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VOLATILE FATTY ACIDS, pH AND MICROBIAL ACTIVITY IN THE RUMEN CONTENTS OF THE COW

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Selostus:

Haihtuvat rasvahapot, pH ja mikrobiston aktiviteetti lehmän pötsin sisällössä

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M A R T T I L A M P I L A

Agricultural Research Centre, Department of Animal Husbandry

Tikkurila, Finland

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To be presented, with the permission of the Faculty of Agriculture and Forestry of the University of Helsinki, for public criticism in Auditorium XII on October 10th, 1964, at 12 o'clock.

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PREFACE

The present work belongs to a series of studies on the rumen which were introduced into the research programme of the Department of Animal Husbandry at the time when Professor Ilmari P o i j ä r v i, Ph.D., was Head of the Department. I am very grateful to him for his cooperative and encouraging attitude which was of great help in the early part of this study. I also wish to express my sincere appreciation to Professor Orvo R i n g, Dr. of Agric. and Forestry, present Head of the Department of Animal Husbandry, who in many ways has helpfully aided in all phases of this work. I am also thankful to my colleages for their assistance.

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Some of the analytical determinations were carried out in the Department of Agricultural Chemistry and Physics. I want to express my thanks to the Head of the Department, Professor Martti S a l o n e n, Dr. of Agric. and Forestry, for allowing the use of the Department's equipment and to Mr. T. K e r ä n e n, Dr. of Agric. and Forestry, and Mr. E. B a r k o f f, Dipl.Eng., for their help in performing the analyses.

The major share of the mathematical treatment of the results was carried out by Mr. T. M o i s i o, Mag.Phil., and Mr. H. V ä l i a h o, Mag.Phil., to whom I extend my thanks.

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Tikkurila, July 21, 1964.

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INTRODUCTION

It is well known at the present time that digestive fermentation in the rumen is accompanied by abundant production of volatile fatty acids. Numerous investigations have clearly shown the great importance of these acids in the nutrition of ruminants.

The investigation of PALOHEIMO *et al.* (1955), based on the lignin-ratio technique, showed that from 85 to 91 per cent of digestible N-free non-lignin organic matter of the fodder disappeared in the proventriculi when cattle is fed on hay. In an another work PALOHEIMO and MÄKELÄ (1960) found the variation of the same percentage on variable diets to be from 58 to 96. In the work of BALCH (1957) it appeared that of the total dry matter disappearing from the alimentary tract, from 43 to 83 per cent disappeared in the reticulo-rumen when determinations based on the lignin-ratio technique were made on samples taken near the reticulo-omasal orifice. Using sheep with a duodenal fistula, HOGAN (1957) found that almost 70 per cent of the digestible dry matter of the ration consisting of hay and a concentrate mixture was lost between the mouth and the duodenum. In the detailed investigation of MARSTON (1948) from 72 to 79 per cent of the combustible energy of cellulose, fermented by rumen microorganisms *in vitro*, was found in the lower volatile fatty acids produced.

When calculations on the total amounts of the volatile fatty acids produced daily in the rumen are made on the basis of the above results, the effect of acid production on the rumen acidity becomes an interesting question.

The effect of volatile fatty acid production on the life conditions of rumen microorganisms has been investigated in many studies, even in earlier times, when fermentative digestion of fodders was considered much less important than it is now known to be. These early studies are the source of many results of reseach on the pH of the rumen which have been repeated in physiology textbooks and which claim that the accumulation of acids in the rumen does not progress to the point of being appreciably harmful to the microbe population (MANGOLD 1929, p. 151; SCHEUNERT and TRAUTMANN 1952, p. 84; DUKES 1953, p. 330). The abundant secretion of saliva with its strong buffering effect was thought to be the reason why on normal diets the pH of the rumen was close to neutral or even on the alkaline side.

Relatively early, however, MARKOFF (1913) calculated from his investigations that the production of acids in the rumen exceeds by a multiple the neutralizing capacity of the alkaline saliva. This apparent contradiction was satisfactorily explained when it was shown that volatile fatty acids are removed from the rumen by absorption through its walls (MCANALLY and PHILLIPSON 1942, BARCROFT *et al.* 1943). When these studies thus indicated that a second apparently efficient mechanism was also operative in stabilizing the rumen pH, the conception of rumen contents neutrality was readily accepted.

Preliminary observations made by the writer on the pH of rumen contents gave reason to undertake further studies on this problem. The rather low pH values found in milk cow on ordinary diets (LAMPILA 1955), together with similar reports mentioned in some earlier investigations, indicated the possibility that a hydrogen ion concentration higher than optimum could retard the microbial activity and thus have an unfavorable effect on the nutrition of the animal. However, the question was complicated by the fact that the pH of the contents in the upper part of the rumen was generally definitely lower and the concentration of volatile fatty acids higher than in the basal part of the organ. A study of the literature revealed that MONROE and PERKINS (1939) had obtained similar results, although the differences were smaller. Determinations made by SMITH (1941) also indicated pH differences between different parts of the rumen. Studies made by P. H. SMITH *et al.* (1956) confirmed these observations and showed distinct difference in pH and volatile fatty acid concentration between the upper and lower parts of the rumen contents in a normal, unfistulated cow.

These various studies give reason to assume that the rumen contents and the conditions prevailing within them cannot be considered as a single, homogenous entity. This is further indicated by the observations that the dry matter content of the ventral sac is clearly less than that of the dorsal sac (BALCH 1950) and that the fermentation of cellulose and other cell wall carbohydrates proceeds more rapidly in the lower part of the rumen (e.g. BALCH and JOHNSON 1950, MILES 1951). The latter fact would seem to agree with the assumption that the differences in pH occur in a range where they have an influence on the fermentative activity of the microorganisms. The results of preliminary investigations made by the writer on this problem supported this assumption (LAMPILA 1959).

