SOME FACTORS AFFECTING INTAKE OF ROUGHAGES BY DAIRY CATTLE

Thesis for the Degree of M. S.

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Bruce I. Veltman

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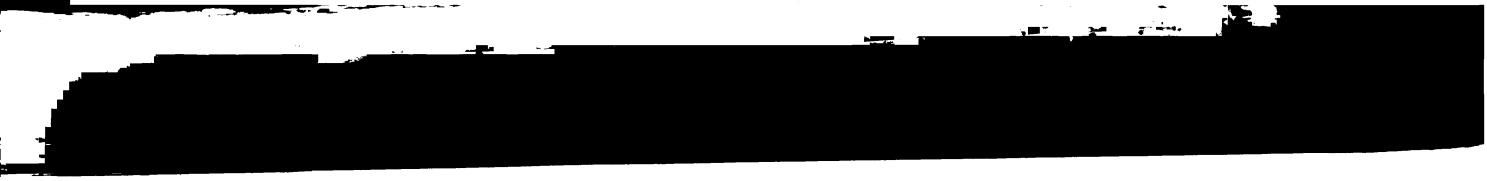

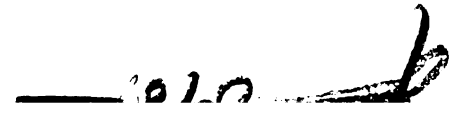

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By

Bruce I. Veltman

AN ABSTRACT OF A THESIS

Submitted to
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ABSTRACT

SOME FACTORS AFFECTING INTAKE OF
ROUGHAGES BY DAIRY CATTLE

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The study herein was conducted to obtain information on the various factors which affect the intake of roughage feeds by dairy cattle.

Intraruminal administration of hay, beet pulp, direct-cut alfalfa silage, and two silage fractions increased total dry matter intake of rumen fistulated cows in every case. Voluntary intake was reduced except when the silage extract fluid was added.

Dried beet pulp administered intraruminally to cows fed grain and hay appeared to be digested rapidly and resulted in no change in rumen pH, whereas, cows fed only hay did not appear to digest beet pulp as rapidly. This resulted in an accumulation of beet pulp in the rumen and a greatly reduced rumen pH. $NaHCO_3$ added into the rumen of one cow restored pH to normal and resulted in a rapid reduction of beet pulp accumulation and increased numbers of rumen microflora.

Rumen ammonia concentrations were higher when cows were fed direct-cut alfalfa silage than when fed alfalfa hay. There was little difference in rumen pH. Higher

concentrations of acetate and a higher acetate-to-propionate ratio was observed in rumen fluid when hay was fed, but butyrate concentrations were higher when silage was fed.

Rumen retention time of dry matter and fiber was reduced as roughage dry matter intake increased. Percent dry matter as well as total dry matter in the rumen increased as roughage dry matter intake increased while total weight of rumen contents remained nearly constant.

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INTRODUCTION

Dairy cattle feeding during recent years has undergone many changes. Development of forage harvesting equipment especially that for making silage has enabled dairymen to completely mechanize harvesting and feeding operations. This has permitted the harvesting of large amounts of forage crops during the relatively short period of optimum quality and yield while at the same time reducing field losses and weather damage. These advances have permitted more efficient feed production and handling, but meanwhile have created other problems.

Observations over a period of years have shown that cows consume larger quantities of hay in terms of dry matter than of silage produced from forage of comparable quality. Because of the economic advantages in harvesting hay crops as silage, a study to investigate factors responsible for the limited consumption of silage may eventually lead to a solution of this problem.

The object of the studies in this report is an attempt to learn more about factors affecting intake of roughages by dairy cattle. Through a better understanding of these factors, investigators will be able to plan a more systematic approach to the problem of limited intake of silage by dairy cattle.

REVIEW OF LITERATURE

Undoubtedly one of the first learning processes of an infant is that the pain of hunger is relieved by the intake of food. Only in relatively recent times have efforts been made to understand the basis and mechanisms responsible for the feeling of hunger and subsequent intake of food. Early theories explaining the sensation of hunger were approached largely through speculation. One early theory considered the stomach to be the exclusive seat of hunger feelings. Gastric contractions were believed to result from the empty stomach and these contractions were said to be responsible for hunger feelings (48). The validity of this theory was seriously doubted by investigators who observed that an animal's intake of food was unaffected when the stomach was removed or denervated making gastric contractions impossible (96, 100). Others according to Anand's review (1) postulated that the sensation of hunger resulted from depletion of body energy reserves. They speculated that within the brain some center sensitive to depletion of these reserves initiated the feeling of hunger causing the animal to search for food.

Roux (96) and others late in the 19th century suggested the idea that hunger was a sensation of general origin involving all or many organs of the body including

the circulating blood and brain centers. More recent and comprehensive investigations with the benefit of modern technology tend to support the theory that regulation of food intake is a central nervous phenomena influenced by various organs of the body (1).

During the 19th century clinicians described cases where obesity in humans has apparently resulted from brain tumors and lesions in the base of the brain (1). Later Keller and his group (62, 63, 64) experimentally produced lesions in the hypophysiohypothalamic region in dogs and cats which resulted in obesity in animals surviving the operation. They established that this obesity was associated with an increased food intake. Hetherington (51), in 1940 with the aid of improved surgical techniques, was able to selectively produce lesions in the hypothalamus without involving the hypophysis. He was able to relate the resulting obesity in rats exclusively to damage of the hypothalamus.

The fact that damage to the hypothalamus was responsible for increased food intake and resulting obesity has prompted intensive investigations to elucidate the role of the hypothalamus in regulation of food intake.

Hetherington (51, 53) observed that bilateral lesions produced in the ventromedial nuclei or immediately lateral to this nucleus were most effective in producing

obesity in rats. Anand and Brobeck (2, 3) observed bilateral lesions restricted to the region lateral to the ventromedial nuclei and sparing the lateral nuclei also produced hyperphagia and obesity. They produced lesions in the extreme lateral portion of the lateral hypothalamus producing complete aphagia and death of the animal by starvation. This effect of lateral lesions occurs whether the ventromedial region is intact or has been previously destroyed. They suggested that axons from the ventromedial region project laterally to produce inhibition in the lateral area.

The observations of these investigators (2, 3) and others not listed lead to the suggestion that the lateral hypothalamic area be designated a "feeding center" and the medial area a "satiety center."

Work has been done with other species indicating that the hypothalamic centers are similarly located and that they have much the same action regardless of species. Anand et al. (5, 34) observed that electrical stimulation in the lateral hypothalamic area in cats produced a marked increase in daily food intake. Larsson (69) produced similar effects in goats by electrically stimulating the lateral hypothalamic area. Hypertonic salt solutions locally injected in the lateral area also produced increased food intake. Local injections of an anesthetic into these

lateral areas caused temporary aphagia in starved goats. Larrson (69) also occasionally produced hyperphagia in goats by electrical stimulation in regions caudolateral to the mammillary body. Stimulation of the lateral hypothalamus produced such effects as licking, swallowing, and chewing. These observations suggest that facilitatory influences from the lateral hypothalamus project caudally into the brain stem and thus bring about augmentation of the feeding reflexes (1).

Feeding reflexes in experimental animals have been shown capable of being carried on independently of higher brain centers. Miller and Sherrington (84) showed decerebrate cats capable of swallowing and even to rejection of certain material placed on the tongue. Dell (35) described the role of the reticular formation in exploration and feeding behavior. The reticular formation, except for the special sensory and motor nuclei, makes up much of the gray matter portion of the brain stem. He stated that blood sugar begins to drop some time after a meal, at which time the circulating adrenaline level rises to augment liberation of glucose from liver glycogen stores at a faster rate. The reserves of glycogen rapidly decline, causing a greater reduction in blood glucose accompanied by a continuing rise in adrenaline levels. Adrenaline is a powerful stimulant to the reticular formation. This

excitation initiates a random locomotor activity while at the same time lowering the threshold to stimulation throughout the reticular formation. At this point, nervous stimulation from higher brain centers (e.g., cerebral) can easily influence the action of the lower reflex centers in the reticulum and give some purposeful action in food seeking behavior to this random activity. It does not seem unreasonable to suggest that the hypothalamus sends inhibitory or excitatory impulses integrating the activity of the feeding mechanisms and perhaps in this way regulates the quantity of food intake.

Though the hypothalamus has been the object of intensive investigation, there is much more to be learned about its functions. Experimental observations to date are very sketchy and incomplete regarding the interworkings between the hypothalamus and higher brain centers.

Evidence suggests that cerebral structures of the frontal and temporal lobes included in the "limbic system" may influence food intake. The limbic system, not well defined, lies superior to the thalamus and hypothalamus and is believed to be connected both directly and indirectly with these bodies. It is believed the limbic system has some integrating control on the functions of the hypothalamus as well as the autonomic system. The studies imply the possibility of both facilitation and inhibition

of feeding behavior from the limbic level. Anand (6) has noted that changes in food intake after limbic lesions were more marked in monkeys than in cats while Anand and Brobeck (4) did not find any change in food intake in rats with similar lesions. This species difference suggests a process of "encephalization" in higher animals at the limbic levels. Other observations suggest the limbic region in higher animals may have more to do with choice or discrimination between different foods than with quantity of food intake. Anand (6) noted monkeys, especially after temporal lobe lesions, lost their discriminatory power between edible and nonedible objects.

The hypothalamus is generally recognized as the center responsible for maintenance of the balance between energy input and expenditure within the animal body (21, 65, 80). This regulatory influence of energy intake is accomplished by inhibitory or excitatory impulses on reflex feeding centers lower in the brain stem and possibly directly to organs involved. Information from several sources throughout the body is continually acting on the hypothalamus which ultimately integrates it into action.

Circulating metabolites of the blood have received considerable attention regarding their influence on the hypothalamus. Blood glucose was the first metabolite to receive attention. Early observers (63, 30) noted blood

glucose levels were high soon after a meal and gradually declined until hunger was evident. Carlson in 1916 (30) advanced the idea that control of food intake was based on the concept that lowering of blood sugar occurring between meals stimulated stomach contractions producing hunger pains which in turn motivated the animal to eat. This theory found wide acceptance for many years among investigators and was used as a basis in designing experiments.

Later, Mayer and colleagues (79, 80, 81) postulated glucoreceptors sensitive to blood glucose levels exerted influence on the "satiety centers" in the hypothalamus and thus regulating the body energy balance through appetite regulation. Mayer and associates conducted several experiments attempting to locate glucoreceptor mechanisms in an effort to establish their theory. Mayer and Marshall (77, 78, 82) demonstrated a rapid uptake of glucose in the hypothalamus as indicated by the accumulation of gold following injections of goldthioglucose. Later studies using goldthioglucose showed the hypothalamus was not exclusive in its high utilization of glucose. After intravenous injections of radio active glucose phosphate, Forssberg and Larsson (41) noted a greater uptake of P^{32} in the hypothalamic feeding center than in other hypothalamic areas of hungry rats. They emphasized such results indicated

only an over-all increased activity in this region and not direct evidence to indicate the hypothalamus being a primary glucoreceptor mechanism center.

Anand (8) implanted electrodes in areas throughout the hypothalamic region of monkeys and cats for recording the electrical activity produced. Blood glucose levels were changed by intravenous infusion of glucose or intravenous injection of insulin. He observed increased electrical activity in the "satiety centers" with the production of hyperglycemia, while electrical activity was reduced in the feeding center. Conversely, hypoglycemia decreased activity in the "satiety centers" with a slight increase noted in the "feeding centers." He did not interpret these findings as convincing evidence of glucoreceptors in the hypothalamus. This evidence does establish, however, a relationship between glucose utilization and activity within hypothalamic areas.

Grossman (47) and Janowitz (60) observed that blood sugar levels per se were not a good indicator in determining appetite. They found hunger did not occur simultaneously with the lowest level of blood sugar, but later when it had started to rise. These same investigators (57, 59) observed in rats and dogs that production of hyperglycemia did not decrease food intake. Grossman (45), experimenting with normal human subjects, observed that

production of hyperglycemia did not suppress food consumption. Mayer (80) suggests, that for studying the influence of blood glucose on appetite, absolute blood glucose levels by themselves do not give a measure of glucose availability. Van Itallie (108) was able to demonstrate a generally reliable representation of glucose utilization by measuring ateriovenous glucose differences. His experiments showed these A-V glucose differences were high in normal humans after a meal and that hunger was always observed as these values approached zero.

The mechanism by which the hypothalamus regulates body energy balance is not clear when one considers blood glucose the only metabolite concerned in this balance. Evidence presented to date does not appear to be in mutual agreement.

Changes in concentrations of other metabolites have been implicated as having a relationship with intake and possibly with energy expenditure. Study of the nonesterified fatty acid (NEFA) fraction of blood plasma has revealed a close inverse correlation between ateriovenous blood glucose differences and (NEFA) levels. These also correlated well with satiety and hunger feelings. The (NEFA) fraction in the blood appears to originate primarily from adipose tissue with the amount in the blood regulated by some lipolytic factor presumably in the

circulation (87). Evidence suggests that a fat mobilizing hormone exists which is believed to be associated with long term food intake regulation and also short term energy mobilization (26). The experiments of Bates (11) showed that the amount of fat mobilized daily was proportional to the size of fat deposits. These more recent findings tend to support the early ideas of Kennedy (66) who postulated that the long range control of food intake was regulated by some element sensitive to varying concentrations of circulating metabolites. He suggested that the hypothalamus might be the receptive sight which in turn exerts its control on appetite.

Mellinkoff (83) correlated appetite with serum amino acids and blood sugar concentrations in normal human subjects given hydrolyzed protein and glucose. Evidence suggested an inverse relationship between serum amino acid concentration and appetite. Little experimental work has been reported in support of this postulated control mechanism except to confirm that such a relationship does exist.

Brobeck in 1948 proposed the hypothesis of thermostatic regulation of feed intake (20). He wrote, "Animals eat to keep warm, and stop eating to prevent hyperthermia." He stated that specific dynamic action of a ration determines the amount of food eaten. The effective regulator was not the energy value of the food but rather the amount

of extra heat released in its assimilation. This signals the hypothalamic mechanisms to adjust the total quantity of food eaten.

Since Brobeck stated his hypothesis much experimental evidence has been accumulating substantiating this principle as being a function in regulation of body energy balance.

Kibler and Brody (23, 67) observed feed intake of cattle was drastically reduced when exposed to high environmental temperatures in which they were unable to adequately dissipate body heat to maintain normal body temperatures. Brobeck (20), studying effects of temperature on rats, found food intake falls with temperature rise to a point where they will not eat due to hyperthermia. Appleman and Delouche (10) observed the feed intake of goats declined slowly as temperatures advanced to 90° F. and then dropped off more abruptly as temperature increased. Feeding stopped when body temperatures rose to 104° F. From these results there appears to be two possible relationships between temperature and feeding: (1) a gradual fall in intake as temperature rises may be a response to stimulation of peripheral thermal receptors without change in central body temperature, and (2) the more abrupt drop in intake may result from central hyperthermia (22). Assimilation of food consumed produces a heat rise in the

body above the postabsorptive level. This has been termed specific dynamic action. Passmore and Ritchie (89) demonstrated the promptness of specific dynamic action in experiments measuring skin temperature of human subjects after a meal. Within one hour there was a detectable rise in temperature. Booth and Strang (18) recorded the elevation of skin temperature which follows a meal on normal and obese human subjects. They attempted a correlation between this heat rise with onset of satiety and suggested the possibility that heat itself produces satiety. The effect of specific dynamic action appears to participate more prominantly in satiety when environmental temperatures are high. This additional heat is much more likely to act upon the central receptor mechanism to bring about more heat elimination and inhibition of food intake. Conversely in a cold environment the animal produces extra heat to maintain body temperature. The specific dynamic action, under these conditions, is then a small portion of the total heat produced and is insufficient to produce a thermal effect (22). MacDonald and Bell (72) observed that intake of milking cows increased 6 to 9 percent when average daily temperatures dropped from 40° F. to 0° F.

Several experiments have been conducted in an effort to understand the mechanism by which temperature affects the central nervous system and ultimately body energy balance.

Magoun et al. (73) showed that local warming of the preoptic area caused mobilization of various heat loss mechanisms. This has been considered the site of a "heat loss center." More recently, Kundt et al. (68) have shown that local cooling of the area induced peripheral vasoconstriction. This evidence indicates the preoptic and rostral hypothalamus area is a "temperature control center." Anderson (9) observed an interesting relationship between heat and cold applied locally in this area and appetite of goats. Eating was initiated by cooling this area in a feed satiated animal, but ceased when temperatures returned to normal. When cooling was continued intermittently for an hour, eating occurred during periods of cooling. Meanwhile, the thermal regulating mechanism of the animal was actuated, resulting in general peripheral vasoconstriction. This allowed body temperature to rise above the temperature at which a goat normally stops eating. In this case the goat kept on eating normally. Warming this area soon after beginning the regular meal caused the animal to stop eating and begin drinking large volumes of water. Peripheral vasodilatation and gradual lowering of body temperature followed. These observations indicate a close relationship between thermo sensitive elements in the preoptic area and rostral hypothalamus and the hypothalamic "feeding center."

It now appears that specific dynamic action acts directly upon cells in or just ahead of the hypothalamus to evoke cutaneous vasodilatation, and this is accompanied by central inhibition of appetite and induction of satiety. This would tend to indicate that under normal conditions the thermostatic regulation is of minor influence, but under heat stress conditions this mechanism exerts a powerful influence on feed intake.

Gastric hunger contractions have been considered to be one of the primary hunger sensations from early times. A consideration of how the gastro-intestinal area fits into present day concepts of the regulation of food intake is of interest.

Early investigators attempted to specifically locate these contractions and determine where they originated. Carlson (30) noted the sensation of hunger pangs was chiefly related to activity in the fundic portion of the stomach and that motility in the pyloric antrum during digestion caused no sensation of hunger. Such contractions may be as great as those occurring in the empty stomach during a hunger period. Action in the fundic portion of the stomach, associated with hunger, may greatly exceed that observed during digestion. Quigley et al. (93) employed the triple balloon technique for a detailed study of hunger sensations. The subject

indicated the feeling of hunger sensations during these tests. Pangs were observed most commonly in the distal region of the stomach; though, at times, when the distress of hunger pangs was greatest, several contractions over the stomach were observed simultaneously. Employing a double balloon in the duodenum along with the stomach balloons, Quigley observed that mild hunger pangs initially originated in the duodenum. As the hunger became more intense, stomach pains predominated.

Following these early investigations, attempts have been made to ascertain the basic factors which regulate hunger contractions and the associated hunger sensations. Carlson (30) stated that the gastric hunger mechanism is primarily automatic or independent of blood changes and central nervous influences. More recently Stunkard et al. (104) and Quigley (94) have demonstrated in human subjects that small ateriovenous glucose differences coincide generally with gastric hunger contractions and hunger sensations. When A-V glucose differences were large along with higher levels of blood sugar, stomach contractions were inhibited as well as hunger sensations. Several investigators (25, 60, 94) found no correlation between absolute levels of blood sugar and spontaneous gastric hunger contractions.

Grossman and Janowitz and associates (58, 59, 98) produced evidence that gastric distension is important in bringing about satiety. Inert bulk placed into the stomach of a dog which had undergone esophagostomy was as effective as food in producing inhibition of eating. On the other hand, Quigley (94) reported food substances, confined to the stomach, did not inhibit hunger contractions; in fact the substances might stretch the stomach and augment hunger contractions. Stomach contractions were inhibited soon after food entered the upper intestine. He also reported evidence that a gastric inhibiting substance released from the intestine was enterogastrone. Janowitz (59) noted that enterogastrone inhibited gastric contractions when given experimentally but did not alter the amount of food eaten. Grossman (46) and Quigley (94) agree it is not essential that animals or humans experience gastric hunger sensations for normal regulation of food intake.

Sharma et al. (99) have recently studied relationships between the hypothalamus and gastric contractions in various experiments by recording electrical activity in the hypothalamus. Inflation of an intragastric balloon increased electrical activity of the "satiety center." They believe this emphasizes the role played by gastric distension in bringing about satiety through

activation of "satiety centers." Glucagon injections, followed by a subsequent rise in blood sugar and A-V differences, resulted in increased activity in the satiety center and simultaneous inhibition of gastric hunger contractions. Glucagon administered after destruction of satiety centers produced a rise in blood glucose but no inhibition of gastric hunger contractions nor any change in electrical activity of the hypothalamic region. This evidence supports the idea that increased activity of satiety regions is the factor which inhibits gastric hunger contractions.

Gastric hunger sensation is but one factor involved in motivation of food intake in the maintenance of life. The exact role of gastric contractions in the hunger state is not clear, although it seems certain that they can be inhibited by the satiety mechanism.

In contrast to the extensive work on feed intake regulation in small animals, there is very little experimental work on factors that influence the intake of farm animals. The mechanisms by which ruminant animals regulate feed intake may be quite different from those in small animals. Ruminants consume bulky feeds which are low in energy, while the simple stomached animals consume feeds high in energy having a minimum of bulk. Food eaten by small animals is broken down in the stomach and

small intestine and assimilated. In ruminants, on the other hand, a large portion of food eaten is broken down by the fermentative action of rumen microflora and absorbed from the rumen before reaching the lower digestive tract. Ruminants, used primarily for meat and milk production, have been bred to consume large quantities of feed. More rapid weight gains in fattening beef cattle and greater milk production in dairy cows has been shown to be related to larger feed intake. Greater feed intake by cattle is of major economic importance to cattlemen as well as the consumer. Little is known, however, about the factors involved in regulating feed intake by ruminants.

Factors Affecting Intake of Roughages by Ruminants

Information on feed intake of dairy cattle up to 1950 was summarized by Blaxter (14) and indicated that the amount of feed intake, measured in terms of dry matter, increased as energy concentration of the ration increased. This was based on feeding grain mixtures in addition to a diet primarily composed of roughages. Experiments conducted by Blaxter and associates (15, 16, 17) since that time have led them to suggest that purely physical factors dominate the regulation of intake of roughages by sheep. Reports by Campling and Balch et al. (27, 28) and Makela

(74) regarding roughage intake by dairy cattle are in agreement with this idea. This appears to be the reverse of the situation of intake regulation in simple stomached species. Data of Mayer (80) showed that rats increase food intake as energy concentration of the ration is reduced in order to maintain a constant caloric intake. Persson and Svensson (90) observed intake of chickens followed the same trend, concluding that this species eats to maintain a constant energy intake. Experiments of Peterson et al. (91) in which they used wood cellulose to increase the bulk in the ration fed to chicks stated: "The primary factor in the voluntary food intake of young chicks appears to be the need for energy." Kennedy (66) suggests that a regulation of food intake of the same general type must occur in humans, based on the very constancy of an adult body weight in man, consuming a wide variety of diets and expending variable amounts of energy.

Illustrating the principle that ruminants consume more high than low quality roughages, Blaxter (17) fed sheep low, medium, and high quality long hay. Quality was determined in digestibility trials and expressed as apparent digestibility. Intake and rate of passage increased with improvement in apparent digestibility.

Campling, Freer, and Balch conducted a series of investigations to study physical factors in the rumen

affecting voluntary intake of hay by cows. In their first report (27) they determined the importance of the amount of digesta in the rumen at different times relative to the time of feeding. Fistulated cows were used and contents were removed at defined times after eating. The cows had been trained to consume the entire daily ration during a relatively short time after feeding, usually three to four hours. In the first experiment, food boluses were removed from the rumen as the hay was eaten during a normal feeding period of three hours. The food removed amounted to 76 to 96% of the normal daily intake. After this time the cows were allowed to eat normally. Eating was prolonged for another three or four hours resulting in an increased total intake, 70 to 85% over the normal daily intake. These results suggested that the accumulation of swallowed food in the rumen exerts a direct and immediate effect on the time the cow ceased to eat hay and on the amount consumed.

In the second experiment, 50 pounds of digesta (rumen contents) containing 7.1 pounds of dry matter or equal to 8.4 pounds of hay were removed from the rumen. The digesta were removed at three different times which were: 1) during the meal, 2) just after the meal, and 3) mid-way between meals. The cows increased their hay

intake 2.4, 6.2, and 0.4 pounds for the three treatments, respectively. The cows never completely compensated for the food removed. The nearest compensation occurred when 50 pounds of rumen contents were removed just after the meal when only 2.2 pounds less hay than normal were consumed. When 50 pounds of digesta, with an estimated dry matter content of 10% (a low estimate), were added to the rumen before a meal the cows decreased their hay intake by 4 to 5.8 pounds. These results indicate that a cow eats to a nearly uniform distension of the rumen or other non-defined conditions therein.

