150	0.160	0.990**	0.180
VFA conc. in rumen fluid	1.130	18.571*	1.970	3.390	14.032**	1.800	19.820	54.250**	1.410**	26.450**	37.730**	2.160
VFA conc. in abomasal fluid	0.060	0.070	0.970	0.060	0.330**	0.042	0.050	0.0003	0.037	0.030	0.343**	0.062
Ammonia conc. in rumen fluid	2.580	8.770**	2.650	1.970	48.280**	1.100	26.270**	33.310**	3.170	37.370**	28.030**	6.150
Ammonia conc. in abomasal fluid	0.300	21.020**	0.400	0.350	9.570**	0.520	2.320*	16.350**	1.040	1.710	1.180	2.600

† degree of freedom were 9, 3 and 27 for times, animals and error, respectively for CC and CP and 11, 3 and 33 for TC and TP for all measurements except that for CC and CP they were 5, 3, 16 and 11, 3, 33 for times, animals and error for abomasal flow rate, respectively.

* significant differences at $p < 0.05$

** significant differences at $p < 0.01$

Appendix 3. Individual steer values for measurements reported in this thesis.

	CC				CP				TC				TP			
Measurement	18	16	15	55	18	16	15	55	18	16	15	55	18	16	15	55
VFI (kg/day)	7.66	8.76	10.92	9.47	8.95	9.14	9.96	7.94	8.20	6.94	7.39	9.18	7.13	5.53	8.23	7.18
Body weight gain (kg/day)	0.70	0.83	0.84	0.87	0.80	0.74	0.86	0.59	0.65	0.64	0.57	0.56	0.68	0.39	0.49	0.64
Total digestion (%)																
Dry matter	81.01	80.00	81.00	80.00	79.00	80.00	79.00	79.00	78.00	78.00	78.01	78.01	77.99	77.01	77.00	77.01
Organic matter	81.76	80.86	81.69	80.79	79.54	80.29	79.52	79.52	79.02	79.02	78.71	78.90	78.41	77.88	77.45	77.51
Energy	80.36	78.79	80.53	79.78	77.65	78.48	77.03	78.27	74.71	75.64	76.53	76.16	75.35	76.29	74.46	73.98
Protein	78.04	77.12	78.76	76.86	77.05	77.46	74.98	76.65	70.79	76.00	75.00	78.06	72.06	70.79	69.88	68.93
Digestion at forestomachs (out of a total value of 100)																
Dry matter	57.04	65.36	64.33	57.73	62.37	56.96	55.27	56.80	52.68	51.37	50.80	47.97	47.02	43.92	44.77	48.33
Organic matter	61.07	68.85	66.70	61.00	63.23	57.99	57.29	57.70	54.16	55.21	54.67	51.67	50.38	45.79	48.59	49.59
Energy	60.19	67.94	60.97	63.59	62.64	60.06	55.30	51.90	49.03	57.67	53.55	50.80	44.88	42.48	47.97	51.05
Protein	-29.50	-15.09	-16.41	-22.62	- 8.48	-15.05	-17.00	-14.51	-11.51	-13.00	-13.97	-10.21	- 8.61	- 9.00	- 8.98	-14.21

continued ...