Cases of overeating, which sometimes occur in practice and which have also been produced experimentally, have shown that large rations of concentrated fodders or their readily fermentable carbohydrate components may lead to a very intensive formation of acids and exceptionally low pH values in the rumen contents (e.g. HUNGATE *et al.* 1952, BROBERG 1960, KROGH 1959, 1960, 1961 a). An extreme consequence of this condition is the destruction of the normal microbe population of the rumen and serious illnes in the animal. It has been claimed that as rations are increased, a lowering of the pH from approxi-

mately neutral to a point which is detrimental to the health of the animal occurs suddenly at a certain limit (KROGH 1959). This, however, conflicts with the knowledge of the buffering action of the rumen contents (TURNER and HODGETTS 1955 b). It also disagrees with experiments in which pH determinations were made in different parts of the rumen (LAMPILA 1955) or in its upper part (BALCH *et al.* 1955, BALCH and ROWLAND 1957). Since high-yielding dairy cows are often fed with food quantities approaching the level of overeating, it is important to know whether the harmful effect of excessive acid accumulation on the microorganisms appears suddenly or gradually. The recognition of the detriments and the finding of means for preventing or reducing them is obviously important in attempts to increase the productivity of the animals.

This investigation is a continuation of earlier works (LAMPILA 1955, 1959; LAMPILA and POIJÄRVI 1959). The purpose was to get a more exact picture of the interrelations between the volatile fatty acid accumulation, pH and the activity of some vital processes of the rumen microbial population in "normal" diets. A "normal" diet, as opposed to "overfeeding" by a forced diet, is meant here one which the animal can cope with easily and manage on from day to day.

An essential part of the work was to study further the differences of pH and volatile fatty acid concentration found between the upper and lower rumen in the earlier works (LAMPILA 1955, LAMPILA and POIJÄRVI 1959, P. H. SMITH *et al.* 1956). The immediate cause of the pH differences was primarily studied by determinations on the neutralizing capacity and concentrations of five mineral ions in the rumen fluid. It was hoped thereby to acquire knowledge on the possibilities of affecting the rumen pH by means of mineral feeding.

The correlation between pH and the fermentative activity of the mixed rumen microbe population was investigated by the *in vitro* method using rumen contents as a substrate in the short-term incubation experiments. The effect of the volatile fatty acid concentration on the fermentative activity was also studied using the same method.

The balance between the reactions producing and consuming ammonia was the second object of this study considering the correlation between the pH and microbial activity. It was thought that this balance would be a good indicator to the possible rôle of the rumen pH by the utilization of nitrogenous substances (cf. PEARSON and SMITH 1943 b).

REVIEW OF THE LITERATURE

I. Acid concentrations and pH in the rumen contents

Under normal feeding conditions the lower volatile fatty acids (VFA) are practically the only organic acids affecting the pH of the rumen contents. Their presence in the rumen was known as early as the last century (BARNETT and REID 1961, pp. 1—2). It was also known then that the alkali bicarbonates in saliva neutralized these acids, with the result that the pH of the rumen contents was mainly dependent upon the relative equivalent concentrations of these two groups of compounds.

In the production of volatile fatty acids, an intermediate product often observed in small amounts — providing that the diet is suitable for its accumulation — is lactic acid (PHILLIPSON 1942, BALCH *et al.* 1955). Its amount and proportion among the total acids may, however, rise considerably if the rations contain abundant starch or sugar (HUNGATE *et al.* 1952, BRIGGS *et al.* 1957, KROGH 1959, 1960, 1961 a). Since lactic acid dissociates more than the volatile fatty acids, it has a greater effect on lowering the pH, a result which may be quite distinct under certain conditions (BRIGGS *et al.* 1957). Many of the bacteria in the normal rumen microflora (reviewed by ANNISON and LEWIS 1961, pp. 38—51) form lactic acid as a final product of their metabolism, but it is generally fermented into fatty acids so rapidly that it does not accumulate. The bacteria capable of metabolizing lactic acid (ANNISON and LEWIS 1961, p. 48), however, are apparently more susceptible to the unfavourable effect of high hydrogen ion concentration than are the *Str. bovis*-type bacteria and the lactobacilli, which produce lactic acid and which become the predominant organisms when the pH is lowered (HUNGATE *et al.* 1952, KROGH 1961 a).

A. Factors affecting acid production and pH in the rumen

The nutrient supply of the rumen microorganisms, which primarily affects their life activities and concurrently the rate of acid production, is dependent on the feeding of the host animal. Neutralization of the acids and their removal from the rumen are, on the other hand, dependent on physiological mechanisms in the animal. These mechanisms are clearly aimed at keeping the acidity of the rumen close to neutral (MASSON and PHILLIPSON 1951). The pH of the rumen contents thus indicates the fluctuations in equilibrium between these two groups of agents with opposed action.

1. Nutritional factors. — Trials with purified diets have shown (e.g. LOOSLI *et al.* 1949, THOMAS *et al.* 1951) that the rumen microbe population is able to exist on a diet containing carbohydrates, nitrogenous compounds and minerals; fats and fat-soluble vitamins added to the diets are apparently not

essential. When the animals are fed on rations which meet their normal nutritional requirements, the basic nutrient needs of the rumen microorganisms are generally also fulfilled. Some species of bacteria require certain growth factors (BARNETT and REID 1961, pp. 49—50), but because of the normal symbiotic relationship in the rumen, these factors are usually not indispensable in the rations.

When the basic nutrient requirements of the microorganisms are satisfied, their fermentation activity is dependent upon the concentration of various compounds in the rumen, and these concentrations vary according to the feeding. Fluctuations in fermentation activity are seen most clearly during the period of indoor feeding, when the concentration of acids regularly rises and the pH decreases after feeding (PHILLIPSON 1942, BALCH *et al.* 1955). These fluctuations become less pronounced or may disappear when the number of feedings is increased (KNOX and WARD 1961) or when there is free access to fodder, such as in pasture (LAMPILA 1955).

Carbohydrates, even as early as the last century, have been known to exhibit differences in their fermentability and intensity of acid formation. When TAPPEINER (1884, p. 120) in his classical work added sugar to a culture of rumen bacteria whose substrate was cellulose, the result was an intense fermentation, while the cellulose remained completely or almost completely intact.

PHILLIPSON and MCANALLY (1942) studied the rate of fermentation of various carbohydrates by adding them in 100-gram doses through the fistula into the rumen of a sheep and subsequently observing the changes in acid concentrations and pH of the contents. The relatively most rapidly fermenting carbohydrates were glucose, fructose and sucrose. The addition of each of these caused a pronounced increase in concentration of volatile fatty acids as well as an abrupt drop in pH and the accumulation of lactic acid. No lactic acid was found after the addition of galactose, lactose or maltose, and in these cases the changes in pH and VFA concentration were slower. The slowest fermentation occurred with starch and cellulose. Because of its bulk, only 50 grams of cellulose was added.