In a third experiment water filled bladders containing up to 100 pounds were placed in the rumen. Intake decreased 0.54 pounds of hay for each 10 pounds of water added. While this was a small decrease, calculations showed that 0.6 pounds of dry matter in digesta, which contain about 10% dry matter, occupy about the same space as 10 pounds of water. When 100 pounds of water were poured directly into the rumen daily, voluntary intake of hay was not changed significantly. Campling et al. concluded from the results of their experiments that changes in intake, due to the transfer of digesta, were due to the dry matter or volume associated with the dry matter in the rumen rather than the water alone. Thomas (106) dripped water into the rumen of heifers with no effect on

intake. Hillman (54) and Thomas et al. (106) found that the addition of water to hay or silage, which was fed to heifers and cows, did not cause an appreciable change in dry matter intake.

All this evidence indicated that a cow eats until the rumen becomes distended to some critical pressure giving rise to a feeling of satiety, and she then stops eating. A reasonable extension of this assumption could be that the amount of food eaten during the following meal may depend upon the rate of breakdown of food in the rumen and its subsequent disappearance from the rumen.

Ingalls (56) observed that sheep fed alfalfa and trefoil hay ate more frequently and consumed more of this forage than when fed brome and reed canary grass. These observations suggest the possibility that the legumes may have been broken down in the rumen more rapidly than the grasses. It also could be that the sheep preferred the taste of the legumes or found them physically easier to handle while eating than the grasses.

Campling et al. (28) determined the amounts of digesta in the rumen at given intervals after a meal. They then attempted to relate the amount of reduction of ingested feed in the rumen after a meal and the quantity of a roughage consumed during the following meal. Rumen fistulated cows were fed hay or straw ad libitum once

daily in part of this experiment. Rate of breakdown of ingested feed in the rumen was estimated from rate of disappearance of cotton thread placed in the rumen. Mean retention time of undigested food residues in the alimentary tract was measured by placing a small amount of stained food particles in the rumen and counting the number of stained particles in subsequent samples of feces. Retention time of the lower digestive tract was determined by introducing milled stained food particles into the abomasum at feeding time. The time required for these particles to appear in the feces was termed lower tract retention time. The amount of digesta in the rumen was measured directly by manually emptying the rumen. Mean voluntary intake was 22 pounds of hay and 10 pounds of straw. The mean digestibility of dry matter was 65% for hay and 45% for straw. Estimated rate of digestion as measured by 25% loss of weight of cotton threads in the rumen was 26 hours for the hay compared to 166 hours for the straw, or six times faster in the hay fed cows. Retention time of undigested residues varied greatly between cows, however, retention time was less in cows with greater feed intake than in those at lower levels of intake. This was true for both hay and straw. These observations led to the suggestion that some cows have characteristically long retention time of undigested residues, and that this

factor may account for individual variations in voluntary intake. The mean amounts of ingesta in the rumen of cows fed hay before feeding was 165 pounds (14.4 lb. D. M.) and 128 pounds (13.6 lb. D. M.) for straw fed cows. This difference in dry matter amounted to only 6%. After eating to the limit of appetite rumen contents of the hay fed cows averaged 250 pounds (27.2 lb. D. M.) and for the straw fed cows 184 pounds (20.2 lb. D. M.). These results indicate that a cow does not eat to a critical distension of the rumen unless this point is different for each roughage. The rate of disappearance of feed from the rumen to some low critical level for a particular feed preceeding the next meal appeared to determine the amount of feed consumed.

Earlier experiments by Campling et al., described above, indicated that a cow ceased to eat when the rumen became distended to some critical point. Under the conditions of this experiment, however, the authors have interpreted the results to indicate that intake was regulated by the amount of "ingesta" in the rumen some time before feeding. Whether intake was regulated by rumen distension or some low critical level of "some factor" in the rumen, the amount of a roughage consumed was directly related to the rate of disappearance of ingested feed in the rumen.

Campling et al. (29) recently reported another experiment in which dry matter intake by cows fed straw alone ad libitum with the addition of urea into the rumen was 40% greater than when no urea was added. These cows were allowed to eat only once daily during a short period as in the experiment described above. Retention time of dry matter in the rumen was reduced during the period of urea administration. The before feeding weight of rumen contents was only 7% greater during urea treatment while dry matter in the rumen was the same for both. After feeding, cows receiving urea had 11% more dry matter in the rumen than those fed straw alone. This was submitted as additional evidence supporting Campling's et al. more recent conclusions.

Makela (75) observed that total weight of rumen contents did not vary extensively between cows fed high and low levels of feed intake. In contrast, dry matter in the rumen tended to increase as intake of dry matter increased. He developed a method of expressing rumen retention time of feed components in the rumen and found with increased feed dry matter intake the retention time of dry matter in the rumen was reduced. Thomas et al. (105) observed little change in the weight of total rumen contents of cows 4 to 5 hours after feeding hay or silage ad libitum or at submaintenance levels. There was,

however, considerably more dry matter in the rumens of cows fed at the higher level of intake. Calculations of dry matter retention time indicated an inverse relationship between dry matter intake and dry matter retention time in the rumen. Evidence from the literature strongly indicates that intake of feed is directly related to the rate of disappearance of feed primarily from the rumen.

At this point a review of some of the factors responsible for differences in rates of time for ingested food to disappear from the rumen may be of interest.

Blaxter (17) fed sheep long hay, medium ground cubed, and finely ground cubed hay from the same source. The before feeding rumen fill was greater in sheep fed long hay than when they were fed medium and fine ground grass cubes. Ad libitum intake of long hay was 1800 grams per day compared with 2400 grams per day for the ground cubed hay. He concluded that changes in physical form of a roughage can modify its passage through the digestive tract. Another explanation could be that the grinding of the hay greatly increased the exposed surface area for more rapid attack by rumen microflora resulting in faster digestion. Makela (88) noted the concentrate portion of a ration consisting of ground grains disappeared much more rapidly from the rumen than hay

fed at the same time. He attributed much of this faster rate to the small particle size of this feed. Another possibility could be that rumen bacteria break down concentrate in preference to hay. Difference in particle size could not explain the more rapid disappearance from the rumen of one hay than another when sheep were fed long hay from two sources. The possibility that one forage was broken down more rapidly by rumen microflora than the other was suggested (16).

Crampton (32) postulated that the simple matter of bulk could not be the cause for differences in voluntary intake of forages as there is little difference in bulk between forages that are eaten in widely different amounts. He suggested that the rate of digestion of the forage or, more specifically, the rate of reduction of rumen load is involved. Therefore the frequency of eating ultimately depends upon the ease and vigor of the attack by microflora on the cellulose and hemicellulose of the ingested forage. He also stated that the rate of digestion may be retarded by circumstances which interfere with the numbers or activity of microflora including excessive lignification (31).

Dehority et al. (33) studied the rate and extent of hemicellulose fermentation by rumen bacteria on forages with respect to stage of maturity by in vitro

technique. It was noted that the extent of hemicellulose fermentation was reduced with advancing maturity. When mature forage was removed from the fermentation, however, and exposed to a ball milling process in which particle size was greatly reduced, and re-exposed to fermentation the extent of hemicellulose fermentation increased. This experiment provides evidence that hemicellulose digestion is influenced by the maturity of the forage and suggests that this effect is the result of lignin forming a physical barrier between the plant hemicellulose and rumen bacteria. Crampton (32) also noted that one species of forage may naturally contain a higher percentage of lignin than another. This may explain why one forage is broken down more rapidly than another and consequently eaten in greater amounts.

Low nitrogen content in forages can be another factor responsible for reduced numbers or activity of rumen microflora and the resultant retarded breakdown of the cellulose portion of forages (31).

A good illustration for this point was brought out in the experiment by Campling et al. (29) when straw alone was fed to fistula cows. Voluntary intake of straw was increased by 40% when 75 grams of urea was placed into the rumen. Mean retention time of food residues was reduced 20% even when intake was restricted to a pretreatment

level. It was also interesting to note that retention time through the remainder of the digestive tract was not changed by this treatment, thereby, emphasizing the role of the rumen in influencing feed consumption. Another experiment, where urea was fed or directly infused into the rumen of sheep and steers fed straw, did not increase the digestibility of the straw, and the addition of urea reduced voluntary consumption of straw 12 to 15% (85). In his review on urea supplementation, Reid pointed out that urea may not increase microbial activity or percent digestion of a feed low in readily available carbohydrates (95).

Crampton suggests that specific mineral deficiencies besides nitrogen or excess bacteriostatic agents in feed may reduce the rate of digestion affecting voluntary intake of roughages (31).

Smart et al. (101) have demonstrated a factor in sericea forage that is inhibitory to rumen cellulolytic activity.

The appetite depressing effects caused by deficiencies of several inorganic elements has been reviewed by Lepkovsky (70). Most of the work in this field has been done with non-ruminant animals and may not apply to ruminants.

Research regarding intermediate products of rumen fermentation and deficiencies of specific metabolites in the rumen fermentation process has not progressed far enough at this time for a comprehensive review to be written regarding relationships between products of rumen fermentation and feed intake of cattle.

Factors Affecting Intake of Legume Silage

Research regarding intake of hay-crop silage compared with hay has been extensively reviewed in the thesis by Hillman in 1959 (54). More recent reports on consumption of silage stored with varying amounts of dry matter have been published (43, 86, 107). Research indicates with few exceptions that cattle consume more pounds of dry matter in the form of hay than silage. There is a tendency for greater consumption of silage as the dry matter percent increases when starting with high quality early cut forages.

Some work has been reported in the literature showing attempts to isolate various factors responsible for the decreased intake of silage. Intravenous infusions of glucose and various volatile fatty acids were performed by Dowden and Jacobson (37) on dairy heifers to study the effects of these metabolites on feed intake. They found infusions of acetic acid and propionic acid, providing

12.5% of daily maintenance requirements for energy, significantly depressed intake. Infusions of glucose, butyric, valeric, hexonic and lactic acids each providing 6.5% of daily requirements had no significant affect on appetite. They suggested the possibility of chemoreceptor response to changes in blood constituents being a mechanism regulating intake of metabolites including these acids. Silage contains significant amounts of these acids and these authors suggested the possibility that these acids may be a factor responsible for reduced intake of silage.

Emery et al. (40) fed corn silage soaked with varying amounts of lactic acid to dairy heifers. They observed a reduced dry matter intake with increasing amounts of U.S.P. lactic acid on the silage. Intake was only slightly depressed, however, when lactic acid salts were fed in one instance. The U.S.P. lactic acid may have contained more impurities and polymers than the lactate salt mixture. This may explain why intake was depressed more when the U.S.P. lactic acid was fed. Rusoff and Randel (97) fed hay soaked with mixtures of major organic acids representative of those found in good and poor silage. They observed heifers consumed significantly more hay soaked with acid mixtures representative of poor silage than of the good silage. They concluded that any decrease in palatability of poor quality silage does not appear to be

due to its characteristic volatile fatty acid content and suggested that some other constituents of silage must be responsible for this decrease.

Thomas et al. (106) conducted a series of trials using dairy heifers to study factors that influence the rate of consumption of alfalfa silage. In one trial of a preliminary nature a wide variety of materials was added to silage fed to heifers. In another trial these materials were placed directly into the rumens of heifers. Several materials fed or administered had no significant effect on total dry matter consumption. However, intake was depressed by addition of such materials as glucosamine, large amounts of lactic acid alone or with other volatile fatty acids, effluent liquid from a silo, various extracts and residues from silage, and ammonium salts. These trials were of an exploratory nature attempting to separate factors responsible for the low intake of silage.

Investigations should be continued exploring fractions of silage to identify the constituents which limit voluntary intake. This limiting effect on intake is probably a metabolic effect rather than a result of palatability. The relationship of metabolic products from silage and/or the rumen with body intake regulation centers should be studied exhaustively to gain a better insight regarding the factors which limit consumption of silage by cattle.

PROCEDURES

Procedure for Intraruminal Treatments

All animals, except cows A103 and 660, used during this experiment were non-lactating non-pregnant mature cows fitted with plastic rumen fistula plugs. The cows were retained in stanchions with individual mangers constructed to minimize feed loss and make possible the measurement of daily feed intake and refusal. Water was available at all times. Most cows were fed hay only two times daily, at 7:30 A. M. and 1:30 P. M. The two lactating cows were fed alfalfa hay plus a grain mixture, and in the other trials one dry cow was fed fresh-cut alfalfa forage. The amount of forage fed was at least 10% in excess of voluntary consumption. The hay fed as well as that refused was weighed, and moisture content was determined for all materials fed or administered. This allowed calculation of dry matter intake. Alfalfa hay of medium quality from the same source was fed throughout these trials.

The experimental feedstuffs were placed in the rumen at twelve hour intervals beginning at morning feeding time. Intraruminal infusions of experimental fluids were made three times daily: during the morning feeding, six, and twelve hours later. The portion for each infusion

was stored in glass jugs suspended above the cow and transferred through rubber tubing into the rumen by way of the fistula. Administration of dry material required 15 minutes each time and liquids required approximately 20 minutes for each infusion. The amount of dry material placed in the rumen was administered to the full capacity of the rumen without causing obvious discomfort to the cow. However, for any one material, a standard amount was usually established so that suitable comparisons could be made between cows.

The daily amounts and materials placed in the rumen ranged from 20 to 30 pounds of fresh-cut high quality mixed alfalfa-grass forage, 10 to 12 pounds of chopped hay (the same material as that fed), and 12 to 15 pounds of beet pulp, 30 to 32 pounds of grass silage, 30 pounds of washed pressed silage, and 35 to 56 pounds of the fluid portion of alfalfa silage. In two special trials larger amounts of this fluid were administered. The effect that each of these materials exerted on voluntary intake was calculated.

Average daily intake 6 days before and after treatment was compared with consumption during the experimental period of 8 days. It was reasoned that elimination of intake on days immediately following a change would give a more true expression of the effect of that treatment on

voluntary intake. Consequently, intake on the first day of treatment, two days for silage fluid and washed pressed silage treatment, and two days following treatment were eliminated from the average.

Procedure for Preparation of the Two Silage Portions

A hand powered hydraulic lard press was employed to express the liquid portion from the solid portion of the direct-cut alfalfa silage used in these experiments. The capacity of the press was 2.65 cu. ft. which held approximately 100 pounds of silage as removed from upright concrete stave silos. A force, up to 160 lbs./sq. inch, was sustained on the material in the press for a period of 45 minutes.

The original silage contained 26.1% dry matter. On the first pressing, called 1st extract, 36 pounds of juice were removed from 100 pounds of silage. Approximately the same amount of water as was in the juice was added to the remaining silage and allowed to stand 6 to 12 hours. This permitted the silage to soak up the water and equilibrate with the soluble portion. The silage was pressed again and this juice was saved separately. This was called 2nd extract. The juice was stored in ten gallon milk cans at a temperature of 40° F. for periods up to seven days.

The dry matter of the silage juice, carbohydrate, and volatile fatty acid contents appear below.

		1st extract	2nd extract
Dry matter	(%)	8.42	4.54
ash	(% of D.M.)	19.53	20.33
carbohydrate	"	0.354	0.14
Volatile fatty acids	(uM/ml.)	440.00	170.21
formic acid	"	8.78	7.32
acetic acid	"	178.15	74.23
propionic & butyric	"	3.14	0.0
lactic & succinic	"	250.50	88.66
pH		4.40	4.45

After the second pressing, the solid material was removed from the press and placed into a garbage can with a perforated bottom. The silage was then flushed with cold water for an hour to remove as much of the remaining soluble material as possible.

After the third pressing, the remaining product averaged 34% dry matter. This was believed virtually exhausted of the normal silage juice constituents as the pH of this expelled fluid on pressing was 5.9 compared to 4.4 and 4.45 for the two previous fluid portions. This product was called washed pressed silage and was stored in a cool place, 40 to 50° F., until used in the trial. Dry matter, fiber, and soluble carbohydrates for the original silage, the washed pressed silage, and hay fed are presented below.

		Silage	Washed pressed silage	Hay
Dry matter	(%)	26.1	34.14	86.92
Fiber	(% of D.M.)	40.47	53.70	39.75
Soluble carbohydrate	"	18.40	21.70	20.20

Procedures for Rabbit Trial

Since the infusion of silage juice into the rumen had caused a change in feed intake by cattle, a feeding trial using rabbits was performed to observe the effects this fraction might have on the intake of this species.

The silage juice (first extract) was incorporated into regular rabbit pellets. This was accomplished by sprinkling the juice over trays of pellets 2 or 3 times daily allowing them to air dry between applications. Caution was taken to prevent the pellets from disintegrating and molding. Sufficient juice was added over a period of a week to make the juice dry matter amount to 5% of the total dry matter of the pellets. The control pellets were sprinkled with water in a similar manner to give them the same physical appearance as the silage sprinkled pellets.

Six half grown male rabbits kept in individual pens in a controlled environment were used in this trial. A preliminary 8 day period was used to establish rate of

intake and body weight gain. The rabbits were assigned to two balanced groups based on intake and body weight. During the next 8 days one group was fed control pellets while the other group was fed pellets soaked with silage juice. The treatments were reversed and the trial continued for 8 more days. Intake and weight gains were measured by four day intervals.

Procedures in the Beet Pulp Study

An exploratory investigation was conducted to study some of the possible causes for results observed in the previous trial when beet pulp had been placed into the rumens of fistulated cows.

In the first period of this experiment, a study was set up to determine whether "cows" adapt to more rapid digestion of beet pulp after it is fed for a period of time, and to determine if the ration affects rate of beet pulp digestion. During the second period a study was conducted to determine the effect of rumen pH on the digestibility or disappearance of beet pulp from the rumen.

In the first part of the adaptation experiment, three fistulated cows previously fed hay ad libitum were used, one of which was also fed 10 pounds of a dairy concentrate mixture daily. During the experiment the cows were continued on ad libitum hay feeding along with beet

pulp placed into the rumen. Voluntary consumption of hay was measured as previously described. An attempt to determine if any adaptation occurred was made by determining the concentration of total soluble carbohydrate and uronic acids on the day before intraruminal beet pulp administration (control day), the 1st day, and the 7th day of treatment. Lower concentrations of carbohydrate and uronides on the seventh day than on the first day would be taken as an indication of adaptation. Rumen volatile fatty acid concentrations were also determined on these days. This was done to observe any changes in volatile fatty acid concentrations associated with adaptation.

Samples of rumen fluid were collected immediately before feeding, 1.5, 2.5, 3.5, and 4.5 hours after morning feeding and intraruminal placement of beet pulp. The procedure for collection and preparation of rumen fluid is explained later in the section on laboratory procedures.

The second part of the first period of this experiment involved an in vitro fermentation using rumen fluid collected from eight cows to determine if beet pulp, used as a substrate, was digested more rapidly by cows receiving beet pulp in their rations than those not receiving beet pulp. This group was again divided into those cows fed hay plus grain and those fed hay only. The cows receiving beet pulp in their ration or intraruminally had

been on this diet for at least two weeks, while those cows not receiving beet pulp had not been fed beet pulp for at least a month prior to this trial.

Initial and post incubation samples were taken from the in vitro fermentation apparatus for measurement of disappearance of total uronides, total carbohydrates, and soluble uronides. Gas production was also measured to indicate relative activity among the samples. Total solids of each fermentation flask were determined for an approximation of substrate digestion during fermentation.

The second period of this exploratory investigation involving intraruminal administration of beet pulp was a continuation of the first phase using the two hay fed cows.

Beet pulp was placed in the rumen for a further period of 11 days. After the third day of the second period, $NaHCO_3$ was placed in the rumen of one cow in addition to beet pulp. Sufficient $NaHCO_3$ was used to return rumen pH to the pretreatment level for that cow. The other cow served as control.

Observations were made on rumen pH, gross visual approximation of the percent beet pulp in the rumen ingesta, and microscopic observations of rumen contents. Microscopic observations were made to obtain a rough approximation of rumen microbial populations.

Procedures for Studying Rumen Characteristics When Cows Were Fed Silage Versus Hay

Several experiments (44, 55, 71, 107) have shown that cows consume somewhat larger amounts of dry matter as hay than as silage when both were made from forage of comparable quality. The possibility that there may be a difference in the way these feeds are broken down in the rumen or that the rate of breakdown may influence the difference in intake of these feeds was considered. This experiment was set up to observe rumen pH, NH_3 concentrations and volatile fatty acid concentrations when cows were fed both hay or silage. This was done in an effort to determine if feeding silage would result in different values of these measurements than when cows were fed hay.

Two rumen fistulated cows, T23 and T19 were fed once daily at three levels of intake beginning with a submaintenance level of approximately 5 pounds of dry matter per day, then 10 pounds per day, and finally ad libitum. These levels will be referred to hereafter as low, medium, and high, respectively. Hay was fed to one animal while silage was fed to the other for a period of two weeks at each level of feeding beginning at the low level. Rumen fluid samples were taken on one day toward the end of each period. The feed to each animal was then reversed

and the trial continued beginning on the low level with another ten days allowed for adjustment at this level. Rumen samples were taken on one day of each period: at 1.5, 2.5, 3.5, 4.5, 6.5, and 12 hours after feeding. The rumen fluid samples were collected and preserved by the method described in the laboratory procedures. T19 went "off feed" when silage was fed at the high level making it necessary to use a substitute animal, Arthur.

Procedures for Laboratory Analysis

Dry matter determination. All samples of feed, materials added into the rumen, and rumen contents for dry matter determination were handled in the following way. Individual samples were weighed out into tared pans and placed in a forced air oven at 80° C. for 48 hours. The samples were then removed and reweighed. The weight difference divided by the original net weight times 100 was expressed as the percent dry matter.

Collection and preparation of rumen fluid samples. The rumen contents were mixed by hand to ensure representative sampling of the entire rumen. If the contents could not be mixed adequately, handfuls of contents were taken from several locations inside the rumen in order to obtain reasonably representative sampling. Handfuls of rumen contents from the various locations in the rumen

were squeezed and the fluid collected in glass jars. These samples were then immediately taken to the barn laboratory where pH was determined within five minutes after removal from the rumen. The samples were then centrifuged at 32,000 times gravity for five minutes to remove solid particles in the fluid. Fifty ml. (milliliters) of this sample were measured into a bottle to which 1 ml. of 50% H_2SO_4 was added. This was stored in a refrigerator for carbohydrate, uronic acid, and volatile fatty acid analyses. Another 10 ml. portion of the sample, for the carbohydrate and uronic acid determinations only, was preserved in 30 ml. of absolute ethyl alcohol and refrigerated until analyzed.

In vitro fermentation. Samples of rumen fluid (500 ml.) strained through a double layer of cheese cloth were obtained from eight cows about three hours after the morning feeding. One hundred fifty ml. of this rumen fluid and 50 ml. of buffer solution were added to the 250 ml. erlenmyer fermentation flask to which 1 gm. (gram) of finely ground beet pulp had been previously added. The buffer solution of anhydrous salts, containing 2.04 gm. KH_2PO_4 plus 4.36 gm. Na_2HPO_4, was made up to 500 ml. with H_2O. This resulted in a 1/10 molar buffer solution with a pH of 7.0. Each flask was then attached to a gas burette and placed in a 39° C. water bath. pH of the rumen

fluid mixture was taken at the beginning of the fermentation. Fifty ml. of each rumen fluid sample were preserved with 1 ml. of 50% H_2SO_4 (v/v) and stored in a refrigerator for glucuronic acid and soluble carbohydrate analysis at a later date. Ten ml. of each rumen fluid sample were preserved with 2 ml. of 6N HCl for total uronic acid and total carbohydrate determination. A blank flask containing 150 ml. of water and 50 ml. of buffer plus 1 gm. of beet pulp was also included in this fermentation trial. Gas production was measured at 0.5, 1, 1.5, 2, and 3 hours after discarding an initial adjustment period of 15 minutes. The flasks were mixed before each reading. After 3 hours of fermentation a 50 ml. aliquot of the ferment was removed and preserved with 10 ml. 6N HCl for final total uronic acid and carbohydrate determination. Another portion was removed and centrifuged for 5 minutes at 1400 times gravity. Fifty ml. of the supernatant were preserved with 1 ml. H_2SO_4 for determination of final soluble uronides and carbohydrates. Another 50 ml. aliquot was placed in a sediment tube and centrifuged at 1400 times gravity for 10 minutes and the volume of the sediment and liquid was immediately read. This volume was used as an estimate of total solids remaining in the fermentation flask. Final pH was also determined after the fermentation in each flask.

Determination of pH. The pH of feed and rumen contents was determined on the Beckman pH meter and glass electrode using standard procedures recommended by the manufacturer.

Carbohydrate determination. A method for hydrolysis of beet pulp was developed prior to the fermentation trial so that samples could be completely solubilized and hydrolyzed to obtain representative amounts of substrate present in the fermentation flasks. In the preliminary experiments 10 ml. portions of 1/10 N, 1 N, and 6 N H_2SO_4 and 1/10 N and 1 N HCl were heated with 0.5 gm. samples of finely ground beet pulp for 30 minutes on a hot water bath. Results of the phenol-sulfuric acid carbohydrate determination (38) indicated a much higher carbohydrate value for the sample hydrolyzed in 1 N HCl. An optimum hydrolysis time of 30 minutes on the hot water bath was established after trying hydrolysis times ranging from 10 to 60 minutes.