	CC				CP				TC				TP			
	18	16	15	55	18	16	15	55	18	16	15	55	18	16	15	55
Reticulorumen Contents																
Volume (l)																
11:00 P.M.	40	40	54	56	40	38	46	46	44	38	70	53	34	28	64	53
8:30 A.M.									33	26	55	38	24	20	48	37
11:00 A.M.	41	41	52	57	38	33	47	47	40	34	65	54	30	25	66	53.5
8:30 P.M.									32	24	56	36	26	18	42	39
Weight (kg)																
11:00 P.M.	33.50	25.50	53.30	45	27.85	33	39	40.10	50.30	34.80	59.50	37.10	30.50	36.50	40	44.50
8:30 A.M.									37.90	26.20	37	25	23	29	32.50	32.50
11:00 A.M.	35	25.80	51.50	42	27.30	28	39	44.50	41.30	34	45.20	35	30	30	40.20	42
8:30 P.M.									38	25	37.30	23.80	24	26	30	33.20
Dry matter (kg)																
11:00 P.M.	5.05	3.97	8.34	5.76	4.11	4.65	6.40	5.31	8.91	5.57	11.77	6.59	5.61	5.45	7.17	7.23
8:30 A.M.									5.02	3.17	5.12	3.30	3.40	2.90	4.13	4.01
11:00 A.M.	5.67	4.15	7.70	5.69	4.69	4.31	6.64	5.94	8.07	6.17	7.15	5.62	6.28	4.10	6.63	6.90
8:30 P.M.									4.88	2.91	4.74	3.30	3.11	2.11	3.83	3.71
Water (l)																
11:00 P.M.	28.44	21.54	44.96	39.24	23.74	28.35	32.60	34.79	41.39	29.23	47.73	30.51	24.89	31.05	32.83	37.27
8:30 A.M.									32.88	23.03	31.88	21.71	19.60	26.10	28.37	28.49
11:00 A.M.	29.33	21.12	43.80	36.31	22.62	23.69	32.36	38.56	33.23	27.83	38.05	29.38	23.72	25.90	33.54	35.10
8:30 P.M.									33.12	23.89	32.56	20.50	20.89	22.09	26.18	29.49

continued ...

	CC				CP				TC				TP			
	18	16	15	55	18	16	15	55	18	16	15	55	18	16	15	55
Reticulorumen Contents																
Density (kg/l)																
11:00 P.M.	0.84	0.64	0.99	0.80	0.70	0.88	0.85	0.87	1.14	0.92	0.85	0.70	0.90	1.30	0.63	0.84
8:30 A.M.									1.15	1.01	0.67	0.66	0.96	1.45	0.68	0.88
11:00 A.M.	0.85	0.63	0.98	0.79	0.72	0.85	0.83	0.95	1.21	1.00	0.85	0.65	1.00	1.20	0.61	0.79
8:30 P.M.									1.19	1.04	0.67	0.66	0.92	1.44	0.71	0.85
Dry matter (%)																
11:00 P.M.	15.10	15.55	15.64	12.79	14.77	14.10	16.41	13.24	17.71	16.00	19.70	17.77	18.40	14.92	17.93	16.24
8:30 A.M.									13.24	12.10	13.83	13.18	14.80	10.01	12.70	12.33
11:00 A.M.	16.19	16.10	14.96	13.54	17.16	15.38	17.03	13.34	19.53	18.16	15.82	16.05	20.94	13.66	16.50	16.43
8:30 P.M.									12.84	11.63	12.70	13.86	12.94	8.10	12.75	11.18
Abomasal flow rate (ml/hr)																
4:00 P.M.	3059	3727	4022	3831	3415	2976	3585	3585	2855	2502	3119	2570	3105	3042	2855	3238
8:00 P.M.	3321	3790	4055	3975	3261	3096	3718	3585	3307	2743	3624	2935	3624	3367	3408	3935
12.00 P.M.	3096	3942	4015	3902	3337	3000	3760	3562	3367	2804	3550	2874	4016	3615	3397	3863
4.00 A.M.	3122	3727	3903	3975	3457	3149	3647	3624	2855	2487	3140	2530	3159	3102	2845	3238
8:00 A.M.	3261	3975	4015	3983	3321	2915	3542	3585	3261	2721	3551	2894	3582	3397	3238	3863
12.00 A.M.	3155	3792	4087	3935	3355	2855	3613	3408	3261	2814	3656	2687	3796	3688	3367	3863

continued ...