In his experiments on the appearance of acute indigestion, KROGH (1959, 1960, 1961 a) studied the effect of sucrose, lactose and starch on fermentation in sheep rumen. The animals were fed twice daily with hay. He added carbohydrate through the fistula, increasing the daily dosage according to a varying plan until symptoms of disturbance appeared. Sucrose produced the symptoms with the smallest doses (200—500 g), lactose required more (700—1200 g), while starch could be given in amounts of as much as 1500 g before symptoms appeared. The maximum dosage of each carbohydrate was apparently inversely correlated to its rate of fermentation. KROGH emphasizes the fact that the change in rumen conditions and microbe population was sudden. In comparing these results with those of PHILLIPSON and MCANALLY (1942), it is difficult to find an explanation — unless perhaps in the experimental technique — for the

fact that the rumen pH remained close to neutral in spite of large amounts of sucrose and lactose (KROGH 1959, 1960).

MCNAUGHT (1951) studied the fermentation of different carbohydrates in short-term *in vitro* trials using the synthesis of cell protein as an indirect indicator and maltose or glucose as a control. Among other results, she found that raw starch from maize and potatoes was a relatively poor substrate, but when cooked it promoted protein synthesis more than did maltose. Also noteworthy were the observations that cellobiose was as good a substrate as fructose or maltose and that the latter was slightly poorer in comparison to L—(—)-arabinose and D—(—)-xylose.

HOWARD (1955) has shown that a pure preparation of wheat flour pentosan can be rapidly broken down in a culture of rumen bacteria. Purified hemicellulose from straw was also readily fermented in the trials of MCANALLY (1942). On the other hand, only 50—60 % of non-purified hemicellulose was broken down in 7 days. It is obvious that in the purifed form, fermentation of such plant constituents may differ considerably from their normal behaviour in plant tissues along with other compounds, such as lignin, which is known to be effective in impeding the fermentation of carbohydrates.

The activity of cellulose fermentation both *in vivo* and *in vitro* is known to be dependent upon the presence of certain other accompanying substances (reviewed by BARNETT and REID 1961, pp. 94—99). For example, HOFLUND *et al.* (1948), among others, observed that sugar at a concentration of 0.1—0.2 % stimulated the breakdown of cellulose *in vitro*, while higher concentrations retarded the process. The effect was similar *in vivo*.

SUDGEN (1953) considered the possible rôle of rumen protozoa in evening out the fermentation peaks by storing carbohydrates in their cells during abundant supply and utilizing these carbohydrates during times of deficiency.

Nitrogenous compounds must occur in sufficient amounts for the optimum fermentation of carbohydrates. When large amounts of readily fermentable sugars or starch are present, the poor fermentation of cellulose (or fibre) appears to be due to a deficiency of soluble nitrogenous substances. In the trials of BURROUGHS *et al.* (1950 b), for example, addition of starch decreased the digestibility of fibre from 60 % to 13 %, but by adding casein it was raised to a level of 53 %. In the *in vitro* trials of BELASCO (1954) the breakdown of cellulose was increased from 19 % to 91 % by the gradual addition of urea to the culture. When sheep were fed on purified rations (HO *et al.* 1962), in which cellulose constituted the main source of carbohydrate, the digestibility of this compound was improved from 53 % to 73 % when the protein content of the diet was increased from 4 % to 8 %.

The larger proportion of the nitrogenous compounds in the normal diets of ruminants consists of proteins. As a result of the proteolytic activity of the rumen microorganisms (SYM 1938, WARNER 1956), proteins are partly broken down into amino acids, which are then in a utilizable form for the bacteria.

Amino acids can be further degraded fermentatively (SHAZLY 1952, SIROTNAK *et al.* 1953), with the formation of ammonia, carbon dioxide and volatile fatty acids.

Most of the 21 groups of rumen bacteria described by BRYANT (1963) possess the ability to utilize ammonia as their sole source of nitrogen. In six of these groups this compound is indispensable. Even if a great part of the nitrogenous end products of protein degradation should be ammonia, the possibilities of its utilization appear to be good. Ammonia however, occurs consistently in the rumen contents, its concentration being dependent upon the balance between processes forming it and those consuming it. Under certain conditions it may have a considerable importance in neutralizing acids (BRIGGS *et al.* 1957).

The susceptibility of different proteins to microbial attack varies considerably. In the experiments of MCDONALD (1954), 40 % of zein was broken down in the rumen, whereas 90 % of casein was degraded (MCDONALD and HALL 1957). On the basis of the ammonia concentrations of the rumen contents, CHALMERS and SYNGE (1950, 1954) came to the conclusion that casein is more readily decomposed than herring meal. MCDONALD (1952) carried out similar trials with casein, gelatin and zein. The addition of the first two compounds caused a distinct rise in the ammonia concentration, but zein had no effect at all. ANNISON *et al.* (1954) observed differences in ammonia accumulation between groundnut meal, casein, herring meal and maize-gluten meal. In *in vitro* trials, ANNISON (1956) compared the susceptibility of different proteins to degradation by using washed rumen bacteria suspensions. He found that casein, soy protein and arachin were broken down readily, whereas bovine albumin, wheat gluten and zein were degraded with difficulty.

Ammonia may also be formed by the degradation of other nitrogenous compounds, such as purines and pyrimidines (JURTSHUK and HUETER 1955, DOETSCH and JURTSHUK 1957, JURTSHUK *et al.* 1958, cited by BARNETT & REID 1961, pp. 111—112), and by the reduction of nitrates (LEWIS 1951 a, b; HOLTENIUS 1957).

Urea has a special significance among the "amide" nitrogen sources, since it is the most commonly employed compound used to replace protein by cheaper nitrogenous substances (reviewed by REID 1953) and since it is secreted via the saliva into the rumen and possibly also directly through the rumen walls (HOUPT 1959). The rapid decomposition of urea into ammonia within the rumen contents has been shown, among others, by LENKEIT and BECKER (1938) and PEARSON and SMITH (1943 a, b). Since no organic acids are formed by its degradation, as are during the fermentation of purines, pyrimidines and amino acids, it has a relatively more pronounced effect in raising the pH. If the ammonia-consuming reactions have been weakened, for example, as a result of scarcity of readily fermentable carbohydrates, a relatively small amount of urea — especially if given suddenly — may raise the pH of the rumen contents far to the alkaline side and cause a resulting toxic effect (CLARK *et al.* 1951).