Uronic acid determination. The modified carbazole reaction of Bitter (13) was used for determination of uronic acids which result from hydrolysis of the pectin in beet pulp. The procedure that was used follows. Six ml. of the sulfuric reagent (195 ml. H_2SO_4 and 5 ml. M $Na_2B_4O_7$) were placed in glass-stoppered test tubes and cooled to approximately 0° C. One ml. of the test sample

containing between 4 and 60 gm. of uronic acid/ml. was added and mixed thoroughly. A set of tubes containing 1 ml. of known concentrations of uronic acid within this range were run with each analysis. The tubes were then heated on a boiling water bath for 15 to 20 minutes and cooled in water to room temperature. Two tenths ml. of the carbazole reagent (0.1% carbazole in ethanol) was added to the tubes, the tubes shaken, heated at 100° C. for a further 10 minutes and kept in the dark for 3 hours. The pink color appeared after the second mixing and developed during the 3 hour period. Optical density was read at 530 mu.

Rumen ammonia determination. The following procedure used for determining rumen ammonia was a modification of the Permutit method (50). Two ml. of rumen fluid sample were measured into a 100 ml. volumetric flask to which 2 gm. of Amberlite IR-120H were added and allowed to stand. A period of at least 10 minutes was allowed so the ammonia in the sample would be absorbed onto the resin. The resin was then washed free of all sample and 2 ml. of 10% NaOH were added to free the ammonia from the resin. Ten minutes were allowed for this reaction. Seventy ml. of water and 2 drops of Gum Ghatti solution were added to the flask followed by the addition of 10 ml. Nessler's Reagent. After mixing the flask was filled to volume.

The optical density of the resulting colored solution was read on a Beckman model B Spectrophotometer at wave lengths ranging from 480 to 520 mu. The frequency that gave an adequate range on the photometer scale on that particular day was used. The optical density reading of the sample was compared to readings obtained from a series of known concentrations containing a range from 0.2 to 2 mg. N/ml. from a standard ammonia solution that had been nesslerized in the same way.

Rumen volatile fatty acid analysis. Volatile fatty acid concentrations in the beet pulp study were determined by the modified method of Wiseman and Irvin (111). This method employed column partition chromatography using Celite columns with alphamine red-R as the internal indicator and acetone-petroleum ether solutions as eluents. Two ml. samples of rumen fluid were mixed with Celite and placed on the prepared column. Various percentages of acetone in petroleum ether (1, 5, 10, 15, 20, 30, and 40%) were used to elute the various organic acids. The acids separated into colored bands as they descended the column and were collected in separate receiving flasks as each band was flushed from the column. The organic acids usually collected from rumen fluid were eluted in the following order: butyric, propionic, acetic, formic, lactic, succinic acid. Columns containing a mixture of known

concentrations of these acids were run at the same time along with a blank column as a check for accuracy. The receiving flasks containing an indicator were titrated for acidity using 0.1 N KOH. The acidity from the blank flask for each acid was subtracted from that of each sample flask to determine the acid equivalents of each acid in the sample.

Rumen volatile fatty acids during the experiment when hay versus silage was fed were determined by gas chromatography. A model SRL Sargent Recorder coupled to an Aerograph model A-600-D "Hy-Fi" gas chromatograph with hydrogen flame ionization detector was employed. The absorbing column was 8 ft., 1/8 inch OD, of stainless steel packed with 20% Carbowax 20M on Chromabsorb W. The chamber was maintained at a temperature of 118° C. and the injection part at 200° C. The nitrogen carrier gas flow rate was 30 ml./min., and the hydrogen gas flow was 25 ml./min. Two to 4 ul. portions of preserved rumen fluid samples (in H_2SO_4) diluted 1:1 with distilled water were injected into the apparatus. Acetic, propionic, and butyric acids were the only acids which gave a sufficient response so that they could be measured. Calculation of the amounts of acids in the samples was done by measuring the area under the curve on the recording chart compared to areas of known amounts when standard acid

solutions were used. Area, expressed in square centimeters, was determined by measuring peak height times peak width at one half peak height. This gave a more accurate measurement than measuring peak height alone.

Measurement of rumen contents. All rumen emptyings were made 4.5 to 5 hours after feeding. Cows were weighed and returned to their stalls. The entire contents of the rumen and reticulum were removed and placed into tared quart jars. The contents were then returned to the rumen. The closed sample jars were washed of extraneous material and weighed to determine net contents. The entire contents of the jars were quantitatively transferred to tared aluminum drying pans using 150 to 200 ml. ethanol. Dry matter determinations were made as previously described.

Fiber analysis. Fiber analysis was done by the acid detergent method described by Van Soest (109).

Two grams of air dry material were weighed into a glass beaker for refluxing. A conventional crude fiber apparatus was used. One hundred ml. of 2% hexadecyltrimethylammonium bromide dissolved in 1 N H_2SO_4 were added in the beaker. Two ml. of decahydronapthalene were added as an antifoamant and to facilitate removal of pigments. The mixture was heated to boiling and refluxed on the fiber apparatus for 60 minutes. The acid-detergent fiber was filtered on a previously weighed sintered glass

crucible, using light suction. The filter mat was lightly stirred while washed with a stream of hot water (90 to 100° C.) until filtrate became free from color and foam. The contents were then washed repeatedly with cold acetone until washings became clear. The crucible was sucked free of acetone and dried in a forced draft oven at 100° C. for 2 hours, cooled in a dessicator and weighed.

Carbohydrate analysis. The phenol-sulfuric acid carbohydrate procedure by Dubois et al. (38) was used for determining carbohydrate in feed analysis, rumen carbohydrate, and carbohydrate in the in vitro fermentation trial. A brief description of this procedure follows.

The filtrate from the acid detergent fiber determination was made up to 2000 ml. with water. Five ml. of this dilution were then made up to 50 ml. by addition of water and 2 ml. were placed in a testube. One ml. of a standard 1% glucose solution in water was made up to 200 ml. Five tenths ml., 1.0, 1.5, and 2.0 ml. of this were measured into testubes and brought up to 2 ml. volume with H_2O. A blank containing 2 ml. H_2O was also included. Five hundredths ml. phenol reagent (80% phenol) was added in each tube and mixed. Using a syringe pipette, 5 ml. concentrated H_2SO_4 were rapidly added to each tube to insure mixing and additionally mixed on a Vortex mixer. After the tubes were cooled to room temperature, optical density

was read at 490 mu on the photometer. A plot was made of the optical density of the glucose standards against concentration and optical density of the sample was used to determine the amount of carbohydrate in the sample.

RESULTS

Results of the various experiments performed during these studies are divided into five sections and appear in the following order: (1) Effects of intraruminal administration of feed materials on dry matter intake. (2) Intake by rabbits fed silage juice treated pellets. (3) Factors affecting rate of beet pulp digestion and effects of beet pulp on rumen characteristics. (4) Rumen characteristics of cows fed hay or direct-cut alfalfa silage. (5) Retention time and other relationships in the rumen affected by rates of intake of roughages.

Effects of Intraruminal Administration of Feed Materials on Dry Matter Intake of Fistulated Cows

Results listing the materials administered and their effects on voluntary intake are given in Table 1. Located in column one of the table is the number of cows in each trial. The kind of feed offered is given in the second column. The material placed in the rumen is given in the third column. Average dry matter intake for six days before, eight days during, and six days after treatment are given in the next three columns. The seventh column shows the change on voluntary intake during

TABLE I

AVERAGE VOLUNTARY AND TOTAL INTAKE OF COWS WHEN SIX FEED MATERIALS WERE PLACED IN THE RUMEN

Number of Cows	Feed	Material placed in rumen	Dry Matter Consumption 6 days before	8 days during	6 days after	Change	D.M. placed into rumen	Difference
			lb/day	lb/day	lb/day	lb/day	lb/day	lb/day
1	Fresh alfalfa	Fresh alfalfa	20.55	17.58	17.15	-1.70	7.0	5.3
1	Hay	Fresh alfalfa	37.27	34.19	37.70	-3.29	4.75	1.46
2	Hay	Hay	27.23	24.48	28.06	-3.17	10.30	7.13
3	Hay	Hay	37.76	32.39	37.63	-5.31	10.44	5.13
	grain (milk production)		38.08	37.11	33.14	+1.50		
4	Hay	Beet pulp	25.61	16.11		-9.50	11.37	1.87
2	Hay	Beet pulp	35.24	31.78	37.61	-4.65	12.70	8.05
	grain (milk production)		32.02	27.99	27.65	-1.85		
2	Hay grain	Alfalfa silage	41.89	35.17	40.75	-6.15	8.26	2.11
4	Hay	Washed pressed silage	25.27	19.56	24.90	-5.52	10.24	4.72
4	Hay	Silage fluid	23.56	25.43	-25.05	+1.12	3.22	4.34
6	Hay	Silage fluid	24.94	25.95		+1.01	3.42	4.43

administration when compared to the average of pretreatment and post-treatment periods. The eighth column gives the amount of material placed in the rumen, and the last column shows the total increase in dry matter intake by the intraruminal administration of feed materials.

Placing these materials in the rumen increased total intake in all experiments as shown in Table 1, however, voluntary intake decreased in most instances.

Voluntary intake was depressed about the same when hay or fresh alfalfa was administered to cows fed hay, yet, total intake increased more when hay was placed in the rumen. More hay dry matter was placed in the rumen than dry matter as fresh alfalfa. When hay was administered to cows fed grain plus hay, their voluntary intake of hay was depressed somewhat more than in cows fed only hay (5.31 vs. 3.17 lb., $P < 0.05$). When beet pulp was administered to cows fed hay, voluntary intake of hay was significantly reduced compared to the addition of hay in the rumen (9.50 vs. 3.17, $P < 0.01$). Three cows fed hay plus grain decreased their voluntary intake 5.31 lb. when 10.44 lb. hay was given intraruminally compared to 4.65 lb. when 12.7 lb. beet pulp was given intraruminally. When 8.3 lb. alfalfa silage dry matter was given intraruminally, the voluntary intake decreased by 6.15 lb. The differences in voluntary intake between these three treatments was not statistically significant.

Two hay fed cows were used in a single reversal trial comparing intraruminal administration of two silage fractions. Administration of washed pressed silage depressed intake of hay more than the fluid portion (-5.52 vs. +1.12, $P < 0.01$). The fluid portion appeared to have a stimulating affect on intake in three out of the four trials. The washed pressed silage depressed intake slightly more than when hay was administered to these cows in an earlier trial (-5.52 vs. -3.17). A coefficient of correlation between change in intake when all materials in Table 1 were considered versus dry matter placed in the rumen was $r = 0.719$, $P < 0.05$.

Results of two single trials, when silage juice was infused in increasing amounts, are shown in Table 2. The intake figures are averages excluding the first day after each increase in fluid infused since voluntary intake was usually depressed on the first day of each change. Voluntary intake of A192 was slightly decreased at all levels of administration of the silage fluid although total intake was increased slightly because of the dry matter in the silage fluid.

Voluntary intake as well as total intake of T26 was increased while given silage fluid infusions up to 28 liters per day, but at the level of 32 liters per day this cow went "off feed." This indicated that the fluid

TABLE 2

EFFECT ON INTAKE WHEN TWO COWS WERE ADMINISTERED SILAGE JUICE

Cow No.	Silage juice	Days administered	Voluntary intake	Total intake
	liters/day		lb/day	lb/day
A192	before	0	23.3	23.30
	12	5	22.9	25.41
	16	5	20.0	23.35
	20	3	22.28	26.47
T26	before	0	33.0	33.00
	20	2	37.0	37.19
	28	3	34.3	40.20
	32	2	16.0	22.70

portion of silage had very little, if any, depressing effect on voluntary intake of hay, and not until large amounts of this fluid were given was intake depressed. This was a result of the cow going "off feed."

Voluntary intake decreased by 9.5 lb. when four cows fed hay were given beet pulp intraruminally (Table 1, item 5). The decrease nearly equalled the beet pulp dry matter added (11.37 lb.) into the rumen. Voluntary intake during the first few days of treatment was not appreciably affected, but became progressively depressed after this initial period to the end of the experimental period. In each case a build-up of beet pulp was noted in the rumen. Gross visual observations revealed an accumulation

of beet pulp in the rumens of these cows from 20 to nearly 100% at the end of the trial. This was an indication that beet pulp placed in the rumen was apparently not being digested as rapidly as the rate of input. Observations on rumen pH showed a definite decline through the treatment period. pH of rumen contents of two cows was 7.05 and 7.18 when observed before feeding on the first day. On the seventh day the pH had dropped to 6.28 and 6.30 before the time of feeding and beet pulp administration. Three to 4 hours after feeding, the pH had dropped below 6.0. pH was depressed to 4.78 in one cow with nearly 100% beet pulp in the rumen.

In the other trial when beet pulp was administered to cows fed grain and hay, such adverse affects were not observed. The data in Table 1, item 6, indicate that there was a small depression in voluntary intake which was about the same as that observed when hay was administered. There was no obvious build-up of beet pulp in the rumen of these cows, and voluntary hay intake was not progressively depressed as in the hay fed cows. Rumen pH did not show the pronounced drop that appeared in the cows fed hay alone. Before treatment rumen pH in one cow was 7.18 while on the 7th day before feeding and treatment the pH was 6.56, also, the total dry matter intake of the cows fed grain plus hay was increased during treatment by

8.05 lb. compared to only 1.87 lb. for cows fed hay. The cows fed hay appeared to decrease voluntary intake to compensate for the additional dry matter administered intraruminally, while the cows fed grain plus hay had no such voluntary compensation.

Milk production decreased both times that the cows fed hay plus grain were given beet pulp even though total dry matter intake increased approximately 8 pounds; however, milk production increased slightly during administration of hay to three cows fed hay and grain.

Intake by Rabbits
Fed Silage-Juice Treated Pellets

Table 3 gives the data on intake and weight gain of each rabbit when fed the treated and control pellets. Analyses of variance of the data are shown in Appendix Table I.

The analysis of feed intake indicates no significant differences, however, there was slightly less consumption of the silage juice treated pellets than of the control pellets (440.4 gm./day vs. 467.6 gm./day). When the rabbits were fed the silage juice soaked pellets, their pellet intake during the first four days was less than in the last four days. This could have been caused by the rabbits not being accustomed to the taste of such

Table 3

DRY MATTER INTAKE AND WEIGHT GAINS BY RABBITS FED PELLETS TREATED WITH SILAGE JUICE VS CONTROL PELLETS

Dry Matter Intake (gm/4 day)

		Rabbit No.	Silage Juice Pellets	Water Pellets
Group 1	1st 4 days	275	297	396
		271	394	543
		272	443	612
		Average	378	517
	2nd 4 days	275	434	391
		271	503	541
		272	595	532
		Average	511	488
Group 2	1st 4 days	258	292	286
		268	504	419
		273	447	416
		Average	414	374
	2nd 4 days	258	292	350
		268	527	558
		273	557	567
		Average	459	492
Average per treatment			440.4	467.6

Weight Gains (gm./treatment)

	Rabbit No.	Silage Juice Pellets	Water Pellets
Group 1	275	108.0	32.5
	271	196.0	109.0
	272	120.0	95.0
	Average	141.3	78.8
Group 2	258	21.0	6.5
	268	69.5	142.5
	273	110.0	164.0
	Average	66.8	104.3
Average per treatment		104.08	96.58

pellets, but after becoming accustomed to them the rabbits ate as much of the silage juice pellets as of the control pellets.

Rabbit 258 would not eat the silage juice treated pellets during the first four day period, however, during the second four days this rabbit ate them fairly well. The intake of the second four days was substituted into the first period of the analysis for this rabbit to avoid unfair biasing of the analysis in favor of the water soaked pellets.

Weight gains averaged slightly higher during the period when silage juice treated pellets were fed than when the control pellets were fed (104.08 gm. vs. 96.58 gm.). This difference was not significant. Apparently the variability in weight gains of rabbits on both rations was too great to show any real differences between the treatments. Results from this trial indicated very little difference in feed dry matter intake or weight gains when rabbits were fed silage juice treated pellets or the control pellets.

Factors Affecting Rate of Beet Pulp Digestion and Effects of Beet Pulp on Rumen Characteristics

A graphic presentation of the percent beet pulp in the rumen, rumen pH, pounds of beet pulp placed in the rumen, and voluntary intake of dry matter of cows used in this investigation is shown in Fig. 1. Concentrations of uronic acids and total soluble carbohydrates in rumen fluid from three cows administered beet pulp intraruminally is shown in Appendix Table II. This includes data from the day before treatment, the first day and the seventh day of treatment. Samples preserved with H_2SO_4 and ethyl alcohol were averaged, since the two samples were found to be closely correlated. Uronic acid concentrations between the two samples had a correlation coefficient of 0.987. A comparison of the means by the "t" test showed that they were not different ($P < 0.01$). The correlation coefficient for concentration of total carbohydrate was 0.98 between samples preserved with H_2SO_4 and ethanol. The mean concentration for the ethanol preserved samples was not significantly different than that of the H_2SO_4 preserved samples ($P < 0.01$). With this evidence that the samples preserved both ways were not different, they were considered as duplicates and averaged. This was considered desirable for greater accuracy. Tables 4 and 5 give the average values of con-

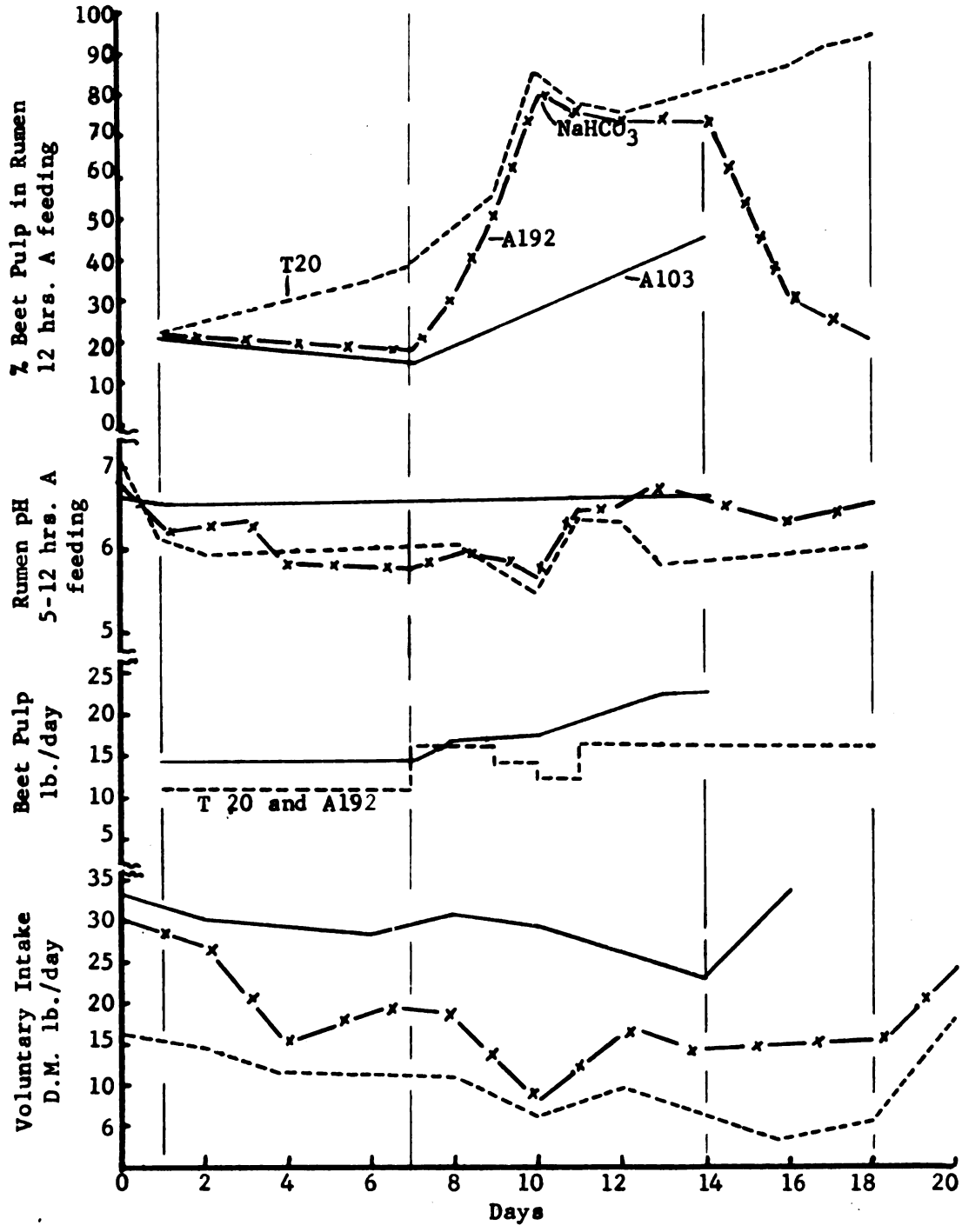


Figure 1. The effects of intraruminal administration of beet pulp on percent beet pulp in rumen, rumen pH, and voluntary dry matter intake of three cows; A103 fed grain and hay, A192 and T20 fed hay.

TABLE 4

RUMEN URONIC ACID CONCENTRATIONS FOR THE THREE DAYS AND SAMPLE TIMES INDICATED IN RELATION TO INTRARUMINAL BEET PULP ADMINISTRATION TO EACH OF THREE COWS

Hours	Cow No.	Control Day	Day 1	Day 7	Average
		mg./ml.	mg./ml.	mg./ml.	mg./ml.
- .1	A103	.136	.120	.101	.119
	A192	.122	.130	.116	.123
	T20	.101	.127	.144	.372
					.122
1.5	A103	.155	.632	.222	.336
	A192	.164	.894	.136	.398
	T20	.102	.507	.715	.441
					.392
2.5	A103	.117	.200	.140	.152
	A192	.168	.290	.157	.205
	T20	.099	.156	.160	.138
					.165
3.5	A103	.131	.154	.126	.137
	A192	.128	.197	.174	.166
	T20	.085	.163	.162	.137
					.147
4.5	A103	.120	.136	.119	.125
	A192	.127	.150	.154	.144
	T20	.085	.152	.170	.135
					.135
Average		.613	1.336	.932	
Average	A103	.132	.248	.142	
	A192	.142	.332	.147	
	T20	.094	.221	.270	

Analysis of Variance Table

Source	Sum of Squares	DF	Mean Square	F Ratio	Sig.
Day	0.1574	2	0.0787	4.575	0.05
Time	0.4582	4	0.1145	6.657	0.01
Cow	0.0085	2	0.0042	0.244	n.s.
D x T	0.3009	8	0.0376	2.186	n.s.
D x C	0.084	4	0.0210	1.22	n.s.
T x C	0.018	8	0.0022	0.128	n.s.
Error	0.2767	16	0.0172		
Total	1.2119	44			

TABLE 5

RUMEN TOTAL SOLUBLE CARBOHYDRATES CONCENTRATIONS FOR THE THREE DAYS AND SAMPLE TIMES INDICATED IN RELATION TO INTRARUMINAL BEET PULP ADMINISTRATION TO EACH OF THREE COWS

Hours	Cow No.	Control Day	Day 1	Day 7	Average
		mg/ml.	mg./ml.	mg/ml.	mg/ml.
-0.1	A103	.661	.660	.757	.693
	A192	.305	.274	.473	.351
	T20	.242	.229	.876	.449
					.497
1.5	A103	.837	6.416	1.920	2.480
	A192	.341	2.080	.582	1.001
	T20	.270	1.332	1.697	1.099
					1.527
2.5	A103	.648	1.690	1.101	1.146
	A192	.479	.783	.584	.615
	T20	.234	.380	.585	.400
					.720
3.5	A103	.720	.968	.807	.832
	A192	.348	.511	.526	.462
	T20	.199	.395	.406	.333
					.542
4.5	A103	.690	1.139	1.031	.953
	A192	.340	.362	.483	.395
	T20	.234	.402	.520	.385
					.578
Average		.437	1.175	.708	
Average	A103	.711	2.175	1.778	
	A192	.362	.802	.530	
	T20	.236	.548	.871	

Analysis of Variance Table

Source	Sum of Squares	D.F.	Mean Square	F Ratio	Signif.
Day	4.1824	2	2.0912	3.7443	0.05
Time	6.7529	4	1.6882	3.0227	0.05
Cow	4.5241	2	2.2620	4.051	0.05
DxT	10.3969	8	1.2996	2.3269	----
DxC	3.9843	4	.9960	1.7833	----
TxC	1.5961	8	.1996	.3573	----
Error	8.9373	16	.5585		
Total	40.3740	44			

centrations in the rumen samples preserved both ways. These tables give average concentrations for each cow on each day of sampling and for times during the day. Analysis of variance is also shown in these tables.