	CC				CP				TC				TP			
	18	16	15	55	18	16	15	55	18	16	15	55	18	16	15	55
pH of the Rumen Fluid																
3:00 P.M.	6.10	6.30	6.10	6.35	5.90	5.75	5.85	5.50	6.05	6.00	6.25	6.40	5.30	5.45	5.55	6.30
6:00 P.M.	6.30	6.40	6.15	6.50	5.90	5.75	5.80	5.80	6.30	6.05	6.30	6.00	5.90	5.70	6.20	6.85
9:00 P.M.	6.30	6.10	6.00	6.40	5.60	6.10	5.70	5.90	6.40	6.15	6.90	6.40	6.70	6.05	6.70	7.10
9:30 P.M.	6.20	6.25	6.00	6.60	5.90	5.90	5.70	5.90	6.40	6.10	6.80	6.30	6.30	6.30	6.20	7.00
10:00 P.M.	6.25	6.35	6.05	6.45	5.80	5.95	5.80	5.90	5.60	5.80	5.30	5.80	6.00	5.90	6.40	6.70
12:00 P.M.	6.30	6.20	6.20	6.45	5.80	6.00	5.70	5.90	5.70	5.70	5.50	5.90	5.20	5.90	5.60	6.10
3:00 A.M.	6.40	6.35	6.20	6.40	5.90	6.15	5.80	5.95	6.20	5.80	6.50	6.30	5.70	5.70	5.60	5.90
6:00 A.M.	6.35	6.25	6.15	6.30	5.90	6.20	5.80	5.80	6.20	5.90	6.55	6.40	6.60	6.10	6.20	6.35
9:00 A.M.	6.40	6.40	6.40	6.35	5.70	6.20	5.85	6.10	6.30	6.20	6.65	6.40	6.90	6.20	6.30	6.65
12:00 A.M.	6.20	6.45	6.30	6.25	5.60	6.40	5.85	5.45	5.70	5.80	5.90	5.70	5.00	5.90	5.40	6.65
VFA Concentration in Rumen Fluid (mmol/100 ml).																
3:00 P.M.	12.86	13.03	17.02	11.30	12.90	17.12	14.44	12.04	14.71	17.00	10.85	14.50	13.07	15.86	13.12	10.37
6:00 P.M.	13.04	10.42	15.76	10.46	15.93	16.50	13.31	11.62	9.47	15.38	10.34	11.88	10.07	13.48	11.99	9.21
9:00 P.M.	15.69	10.00	15.78	10.76	16.98	16.31	16.43	11.11	7.94	13.67	8.42	10.02	10.34	12.16	6.94	8.66
9:30 P.M.	11.18	13.21	15.16	11.92	15.94	14.19	14.78	14.89	10.43	15.64	10.58	11.15	13.47	13.59	10.95	10.63
10:00 P.M.	11.97	11.84	14.32	10.51	13.85	17.08	12.63	11.82	12.13	17.98	12.80	14.21	14.04	16.62	13.13	12.91
12:00 P.M.	14.77	11.26	13.73	12.77	13.87	15.28	14.21	12.90	15.76	21.20	13.05	18.44	18.77	17.30	14.02	14.89
3:00 A.M.	14.06	14.18	13.30	13.79	14.02	18.48	14.46	16.50	15.88	15.21	10.63	12.92	12.55	15.17	11.35	10.73
6:00 A.M.	12.62	11.82	13.52	12.80	12.79	15.43	13.60	14.04	11.73	15.00	12.30	11.99	12.15	12.96	11.23	9.93
9:00 A.M.	15.69	12.75	14.39	11.67	12.48	14.74	13.49	11.34	7.81	12.99	9.60	9.58	7.35	12.77	6.59	8.51
12.00 A.M.	12.05	11.77	14.92	12.73	15.28	16.85	13.65	15.98	15.24	18.77	14.13	15.67	22.61	19.22	14.86	13.71

continued ...