McDONALD (1948) observed that ammonia nitrogen consistently made up a large share of the total nitrogen content in the rumen liquor of grazing sheep. In a typical case, when a sheep was put to pasture after fasting overnight, the ammonia concentration of its rumen liquid increased in 6 hours from 8 to 35 mg %. In the studies of JOHNS (1955), when sheep were kept on high-production pasture having a protein content of 31 %, the ammonia concentration of the rumen attained high values, with a maximum of 139 mg %. When the animals were fed on hay with a protein content of 17.5 %, the ammonia concentration varied from 22 to 30 mg %. In the investigations of HEAD and ROOK (1955) the ammonia concentration in the rumen of cows kept on pasture was at a continuously high level of 40—60 mg %. During the period of indoor feeding, the usual level ranged from 10 to 20 mg % with peak values up to 30 mg % after feeding. Similar ammonia concentrations were also found in cows at pasture in the studies of ORTH and KAUFMAN (1958). When the crude protein content of the pasture grass decreased from 17 to 14 %, the ammonia content in the rumen diminished from about 20 to 5 mM/l.

BRIGGS *et al.* (1957) carried out numerous trials with fistulated sheep in which determinations were made of the rumen ammonia concentrations as well as amounts of total VFA, lactic acid and pH on eleven different types of diets. In the diets which did not contain protein concentrates, the maximum ammonia levels ranged from 16 to 56 mg %. The maximums on diets containing protein concentrates were generally within the range 57—86 mg %. When large amounts of casein were added, the maximum values rose to as much as 223 mg %. The production of acids was at the same time so abundant that even in this case the pH remained on the acidic side.

In the trials of BUTZ *et al.* (1958) the ammonia level in the rumen contents of two cows ranged from 5.5 to 11.5 mg % when the animals received hay containing 305 g crude protein. Two other cows which were given 6 kg of concentrates containing 1 570 g crude protein, showed maximum values after feeding of 24.8 and 26.7 mg %. Similar average maximum ammonia concentrations, approximately 12—16 me./l, were also observed in the trials of LAMPILA (1960) when a fistulated cow was fed on hay, concentrates and differently prepared silages.

The effect of minerals in relation to the problem of acidity in the rumen may manifest itself, in the first place, as an influence of the nutrition of the rumen microbes (reviewed by BARNETT and REID 1961, pp. 170—207). However, the mineral requirements of rumen microorganisms and the optimum concentrations of various elements are still very incompletely understood. On the other hand, the significance of minerals in neutralizing acids and in buffering the rumen contents is well known. The main question in this connection is primarily only how large is the direct influence of ingested minerals on the pH of the rumen in comparison to the effect of salivary secretion and to what extent both of these factors are dependent upon the supply of minerals.

BURROUGHS *et al.* (1950 a) and SWIFT *et al.* (1950) were the first workers to establish the favourable effect of alfalfa ash on digestion. The advantageous influence was mainly seen as improved digestion of fibre when the roughage in the diet consisted principally or entirely of ground corn cobs. The assumption of BURROUGHS *et al.* (*loc. cit.*) that the alfalfa ash supplied certain required minerals, primarily trace elements, is considered to be correct (BARNETT and REID 1961, pp. 170—176). Certain workers (CHAPPEL *et al.* 1952, 1955), however, have paid attention to the alkalinity of alfalfa ash assuming that this would have a favourable effect on digestion acting by means of the rumen pH.

Investigations on the mineral concentrations of the rumen liquid have shown that sodium and potassium ions are the most important in neutralizing acids (BAILEY 1961 b). The close correlation in concentrations of these elements between the saliva and the rumen liquid emphasizes the primary significance of salivary secretion in the neutralization of the rumen contents. Since mastication, both during eating and in ruminating, greatly increases the salivary secretion (BAILEY 1961 a), the physical character of the fodder is an important factor. For example, the studies of GORDON (1958 a, b) showed that rumination was considerably decreased on a diet exclusively consisting of concentrates or when the coarse fodder had been ground. In the cases where alfalfa ash was observed to be advantageous, the diets were apparently such that rumination may have been less than normal. Thus in these cases there were perhaps better conditions for a direct effect of the added alkaline minerals in increasing the pH of the rumen liquid than on normal diets.

The positive effect of alkaline mineral mixtures in promoting appetite and growth has been distinctly noticeable when animals were fed on purified diets (MATRONE *et al.* 1957, 1959) in which rumination is much less than normal (OLTJEN *et al.* 1962 a). When OLTJEN *et al.* (1962 b) gave an alkaline mineral mixture to one of two identical twin steers fed on a purified diet, the pH as well as the concentration of thiamin and riboflavin in the rumen liquid of one steer was higher than that of the second steer which received an acidic mineral mixture.

The low concentrations of calcium and magnesium in the rumen fluid found in the studies of GARTON (1951) give an indication that these elements participate only slightly in buffering the rumen liquid.

In the investigations of GARTON (*loc. cit.*) variations in the phosphorus content of the rumen liquid were found due to diet. BAILEY (1961 b) was not able to observe any correlation between the phosphorus concentration of the saliva and that of the rumen liquid. CLARK (1953) observed that phosphorus deficiency caused statistically significant decreases in its concentration in the blood and saliva. The phosphorus level in the rumen liquid also diminished but there was no statistically significant change.

AMMERMAN and THOMAS (1952) studied the buffering capacity of extracts made from Ladino, alfalfa and bluegrass hay and found that the latter had the

strongest buffering action. When fresh bluegrass or hay made from it was fed to animals, the pH of the rumen liquid was higher than when corresponding forms of Ladino or alfalfa were administered.

In the studies of BRIGGS *et al.* (1957) there were rather large variations in the relation between pH and concentration of volatile fatty acids on different diet types. It appears that some of these variations may have been due to differences in mineral composition of the diets, although other factors contributed at the same time.