Uronic acids in the rumen were higher ($P < 0.05$) on day one of treatment than on control day for all cows (1.336 vs. 0.613, Table 4). These averages include the -.1 hour sample. At 1.5 hours after feeding, rumen uronic acids were much higher on day one than on control day for all cows. However, on day 7, these concentrations were near control levels for A192 and A103, but had increased in T20. Average rumen uronic acid concentrations were lower for A103 and A192 on the seventh day post-treatment than on the first day of treatment. These values indicated that these 2 cows were breaking down the pectins in beet pulp more rapidly on the seventh day than on the first day.

A positive coefficient of correlation between uronic acid and total carbohydrate values determined from rumen fluid samples preserved in H_2SO_4 was 0.6424 ($P < 0.01$).

Concentration of total soluble carbohydrate in rumen fluid on day one of treatment was higher than on the control day for all cows (1.175 > 0.437, $P < 0.05$). Total soluble carbohydrate values were also higher on day

one than on day seven for cows A103 and A192 (2.175 and 0.802 vs. 1.778 and 0.530). These comparisons indicated that there was a more rapid disappearance of total carbohydrates on the seventh day of treatment than on the first day for these two cows. This could be interpreted to indicate some type of adaptative process was taking place in these two cows. Total soluble carbohydrate values were higher on day seven for T20 than on day one indicating that this "cow" did not adapt to more rapid digestion of beet pulp (0.871 › 0.548).

Visual observation of beet pulp in the rumen contents confirmed the above findings. The top graph of Fig. 1 shows that beet pulp appeared to be accumulating in the rumen of T20 by the seventh day while the amounts were declining for A103 and A192.

Rumen volatile fatty acid concentrations listed in Table 6 show no particular trends associated with the beet pulp treatment. The acetate-to-propionate ratio likewise shows no trend. The lactate concentration was highest on day one for A103 while for A192 and T20 the lactate concentration was highest on the seventh day.

The amount of beet pulp administered to A103 was increased to 16 pounds on the seventh day of treatment and increased to 22 pounds per day on the thirteenth and fourteenth days to observe the build-up of beet pulp in

TABLE 6

RUMEN VOLATILE FATTY ACID CONCENTRATIONS BEFORE FEEDING AND AVERAGE CONCENTRATIONS AFTER FEEDING FOR THE THREE DAYS IN RELATION TO INTRARUMINAL BEET PULP ADMINISTRATION TO EACH OF THREE COWS

Cow	Day	Hour	Acetate	Propionate	Lactate	Ac./Prop.	Total Acids
			molar %	molar %	molar %		uM/ml.
A103	7	0	72	16	--	4.50	107.8
	0	1.5-3.5	71	16	.07	4.44	100.5
	1	" "	71	17	.15	4.18	108.4
	7	" "	68	22	.09	3.05	138.7
A192	0	0	69	19	.06	3.63	71.5
	1	0	60	23	.06	2.61	60.1
	7	0	67	19	.08	3.53	125.4
	0	1.5-4.5	69	21	.08	3.29	136.5
	1	" "	73	15	.08	4.87	141.7
	7	" "	72	18	.14	4.00	140.0
T20	0	0	68	21	.05	3.24	64.2
	1	0	65	23	.05	2.83	74.0
	7	0	65	23	.05	2.83	133.5
	0	1.5-4.5	58	33	.03	1.76	92.8
	1	" "	68	24	.17	2.83	114.0
	7	" "	70	18	.41	3.88	145.0

the rumen. The top graph of Fig. 1 shows that on the tenth day the rumens of both cows contained about 80% beet pulp. Rumen pH at this point was considerably depressed. On the ninth day low pH readings of 5.0 and 5.45 were observed in A192 and T20, respectively.

Microscopic observation of rumen fluid samples from these cows revealed that the protozoa population seemed to be sensitive to low pH in the rumen. Table 7 shows the microflora changes in the rumens of A192 and T20 on days that microscopic observations were made. By the tenth day no protozoa were visible in the rumen samples from A192, while a greatly reduced number was visible in samples from T20. On the 12th day protozoa numbers in the rumen of T20 had returned to normal. A slight reduction in the percent of beet pulp in the rumen ingesta and a rise in pH was also noted at this time. This trend was only transient, however. On the thirteenth day total microflora numbers had reduced considerably accompanied by an increased accumulation of beet pulp in the rumen and a decreased rumen pH. This condition continued to progress for the next five days until the end of the experiment.

On the tenth day $NaHCO_3$ was added in the rumen of A192 to bring the pH up to 6.4 - 6.6. This was a normal post feeding pH for this cow before initiation of the

TABLE 7

GENERAL MICROSCOPIC OBSERVATIONS OF RUMEN MICROFLORA OF TWO COWS WHILE RECEIVING LARGE AMOUNTS OF BEET PULP FOR ELEVEN DAYS

Day of treatment	Protoza		Short rods		Total numbers	
	A192	T20	A192	T20	A192	T20
6	2*	3	3*	3	3*	3
9	0	1	2	2	2	3
11	0	1	2	2	2	2
12	1	3	2	3	2	3
13	3	3	3	3[a]	3	2
16	3	2	3	2	3	1

* 3 normal numbers

2 reduced numbers

1 very few

0 none visible

[a] Large numbers of short rods were clumped around feed particles.

beet pulp treatment. Beet pulp accumulation in the rumen of A192 was not reduced markedly until after the fourteenth day. Rumen microflora numbers did not return to normal until the thirteenth day and remained normal through the remainder of the treatment period. After the fourteenth day the percent of beet pulp in the rumen rapidly decreased. This seemed to be associated with the increased rumen pH and normal rumen microflora numbers. The chart at the bottom of Fig. 1 shows that voluntary dry matter intake returned to the pretreatment level in two days for cows A103 and T20, while A192 had not yet reached this level on the second day after the end of treatment.

The results of this trial suggest that rumen microflora, especially protozoa, are sensitive to low rumen pH. This is in agreement with published information (92). It was observed that both numbers and activity of microflora were greatly reduced when the pH decreased to 5.5 or below for a portion of the day. Under these conditions there was a visible increase in percentage of beet pulp in the rumen ingesta. This could be the result of a decreased rate of breakdown of the beet pulp. More work is necessary in order to understand why addition of beet pulp depressed the pH in the rumen of cows fed only hay while in that of cows fed grain and hay there was very little change in rumen pH.

Data giving changes in pH, carbohydrates, and uronides when rumen fluid from different cows was added to a beet pulp substrate and fermented in vitro are shown in Table 8. Rumen fluid samples were obtained from cows A192 and T20 on the 17th day of intraruminal administration of beet pulp; two cows, 174 and 188, fed hay, grain, and beet pulp for a month; two cows, K139 and T26, fed only hay; and two other cows, A103 and T3, fed hay and grain. The last four cows had not received beet pulp for at least a month prior to the trial.

Analyses of variance of the results of the in vitro fermentation trial are given in Table 9. In the analysis of variance table when samples from cows receiving beet pulp were compared to those not receiving beet pulp, the groups were subdivided into cows fed hay and cows fed grain. This was considered as four treatments. In the other analyses samples from cows fed hay and grain were compared to samples from cows fed hay and considered as two treatments.

pH changes from the initial to the final pH were not significantly different in fermentations from cows receiving beet pulp and those not receiving beet pulp.

Total solids remaining were used as an indication of beet pulp substrate disappearance. These values did not show any consistant difference between fermentations

TABLE 8

SUMMARY OF RESULTS OF BEET PULP IN VITRO FERMENTATION TRIAL USING RUMEN FLUID FROM COWS FED HAY, WITH AND WITHOUT BEET PULP AND COWS FED GRAIN + HAY, WITH OR WITHOUT BEET PULP

	Cows fed hay				Cows fed hay and grain			
	A192	T 20	K139	T26	A103	T3	174	188
	Beet pulp intraruminally						feed beet pulp	
Original pH	6.54	5.91	6.72	6.60	6.48	6.53	7.05	7.08
Final pH	5.88	5.70	6.21	5.99	5.82	6.02	6.34	6.37
Total solids remaining (%)	17.0	14.0	13.5	15.0	9.0	14.5	8.0	8.5
Gas produced	120 ml	62 ml	86 ml	86 ml	112 ml	120 ml	114 ml	84 ml
Total carbohydrates								
in original R.F. g.	1.14	1.35	.73	.90	2.36	2.63	1.48	.98
in subst. g.	.85	.85	.85	.85	.85	.85	.85	.85
total g.	1.99	2.20	1.58	1.75	3.21	3.48	2.33	1.83
remaining g.	1.54	1.66	1.15	1.30	2.00	2.46	1.70	1.13
disappearance g.	.45	.54	.43	.45	1.21	1.02	.63	.70
Disappearance %	22.6	24.5	27.2	25.7	37.7	29.3	27.0	38.3
Total uronides								
in original R.F. mg.	99.5	100.0	57.3	71.3	137.0	168.6	105.0	72.6
in subst. mg.	104.0	104.0	104.0	104.0	104.0	104.0	104.0	104.0
total mg.	203.5	204.0	161.3	175.3	241.0	272.6	209.0	176.6
remaining mg.	161.0	137.4	140.0	127.6	198.0	190.0	132.6	143.6
disappeared mg.	42.5	66.6	21.3	47.7	43.0	82.6	76.4	33.0
Disappearance %	24.3	32.6	13.2	27.2	17.8	30.3	36.5	18.7
Soluble uronides								
in original R.F. mg.	32.7	27.6	27.4	32.4	27.5	22.5	19.8	17.1
in subst. mg.	48.0	48.0	48.0	48.0	48.0	48.0	48.0	48.0
total mg.	80.7	75.6	74.4	80.4	75.5	70.5	67.8	65.1
remaining mg.	27.4	20.4	23.6	27.8	23.2	13.9	14.4	13.1
disappeared mg.	53.3	55.2	50.8	52.6	52.3	56.6	53.4	52.0
Disappearance %	65.7	73.0	68.3	65.4	69.3	80.2	78.8	79.9

TABLE 9
ANALYSES OF VARIANCE OF RESULTS OF THE BEET PULP IN VITRO FERMENTATION TRIAL

	A192	T20	K139	T26	A103	T3	174	188
Change in pH	-.66	-.21	-.51	-.61	-.66	-.51	-.71	-.71

Beet pulp vs no beet pulp

Source	DF	S. Squares	Mean Squares	F Ratio	Signif.
Treatment	3	0.0763	.0254	.8668	n.s.
Residual	4	0.1175	.0293		
Total	7	0.1938			

Total solids Remaining	17.0	14.0	13.5	15.0	9.0	14.5	8.0	8.5

Grain + hay fed vs hay fed alone

Treatment	1	47.53	47.53	8.22	0.05
Residual	6	34.69	5.78		
Total	7	82.22			

Beet pulp vs no beet pulp

Treatment	3	2.53	.843	.04	n.s.
Residual	4	79.69	19.92		
Total	7	82.22			

Gas produced	120 ml.	62 ml.	86 ml.	86 ml.	112 ml.	120 ml.	114 ml.	84 ml.

Grain + hay fed vs hay fed alone

Treatment	1	722.0	722.0	1.748	n.s.
Residual	6	2478.0	413.0		
Total	7	3200.0			

Total CHO % Disappear	22.6	24.5	27.2	25.7	37.7	29.3	27.0	38.3

Grain + hay fed vs hay fed alone

Treatment	1	129.09	129.09	6.88	.05
Residual	6	112.51	18.75		
Total	7	241.60			

Beet pulp vs no beet pulp

Treatment	3	139.55	46.52	1.8235	n.s.
Residual	4	102.05	25.51		
Total	7	241.60			

Total Uronides	24.3	32.6	13.2	27.2	17.8	30.3	36.5	18.7

Beet pulp vs no beet pulp

Treatment	3	85.17	28.39	.3079	n.s.
Residual	4	368.99	92.20		
Total	7	454.16			

Soluble Uronides % Disappear	65.7	73.0	68.3	65.4	69.3	80.2	78.8	79.9

Grain + hay fed vs hay fed alone

Treatment	1	160.25	160.25	8.1345	0.05
Residual	6	118.25	19.7		
Total	7	278.50			

from cows receiving beet pulp and those not receiving beet pulp (13.4% vs. 11.9%). There was, however, less substrate remaining in the fermentations using rumen fluid from cows fed grain than by using rumen fluid from cows fed hay (10.0% > 14.9%, $P < 0.05$).

Gas production, which was an indication of fermentation activity, was too variable between samples to show any trend, but gas production was much less in the fermentation flask representing cow T20 than from any other flask.

Disappearance of total carbohydrates was greater in fermentations using rumen fluid from cows fed grain than in those using rumen fluid from cows fed hay (33.1% > 25.0%, $P < 0.05$). There was not a significantly greater disappearance of total carbohydrates using rumen fluid from cows fed beet pulp than by those not receiving beet pulp (28.3% vs. 30.0%).

Disappearance of total uronides was too variable to show any trend. In spite of a difference of 6% between cows receiving beet pulp and those not receiving beet pulp (28.2 vs. 22%) the variability was too great to be statistically significant.

Disappearance of soluble uronides was greater in fermentations using rumen fluid from cows fed grain than in those using rumen fluid from cows fed hay (77.05% > 68.1%,

$P < 0.05$). The differences between fermentation flasks from cows receiving beet pulp and those not were not significantly different (74.3% vs. 70.8%).

These results indicated that in general rumen fluid from cows receiving beet pulp did not ferment beet pulp more rapidly than rumen fluid from cows not receiving beet pulp. These results do show, however, that rumen fluid from cows fed grain digest beet pulp more rapidly than rumen fluid from cows fed only hay. The results of the in vitro fermentation failed to show a difference in the rate of fermentation of beet pulp between cows A192 and T20. Visual observation indicated that beet pulp was disappearing more rapidly from the rumen of A192 which had been receiving $NaHCO_3$ for seven days before this sample was obtained.

Rumen Characteristics of Cows Fed Hay or Direct-cut Alfalfa Silage

Data on rumen characteristics of two cows fed hay or direct-cut alfalfa silage each at three different levels in a reversal experiment are shown in Tables 10, 11, 12, and 13; and in Appendix Tables III, IV, and V; and in Figs. 2 and 3.

Rumen pH, NH_3, and volatile fatty acid concentrations were determined and analyzed statistically by

TABLE 10

RUMEN PH IN COWS FED SILAGE VS HAY AT THREE LEVELS AND SIX TIMES DURING THE DAY

Hrs. After Feeding	Low Intake				Medium Intake				High Intake				Average
	Hay		Silage		Hay		Silage		Hay		Silage		
	T19	T23	T19	T23	T19	T23	T19	T23	T19	T23	T19	T23	
1.5	6.95	7.05	7.20	6.50	6.89	6.88	6.82	6.50	6.68	6.90	6.83	6.69	6.89H 6.76S
2.5	6.95	7.18	7.05	6.60	6.88	6.86	6.80	6.51	6.71	6.85	6.50	6.60	6.90H 6.68S
3.5	6.85	6.98	7.06	6.55	6.86	6.65	6.77	6.43	6.58	6.74	6.74	6.62	6.78H 6.71S
4.5	6.78	7.07	7.14	6.53	6.77	6.70	6.73	6.35	6.56	6.77	6.71	6.70	6.77H 6.69S
6.5	6.55	7.02	7.09	6.50	6.78	6.58	6.74	6.26	6.55	6.66	6.35	6.65	6.69H 6.60S
12.0	6.37	7.11	6.99	6.62	6.80	6.82	6.60	6.30	6.12	6.43	6.05	6.49	6.61H
Average	6.74	7.07	7.09	6.55	6.83	6.75	6.74	6.39	6.53	6.72	6.53	6.62	6.51S
Average		6.90		6.82		6.79		6.56		6.62		6.57	

Analysis of Variance Table (Rumen pH)

Source	D.F.	Sum of Squares	Mean Square	F	Signif.
Feed	1	.26	.26	26.1	.01
Time	5	.581	.1162	11.7	.01
Animal	1	.0695	.0695	6.9	.05
Level	2	.856	.428	43.0	.01
F x T	5	.047	.0094	.9	n.s.
F x A	1	.7565	.7565	76.1	.01
F x L	2	.095	.0475	4.8	.05
T x A	5	.16295	.03259	3.2	n.s.
T x L	10	.2533	.02533	2.5	n.s.
A x L	2	.4027	.20135	20.2	.01
F x T x A	5	.01788	.003576	.3	n.s.
F x T x L	10	.09035	.00905	.9	n.s.
F x A x L	2	.48921	.244605	24.6	.01
T x A x L	10	.0882	.0882	8.8	.01
Error	10	.09931	.009931		
Total	71	4.2689			

TABLE 11

RUMEN NH_3 IN COWS FED SILAGE VS HAY AT THREE LEVELS SIX TIMES DURING THE DAY

Hrs. After Feeding	Low Intake				Medium Intake				High Intake				Average
	Hay		Silage		Hay		Silage		Hay		Silage		
	T19 mg/ml	T23	T19	T23	T19 mg/ml	T23	T19	T23	T19 mg/ml	T23	T19	T23	
1.5	.297	.249	.319	.443	.328	.479	.431	.589	.385	.431	.408	.613	.361H .467S
2.5	.376	.216	.375	.425	.273	.364	.229	.698	.346	.327	.370	.692	.317H .464S
3.5	.376	.418	.363	.446	.238	.334	.249	.619	.322	.273	.364	.350	.326H .390S
4.5	.208	.078	.350	.407	.170	.252	.227	.747	.234	.162	.212	.480	.184H .403S
6.5	.337	.016	.216	.344	.083	.114	.218	.769	.212	.088	.255	.467	.141H .379S
12.0	.089	.066	.018	.236	.117	.216	.200	.347	.140	.079	.234	.392	.117H
Average	.280	.174	.274	.385	.202	.293	.259	.6281	.2731	.227	.307	.499	.237S
Average		.214		.329		.247		.443		.250		.403	

Analysis of Variance Table (Rumen NH_3)

Source	D.F.	Sum of Squares	Mean Square	F	Signif.
Feed	1	0.40635	.40635	64.4	.01
Time	5	0.4822	.09644	15.2	.01
Animal	1	0.1858	.1858	29.4	.01
Level	2	0.05896	.02948	4.6	.05
F x T	5	0.06406	.01281	2.0	--
F x A	1	0.26825	.26825	44.4	.01
F x L	2	0.0266	.0133	2.1	--
T x A	5	0.00524	.00104	.1	--
T x L	10	0.07454	.00745	1.1	--
A x L	2	0.16389	.08194	13.0	.01
F x T x A	5	0.06446	.01289	2.0	--
F x T x L	10	0.02061	.00206	0.3	--
F x A x L	2	0.00338	.00169	0.2	--
T x A x L	10	0.07412	.00741	1.1	--
Error	10	0.06303	.00630		
Total	71	1.96152			

TABLE 12. AVERAGE RUMEN VOLATILE FATTY ACID CONCENTRATIONS FOR COWS FED HAY VS. SILAGE

Hrs.A.feed	1.5		2.5		3.5		4.5		6.5		12.0	
	Hay uM/ml.	Silage uM/ml.	Hay uM/ml.	Silage uM/ml.	Hay uM/ml.	Silage uM/ml.	Hay uM/ml.	Silage uM/ml.	Hay uM/ml.	Silage uM/ml.	Hay uM/ml.	Silage uM/ml.
Acetate	64.0	62.0	65.0	62.0	65.0	61.0	64.0	61.0	79.0	67.0	78.0	63.0
	63.0		64.0		63.0		63.0		73.0		71.0	
	Low level				Medium level				High level			
	Hay	Silage			Hay	Silage			Hay	Silage		
	53.6	55.5			69.0	66.6			85.5	66.6		
	54.5				67.8				76.0			
Propionate	14.0	16.0	14.0	16.0	13.0	15.0	13.0	15.0	13.0	15.0	17.0	17.0
	15.1		14.9		14.0		13.8		16.8		15.3	
	Low level				Medium level				High level			
	Hay	Silage			Hay	Silage			Hay	Silage		
	10.8	13.6			13.5	17.0			18.9	16.2		
	12.2				15.2				17.6			
Butyrate	4.0	6.0	4.3	6.3	3.8	6.0	3.8	6.2	5.8	3.7	5.8	6.8
	5.0		5.33		4.91		5.0		6.5		6.33	
	Low level				Medium level				High level			
	Hay	Silage			Hay	Silage			Hay	Silage		
	2.75	3.33			3.92	7.58			7.12	8.33		
	3.04				5.75				7.75			

TABLE 13 . AVERAGE ACETATE-TO-PROPIONATE AND 2-TO-3 CARBON VFA RATIOS FOR COWS FED HAY VS SILAGE

Hrs.A.feed	1.5		2.5		3.5		4.5		6.5		12.0	
	Hay	Silage	Hay	Silage	Hay	Silage	Hay	Silage	Hay	Silage	Hay	Silage
Acetate P. Propionate	4,48	3.92	4.74	4.01	5.27	4.08	5.09	4.24	4.88	4.14	5.20	4.38
	4.200		4.373		4.672		4.662		4.509		4.790	

Low level		Medium level		High level	
Hay	Silage	Hay	Silage	Hay	Silage
5.06	4.13	5.20	3.99	4.58	4.28
4.589		4.588		4.426	

2/3 Carbon	1.5		2.5		3.5		4.5		6.5		12.0	
	Hay	Silage	Hay	Silage	Hay	Silage	Hay	Silage	Hay	Silage	Hay	Silage
	5.01	4.69	5.33	4.71	5.85	4.74	5.53	5.07	5.47	5.10	5.89	5.25
	4.849		5.018		5.295		5.300		5.287		5.570	

Low level		Medium level		High level	
Hay	Silage	Hay	Silage	Hay	Silage
5.53	4.63	5.75	4.90	5.25	5.24
5.0837		5.3279		5.2487	

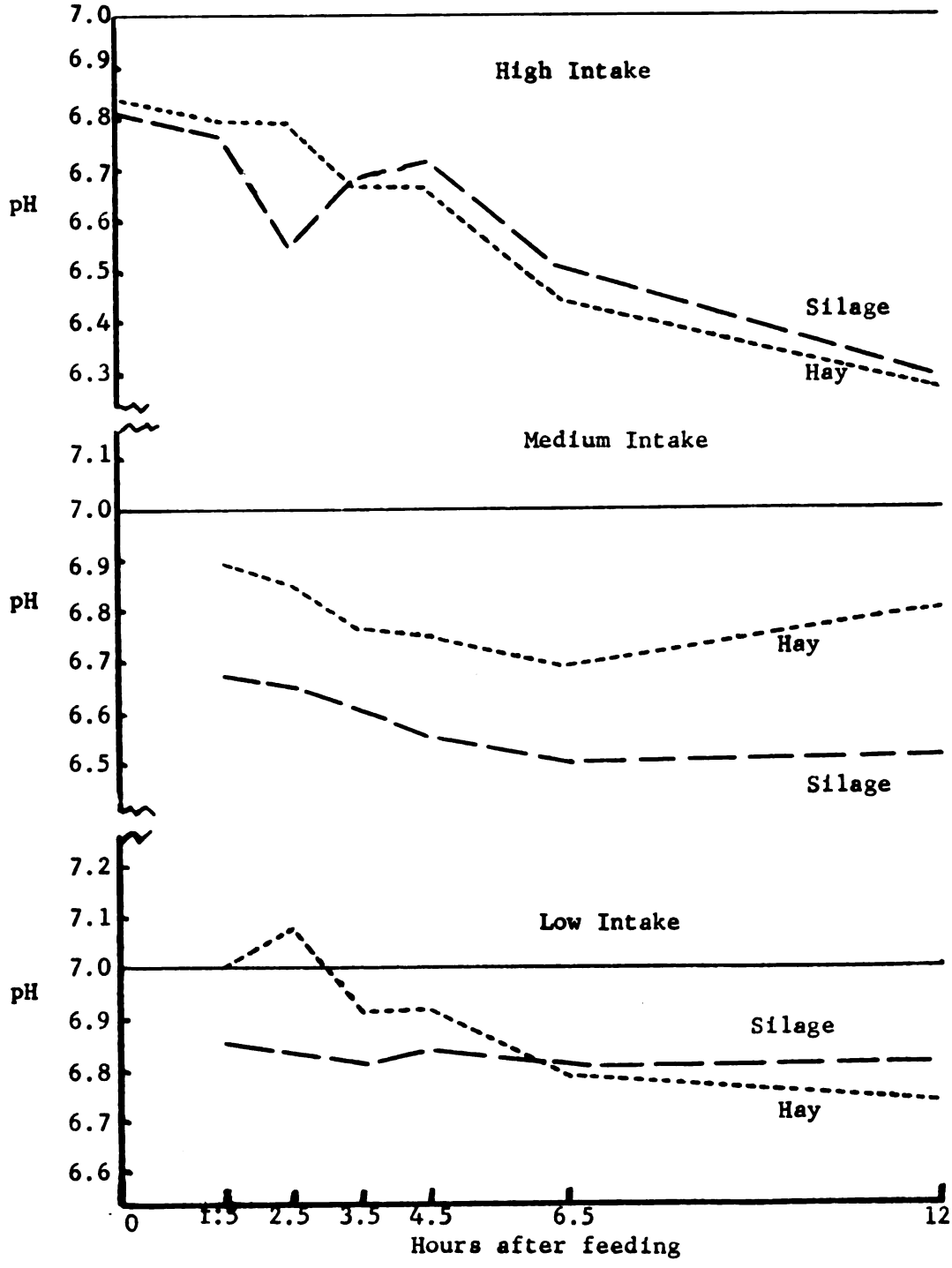


Figure 2. Average postprandial rumen pH of two cows fed alfalfa hay or direct-cut alfalfa silage at three levels; 5 lb. D.M./day, 12 lb. D. M./day, and ad libitum.