	CC				CP				TC				TP			
	18	16	15	55	18	16	15	55	18	16	15	55	18	16	15	55
Osmolality of Rumen Fluid (mosmol/l)																
3:00 P.M.	502	635	510	670	720	575	690	600	570	660	685	530	735	525	695	737
6:00 P.M.	508	671	545	640	710	580	695	595	525	593	600	651	645	550	660	727
9:00 P.M.	520	686	535	610	728	568	730	586	480	570	466	548	500	520	510	530
9:30 P.M.	510	685	570	620	750	590	750	610	620	680	691	694	805	710	742	782
10:00 P.M.	509	638	580	650	710	583	720	590	630	683	685	700	750	600	697	728
12:00 P.M.	515	688	520	690	715	591	710	640	560	675	657	655	790	586	701	740
3:00 A.M.	503	650	560	685	710	596	765	620	476	640	630	620	775	560	720	715
6:00 A.M.	507	635	530	675	705	587	706	596	520	650	645	608	675	530	690	690
9:00 A.M.	510	620	540	682	746	578	720	606	490	645	512	550	530	493	510	495
12:00 A.M.	509	655	550	620	732	590	735	630	590	680	680	693	760	620	720	725
Plasma Osmolality (mosmol/l)																
3:00 P.M.	283	283	284	270	273	285	282	278	286	256	280	282	276	282	265	270
6:00 P.M.	282	278	285	268	275	284	280	280	285	256	278	281	265	275	246	283
9:00 P.M.	285	277	286	266	276	283	281	281	278	250	270	283	264	265	247	282
9:30 P.M.	286	278	283	272	278	283	278	278	290	291	290	292	291	283	306	295
10:00 P.M.	282	278	285	271	277	285	281	282	286	280	285	290	285	280	312	290
12:00 P.M.	284	280	287	268	280	283	281	276	283	265	270	287	285	283	295	282
3:00 A.M.	283	278	282	272	276	282	282	281	276	271	275	282	274	273	290	282
6:00 A.M.	284	280	284	267	278	283	283	280	280	270	263	283	270	278	283	284
9:00 A.M.	282	280	283	273	275	284	282	282	277	273	260	275	260	273	280	283
12:00 A.M.	284	282	285	271	278	283	283	279	285	283	282	283	273	281	285	280

continued ...

	CC				CP				TC				TP			
	18	16	15	55	18	16	15	55	18	16	15	55	18	16	15	55
Ammonia Concentration in Rumen Fluid (mg/100 ml)																
3:00 P.M.	9.10	8.00	11.25	7.50	5.00	8.50	9.00	8.00	7.00	5.00	4.20	4.65	6.75	7.00	7.25	5.60
6:00 P.M.	9.00	6.00	8.50	8.50	3.50	5.50	8.50	7.50	6.75	7.50	7.60	8.00	4.00	7.25	8.25	8.70
9:00 P.M.	9.50	11.75	9.50	7.00	4.25	4.50	7.50	9.00	4.75	7.00	8.50	9.90	6.50	8.00	10.15	11.90
9:30 P.M.	7.30	9.75	9.00	8.00	3.75	7.40	9.00	7.50	8.00	7.50	9.60	12.50	7.25	15.50	15.25	12.50
10:00 P.M.	5.00	10.50	8.00	7.00	5.00	5.20	9.50	9.50	10.00	13.50	9.40	15.90	14.75	16.00	13.50	10.20
12:00 P.M.	9.50	10.00	8.25	8.00	6.00	5.30	8.00	9.50	7.50	4.50	5.60	9.90	6.75	12.25	3.50	5.20
3:00 A.M.	12.20	9.25	8.25	7.00	4.50	6.90	8.50	10.50	6.30	5.75	6.50	6.10	5.50	6.50	7.50	6.70
6:00 A.M.	12.50	9.40	10.75	7.50	4.75	5.50	10.02	10.50	4.25	6.50	4.20	9.90	6.25	5.25	8.75	5.50
9:00 A.M.	12.20	8.90	9.25	8.00	4.25	9.00	10.02	10.75	6.25	7.00	8.30	8.60	7.00	8.75	9.75	8.10
12:00 A.M.	12.60	8.25	10.75	8.50	5.00	7.40	10.50	9.00	7.25	7.50	5.80	11.35	1.25	14.50	4.00	4.90

End.