2. Physiological factors. — Rumination and salivary secretion are closely related processes, both of which effectively stimulate the microbial digestion of fodders. Mastication reduces the size of the food particles, thus increasing the surface area attacked by the rumen bacteria. In addition, the finely-masticated food particles can more easily pass through the reticulo-omasal opening and subsequently enter the lower parts of the digestive tract (BALCH 1958 a). The increased salivary secretion during rumination (BAILEY 1961 a) also promotes the neutralization of acids.

HAUBNER (1837; cited by MANGOLD 1929, p. 113) was apparently the first investigator to point out the significance of saliva in ruminant digestion. He observed that preventing the entrance of saliva into the rumen resulted in the contents of this organ becoming a dryish, solid mass, where fermentation ceased.

With reference to the experimental results of COLIN and to the later results of ELLENBERGER and SCHEUNERT, MANGOLD (1929, p. 118) stated that an adult cow secretes about 56 kg of saliva in 24 hours. Approximately 40 kg was estimated to be secreted during eating and rumination, while 16 kg were secreted during rest.

MARKOFF (1913, p. 40) analyzed saliva samples collected from the mouth of cattle and calculated that the neutralizing capacity of 50 kg of saliva corresponds to approximately 300 grams of sodium carbonate. On the basis of calculations from his fermentation experiments he estimated (p. 67) that this amount was sufficient to neutralize only about one-seventh of the acids formed in the rumen in 24 hours.

MCDOUGALL (1948) performed the first consistent and detailed investigation on the mineral composition of ruminant saliva. According to his results, the ash of sheep mixed saliva — which amounted to 0.7—0.9 % of the saliva — contained sodium as its principal component. The content of sodium varied from 370 to 462 mg per 100 ml.

BALCH (1958 b) studied salivary secretion in fistulated cattle during the time of feeding by catching food boluses as they entered the rumen and determining the increase in water content of the food. He concluded from these studies that in many diets, salivary secretion during the time of eating must exceed 100 lb. daily. When the animals were fed on hay, they secreted saliva amounting to 4.3—5.7 times the weight of the ingested hay, whereas on concentrated diet the amount was only 1.2—1.5 times that of the food. The saliva

secretion per unit weight of dry matter was 5.3 times that of the food when grass was given and 3 times when the food consisted of mangolds.

Using the same experimental method, BAILEY (1961 a) observed that the amount of saliva secreted at ingestion of different kinds of fodder was partly dependent upon differences in rate of saliva secretion. Mainly, however, such variations in the amount of saliva were due to differences in rapidity of eating. Tentative estimates suggested that the average daily secretion of four cows on five different diets varied from 98 to 190 litres. These values greatly exceed those estimated in previous studies, 50—60 litres daily. The rate of saliva secretion during eating was 2—4 times and during rumination about 2 ½ times that during rest.

In this same series of studies, BAILEY and BALCH reported that the composition of saliva varies in a certain manner according to the rate of secretion (1961 a) and gave figures on the average composition of the mixed saliva on a hay diet (1961 b). The bicarbonate content indicating the neutralizing capacity of the saliva was found in this case to be 126 me. per litre. This figure, as well as the contents of the other components, is similar to that found in parotid saliva. Urea nitrogen, amounting to 1.3—14.4 mg per 100 ml, averaged 77 % of the total salivary nitrogen. The concentration of urea in the saliva was clearly correlated to its concentration in the blood plasma, amounting constantly to over 65 % of the latter. In the final paper of the series, BAILEY (1961 b) presents results showing the interdependence of the mineral compositions of saliva and rumen contents. In addition, he gives estimates on the total secretions of various minerals.

The passage of molecules and ions through the rumen wall is a relatively new aspect in the physiology of ruminants. The introduction of this subject had a profound influence on investigations concerning the fermentative digestion of fodders.

The absorption of volatile fatty acids was first shown by MCANALLY and PHILLIPSON (1942), but the permeability of the rumen wall and the probability of VFA absorption through it had already become evident in some previous investigations (TRAUTMANN 1933, DAVEY 1936, RANKIN 1942, PHILLIPSON and MCANALLY 1942). This observation was confirmed in the studies of BARCROFT *et al.* (1943) and DANIELLI *et al.* (1945).

The experiments of DANIELLI *et al.* (*loc. cit.*) showed that the rate of absorption from an acidic solution is much higher than from an alkaline solution and that reducing the pH increases the absorption of butyric acid more than that of other acids. Similar findings were made by PFANDER and PHILLIPSON (1953) and by TSUDA (1957). In the trials of GRAY (1948), absorption from an acidic solution was rapid, while an alkaline medium appeared to prevent the process. The results of BLOOMFIELD *et al.* (1963) confirmed those of GRAY in respect that no absorption occurred from an alkaline solution, or if so, it was very slight. The experimental results of MASSON and PHILLIPSON (1951) are

further proof of the pH-stabilizing effect of the rumen wall and its permeability. When an alkaline solution of volatile fatty acids was introduced into the rumen, the pH of the solution dropped rapidly, tending to approach that of the blood. This decrease in pH was due to the passage of chloride and bicarbonate into the rumen.

The effect of concentration of volatile fatty acids on their absorption was evident in the studies of ANNISON *et al.* (1957), which showed that under normal feeding conditions there was a positive correlation in concentration between the rumen contents and the portal blood. HOGAN (1961) observed that at a pH of 6.5 an increase in acid concentration enhanced the absorption of acids and that the effect was even more pronounced at a pH of 4.5.

According to the investigations of HUETER *et al.* (1956) lactic acid may be absorbed from the rumen, since the addition of sodium lactate, either alone or together with calcium lactate, produced a rapid and marked rise in the lactic acid concentration of the blood. Calcium lactate alone, however, did not have such an effect, even though the concentration of lactic acid in the rumen liquid rose very markedly.

MCDONALD (1948) established the passage of ammonia through the rumen wall; he observed that the introduction of ammonium acetate into an empty sheep rumen caused an increase in ammonia concentration in its venous blood. In the trials of LEWIS *et al.* (1957 a) positive correlation was found in ammonia concentration between the rumen contents and the portal blood. This effect occurred when the ammonia concentration in the rumen liquid was 60 mmol./l or higher. According to the experiments of HOGAN (1961), at a pH of 6.5 increasing the concentration gradient and the absorption of volatile fatty acids resulted in an augmentation of the ammonia absorption, but at a pH of 4.5 neither of these two factors had any visible effect. In the work of BLOOMFIELD *et al.* (1963) no absorption of ammonia from an acidic solution was noted, whereas it was abundant from an alkaline solution.