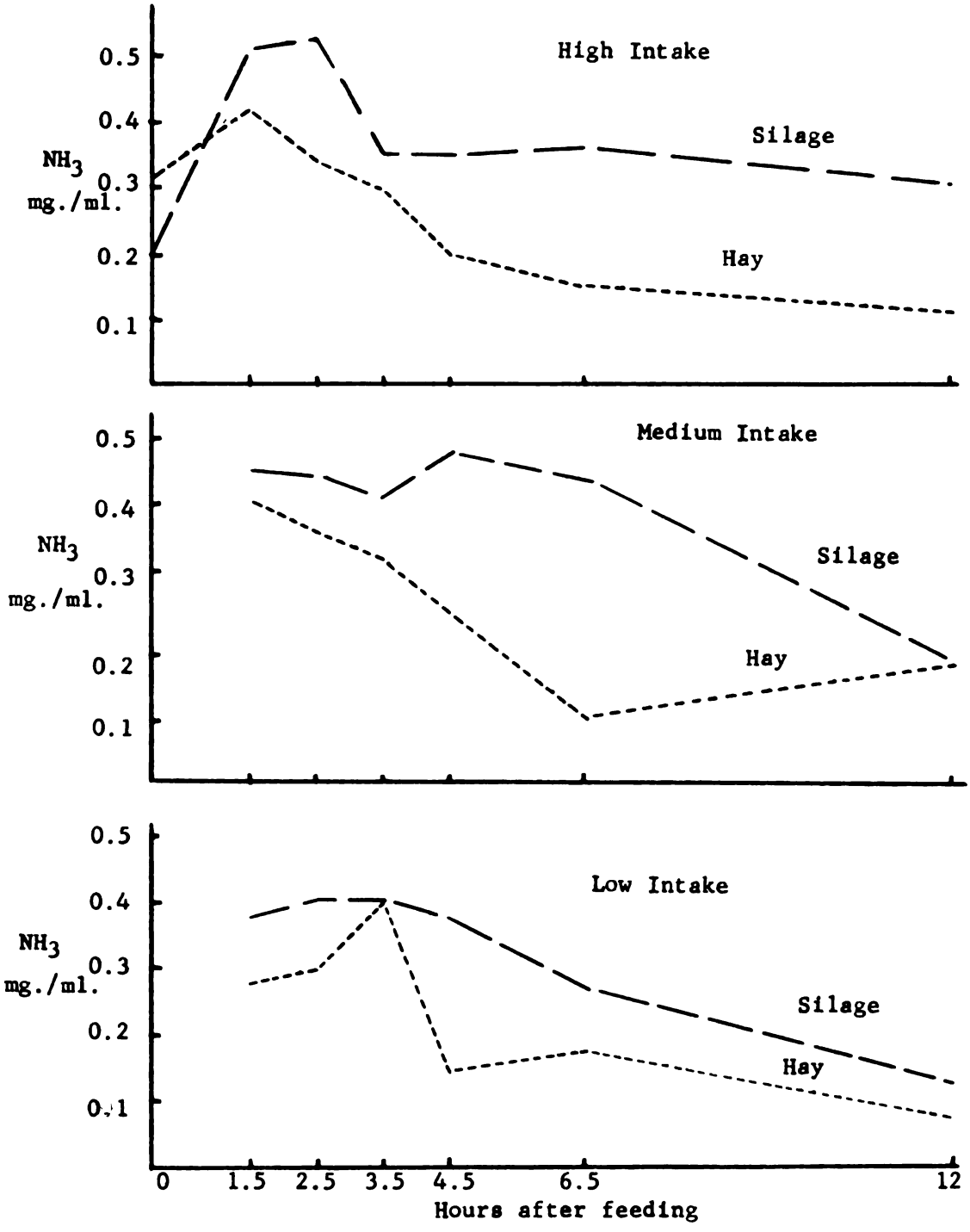


Figure 3. Average postprandial rumen ammonia concentrations of two cows fed alfalfa hay or direct-cut alfalfa silage at three levels; 5 lb. D.M./day, 12 lb. D.M./day, and ad libitum.

analysis of variance and are given in Tables 10 and 11. The usefulness of these analyses for interpreting the results of this experiment were somewhat limited. Rumen fluid samples were taken only one day on each level of feeding and the use of only two animals limited the number of observations for establishing average concentration values on any particular level of feeding of hay or silage. The time element in carrying out the sequences of feeds and feeding levels also may have confounded results somewhat. Aside from this, another animal was substituted for T19 on the high level of silage feeding and this may have complicated results at the high level of feeding. T19 at this time whould not consume silage in high amounts because of some "off feed" condition.

Rumen pH values, shown in Table 10 and Fig. 2, declined at much the same rate during the day when the cows were fed hay or silage. The pH values at 1.5 and 2.5 hours after feeding were higher when the cows were fed hay than when fed silage at all levels of intake. The average pH of the rumen was higher for cows fed hay than for cows fed silage. The analysis of variance given in Table 10 showed that rumen pH was influenced significantly ($P < 0.01$) by several items.

Rumen ammonia concentrations shown in Table 11 and Fig. 3 were consistantly higher throughout the day when cows were fed silage than when fed hay. This occurred at

all levels of feeding. On the medium and high levels of silage feeding the NH_3 concentrations reached their peak 2 to 2.5 hours after feeding, and a smaller peak was noted 5 to 6 hours after feeding. When hay was fed at medium and high levels, NH_3 concentrations were highest 1.5 hours after feeding and continued to decline gradually throughout the day. The analysis of variance given in Table 11 showed that rumen ammonia concentrations were affected significantly ($P < 0.01$) by several items.

Rumen volatile fatty acid concentrations shown in Appendix Table III had a rather large range of values between cows on the different levels of feeding and showed no consistant differences in amounts when cows were fed hay or silage. Concentrations of acetate, propionate, and butyrate appeared to increase as the feed intake increased. These average concentrations in rumen fluid are shown in Table 12. Concentrations of these acids were not significantly different since individual animals responded differently at the three levels of intake. The results of the analysis of variance of these volatile acids are shown in Appendix Table IV. The concentration of butyrate was consistantly higher when silage was fed at all levels than when hay was fed. The average acetate-to-propionate ratio was consistantly higher when cows were fed silage at all levels and at all times of the day.

The 2-to-3 carbon ratio was calculated assuming that a mole of butyrate is broken down into two moles of acetate. The 2-to-3 carbon ratio averaged higher in fluid from animals fed hay at the low and medium levels of feeding, while at the high level there was no difference in this ratio when hay or silage was fed. Averages of the acetate-to-propionate ratio and 2-to-3 carbon ratio for the three levels of feeding and six times of the day are given in Table 13. Analyses of variance for these ratios are shown in Appendix Table V. These ratios appeared to be significantly affected by differences between animals as well as differences between the hay and silage.

Retention Time and Other Relationships in the Rumen Affected by Rates of Intake of Roughages

The combined data representing 32 rumen emptyings using eight rumen fistulated non-lactating cows and given six different roughage rations are located in Appendix Table VI. Rumen retention times of roughage dry matter and fiber over a wide range of intake are shown in Figs. 4 and 5.

The ratio of dry matter in the rumen divided by daily dry matter intake was used as a measure of rumen retention time. The data indicate that retention time

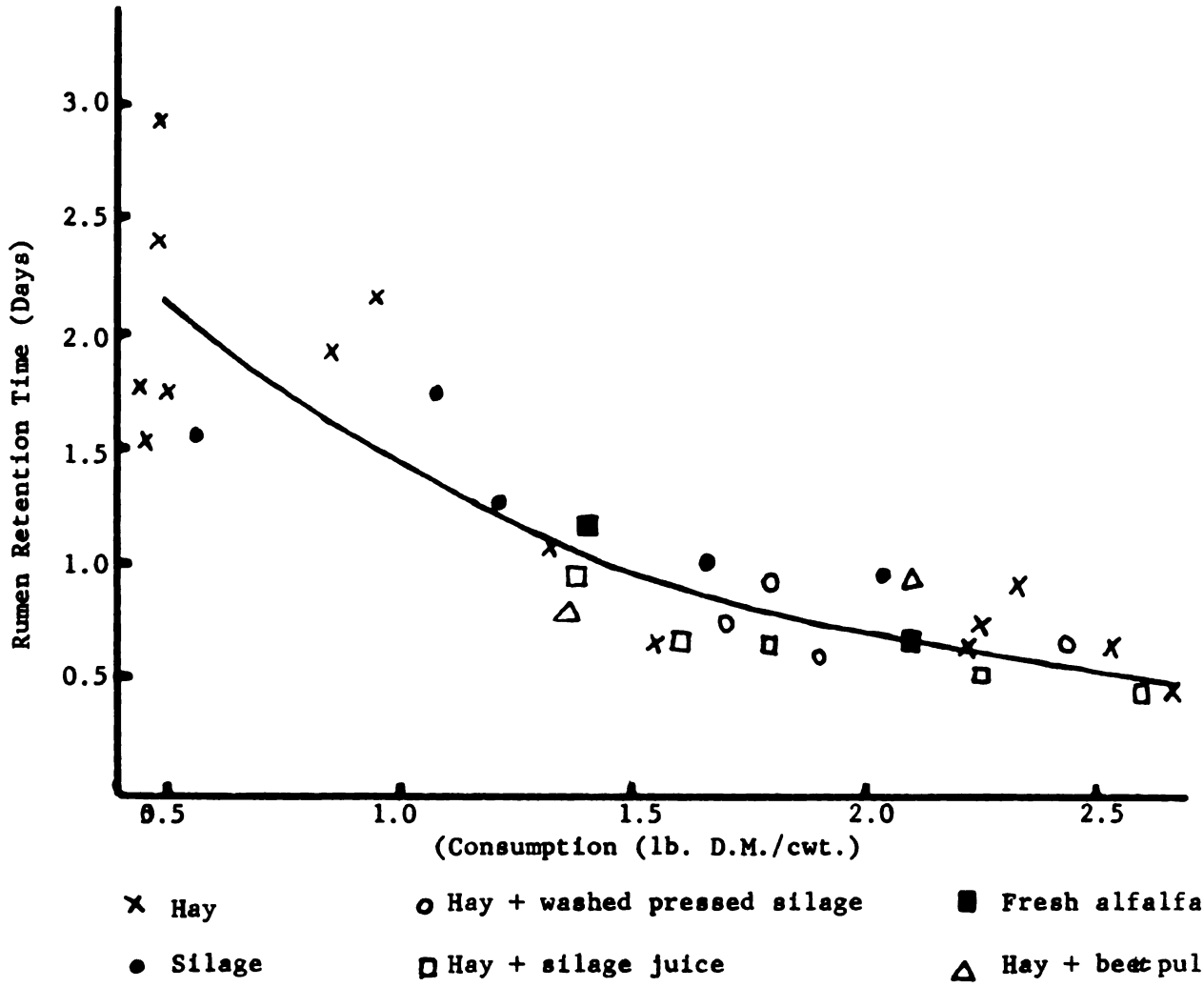


Figure 4. Relation between rate of dry matter intake and rumen retention time calculated as lb. D.M. in the rumen divided by daily dry matter intake.

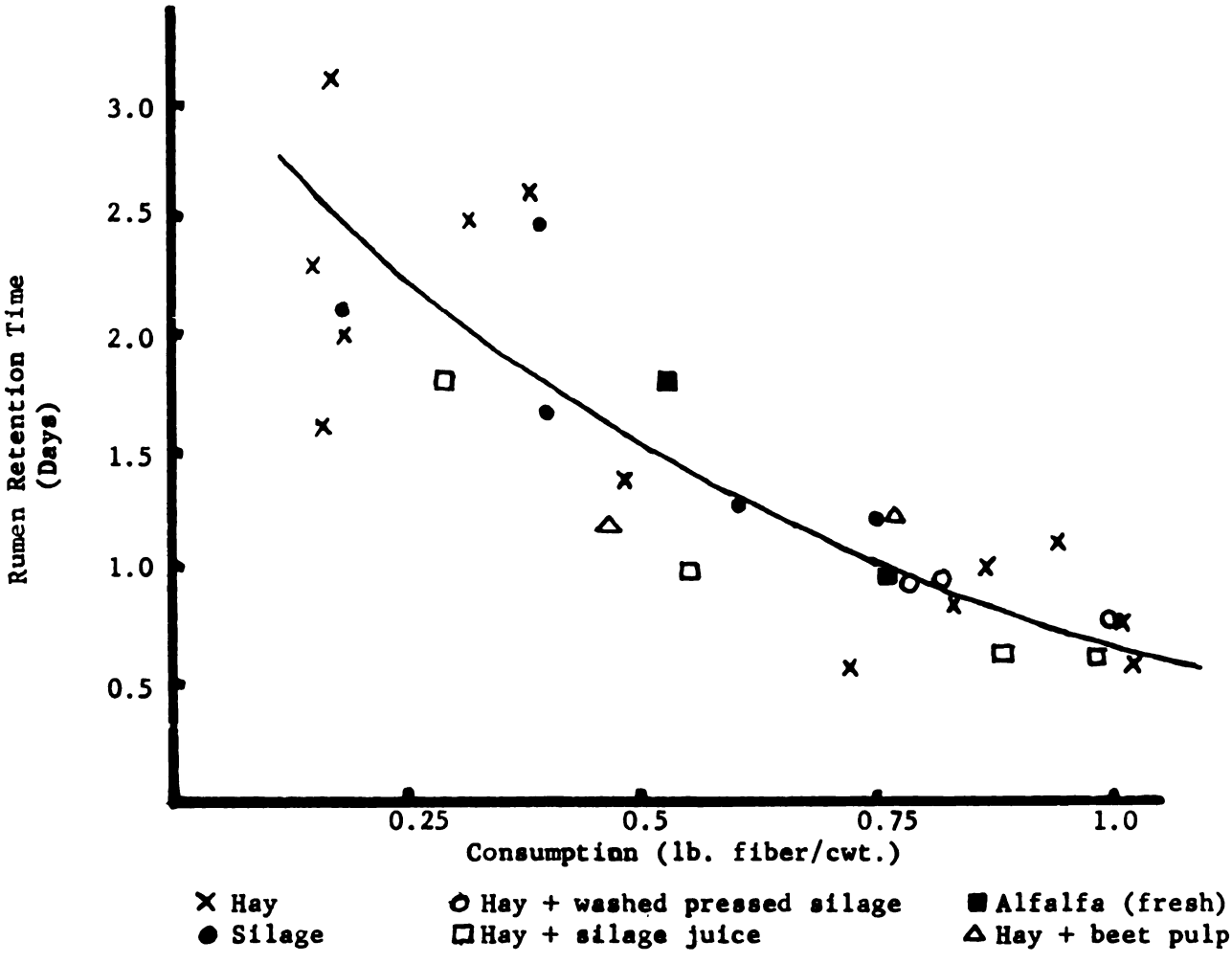


Figure 5. Relationship between rate of fiber intake and rumen retention time calculated as lb. fiber in the rumen divided by daily fiber intake.

decreased as dry matter intake increased. For example, when dry matter intake was limited to 0.5 lb./cwt. per day, mean retention time was approximately two days. When dry matter intake increased above 1.5 lb./cwt. per day, mean rumen retention time was less than one day. A line representing the relationship between retention time and intake was drawn free-hand in Fig. 4. It is interesting to note that all points regardless of type of forage fed are reasonably close to the free-hand drawn line; also, the relationship was not linear over the range of intake.

Fiber retention time was calculated by dividing fiber content of the rumen by daily fiber intake. The retention time of fiber in the rumen decreased as fiber intake increased. This is shown in Fig. 5. Fiber retention time was correlated with dry matter retention time ($r = 0.9755$, $P < 0.01$, Appendix Table VII). Rumen retention time for dry matter was 1.5 days when 1 lb. of dry matter per cwt. was fed daily. This value was more than the similar value of 0.75 days for retention time of fiber when 1 lb. of fiber per cwt. was fed daily ($P < 0.01$). Dry matter intake was greater than fiber intake for all feeds ($P < 0.01$) and was significantly correlated ($r = 0.9736$, Appendix Table VII).

Even though the cows were paired with themselves their intakes while on low, medium, and high levels of hay or silage were not as close together as desired. Consequently, five arbitrary pairs having the same dry matter intake per cwt. of hay and silage were selected. Intake and rumen characteristics of five cows fed silage and five cows fed hay are shown in Appendix Table IX. Rumen dry matter retention time of the cows fed hay was highly correlated with retention time of the cows fed silage over the wide range of feed dry matter intake ($r = 0.997$, $P < 0.01$). A "t" test of mean retention times of the hay fed group compared with the silage fed group indicated that the retention times were not different ($P = 0.5$).

The retention time of fiber in the rumen was compared between cows fed silage and cows fed hay, shown in Appendix Table IX, and found to be correlated ($r = 0.994$, $P < 0.01$). The "t" test of mean retention times of these groups showed no significant difference ($P > 0.7$).

Appendix Table X gives data on rumen retention time of five cows fed hay compared to five cows fed hay with silage juice infused intraruminally at comparable levels of intake. Rumen retention time of dry matter in the cows fed hay compared to the dry matter retention time of the cows fed hay with silage juice added in the

rumen was correlated r = 0.816, approaching P = 0.05. A comparison of mean retention times of these groups indicated that they were not statistically different (P = 0.7).

Appendix Table XI shows a comparison between four cows fed hay plus washed pressed silage (WPS) and four cows fed hay plus silage juice. Retention time of dry matter in cows fed hay and WPS was not significantly correlated with dry matter retention time of the cows fed hay plus silage juice. Intakes of these cows were not well matched over the range of dry matter intake. This may have been the cause of a low coefficient of correlation. A comparison between mean retention times of each group indicated they were not different (P > 0.5).

In all comparisons the infusion of silage juice appeared to reduce retention time, but the reduction lacked statistical significance.

Relationships found between dry matter intake per cwt. versus percent dry matter in the rumen, dry rumen contents as percent of body weight, and rumen contents (wet) as a percent of body weight from data in Appendix Table VI are shown in Figs. 6, 7, and 8, respectively.

The percent dry matter in the rumen showed a positive relationship with dry matter intake. A fairly wide range in percent dry matter was observed between cows at any given dry matter intake, however, the points shown in

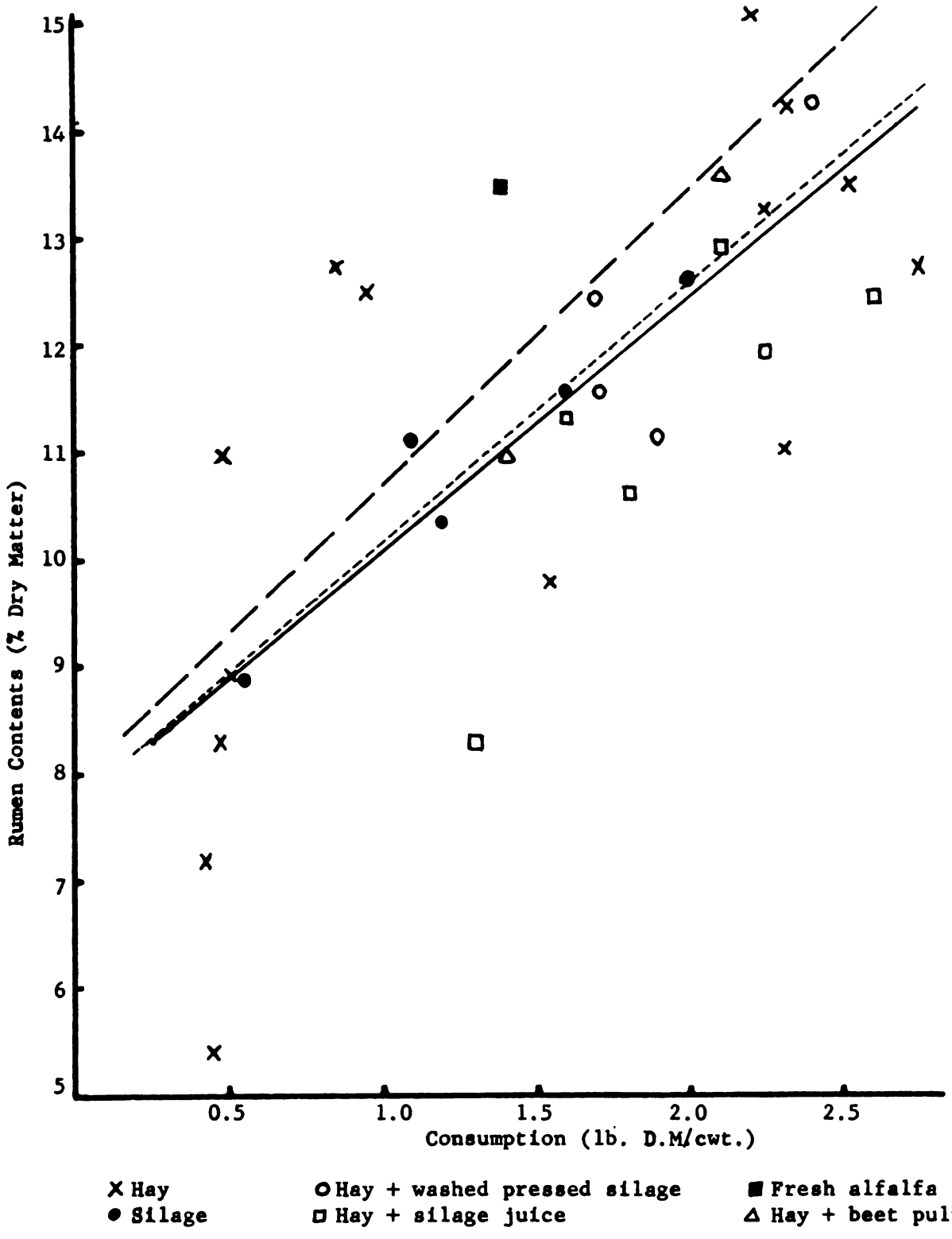


Figure 6. Relationship between rate of dry matter intake and percent dry matter in the rumen. The solid line represents all feeds. The long-dashed line represents 5 cows fed hay paired by rate of intake with 5 cows fed silage given the short-dashed line.

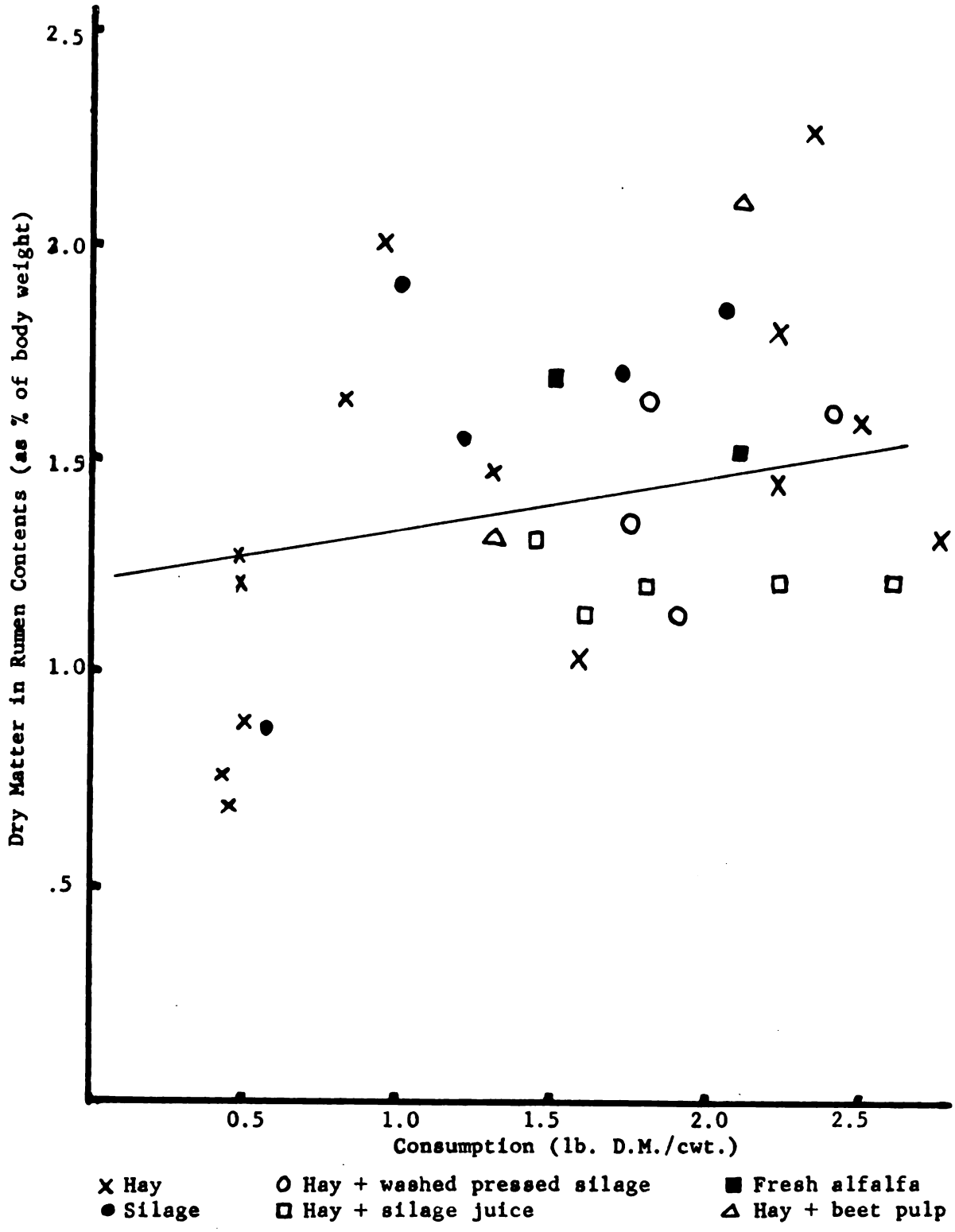


Figure 7. Relationship between rate of intake and dry matter in rumen contents as percent of body weight.

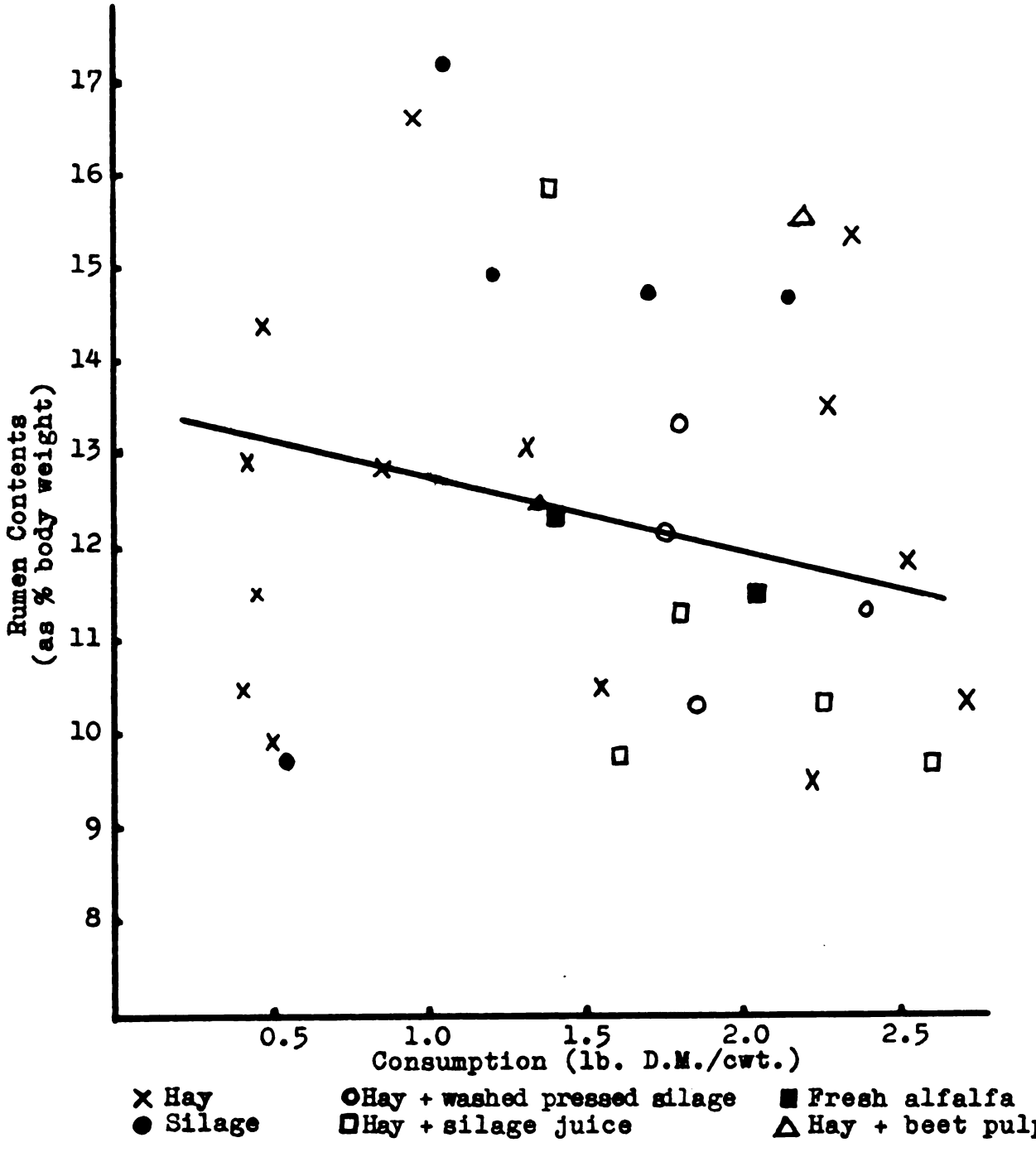


Figure 8. Relationship between rate of intake and total weight of rumen contents as percent of body weight.

Fig. 6 show a definite tendency toward a higher percent dry matter in the rumen with increasing dry matter intake. The "b" term, which is an expression of the relationship between the Y and the X axis, was 2.3. For example, for one pound of dry matter increase there is an increase of 2.3 percentage units dry matter in the rumen.

Dry matter in rumen contents as a percent of body weight showed a tendency to be related to dry matter intake in Fig. 7. The "b" term for the regression line in Fig. 7 indicated that for an increase of one pound dry matter intake the dry matter in rumen contents as a percent of body weight increased 0.148%. There was some indication that different forages have somewhat different relationships of dry matter in the rumen to dry matter intake, but with so few points for any given forage it was decided that such relationships may not be meaningful. A correlation between dry matter in rumen contents as percent of body weight and dry matter intake using all cows fed the various forages indicated that a positive relationship existed, however, this was not significant (Appendix Table VIII).

Fig. 8 shows the relationship between wet weight of rumen contents as percent of body weight and dry matter intake. The "b" term from the regression formula in Appendix Table VIII indicates a negative relationship. Total

rumen contents as percent body weight decreased 0.41% for each increase of one pound of dry matter intake. The coefficient of correlation was not statistically significant.

DISCUSSION

The data presented in Table 1 show that total intake of the cows was increased in every instance by the addition of various feed stuffs into the rumen. This is confirmation of work reported by Campling and Balch (27) who observed an increase in total intake when 50 pounds of rumen ingesta were added into the rumens of cows. This indicates that something has limited voluntary intake and that by force feeding an animal the digestive tract must handle more feed. It appears that the ability of the digestive tract to digest the extra feed may not be the only factor in limiting voluntary intake of roughage feeds. Other factors must contribute to the regulation of feed intake. Little work has been done to determine what other factors may participate in the regulation of feed intake of cattle. Possibly unidentified metabolites in the circulating blood after feeding may have some effect on satiety. Workers have shown that blood sugar levels are rather constant and show no particular relationship to the times when cows eat (54). The role of non-esterified fatty acids in the blood of the bovine in relation to hunger has not been reported. The possibility that the increased activity of microflora in the rumen after feeding and the subsequent slight temperature rise has been suggested as

another factor in producing satiety after eating. These factors and others not yet considered may all have a role in regulation of feed intake by cattle.

The results of this trial and those of Campling and Balch do not appear to be entirely consistant with the generally accepted idea stated by Blaxter (14) that intake of ruminants is entirely based on the rate of disappearance of ingested feed from the rumen. This may be a major factor, but there is reason to believe from other evidence presented here that other factors must be involved in the regulation of feed intake by ruminants.

When Campling and Balch (27) removed 50 pounds of rumen ingesta from the rumen, they observed that the cows did not eat enough additional feed to compensate for the dry matter in the ingesta removed. If rumen fill is the only means by which intake of roughage is regulated, it seems that the cows in this experiment would have completely compensated for the material removed.

In the trials reported in this paper, certain feeds produced greater differences in total intake than others. For example, the addition of washed pressed silage resulted in less increase in total intake than hay when comparable amounts of dry matter from these feeds were placed in the rumens of cows fed hay. This could possibly be explained on the basis that the washed pressed silage, devoid of much of the readily digestible material,

was digested more slowly in the rumen than hay and thereby the dry matter was retained in the rumen longer when washed pressed silage was added than when hay was added in the rumen. Fig. 4 shows that the retention time of dry matter in washed pressed silage administered to four cows was well within the range of the retention time expected when hay was fed at the same levels of dry matter intake. These data are limited but from these observations it does not appear that washed pressed silage was retained longer than the hay dry matter in the rumen. Possibly factors in the washed pressed silage other than slower breakdown were responsible for the small increase in total intake. There is a possibility that products of fermentation during the ensiling process may be responsible for the effect on total intake.

Total intake increased less for cows fed grain and hay than with cows fed hay only when hay was given intraruminally to both. This is shown by data given in Table 1. Kamstra and Miller (61) noted in vitro digestion of cellulose was reduced in rumen fluid from sheep and steers after changing the diet from hay to grain and hay. This is taken as evidence that the cellulose in hay was broken down more slowly in the rumen of cows fed grain and hay than in cows fed hay only. This may not be the only reason for the difference in total intake of these cows. It

is possible, too, that the addition of grain in the diet may have produced some effect on the satiety mechanism responsible for the somewhat less total intake increase of the cows fed grain.

The administration of silage juice (Table 1) resulted in a significantly greater increase in total intake per pound of dry matter added in the rumen than any of the other materials added in cows fed hay. Thomas et al. (106) reported decreased intake when silage juice was infused into heifers. Their trials lasted only three days. In the eight day trials reported in this paper, voluntary intake of hay decreased only on the first day of infusions and increased in most cases through the remainder of the trials. There is a good possibility that this material increased the rate of breakdown of feed in the rumen allowing a greater total intake. The ash content of the silage juice was rather high, 8.42% (page 37). There is a possibility that the mineral content of this ash had some stimulating effect on microflora activity in the rumen resulting in a more rapid breakdown of feed. Borroughs et al. (19) observed that a complex salt solution increased in vitro cellulose digestion in rumen fluid. Fig. 4 shows that dry matter retention time was consistantly less when silage juice was added than for dry matter in most other feed materials administered. Appendix Tables

X and XI both show that dry matter retention time was less when silage juice was administered than dry matter retention time for hay or washed pressed silage when fed at comparable levels. This does not exclude the possibility of other factors in silage juice affecting intake of the cows.

When large amounts of silage juice were added in the rumen of one cow and another not previously reported, these cows went "off feed." It is possible that some factor(s) in large amounts of silage juice disturb normal rumen microflora activity or the ion balance in the rumen which may be responsible for the observed anorexia. Hillman (54) observed cows fed large amounts of silage as the only feed occasionally went "off feed" during his experiments. The cow T19 in one trial reported here almost completely ceased eating silage while receiving silage ad libitum. No work has been reported in the literature attempting to explain the reasons why cows go "off feed" occasionally when fed silage. The results from intraruminal administration of both solid and liquid portions of silage in these experiments indicate the factor(s) may be located in the fluid portion of silage.

Data of preliminary trials in which rabbits were fed regular rabbit pellets treated with silage juice failed to show an increased intake of these pellets over

the control (Table 3). Perhaps subsequent trials using larger numbers of rabbits fed for longer periods on pellets containing a range of juice dry matter concentrations may more accurately demonstrate whether or not the silage juice portion influences feed intake in rabbits.

The study on beet pulp reported here was an attempt to determine if "cows" adapt to more rapid digestion of beet pulp, and what effect ration has on adaptation. Adaptation, as it was used here, was indicated by the faster rate of digestion and utilization of beet pulp in the rumen after seven days of feeding beet pulp compared with the rate on the first day of feeding.

Beet pulp contains a greater amount of pectic substances than other feeds commonly fed to cows. According to two reports in the literature, beet pulp contains an average of 11% pectic substances (24). Upon hydrolysis, these pectic substances are primarily broken down to uronides. Analysis of beet pulp used in the in vitro fermentation trial in this report showed that it contained 10.4% uronides. The method used for laboratory analysis of uronides in this report gave variable results. Recoveries of known amounts of uronides added to samples ranged from 46 to 67%. With such low and variable recoveries, it was decided to perform analysis for soluble carbohydrates in rumen fluid since carbohydrates are

primary products of beet hydrolysis. Analysis of total carbohydrates in beet pulp was 85% (Table 8) which agreed closely with values in other reports. Recoveries of carbohydrates added to samples ranged from 95 to 105%. This was believed to give a more reliable indicator of concentrations of products of beet pulp hydrolysis than the uronide analysis. A report found in the review by Brown (24) after these analyses were made indicated that dried beet pulp contained at least 24% galacturonic acid. The investigators in this report used a pectic enzyme for beet pulp hydrolysis. This may explain the variable results obtained in the uronide analysis in this report.

Uronic acid concentration in the rumen was used as one measurement of beet pulp disappearance. It was assumed that hydrolysis of beet pulp in the rumen would yield uronic acid and that this product would be utilized by the micro-organisms in the rumen fluid more rapidly with increased adaptation to utilization of beet pulp.

Total carbohydrate concentrations in the rumen were used as another measurement of adaptation. It was also assumed that hydrolysis of beet pulp in the rumen would yield soluble carbohydrates which would be utilized more rapidly with increased adaptation to digestion of beet pulp.

Gross visual observation of the amount of beet pulp in the rumen was used as another estimate of adaptation to the digestion of beet pulp in the rumen.

Cow A103, fed grain and hay, appeared to digest beet pulp more rapidly at the end of seven days of beet pulp administration than on the first day as indicated by lower rumen concentrations of uronides and total soluble carbohydrates on the seventh day than on the first day of beet pulp administration (Tables 4 and 5). Visual observation also indicated somewhat less beet pulp in the rumen 12 hours after feeding on the seventh day than on the first day of treatment (Fig. 1). It is questionable if the total amount of volatile fatty acids or percentages of individual volatile fatty acids (Table 6) indicated any adaptation in the rumen of this cow except that the lactate concentration on the seventh day had nearly returned to the pretreatment level. Voluntary intake of feed dry matter was nearly as high on the seventh day as on the first day of treatment. Rumen pH remained at the pretreatment level throughout the seven days of the trial.

The results of the in vitro fermentation trial (Tables 8 and 9) show some indication that beet pulp was digested somewhat more rapidly in rumen fluid from cows fed grain and beet pulp than in rumen fluid from cows fed grain but not receiving beet pulp. The solids

remaining at the end of the fermentation were less when using rumen fluid from cows fed grain and beet pulp than when using rumen fluid from cows fed grain without beet pulp. There was also a slightly increased disappearance of total uronides than with rumen fluid from cows fed grain without beet pulp. This is additional evidence that cows fed grain and beet pulp for a period of time digest beet pulp more rapidly than cows which have not been fed beet pulp.

In the trial when the two cows fed hay were given beet pulp to determine if they could adapt to more rapid digestion of beet pulp, A192 digested beet pulp more rapidly on the 7th day than on the 1st day of administration.

Rumen concentrations of uronides and soluble carbohydrates were lower on the 7th day of beet pulp administration than on the first day in A192 (Tables 4 and 5). This was taken as an indication that beet pulp was being metabolized more rapidly on the seventh than on the first day. Visual observation of rumen contents 12 hours after feeding (Fig. 1) showed very little change from the first to the seventh day. Other indications showed that conditions in the rumen were not returning to normal. Lactic acid concentrations in rumen fluid on the seventh day increased over the first day level (Table 6). Rumen pH,

which had dropped on the first day of treatment was further depressed on the 7th day. Voluntary intake of hay was diminished, although it showed some improvement on the 7th day.

Evidence indicated cow A192 was adapting to a more rapid beet pulp digestion by the seventh day. The amount of beet pulp administered in this trial may have been more than could be handled in the rumen without causing marked changes in rumen pH and lactic acid concentrations when hay was the only other feed given to this cow.

Cow T20 on the other hand showed no indication of digesting beet pulp more rapidly on the 7th day of administration. Rumen uronide and soluble carbohydrate concentrations (Tables 4 and 5) were higher on the seventh than on the first day of treatment. Lactic acid concentrations in the rumen were considerably higher on the seventh than on the first day (Table 6). Percent beet pulp in the rumen increased markedly by the seventh day. Rumen pH, which had dropped on the first day of treatment, showed no indication of returning to the pretreatment level on the seventh day. Voluntary intake of hay declined through the treatment period (Fig. 1). All of these indications showed that this cow was not adapting to digesting beet pulp while A192 demonstrated some adaptation.

It was noted in Fig. 1 that dry matter intake before the trial was considerably less for T20 than for A192. Both cows, however, received the same amount of beet pulp in the treatments. The suggestion was made above that rumen conditions in A192 may have been changed by the large amounts of beet pulp administered. In T20, beet pulp made up a larger proportion of intake, perhaps aggrevating the changes in the rumen even more than in A192 and thus causing a more unfavorable environment for rapid digestion of beet pulp.

The in vitro fermentation of rumen fluid from both of these cows showed that the fluid from T20 digested the beet pulp substrate as rapidly, perhaps somewhat more rapidly, than rumen fluid from A192. In vivo, however, after receiving $NaHCO_3$ in the rumen, A192 was apparently digesting beet pulp much more rapidly than T20 (Fig. 1). A possible explanation for these results was that the buffer solution in the in vitro fermentation mixture raised the pH and thus enhanced conditions for microflora activity in the rumen fluid from T20 to digest beet pulp more rapidly than was occurring in vivo. Microscopic observations of rumen samples indicated greatly reduced microflora numbers on days when rumen pH was low (Table 7). Based on the results of these studies it is questionable if cows fed hay can adapt to the digestion of beet pulp in

quantities as large as those used in these trials. In contrast to the cows fed hay, cow A103, fed grain, demonstrated ability to digest rather large amounts of beet pulp (Fig. 1). Results of the in vitro fermentation trials (Tables 8 and 9) showed further evidence that the beet pulp was digested more rapidly in the rumen fluid from cows receiving grain than in rumen fluid from cows fed hay. There was a significantly greater reduction in solids and a greater percent disappearance of total carbohydrate as well as soluble uronides in rumen fluid fermentations from cows fed grain plus hay than from those fed only hay.

Makela fed beet pulp and hay to cows and also swedes and hay. Swedes are a root crop similar to sugar beets. He observed that when beet pulp and hay were fed the carbohydrate portion of the rumen ingesta had a longer retention time than when hay and swedes were fed. He also cited earlier data comparing rumen retention time of carbohydrates of cows fed grain, hay, and beet pulp to cows fed hay and beet pulp and found that the former had a shorter retention time of the carbohydrates than the latter (76).

This information provides evidence that beet pulp is not digested as rapidly in the rumen of cows fed hay and beet pulp as when cows are fed hay, grain, and beet

pulp. All this evidence indicates that "cows" fed grain digest beet pulp more rapidly and also adapt to digesting larger quantities than cows fed only hay. Perhaps the rumen microflora in cows fed grain have to change less to adapt to digesting beet pulp than rumen microflora in cows fed hay.

When larger amounts of beet pulp were administered to A192 and T20, the rumen pH was greatly reduced which appeared to have a considerable influence on rumen microflora activity (Fig. 1). Microscopic observations (Table 7) indicated a reduction in total numbers when pH was greatly reduced. It seems logical to conclude that reduced digestive activity would be associated with fewer numbers. When $NaHCO_3$ was added in the rumen of A192 to reduce the acidity, total numbers of microflora increased. Apparently digestive activity increased at the same time and resulted in the increased rate of disappearance of beet pulp in the rumen along with increased hay intake by A192 (Fig. 1).

For some yet unexplained reason pH is considerably reduced in cows fed hay and receiving beet pulp. This may be one of the main reasons for the apparent slower digestion of beet pulp in the rumens of the cows in these experiments.

Table 1 shows milk production declined when beet pulp was added in the rumen in two cows while milk production increased when hay was added in the rumen of cows fed

hay and grain in three cows. The difference in milk production response to these treatments cannot be explained on the basis of dry matter intake. The addition of beet pulp increased dry matter intake more than the hay. The possibility that addition of beet pulp changed rumen microflora activity to the production of other less efficient intermediate metabolites was considered. The volatile fatty acid concentrations shown in Table 6 indicated a higher proportion of propionate to acetate concentration in the rumen of A103 after seven days of beet pulp treatment compared to concentrations found in the rumen the day before treatment. The total volatile fatty acid concentrations (Table 6) increased from 100 uM/ml. on the 1st day to 139 uM/ml. of rumen fluid after seven days of treatment. Such a change in total concentrations of acids is often associated with increased milk production. The possibility of a change in intake of protein from the feed was considered. Calculations from estimates of protein content of feed intake showed that before administration of beet pulp the ration contained 14.47% crude protein while during administration of beet pulp the feed, including the beet pulp, averaged 13.95% crude protein. Average protein figures for beet pulp appearing in the literature were 8.8% (24). This small change in protein seems unlikely to have caused the drop in milk production. From

the information available, the drop in milk production resulting from administration of beet pulp cannot be explained.

Throughout various parts of these studies attempts have been made to find ways that silage may behave differently in the rumen of cows than when hay is fed. This has been done in an effort to discover possible causes for the somewhat reduced dry matter intake of silage than hay.

The single reversal trial reported here in which hay and silage each were fed to cows revealed only a few differences. Perhaps the most outstanding difference was the consistantly higher rumen ammonia concentrations found when silage was fed than when hay was fed (Fig. 3 and Table 11). El-Shazly (39) observed higher rumen ammonia concentrations throughout the day in sheep when silage was fed than when hay from the same source was fed. Williams and Christian (110) fed sheep ten different direct-cut grass silages. They observed that ruminal ammonia levels in the sheep were higher just after feeding when silage containing greater amounts of ammonia and residual nitrogen was fed than when silages containing less ammonia and residual nitrogen was fed. It is known that during the silage fermentation process a portion of the plant material is broken down by the action of microorganisms resulting in the production of varying amounts

of ammonia and non-protein nitrogen. It appears logical then that more ammonia would be found in the rumen of cows after eating silage than hay since hay does not go through a fermentation process in which protein nitrogen is broken down to ammonia as in silage. The ammonia in the silage apparently raised the ammonia concentration in the rumen at feeding time and perhaps was not efficiently eliminated from the rumen or utilized as well by rumen microflora as the protein nitrogen thus resulting in the prolonged higher concentrations than when hay was fed. This does not explain the higher concentrations found at the medium and high levels of feeding 4 to 6 hours after feeding (Fig. 3).

The higher concentrations of rumen ammonia found in this study when silage was fed than when hay was fed may be associated with higher concentrations of other non-protein nitrogen breakdown products such as those found in the investigations of Williams and Christian (110). It is possible that ammonia and/or other non-protein nitrogen compounds associated with higher amounts of ammonia in silage may be responsible for limiting consumption of this feed. Thomas et al. (106) observed intake of heifers was depressed when ammonium salts, glucosamine, and urea were placed in the rumen. The silage used in this trial was not analyzed for ammonia content but appeared to be of very good quality as judged by appearance and a

desirable odor with no indication of ammonia present and by acceptance by heifers in another trial. Perhaps other silage of lower quality and less acceptable may contain greater amounts of ammonia and non-protein nitrogen compounds which could be responsible for the limited intake. Ammonia and other non-protein nitrogen compounds should be thoroughly investigated to determine if such compounds are responsible for the limited intake of hay crop silage.

The rumen volatile fatty acid concentrations in cows fed hay or silage were so variable in the experiments reported here (Table 11) that even the averages are of questionable significance. A given cow on different levels of the same feed as well as the same feed fed to both cows gave inconsistant concentrations, however, keeping in mind the questionable reliability of these average figures certain differences were seen when silage or hay was fed (Table 12). When silage was fed, average butyrate concentrations were higher throughout the day on all levels of feeding. Propionate also averaged slightly higher during the first 6.5 hours after feeding when silage was fed, however, acetate concentrations averaged higher when hay was fed. The acetate-to-propionate ratio and the 2-to-3 carbon ratios were higher when hay was fed (Table 13).

Rumen pH values (Fig. 2 and Table 10) showed basically the same trends through the day on the three levels of feeding when silage or hay was fed. This evidence indicates that even though the silage was acidic (pH, 4.4) it did not appear to cause a marked difference in rumen pH 1.5 hours after feeding. In general there was no appreciable difference in pH when either hay or silage was fed throughout the day.