According to HOUPT (1959) urea may be transported through the rumen wall into the rumen, where it is rapidly converted into ammonia.

The passage of mineral ions through the rumen wall was first indicated in the studies of DANIELLI *et al.* (1945). These workers reported that when an alkaline solution of sodium salts of volatile acids is introduced into the rumen, sodium ions are absorbed approximately in roughly equal equivalent amounts as the anions. Under similar conditions in the experiments of MASSON and PHILLIPSON (1951), chloride and bicarbonate passed into the rumen. SPERBER and HYDEN (1952) showed that sodium and chloride passed from Pavlov's pouch into the blood, while potassium moved in the opposite direction. No transfer of phosphate could be observed in their trials. On the basis of venous-arterial difference, PARTHASARATHY and PHILLIPSON (1953) established that sodium and potassium were absorbed from the rumen when their concentrations in the rumen liquid exceeded the corresponding concentrations in the blood. Chloride

also was absorbed when its concentration was 135 mg/100 ml or higher, while at lower levels the transfer was in the reverse direction. The results of DOBSON (1955, 1959) indicated absorption of sodium from the rumen against both an electrochemical and a concentration gradient. Studies carried out by SCARISBRICK and EWER (1951) on sheep showed that transfer of phosphate through the rumen is possible.

The contractions of the reticulo-rumen have been the subject of many early investigations (cited by MANGOLD 1929, pp. 139—147 and by DUKES 1953, pp. 311—313). MANGOLD (pp. 154—155) emphasizes the fact that they produce sufficient mixing of the rumen contents to keep them homogenous and at the same pH in all parts. He basis this conclusion on the results of his studies carried out together with FERBER on a ram. On the other hand, other workers such as PEARSON and SMITH (1943 a) and CARROL and HUNGATE (1954) have observed that the rumen contents of the cow are inhomogenous. The latter found that in most cases the VFA concentration was definitely higher in the solid than in the liquid fraction. They concluded that if the formation of acids takes place primarily in the solid fraction, which can be considered obvious, then the acid concentration in this fraction is also occasionally higher than in the liquid.

According to determinations made by BALCH (1950), the water content of the contents of the fistulated bovine rumen was clearly greater in the lower than in the upper part. This also shows that in spite of the rumen contractions, its contents are not homogenous. The tendency of the rumen material to concentrate in the upper parts is obviously attributable to the adhering of small gas bubbles to the material and the resulting change in its specific gravity, producing a buoyancy effect. This phenomenon can be clearly seen in fermenting cultures of rumen material or other solid substrates.

MONROE and PERKINS (1939) found that the pH was lower in the upper part of bovine rumen contents than in the lower part. A similar difference in pH, but even more distinct, was evident in the work of LAMPILA (1955). In this work there also appeared marked differences in VFA concentration between the upper and lower part of the rumen. These differences agreed with the conclusions of CARROL and HUNGATE (1954), with the variations in water content found by BALCH (1950) taken into consideration. P. H. SMITH *et al.* (1956) even found other differences between the upper and lower rumen, interpretable as being due to stratification of the contents. The occurrence in their work of differences in pH and VFA concentration in a normal cow implies that stratification with its accompanying consequences does not seem to be an artificial condition resulting from fistulation.

B. Studies on pH and acid concentrations

According to the earliest investigations (cited by KNOTH 1928 and by MANGOLD 1929, pp. 154—156) the pH of the rumen contents varied only slightly on both sides of neutral, but values even in excess of pH 9 were also reported. On the other hand, it was known that on an exclusive diet of concentrates the pH could go down as far as 5.4.

KICK *et al.* (1938) observed a diurnal variation in the pH, ranging from 5.5 to 7.7 and dependent upon the type of feeding and on the time after it. The highest values occurred with exclusive feeding of alfalfa hay, while adding corn to the rations caused a drop in the pH.

In the studies of MONROE and PERKINS (1939), a fistulated steer and cow were used and samples were taken from the upper and lower parts of their rumen contents. After feeding, there was a marked decrease in pH in the upper part of the contents, with the result that a distinct difference in pH appeared between the upper and lower parts. During indoor feeding the pH varied within the range 6.58—7.40, while the range was 5.91—6.92 when the animals were at pasture and also received supplementary grain mixture.

HALE *et al.* (1940) determined the pH of samples representing the entire rumen contents of a cow and found the pH to decrease during a period of six hours after the beginning of feeding. When the animal was fed on alfalfa hay only, the average pH was 6.82. The quantity of hay had no appreciable effect on the course of the pH curve. When soy bean oil meal and silage were added to the rations, the pH was lowered close to pH 6.

In the trials of WEGNER *et al.* (1941) with a fistulated heifer fed on a diet of hay, grain mixture and silage, the pH of the rumen contents varied from 6.30 to 7.40. When 1 % urea was added to the diet, the pH in combined samples collected from different parts of the rumen varied from 6.50 to 7.55. The highest values were measured 15 hours after feeding.

SMITH (1941) carried out *in vivo* determinations on the pH of the rumen contents of a cow fed on hay and found the average daily value to be about 6.3. When the hay ration was supplemented with 20 pounds of molasses beet pulp, the average was about pH 6.0. The total fluctuations in pH of two cows on these two diets were from 5.29 to 6.85. Small differences in pH were found between different parts of the contents, with the lowest values appearing in the rear and middle areas. *In vitro* determinations made at the same time gave consistently higher results than the measurements *in vivo*.

PHILLIPSON (1942) studied the pH and acid concentrations of sheep rumen contents under different dietary conditions by drawing samples through the fistula by means of a pipetting method. During indoor feeding the average pH was around 6.5 and the average VFA concentration ranged from 75.9 to 91.9 me./l. Mangolds and cabbage resulted in rapid fermentation with a drop in pH and temporary accumulation of lactic acid in the rumen liquid. At

pasture, the pH was distinctly lower, averaging about 6.1, and the VFA concentration was correspondingly higher, averaging 140.5 me./l.