As mentioned earlier, comparative retention time for silage and hay dry matter fed to the cows in this trial was not measured in all cases; however, a comparison of five pairs of cows fed hay and silage over a wide range of closely matched dry matter intakes are shown in Appendix Table IX. Retention time of dry matter and fiber was very similar over the range of intake for cows fed either hay or silage. A relationship of percent dry matter in rumen contents versus dry matter intake (Fig. 6) showed that the dry matter percent in the rumen was slightly higher when hay was fed than when silage was fed. These findings are in agreement with those of Thomas et al. (105) when they compared dry matter intake and percent dry matter in the rumen when silage and hay were fed to cows. When silage or hay was placed in the rumens of cows fed grain and hay, the effects on voluntary intake

as well as total intake were very similar (Table 1).

Silage fed from the same silo as used in the hay versus silage trial reported herein was well liked by the heifers in another feeding trial. The average consumption was very close to that of comparable hay fed during the trial. The differences noted when comparing hay to a silage of high quality might not be as large as differences when comparing this same hay to a silage of low quality or acceptability. A trial using a less well accepted silage compared to hay may show more significant differences in rumen pH, volatile fatty acid concentrations and ammonia in the rumen. From the results of rumen ammonia differences in this trial, it would be advisable to run analyses on other non-protein nitrogen compounds in the silage as well as in the rumen when animals were fed silage compared with animals fed hay. In this way possibly factors responsible for limiting intake of silage can be isolated.

Makela (75, 76) measured the rate of disappearance or retention time of ingested feed in the rumen by measuring rumen contents four hours after feeding. He found the retention time of dry matter in the rumen increased as dry matter intake was decreased, and that this relationship was curvilinear. Thomas et al. (105) found a similar relationship between rumen retention time and dry matter

intake when cows were fed hay or silage. This group also measured rumen contents approximately four hours after feeding. They emptied the rumens of fistulated cows to obtain their data as was the case in this report, while Makela, above, slaughtered cows to measure rumen contents in obtaining his data.

In the study reported here (Fig. 4) results very similar to the above work were reported. It was interesting to note that regardless of the roughage fed the retention time for dry matter at any intake was reasonably close to the hand-drawn line, however, at lower dry matter intakes there was a greater range in retention time.

In this study rumen retention time of fiber was also determined. Similarly as with dry matter, increased fiber intake resulted in a reduced time for fiber to be retained in the rumen (Fig. 5). It should be noted that the fiber portion associated with the dry matter of any forage fed had a somewhat longer retention time in the rumen. For example, the retention time for intake of 1 lb. dry matter per cwt. was 1.4 days while the retention time for the fiber associated with the dry matter of hay and silage (approximately 40% or 0.4 lbs.) was 1.8 days. This was probably due to the fact that the dry matter portion was more readily digested than the fiber portion

of a feed. In the time between feed consumption and when the rumen contents were measured 4.5 to 5 hours later, a greater proportion of the more readily digestible dry matter was digested and had left the rumen than the fiber which was digested more slowly. Relationships such as those between retention time of dry matter and fiber for a given roughage may become useful in evaluation of roughages.

Thomas et al. (105) noted that the following relationships existed between dry matter intake and rumen contents: as dry matter intake increased the percent dry matter in the rumen increased, as dry matter intake increased dry matter in the rumen as percent of body weight increased somewhat, and as intake increased the weight of total rumen contents (wet) as a percent of body weight remained nearly constant, although, showing a slight tendency to increase.

Using data from Makela (76) the same relationships were seen to exist. The relationships reported here (Figs. 6, 7, and 8) were very similar to the above cited work with one possible exception. In this study a slight negative, but non-significant correlation was seen between weight of rumen contents as percent of body weight and dry matter intake.

Measurement of rumen retention time of feeds and feed constituents such as carbohydrate, dry matter, fiber, and lignin can be very useful in estimating the relative digestion rates or the digestibility of different feeds. Very likely a more thorough study of relationships as those given above can be useful "tools" in understanding differences in feeds and aid in the effort to increase efficiency of feed utilization for more efficient livestock production. This is, of course, the ultimate goal in most nutrition work with livestock.

SUMMARY

Rumen fistulated cows fed hay and two lactating cows receiving grain were administered hay, beet pulp, direct-cut alfalfa silage, and two silage fractions, silage juice, and washed pressed silage, through their rumen fistulas. Total and voluntary dry matter intake was measured six days before and after and eight days during administration of these feedstuffs. Total dry matter intake was increased in every case which ranged from 1.43 to 8.05 lb./day. Voluntary intake decreased 1.70 to 9.50 lb./day except during administration of the silage juice fraction when voluntary intake increased 1.01 to 1.12 lb./day.

Dry matter intake of rabbits was not significantly changed when pellets impregnated with silage juice were fed compared to control pellets.

Dried beet pulp was intraruminally administered to two cows fed hay and one cow fed hay and grain to determine whether beet pulp introduced into the diet was digested more rapidly on the seventh than on the first day. Concentrations of uronic acids and total carbohydrates in rumen fluid were determined before feeding and four times of the day after feeding. Lower concentrations of these constituents on the seventh day compared to the

first day were considered as an indication of adaptation. The cow fed hay and grain appeared to have adapted to a more rapid digestion of beet pulp on the seventh day. One cow fed hay showed some adaptation while the other cow did not appear to show any adaptation.

Another experiment was conducted to determine whether cows fed hay and grain digest beet pulp more rapidly than cows fed only hay. In an in vitro fermentation trial with beet pulp as substrate using rumen fluid from four cows fed hay and grain and four cows fed hay there was greater disappearance of substrate, total carbohydrate, and soluble uronides ($P < 0.05$) using rumen fluid from cows fed hay and grain than fluid from cows fed only hay.

Intraruminal administration of beet pulp to a cow fed hay and grain and cows fed hay resulted in an accumulation of beet pulp in the rumens of cows fed hay and also a reduction in rumen pH while the cow fed grain and hay did not show an increased percent beet pulp in the rumen and pH was not depressed. While beet pulp was accumulating in the rumens of the two cows receiving hay and beet pulp, $NaHCO_3$ was added into the rumen of one cow to restore pH to normal and the other cow served as control. Beet pulp continued to accumulate in the rumen of the control cow while a marked reduction resulted in

the rumen of the $NaHCO_3$ treated cow. General microscopic observations of rumen fluid revealed that a reduction in total numbers of microflora, especially protozoa, was associated with low rumen pH.

Rumen pH, NH_3, and volatile fatty acid concentrations in two cows fed hay or direct-cut alfalfa silage at three levels were determined six times during the day to observe differences in the concentrations when animals were fed these roughages. Rumen pH was slightly higher 1.5 hours after feeding when hay was fed but followed the same trend through the remainder of the 12 hours as when silage was fed. The greatest differences were higher concentrations of rumen NH_3 when silage was fed at all levels and times of the day. Volatile fatty acid concentrations were variable although average acetate concentrations and acetate-to-propionate ratios were higher when hay was fed, and butyrate was higher when silage was fed.

Rumen emptyings were performed during the above trials 4.5 to 5 hours after morning feeding. Rumen dry matter, fiber, percent dry matter in the rumen, and total weight of rumen contents were determined. Rumen retention time of dry matter expressed in days was calculated by dividing daily dry matter intake by dry matter in the rumen and similarly for daily fiber intake. Retention time of dry matter was reduced as dry matter intake

increased. A similar relationship was noted between fiber intake and fiber retention time. As dry matter intake increased percent dry matter in the rumen and rumen dry matter as percent of body weight increased, while there was a tendency for total weight of rumen contents as percent of body weight to decrease slightly.

LITERATURE CITED

(1) Anand, B. K. Nervous regulation of food intake. Physiological Reviews, 41:677. 1961.

(2) Anand, B. K., and J. R. Broebeck. Hypothalamic control of food intake in rats and cats. Yale J. and Med., 24:123. 1951.

(3) Anand, B. K., and J. R. Broebeck. Localization of a "feeding center" in the hypothalamus of the rat. Proc. Soc. Exptl. Med., 77:323. 1951.

(4) Anand, B. K., and J. R. Broebeck. Food intake and spontaneous activity of rats with lesions in the amygdaloid nuclei. J. Neurophysiol., 15:421. 1952.

(5) Anand, B. K., and S. Dua. Feeding response induced by electrical stimulation of hypothalamus in cat. Indian J. Med. Res., 43:113. 1955.

(6) Anand, B. K., S. Dua and G. S. Chhina. Higher nervous control of food intake. Indian J. Med. Res., 46:277. 1958.

(7) Anand, B. K., S. Dua and K. Shoenberg. Hypothalamic control of food intake in cats and monkeys. J. Physiol., 127:143. 1955.

(8) Anand, B. K., S. Dua and B. Singh. Electrical activity of the hypothalamic feeding centers under the effect of changes in blood chemistry. Electroencephalog. and Clin. Neurophysiol., 13:54. 1961.

(9) Anderson, B., and B. Larsson. Influence of local temperature changes in the preoptic area and rostral hypothalamus on the regulation of food and water intake. Acta Physiol. Scand., 52:75. 1961.

(10) Appleman, R. D., and J. C. Deloche. Behavioral, physiological, and biochemical responses of goats to temperature 0° to 40° C. J. Animal Sci., 17:326. 1958.

(11) Bates, M. W. Fat metabolism in obesity. Am. J. Physiol., 180:309. 1955.

(12) Bell, J. M. A comparison of fiberous feedstuffs in non-ruminant rations: effects on growth responses, digestibility, rates of passage, and ingesta volume. Canadian J. Animal Sci., 40:71. 1960.

(13) Bitter, T., and R. Ewins. A modified carbazole reaction for uronic acids. Biochem. J., 81:43. 1961.

(14) Blaxter, K. L. Energy feeding standards for dairy cattle. Nutr. Abstr. and Rev., 20:1. 1950.

(15) Blaxter, K. L. The voluntary food intake of sheep. Proc. Nutr. Soc. 19(2); 30-31. 1960.

(16) Blaxter, K. L., N. Graham, and F. W. Wainman. Some observations on the digestibility of food by sheep and on related problems. Brit. J. Nutr., 10:69. 1956.

(17) Blaxter, K. L., F. W. Wainman, and N. D. Wilson. The regulation of food intake by sheep. Animal Production, Bull. 3, Part 1, p. 51. 1961.

(18) Booth, G., and J. M. Strang. Changes in temperature of skin following ingestion of food. A. M. A. Arch. Intern. Med., 57:533. 1936.

(19) Borroughs, W., H. G. Headley, R. M. Bethke, and P. Gerlaugh. Cellulose digestion in good and poor quality roughages using an artificial rumen. J. Animal Sci., 9:214. 1950d.

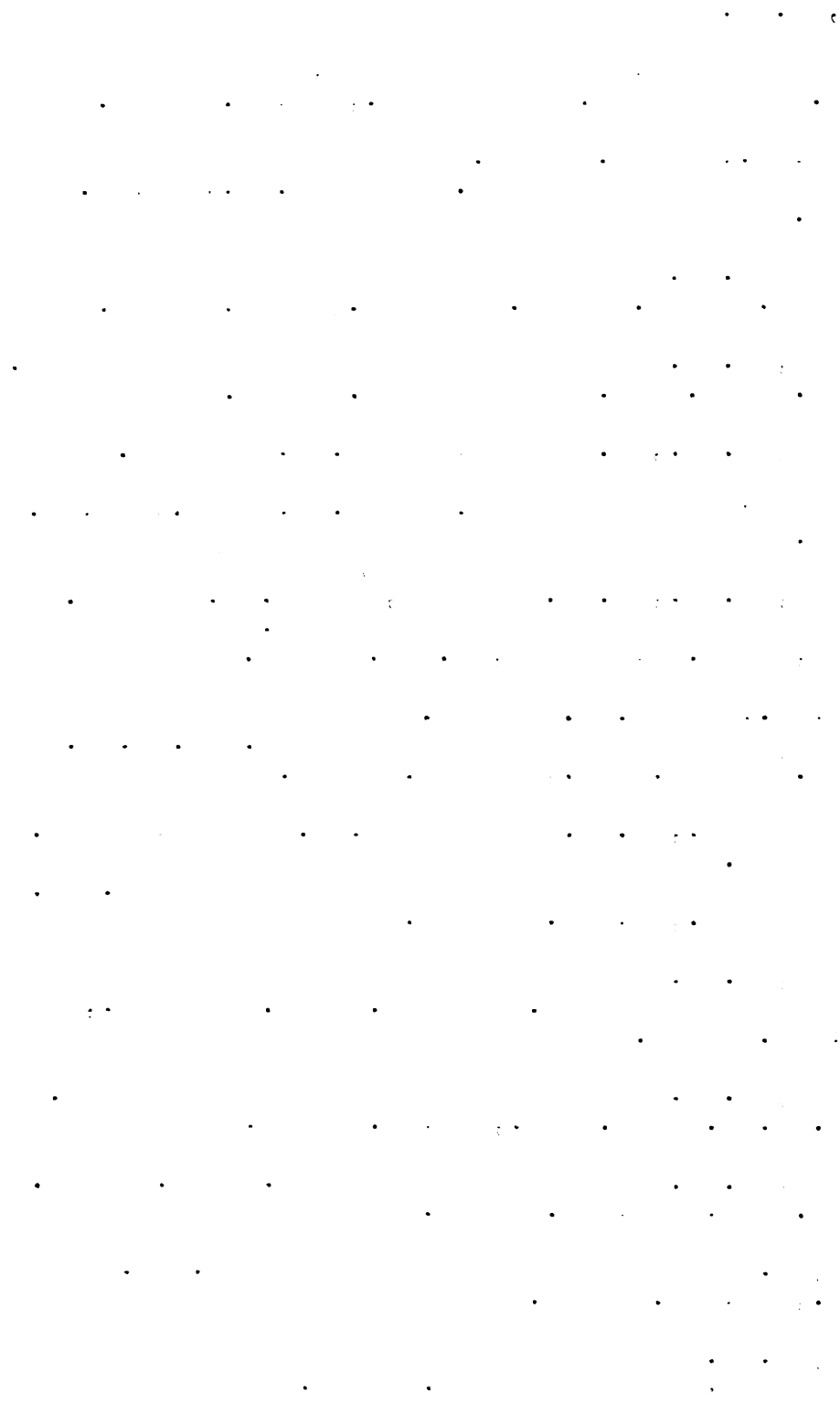
(20) Brobeck, J. R. Food intake as a mechanism of temperature regulation. Yale J. Biol. and Med., 20:545. 1948.

(21) Brobeck, J. R. Neural regulation of food intake. Ann. N. Y. Acad. Sci., 63:44. 1955.

(22) Brobeck, J. R. Food and temperature. Rec. Progr. Horm. Res., 16:439. 1960.

(23) Brody, S. Climatic physiology of cattle. J. Dairy Sci., 39:715. 1956.

(24) Brown, L. D. Composition and nutritive value of beet pulp. (Unpublished). 1961.



(25) Bulatao, E., and A. J. Carlson. Contributions to the physiology of the stomach. Influence of experimental changes in blood sugar level on gastric hunger contractions. Am. J. Physiol., 59:107. 1924.

(26) Cahill, G. F. Extracts of (fat mobilizing substance) found in human urine. Endocrinology, 69:648. 1961.

(27) Campling, R. C., and C. C. Balch. Factors affecting intake of food by cows. (1) Preliminary observations on the effect, on the voluntary intake of hay, of changes in the amount of the reticuloruminal contents. Brit. J. Nutr., 15:523. 1961.

(28) Campling, R. C., and C. C. Balch. Factors affecting voluntary intake of food by cows (2). Brit. J. Nutr., 15:531. 1961.

(29) Campling, R. C., and M. Freer. Factors affecting voluntary intake of food by cows. The effect of urea on the voluntary intake of oat straw. Brit. J. Nutr., 16:115. 1962.

(30) Carlson, A. J. The control of hunger in health and disease. University of Chicago Press, Chicago. 1916.

(31) Crampton, E. W. Interrelationships between digestible nutrient and energy content, voluntary dry matter intake, and the overall feeding value of forages. J. Animal Sci., 16:546. 1957.

(32) Crampton, E. W., E. Donefer, and L. E. Lloyd. A nutritive value index for forages. Proc. of the 8th Int. Grassland Congr., p. 462. 1960.

(33) Dehority, B. A., R. R. Johnson, and H. R. Conrad. Digestibility of forage hemicellulose and pectin by rumen bacteria in vitro and the effect on lignification thereon. J. Dairy Sci., 45:508. 1962.

(34) Delgado, J. M. R., and B. K. Anand. Increase of food intake induced by electrical stimulation of the lateral hypothalamus. Am. J. Physiol., 172:162. 1953.

(35) Dell, P. C. Translation of bodily needs into behavior. Ciba Foundation Symposium--Neurol. basis of behavior. 1958.

(36) Dixon, J. W., and F. J. Massey, Jr. Introduction to statistical analysis. 2nd ed. McGraw-Hill Book Co., New York. 1957.

(37) Dowden, D. R., and D. R. Jacobson. Inhibition of appetite in dairy cattle by certain intermediate metabolites. Nature, 188:148. 1960.

(38) Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. Colorimetric method for determination of sugars and related substances. Analytical Chem., 28:350. 1956.

(39) El-Shazly, K. Degradation of protein in the rumen of the sheep. I. Some volatile acids including branched chain isomers found in vivo. II. Action of rumen micro-organisms on amino acids. Biochem. J., 51:640. 1952.

(40) Emery, R. S., L. D. Brown, C. H. Huffman, T. R. Lewis, J. P. Everett, and C. A. Lassiter. Comparative feeding value of lactic acid and grain for dairy cattle. J. Animal Sci., 20:159. 1961.

(41) Forssberg, A., and S. Larsson. On the hypothalamic organization of the nervous mechanism regulating food intake. II. Studies of isotope distribution and chemical composition in the hypothalamic region of hungry and fed rats. Acta Physiol. Scand., 32, Suppl. 115, pt. II. 1954.

(42) Foster, M. Textbook of Physiology. 4:285. 1891. Cited by Anand (1).

(43) Gordon, C. H., J. C. Derbyshire, W. C. Jacobson, E. A. Kane, and C. G. Melin. Comparisons of unsealed and plastic sealed silages for preservation efficiency and feeding value. J. Dairy Sci., 44:1113. 1961.

(44) Graves, R. R., and J. R. Dawson. Relative values for milk production of hay and silage made from immature pasture herbage. U.S.D.A. Tech. Bull. 649. 1938.

(45) Grossman, M. I. Integration of current views on regulation of intake. Annals of N. Y. Acad. Sci., 63:76. 1955.

(46) Grossman, M. I., G. M. Cummins, and A. C. Ivy. The effect of insulin on food intake after vagotomy and sympathectomy. Am. J. Physiol., 149:100. 1947.

(47) Grossman, M. I., and I. F. Stein. Vagotomy and the hunger-producing action of insulin in man. J. Appl. Physiol., 1:263. 1948.

(48) Haller, A. Fames et Sitis. Elementa Physiol. 6:185. 1776. Cited by Anand (1).

(49) Harris, L. E., and H. H. Mitchell. Urea added to a low-N ration. J. Nutr., 22:167. 1941.

(50) Hawk, P. B., B. L. Oser, and W. H. Summerson. Practical physiological chemistry. 13th ed. The Blakiston Co., New York. p. 890. 1954.

(51) Hetherington, A. W. Hypothalamic lesions and adiposity in the rat. Anat. Record, 78:149. 1940.

(52) Hetherington, A. W. The production of hypothalamic obesity in rats already displaying chronic hypopituriarism. Am. J. Physiol., 140:89. 1943.

(53) Hetherington, A. W. Non-production of hypothalamic obesity in the rat by lesions rostral or dorsal to the ventro-medial hypothalamic nuclei. J. Comp. Neurol., 80:33. 1944.

(54) Hillman, D. Appetite studies in dairy cattle: grass silage vs. hay. Ph.D. Thesis, Michigan State University, East Lansing, Michigan. 1959.

(55) Hodgson, R. E., and J. C. Knott. The feeding value of alfalfa hay compared with mixed hay and grass silage as a ration for dairy cattle. State College of Washington, Agr. Exp. Sta. Bull. 386. 1940.

(56) Ingalls, J. R., J. W. Thomas, and M. B. Tesar. Intake, digestibility, and animal gain of three different cuttings of birdsfoot trefoil, alfalfa, bromegrass, and reed canarygrass. J. Dairy Sci., 45:692. 1962.

(57) Janowitz, H. D., and M. I. Grossman. Effect of parentenal administration of glucose and protein hydrolysate on food intake in the rat. Am. J. Physiol., 155:28. 1948.

(58) Janowitz, H. D., and M. I. Grossman. Factors affecting intake of normal dogs with esophagostomy and gastric fistula. Am. J. Physiol., 159:143. 1949.

(59) Janowitz, H. D., and M. I. Grossman. Effect of prefeeding, alcohol and bitters on food intake of dogs. Am. J. Physiol. 164:182. 1951.

(60) Janowitz, H. D., and A. C. Ivy. Role of blood sugar levels in spontaneous and insulin-induced hunger in man. J. Appl. Physiol., 1:643. 1949.

(61) Kamstra, L. D., and N. T. Miller. Ruminal variation in pH, temperature and in vitro activity as affected by ration change and season. Proc. S. D. Acad. Sci. 39:84. 1960.

(62) Keller, A. D., and W. Noble. The separation of the heat loss and the heat production mechanisms in chronic preparations. Am. J. Physiol., 113:78. 1935.

(63) Keller, A. D., and W. Noble. Further observations on enhanced appetite with resultant adiposity following removal of the posterior lobe of the hypophysis. Am. J. Physiol., 116:90. 1936.

(64) Keller, A. D., W. Noble, and J. W. Hamilton, Jr. Effects of anatomical separation of the hypophysis from the hypothalamus in the dog. Am. J. Physiol., 117:467. 1936.

(65) Kennedy, G. C. The hypothalamic control of food intake in rats. Proc. Roy. Soc. (London) B 137:535. 1950.

(66) Kennedy, G. C. The role of depot fat in the hypothalamic control of food intake in the rat. Proc. Roy. Soc. (London) B 140:578. 1953.

(67) Kibler, H. H., and S. Brody. Environmental physiology, VII. Influence of temperature, 50-5° F. and 50-95° F., on heat production and cardiorespiratory activities of dairy cattle. Mo. Agr. Exp. Sta. Res. Bull., 450:1. 1949.

(68) Kundt, H. W., K. Bruck, and H. Hensel. Das Verhalten der Hautdurchbluntung bei Kühlung des vorderen Hypothalamus. Naturwissenschaften, 44:496. 1957.

(69) Larsson, S. On the hypothalamic organization of the nervous mechanism regulating food intake. Acta Physiol. Scand., 32, Suppl. 115, pt. 1. 1954.

(70) Lepkovsky, S. The physiological basis of voluntary food intake (appetite?). Advances in Food Res., 1:105. 1948.

(71) Little, C. J., A. J. Norden, and A. R. Wolcott. Baled hay (field cured) compared with hay crop silage for winter feeding of dairy cows. Mich. Agr. Exp. Sta. Quart. Bul., 36:2. 1953.

(72) MacDonald, M. A., and J. M. Bell. Effects of low fluctuating temperatures on farm animals. Can. J. Animal Sci., 38:148. 1958.

(73) Magoun, H. W. and J. R. Brobeck. Activation of heat loss mechanisms by local heating of the brain. J. Neurophysiol. 1:101. 1938.

(74) Makela, A. The rate of passage of food in the digestive tract of ruminants. J. Sci. Agr. Soc. Finland, 24:165. 1952.

(75) Makela, A. Studies on the question of bulk in the nutrition of farm animals with special reference to cattle. Soumen Maataloustieteellisen Seuran Julkaisuja. 85 Acta Agralica Fennica. 1956.

(76) Makela, A. On the influence of diet on the retention time of food in the digestive tract of cows. Maataloustieteellinen Aikakauskirja, 32:39. 1960.

(77) Marshall, N. B., and J. Mayer. Energy balance in goldthioglucose obesity. Am. J. Physiol., 178:271. 1954.

(78) Marshall, N. B., R. J. Barnett, and J. Mayer. Hypothalamic lesions in goldthioglucose injected mice. Proc. Soc. Exptl. Biol. Med., 90:240. 1955.

(79) Mayer, J. Glucostatic mechanisms of regulation of food intake. New Engl. J. Med., 249:13. 1953.

(80) Mayer, J. Regulation of energy intake and the body weight: The glucostatic theory and the lipostatic hypothesis. Ann. N. Y. Acad. Sci., 63:15. 1955.

(81) Mayer, J. Correlation between metabolism and feeding behavior and multiple etiology of obesity. Bul. N. Y. Acad. Med., 33:744. 1957.

(82) Mayer, J., and N. B. Marshall. Specificity of gold-thioglucose for ventromedial hypothalamic lesions and hyperphagia. Nature, 178:1399. 1956.

(83) Mellinkoff, S. M. Relationship between serum amino acid concentration and fluctuations in appetite. J. Appl. Physiol., 8:535. 1956.