In the trials of MYBURGH and QUIN (1943) the pH of fistulated sheep rumen contents was slightly alkaline when only hay was given. When supplements of crushed maize were given, the acidity increased. The lowest pH values, 5.3—6.2, were found on a diet consisting exclusively of crushed maize, which led to loss of appetite and symptoms of digestive disturbance.

In the studies of HUNGATE *et al.* (1952) the lowest pH values, 4.1—4.7, were measured when excessive amounts of grain or sugar were introduced into the rumen of fistulated sheep. At the same time there was an abundant accumulation of lactic acid. Similar experiments were also conducted by SCARISBRICK (1954), DAIN *et al.* (1955), BROBERG (1960) and KROGH (1959, 1960, 1961 a), who obtained analogous results as regards pH and lactic acid content.

CASON *et al.* (1954) observed slight differences in the pH curves when fistulated cattle were fed on three different kinds of hay. A significant positive correlation was found between the ash content of the ingesta and the pH of the rumen.

BALCH *et al.* (1955) studied the pH and acid concentrations in the rumen contents of two fistulated cows. They found definite fluctuations which were dependent upon the type of feeding and a negative correlation between pH and VFA concentration in samples taken from the upper part of the rumen contents. On a diet consisting of 16 lb. hay and 20 lb. concentrates, the pH varied from 5.4 to 7.0 and the concentration of volatile fatty acids from 183 to 106 me./l. The lowest pH values (minimum 4.3) and the highest acid levels (maximum 200 me./l) were found when the rations contained 2 lb. hay and 24 lb. concentrates. Lactic acid could be detected during a short period after feeding. Differences in VFA concentration between various parts of the rumen contents were not observed.

LAMPILA (1955) measured the rumen pH of a fistulated cow by the *in vivo* method and observed that the average pH in the lower parts was definitely higher than in the upper part. The greatest difference in average values occurring at one time between these sampling areas was 1.1 pH units. The concentration of volatile fatty acids was inversely related to the pH values. The minimum pH values recorded after feeding in the upper part of the rumen contents varied within the range 5.3—5.8, while in the lower part they were as a rule above 6.

A similar difference in pH and VFA concentration between the upper and lower parts of the rumen was also found in the studies of P. H. SMITH *et al.* (1956). These determinations were made in a normal cow, from whose rumen samples were taken immediately after a fistula had been opened.

WILLIAMS and CHRISTIAN (1956) observed a highly significant positive correlation between rumen VFA concentration and feed intake when 400—1000 g dried grass was fed to 12 mature sheep. The quantity of rations had no effect on the pH, which was in the range 6.3—7.1.

BALCH and ROWLAND (1957) studied the variations in pH and organic acid concentration in fistulated cows using the same method as BALCH *et al.* (1955). The largest difference in VFA concentration between different parts of the rumen contents was 1.1 mM %. On diets corresponding to normal farm feeding practice, the pH varied from 5.07 to 7.00 and the VFA concentration inversely from 16.5 to 8.1 mM %. On diets containing exceptionally little hay, or if the hay was ground, the pH minimum was lower (4.30) and the VFA maximum higher (21.3 mM %). Usually only traces of lactic acid appeared in the contents, but rations containing large amounts of flaked maize caused the maximum concentration of lactic acid to increase to 95—270 mg % two hours after feeding.

BRIGGS *et al.* (1957) investigated the pH and acid concentration of fistulated sheep rumen contents on eleven different types of diet. The pH varied within the range 7.30—4.35, while the volatile acids amounted to 14—242 mM/l. The content of lactic acid, measured on 7 different diet types, was from 0 to 169 mM/l. Lactic acid accumulated in the rumen only when the rations contained ample amounts of soluble carbohydrate or starch. When its concentration exceeded 20 mM/l, the pH was invariably less than 5.0. The authors conclude that the pH rarely falls below this value when there is no lactic acid in the rumen contents. The correlation between pH and VFA concentration varied considerably, depending upon the diet. Such variation was partly due to the accumulation of ammonia on high-protein rations. When lactic acid occurred abundantly, there was a correlation between its concentration and the pH.

C. Sources of error in the determinations

KNOTH (1928) observed that the escape of carbon dioxide from rumen samples caused an increase in their pH with resulting errors in the measurements. Samples taken with a stomach tube had clearly higher pH values than those taken through a fistula, evidently as a result of inclusion of saliva in the rumen sample. Both errors have repeatedly been pointed out in later papers, but especially in earlier studies one or both of them have not been taken into consideration and obviously erroneous results have consequently been obtained.

TURNER and HODGETTS (1955 a, b) made a closer study of the effect of carbon dioxide on the buffering action of the rumen contents and of the effect its escape exerts on the pH. When the concentration of volatile fatty acids was comparatively low and the contribution of bicarbonate to the buffering action correspondingly high, the pH of the sample varied from 6.55 to 8.44 when the pCO_2 was varied from 580 to 0.2 mm Hg (1955 b). A pCO_2 change of this magnitude may occur in a rumen sample while it is standing in contact with the atmosphere. When the pH was lower than 5.5 (i.e., in the range of phosphate and acetate buffering action) carbon dioxide no longer had any significant effect.

In most investigations, the pH and acid concentration of samples taken from the rumen contents were determined on samples consisting of free liquid. According to CARROL and HUNGATE (1954) the concentration of acids in such a fluid is lower than that found for fluid retained by solid food particles. Consequently, results obtained by such measurements, while not actually erroneous, obviously are not representative of the entire rumen contents.

After heavy feeding, the rumen contains only small amounts of free liquid (NEVENS, cited by DUKES, 1953, p. 310) and such liquid can only be obtained from the bottom of the rumen, where it is in contact with the absorbing surface. According to results stated by LAMPILA (1955), and SMITH *et al.* (1956), the concentration of volatile acids in this liquid is distinctly lower than in the fluid obtained from the upper parts of the contents, and the pH is correspondingly higher. These observations serve to confirm the idea that analyses covering only one part of the rumen contents give only an incomplete picture of the conditions existing in the entire rumen.