(84) Miller, F. R., and C. S. Sherrington. Some observations on the bucco-pharyngeal stage of reflex deglutition in the cat. Quart. J. Exptl. Physiol. 9:147. 1916.

(85) Minson, D. J., and W. J. Pigden. Effect of a continuous supply of urea on utilization of low quality forages. J. Animal Sci., 20:962. 1961.

(86) Moore, L. A., and J. W. Thomas. The acceptability of grass/legume silage by dairy cattle. Proc. of the 8th Grassland Congr., p. 701. 1960.

(87) Nutr. Rev. editors. Intracellular free fatty acid pool in adipose tissue. Nutr. Rev., 20:131. 1962.

(88) Paloheimo, L., and A. Makela. Further studies on the retention time of food in the digestive tract of cows. Acta Agralia Fennica, 94:2. 1959.

(89) Passmore, R., and F. J. Ritchie. The specific dynamic action of food and the satiety mechanism. Brit. J. Nutr., 11:79. 1957.

(90) Persson, B. S., and S. A. Svensson. Rate of feed passage in poultry. Sonderdruck aus Archiv für Geflügelkunde Heft 5, 24. 1961.

(91) Peterson, D. W., C. R. Graw, and N. F. Peek. Growth and food consumption in relation to dietary levels of protein and fiberous bulk. J. Nutr., 52:241. 1954.

(92) Purser, D. B., and R. J. Moir. Ruminal flora studies in the sheep. IX. The effect of pH on the celiate populations of the rumen in vivo. Australian J. Agr. Res., 10:555. 1959.

(93) Quigley, J. P., V. Johnson, and E. I. Solomon. Action of insulin on the motility of the gastrointestinal tract. Am. J. Physiol., 90:89. 1929.

(94) Quigley, J. P. The role of the digestive tract in regulating the ingestion of food. Annals N. Y. Acad. Sci., 63:6. 1955.

(95) Reid, J. T. Urea as a protein replacement. J. Dairy Sci., 36:955. 1953.

(96) Roux, J. Maladies due tube digestive. Paris, 1907. Cited by Anand (1).

(97) Rusoff, L. L., and P. F. Randel. Palatability studies of organic acid mixtures characteristic of good and poor quality grass silage. J. Dairy Sci., 45:665, p. 7. 1962.

(98) Share, I., E. Martyniuk, and M. I. Grossman. Effect of prolonged intragastric feeding on oral food intake in dogs. Am. J. Physiol., 169:229. 1952.

(99) Sharma, K. N., B. K. Anand, S. Dua, and B. Singh. Role of stomach in regulation of activities of hypothalamic feeding centers. Am. J. Physiol., 201:593. 1961.

(100) Sherrington, C. S. Textbook of Physiology. E. P. Sharpey-Schafer, ed., Edinburgh:Pentland. 1900.

(101) Smart, W. W. G., T. A. Bell, N. W. Stanley, and W. A. Cope. Inhibition of rumen cellulase by an extract from cericea forage. J. Dairy Sci. 44:1945. 1961.

(102) Snedecor, G. W. Statistical Methods. 5th ed. Iowa State College Press, Ames, Iowa. 1956.

(103) Strominger, J. L., and J. R. Brobeck. A mechanism of regulation of food intake. Yale J. Biol. and Med., 25:383. 1953.

(104) Stunkard, A. J., T. B. Van Itallie, and B. B. Reis. The mechanism of satiety: effect of glucagon on gastric hunger contractions in man. Proc. Soc. Exptl. Biol. Med., 89:258. 1955.

(105) Thomas, J. W., J. R. Ingalls, M. Yang, and B. S. Reddy. Effect of ad libitum or equalized feeding of alfalfa hay or silage on rumen contents and its characteristics. Michigan State University. J. Dairy Sci. 1961.

(106) Thomas, J. W., L. A. Moore, M. Okamoto, J. F. Sykes. A study of factors affecting rate of intake of heifers fed silage. J. Dairy Sci., 44:1471. 1961.

(107) Thomas, J. W., L. A. Moore, and J. F. Sykes. Further comparisons of alfalfa hay and alfalfa silage for growing dairy heifers. J. Dairy Sci., 44:862. 1961.

(108) Van Itallie, T. B. Arteriovenous glucose differences, metabolic hypoglycemia and food intake in man. Am. J. Clin. Nutr., 1:208. 1953.

(109) Van Soest, P. J. A rapid method for the determination of fiber and lignin using detergent. U.S.D.A. A.R.S. (mimeo). J. Dairy Sci. 1961.

(110) Williams, V. J., and K. R. Christian. Concentrations of end-products and morphological types of rumen bacteria in silage-fed sheep. New Zealand J. of Agr. Res., 2:387. 1959.

(111) Wiseman, H. G., and H. M. Irvin. Determination of organic acids in silage. Agr. and Food Chem., 5:213. 1957.

APPENDIX

APPENDIX TABLE I

ANALYSIS OF VARIANCE TABLES OF RESULTS WHEN RABBITS WERE FED SILAGE JUICE TREATED PELLETS VS CONTROL PELLETS

Grams dry matter intake by four day intervals by rabbits fed silage juice treated pellets vs control pellets

Analysis of Variance Table

Source	D.F.	Sum of Squares	Mean Square	F	Signif.
Treatments	1	4428.166	4428.166	.41	n.s.
Periods	1	26533.5	26533.5	2.49	"
Groups	1	9048.166	9048.166	.85	"
T x P	1	2903.994	2903.994	.27	"
T x G	1	5765.998	5765.998	.54	"
T x G x P	1	20768.172	20768.172	1.95	"
Error	16	170453.34	10653.3		
Total	23	241192.0			

Grams weight gains by eight day intervals of rabbits fed silage juice treated pellets vs control pellets

Analysis of Variance Table

Source	D.F.	Sum of Squares	Mean Square	F	Signif.
Treatments	1	468.78	468.78	.14	n.s.
Groups	1	1800.78	1800.78	.54	"
T x G	1	7500.00	7500.00	2.27	"
Error	8	26432.14	3304.0		
Total	11	36201.7			

APPENDIX TABLE II

RUMEN URONIC ACID AND TOTAL SOLUBLE CARBOHYDRATE CONCENTRATION DURING INTRARUMINAL BEET PULP ADMINISTRATION

Day	Uronic Acid					Total Carbohydrate				
	Hours after feeding					Hours after feeding				
	-.1	1.5	2.5	3.5	4.5	-.1	1.5	2.5	3.5	4.5
	(ugm./ml.)					(ugm./ml.)				
A103				H_2SO_4 Preserved						
0	.152	.169	.128	.140	.125	.570	.810	.680	.764	.740
1	.130	.696	.222	.166	.146	.690	7.200	1.790	.600	1.110
7	.116	.241	.152	.142	.133	.800	1.920	1.130	.846	.920
				Ethanol Preserved						
0	.121	.142	.106	.122	.115	.752	.864	.616	.677	.650
1	.110	.568	.178	.143	.126	.630	5.632	1.590	1.336	1.168
7	.087	.204	.129	.110	.105	.715	1.930	1.072	.768	.852
A192				H_2SO_4 Preserved						
0	.133	.185	.189	.139	.123	.395	.388	.530	.395	.378
1	.145	.965	.334	.214	.165	.265	2.081	.775	.510	.390
7	.135	.169	.188	.189	.180	.490	.560	.596	.497	.487
				Ethanol Preserved						
0	.112	.144	.147	.118	.131	.216	.294	.428	.301	.302
1	.116	.824	.247	.180	.135	.284	2.080	.792	.512	.334
7	.098	.103	.126	.160	.128	.456	.604	.572	.556	.480
T20				H_2SO_4 Preserved						
0	.108	.107	.113	.090	.085	.308	.340	.308	.242	.300
1	.144	.575	.176	.183	.170	.237	1.252	.373	.380	.373
7	.155	.766	.180	.144	.135	1.180	1.610	.575	.408	.445
				Ethanol Preserved						
0	.095	.098	.085	.081	.085	.176	.200	.160	.156	.168
1	.110	.440	.136	.144	.134	.222	1.413	.388	.410	.432
7	.134	.664	.140	.180	.205	.572	1.784	.596	.404	.596

H_2SO_4 Preserved

Ave. 0.2161 St. Dev. $\pm$ 0.1694 Ave. 1.0747 St. Dev. $\pm$ 0.935

Ethanol Preserved

Ave. 0.1724 St. Dev. $\pm$ 0.1414 Ave. 1.0089 St. Dev. $\pm$ 0.936

"t" P < .05 n.s. P < .05 n.s.

Correlation Coeff. H_2SO_4 vs. ethanol 0.9874 (P < 0.01)

r = 0.980 (P < 0.01)

APPENDIX TABLE III

RUMEN VOLATILE FATTY ACID CONCENTRATIONS IN COWS FED HAY AND SILAGE

Time after feed	Hay Acet.	Prop.	Buty.	Acet./Prop.	2:3 Carbon	Silage Acet.	Prop.	Buty.	Acet./Prop.	2:3 Carbon
	(mM/ml.)					(mM/ml.)				
T19 Low Level										
1.5	.053	.012	.003	4.42	4.92	.045	.015	.002	3.00	3.27
2.5	.057	.012	.003	4.75	5.25	.048	.013	.003	3.69	4.15
3.5	.058	.011	.003	5.27	5.82	.045	.012	.002	3.75	4.08
4.5	.052	.012	.004	4.33	5.00	.047	.011	.003	4.27	4.82
6.5	.069	.016	.006	4.31	5.06	.053	.011	.003	4.80	5.36
12.0	.093	.018	.005	5.16	5.72	.045	.012	.002	3.75	4.08
T23 Low Level										
1.5	.044	.009	.001	4.89	5.12	.073	.018	.004	4.05	4.50
2.5	.048	.010	.002	4.80	5.20	.060	.015	.003	4.00	4.40
3.5	.045	.009	.002	5.00	5.44	.068	.016	.004	4.25	4.75
4.5	0039	.007	.001	5.57	5.86	.066	.016	.005	4.12	4.75
6.5	.048	.008	.002	6.00	6.50	.060	.014	.004	4.20	4.86
12.0	.037	.006	.001	6.17	6.50	.056	.010	.005	5.60	6.60
T19 Medium Level										
1.5	.055	.012	.003	4.58	5.08	.070	.020	.011	3.50	4.60
2.5	.049	.009	.002	5.44	5.89	.064	.017	.010	3.76	4.94
3.5	.046	.007	.001	6.57	6.86	.071	.019	.010	3.73	4.79
4.5	.046	.009	.001	5.11	5.33	.067	.019	.010	3.53	4.58
6.5	.051	.010	.003	5.10	5.70	.061	.019	.012	3.63	5.42
12.0	.058	.011	.004	5.27	6.00	.076	.022	.011	3.45	4.45
T23 Medium Level										
1.5	.088	.018	.005	4.89	5.44	.061	.013	.004	4.69	5.41
2.5	.089	.019	.006	4.68	5.32	.063	.016	.005	3.94	4.56
3.5	.089	.017	.006	5.20	6.00	.060	.013	.004	4.60	5.23
4.5	.089	.017	.005	5.20	5.82	.066	.015	.004	4.40	4.93
6.5	.093	.019	.006	4.89	5.53	.073	.018	.005	4.05	4.66
12.0	.075	.014	.005	5.36	6.07	.059	.013	.005	4.54	5.31
T19 High Level						(Arthur)				
1.5	.073	.018	.007	4.05	4.83	.052	.015	.007	3.47	4.40
2.5	.066	.015	.006	4.40	5.20	.067	.021	.010	3.19	3.67
3.5	.071	.017	.005	4.18	4.76	.064	.020	.010	3.20	3.70
4.5	0.80	0.16	.007	5.00	5.87	.059	.014	.008	4.20	5.36
6.5	.113	.025	.009	4.52	4.84	.076	.025	.011	3.04	3.92
12.0	.101	.021	.009	4.80	5.66	.069	.019	.011	3.63	4.79
T23 High Level										
1.5	.069	.017	.005	4.05	4.65	.072	.015	.008	4.80	5.97
2.5	.083	.019	.007	4.37	5.10	.071	.013	.007	5.46	6.54
3.5	.081	.015	.006	5.40	6.20	.059	.012	.006	4.92	5.91
4.5	.080	.017	.005	4.70	5.29	.064	.013	.007	4.92	6.00
6.5	.102	.023	.009	4.43	5.22	.072	.014	.008	5.14	6.43
12.0	.107	.024	.011	4.46	5.37	.074	.014	.007	5.29	6.29

APPENDIX TABLE IV

ANALYSIS OF VARIANCE OF RUMEN VOLATILE FATTY ACID CONCENTRATIONS FOR COWS FED HAY VS SILAGE AT THREE LEVELS AND SIX TIMES DURING THE DAY

Acetate

Source	D.F.	Sum of Squares	Mean Square	F	Signif.
Feed	1	.000755	.000755	27.2	0.01
Time	5	.00130	.00026	9.4	0.01
Animal	1	.000584	.00058	20.9	0.01
Level	2	.00565	.002825	101.6	0.01
F x T	5	.000499	.000099	3.6	0.05
F x A	1	.000008	.000008	----	----
FxL	2	.001445	.000722	25.9	0.01
T x A	5	.000575	.000115	4.1	0.05
T x L	10	.001085	.000108	3.9	0.05
A x L	2	.000903	.000451	16.2	0.01
F x T x A	5	.000297	.000059	2.12	----
F x T x L	10	.000693	.0000693	2.49	----
F x A x L	2	.00475	.00237	85.2	0.01
T x A x L	10	.000647	.000065	2.34	----
Error	10	.000278	.0000278		
Total	71	.019469			

Prppionate

Source	D.F.	Sum of Squares	Mean Square	F	Signif.
Feed	1	.025	.025	8.3	0.05
Time	5	.070	.014	4.7	0.05
Animal	1	.011	.011	3.6	----
Level	2	.348	.174	58.0	0.01
F x T	5	.024	.0048	1.7	----
F x A	1	.056	.056	18.7	0.01
F x L	2	.137	.0685	22.8	0.01
T x A	5	.043	.0086	2.8	----
T x L	10	.085	.0085	2.8	----
A x L	2	.052	.026	8.7	0.01
F x T x A	5	.014	.0028	.9	----
F x T x L	10	.045	.0045	1.5	----
F x A x L	2	.318	.159	53.0	0.01
T x A x L	10	.077	.0077	2.7	----
Error	10	.030	.0030		
Total	71	1.335			

Appendix Table IV concluded

Butyrate

Source	D.F.	Sum of Squares	Mean Square	F	Signif.
Feed	1	58.680	58.680	43.7	0.01
Time	5	30.736	6.147	4.6	0.05
Animal	1	21.014	21.014	15.6	0.01
Level	2	268.028	134.014	99.7	0.01
F x T	5	4.074	.814	.6	----
F x A	1	26.125	26.125	19.4	0.01
F x L	2	32.195	16.09	12.0	0.01
T x A	5	.74	.148	----	----
T x L	10	13.476	1.35	1.0	----
A x L	2	1.698	.849	----	----
F x T x A	5	9.95	1.99	----	----
F x T x L	10	6.30	.630	----	----
F x A x L	2	138.75	69.375	51.5	----
T x A x L	10	6.798	.6798	----	----
Error	10	13.436	1.3436		
Total	71	632.0			

APPENDIX TABLE V

ANALYSIS OF VARIANCE OF ACETATE-TO-PROPIONATE AND 2-TO-3 CARBON VFA RATIOS FOR COWS FED HAY VS SILAGE AT THREE LEVELS AND SIX TIMES DURING THE DAY

Acetate-to-Propionate

Source	D.F.	Sum of Squares	Mean Square	F	Signif.
Feed	1	11.97	11.97	53.4	0.01
Time	5	2.88	.576	2.6	----
Animal	1	6.00	6.00	26.7	0.01
Level	2	.43	.21	.9	----
F x T	5	.67	.13	.6	----
F x A	1	2.71	2.71	7.6	0.05
F x L	2	2.54	1.27	5.7	0.05
T x A	5	.60	.12	.5	----
T x L	10	2.44	.244	1.1	----
A x L	2	1.36	.68	3.0	----
FTA	5	1.24	.25	1.1	----
FTL	10	.62	.062	.3	----
FAL	2	2.28	1.14	5.1	0.05
TAL	10	2.16	.216	.9	----
Error	10	2.24	.224		
Total	71	40.137			

2-to-3 Carbon Ratio

Source	D.F.	Sum of Squares	Mean Square	F	Signif.
Feed	1	6.119	6.12	29.1	0.01
Time	5	3.81	.762	3.6	0.05
Animal	1	5.287	5.29	25.2	0.01
Level	2	.74	.37	1.7	----
F x T	5	1.21	.24	1.1	----
F x A	1	2.65	2.65	12.6	0.01
FxL	2	3.06	1.53	7.3	0.05
T x A	5	.78	.16	.7	----
T x L	10	2.85	.285	1.3	----
A x L	2	2.71	1.35	6.4	0.05
F x T x A	5	1.61	.322	1.5	----
F x T x L	10	.61	.061	.3	----
F x A x L	2	2.27	1.13	5.4	0.05
T x A x L	10	4.30	.43	2.0	----
Error	10	2.11	.211		
Total	71	40.12			

APPENDIX TABLE VI

DATA OF RUMEN EMPTYINGS

Cow No.	D.M. Intake	Rumen Contents Wet	Rumen Contents D.M.	Rumen D.M. / Intake D.M.	% D.M. in rumen	Fiber intake	Rumen fiber / intake fiber
	(lb/cwt.)	(% body wt.)			(%)	(lb/cwt.)	
Hay fed							
T26	2.527	11.959	1.608	.636	13.45	1.080	.780
T26	2.757	10.270	1.312	.475	12.78	1.178	.569
K139	1.326	13.060	1.440	1.086	11.03	.482	1.428
T19	.423	10.460	.753	1.779	7.20	.154	2.333
T23	.446	12.910	.684	1.535	5.30	.162	1.630
T23	.484	14.393	1.194	2.467	8.30	.178	3.149
T19	.429	11.507	1.263	2.945	10.97	.158	4.356
A192	2.258	13.479	1.782	.789	13.22	.866	1.040
T20	1.551	10.526	1.029	.663	9.78	.588	.726
T26	2.229	9.427	1.427	.640	15.14	.845	.828
T19	.842	12.789	1.630	1.934	12.74	.319	2.493
T23	.501	9.902	.884	1.766	8.93	.186	2.083
T23	.952	16.666	2.082	2.186	12.49	.383	2.615
T23	2.333	15.306	2.185	.937	14.28	.940	1.248
Silage fed							
T23	1.216	14.898	1.539	1.265	10.33	.403	1.674
T19	.557	9.812	.868	1.562	8.87	.184	2.176
Art	1.084	17.044	1.907	1.760	11.19	.395	2.487
T19	1.670	14.677	1.708	1.023	11.64	.609	1.332
Art	2.042	14.685	1.856	.971	12.64	.745	1.248
Hay + Silage juice							
T26	2.391	10.360	1.234	.517	11.94	.891	.666
T20	1.791	11.200	1.190	.665	10.65	.550	.980
A192	1.383	15.737	1.310	.946	8.33	.292	1.799
T26	2.602	9.726	1.200	.460	12.34	.986	.624
T20	1.622	9.805	1.121	.691	11.43	----	----
Hay + Washed Pressed Silage							
T20	1.790	13.140	1.634	.913	12.44	.815	.963
T26	2.421	11.270	1.611	.665	14.29	1.004	.784
T20	1.758	12.040	1.344	.764	11.16	.786	.904
T26	2.406	10.244	1.130	.470	11.03	----	----
Fresh Cut Alfalfa							
K139	2.117	11.417	1.474	.696	12.91	.767	.970
K139	1.435	12.360	1.663	1.158	13.49	.523	1.637
Hay + Beet Pulp							
T20	1.384	12.442	1.354	.980	10.89	.464	1.213
A192($NaHCO_3$)	2.180	15.360	2.103	.964	13.69	.763	1.296

APPENDIX TABLE VII

RELATIONSHIPS BETWEEN DRY MATTER AND FIBER IN THE RUMEN, RETENTION TIME AND INTAKE

Dry matter retention time vs fiber retention time
(N = 30)

$r = 0.975$, $P < 0.01$ $\quad$ $t = 1.847$, $P > 0.95$

Dry matter intake (lbs./cwt.) vs fiber intake (lb./cwt.)
(N = 30)

$r = 0.9736$, $P < 0.01$ $\quad$ $t = 6.658$, $P > 0.99$

APPENDIX TABLE VIII

RELATIONSHIPS BETWEEN DRY MATTER INTAKE AND RUMEN CONTENTS

Dry matter intake (lb./cwt.) vs percent dry matter in rumen
(N = 32)

$r = 0.74$, $P < 0.01$ $\quad$ $Y = 7.8 + 2.3X$
std. error of est. $\pm$ 1.497

Dry matter intake (lb./cwt.) vs dry matter in rumen as percent of body weight
(N = 32)

$r = 0.28$, $P < 0.05$ $\quad$ $Y = 1.19 + 0.148X$
std. error of est. $\pm$ .3728

Dry matter intake (lb./cwt.) vs weight of rumen contents (wet) as percent of body weight
(N = 32)

$r = -0.15$, $P < 0.05$ $\quad$ $Y = 13.2 - 0.47X$
std. error of est. $\pm$ 2.232

Dry matter intake (lb./cwt.) vs percent dry matter in rumen
(N = 5) cows fed silage

$r = 0.944$, $P < 0.05$ $\quad$ $Y = 7.827 + 2.365X$
std. error of est. $\pm$ 0.539

Dry matter intake (lb./cwt.) vs percent dry matter in rumen
(N = 5) cows fed hay

$r = 0.7345$, $P < 0.05$ $\quad$ $Y = 7.865 + 2.753X$
std. error of est. $\pm$ 1.93

APPENDIX TABLE IX

COMPARING RUMEN RETENTION TIME OF FIVE COWS FED HAY VS FIVE COWS FED SILAGE AT SIMILAR LEVELS OF DRY MATTER INTAKE

Cows fed hay						Cows fed silage					
Cow	DM intake	DM Retention time	Fiber Intake	Fiber Retention time	DM in Rumen	Cow	DM Intake	DM Retention time	Fiber Intake	DM Retention Time	DM in Rumen
	(lb/cwt.)	(days)	(lb/cwt.)	(days)	(%)		(lb/cwt.)	(days)	(lb/cwt.)	(days)	(%)
T26	2.229	.640	.8456	.828	15.14	ART	2.062	.971	.745	1.248	12.66
T20	1.551	.663	.5885	.726	99.78	T19	1.670	1.023	.6096	1.332	11.64
K139	1.326	1.086	.4823	1.428	11.03	T23	1.216	1.265	.4031	1.674	10.33
T23	.952	2.186	.3837	2.615	12.49	ART	1.084	1.760	.3955	2.487	11.19
T23	.501	1.766	.1860	2.083	8.94	T19	.557	1.562	.1840	2.176	8.87
mean	1.3118	1.2682	.4972	1.536	11.476		1.3138	1.316	.4675	1.7834	10.934

Rumen dry matter retention time of cows fed hay vs cows fed silage r = 0.997, P 0.01 "t" = 0.14, P = 0.5

Rumen fiber retention time of cows fed hay vs cows fed silage r = 0.994, P 0.01 "t" = 0.568, P 0.7

APPENDIX TABLE X

INTAKE AND RUMEN RETENTION TIME COMPARING FIVE COWS FED HAY WITH FIVE COWS FED HAY PLUS SILAGE JUICE

Fed Hay			Fed hay + silage juice		
Cow	DM Intake (lb/cwt)	Retention time (days)	Cow	DM Intake (lb/cwt)	Retention time (days)
T26	2.757	.476	T26	2.602	.460
A192	2.258	.789	T26	2.391	.517
T26	2.229	.640	T20	1.791	.666
T20	1.551	.663	T20	1.622	.691
K139	1.326	1.086	A192	1.383	.946
mean	2.0242	.7308		1.958	.656

Rumen dry matter retention time of cows fed hay vs cows fed hay plus silage juice

r = 0.82 n.s. "t" = 0.56 P = 0.7

APPENDIX TABLE XI

INTAKE AND RUMEN RETENTION TIME COMPARING FOUR COWS FED HAY ADMINISTERED WASHED PRESSED SILAGE VS FOUR COWS FED HAY ADMINISTERED SILAGE JUICE

Fed Hay + washed pressed silage			Fed hay plus silage juice		
Cow	DM Intake (lb/cwt)	Retention time (days)	Cow	DM Intake (lb/cwt)	Retention time (days)
T20	1.758	.7644	T20	1.622	.6910
T20	1.790	.913	T20	1.791	.6659
T26	2.406	.470	T26	2.391	.5174
T26	2.421	.6653	T26	2.602	.4600
mean	2.094	.7032		2.102	.5836

Rumen dry matter retention time of cows fed hay administered washed pressed silage vs silage juice

r = 0.46 n.s. "t" = 0.78 P = 0.7