II. The effect of pH on the microbes in the rumen

It has been suggested in numerous papers that the accumulation of volatile fatty acids in the rumen contents or in cultures of rumen bacteria has lessened the vital processes of the microorganisms. However, the author is not aware of any study in which this problem has been investigated separately from the associated change in hydrogen ion concentration. Therefore, only papers dealing with this latter factor are discussed in this section.

A. The effect of pH on the viability of the rumen microorganisms

The earliest studies made on this problem concerned protozoa, since because of their relatively large size and distinct morphological features they were easier to study visually than bacteria. SCHUBERG (1888, cited by KNOTH 1928) was apparently the first to point out that acidity impedes the growth of protozoa in cultures. KNOTH (*loc. cit.*) investigated this matter and observed that Ophryoscolex ciliates died immediately when placed in a medium having a pH of 5.0. Gradually increasing to pH 6.8 lengthened the lifespan of the protozoa, but still higher pH values reduced it once more.

PURSER and MOIR (1959) studied the effect of pH on rumen protozoa with fistulated sheep which were fed on rations of lucern hay, oat chaff and linseed meal. The minimum pH value, 5.66, appeared 2—4 hours after feeding. Addition of glucose caused it to decrease still further to 5.40. Only oligotrich ciliates were found in the rumen. Their reproduction was strongly inhibited at the lowest pH, and their numbers decreased as strongly as to one-third of the level before feeding. There was a positive correlation between minimum pH and protozoa concentration in the pH range of about 5.25—5.85.

KROGH (1959) observed that at a pH of about 5.5 or lower, protozoa disappeared from the rumen when the sheep were given excessive amounts of sucrose.

In the studies of CHRISTIANSEN *et al.* (1962) the small protozoa species (*Entodinium* and *Dasytricha*) became non-viable at about pH 5.5 *in vitro*. Larger species, (*Diplodinium, Isotricha, Epidinium* and *Ophryoscolex*) were able to live at a pH of 4.5 and even lower. On the basis of *in vivo* observations, the authors were of the opinion that under natural conditions the latter were less well able to tolerate a low pH than the smaller species. At the highest pH level studied, 8.5, the protozoa appeared to thrive relatively well.

In the investigations of HUNGATE *et al.* (1952), a marked decrease in pH produced by large amounts of carbohydrate in the rations, caused the death of protozoa and their disappearance from the rumen contents. Around pH 5.5 typical fibre-fermenting bacteria also began to disappear. In the lowest pH range (4.1—4.7) organisms of the *Str. bovis* type were predominant.

A distinct change in the bacterial composition of the rumen was also apparent in the studies of KROGH (1959, 1960, 1961 a, b) when a marked decrease in pH was brought about by large rations of sugar or starch. Gram-positive streptococci (*Str. bovis*) showed a pronounced initial increase when the pH began to drop, but as the acidity increased they soon disappeared. In the lowest pH range (down to 3.8) the rumen contained only lactobacilli and yeasts, which did not occur at "normal" pH levels.

The effect of hydrogen ion concentration on the viability of bacteria can be assessed to a certain extent on the basis of the extent of pH decrease occurring in their pure cultures. BRYANT and BURKEY (1953) investigated 10 different types of bacterial strains appearing in large numbers in the rumen, which were cultured for one week on a substrate of glucose or cellobiose. In cultures from eight strains, the lowest ultimate pH varied from 5.1 to 5.7. The ultimate values of the other two strains were 5.9 and 6.4. This method, however, is not completely reliable for making conclusions, since other factors may inhibit the growth of bacteria before the minimum pH is reached.

B. The effect of pH on fermentative activity

MYBURGH and QUIN (1943) studied the fermentation of glucose *in vitro* by using an inoculum of sheep rumen bacteria and taking the rate of gas formation as a measure of the fermentative activity. The pH of the culture was varied from 4.36 to 8.35. Gas formation was most active in the pH range 6.80—7.80 and decreased more rapidly on the acidic side of this range.

CHENG *et al.* (1955) used washed rumen bacteria suspensions in studying the effect of pH on the fermentation of cellulose in the range 5.6—8.4. Optimum digestion was found in the range 6.8—7.6. At the highest pH, 8.4, the efficiency

of fermentation was about 80 % of the optimum, while at a pH of 5.6 the activity was only one-half the optimal.

In the investigation by LAMPILA (1959) the rate of formation of volatile fatty acids was highest in the pH range 6.2—6.7, falling off steeply when the acidity increased; these trials were carried out with rumen contents which were incubated under conditions of controlled pH.

Results from the investigations of LAMPILA and POIJÄRVI (1959) showed that a drop in pH within the range 6.85—5.30 slowed the formation of acetic and propionic acids but not that of butyric and valeric acids. The rumen contents used as substrate were taken from a cow which had been fed on large amounts of mangolds.

Protozoic fermentation producing volatile fatty acids was most active at pH 7.0 in the work of CHRISTIANSEN *et al.* (1962). At the highest pH, 8.5, acid formation was reduced to about 2/3 and at pH 5.0 to about 1/3 of the maximum.

C. The effect of pH on synthetic activity

Since synthetic and catabolic reactions occur simultaneously in the rumen, it is difficult to study separately one of these sets of reactions and the factors influencing it. The net sum of both reaction types, however, is usually easy to determine, and this is most essential with regard to the nutrion of the host animal.

In the studies of WEGNER *et al.* (1940) the net synthesis of protein was optimal in the pH range 5.5—7.0, as estimated on the basis of the ammonia content of the microbial cultures when inorganic nitrogen was the nitrogen source. In similar studies, PEARSON and SMITH (1943 b) found it optimal in the pH range of 6.3—7.4.

In the work of LAMPILA (1959) the net consumption of ammonia during incubation of rumen contents appeared to increase when the pH increased from 5.8 to 6.7. At lower pH levels the concentration of ammonia in the contents increased.

OLTJEN *et al.* (1962 c) carried out studies using a purified diet and two identical twin steers, giving alkaline mineral mixture to one of them and acidic mineral mixture to the other. The first animal had a higher pH in the rumen contents and more thiamin and riboflavin per gram of dry matter than the second.

D. Optimum pH values for certain reactions of rumen microbes and their enzymes

SYM (1938) isolated from bovine rumen contents a proteinase preparation which hydrolyzed casein. The pH optimum for its activity was found to be 7.3.

WARNER (1956) observed that the proteolytic activity of rumen bacteria was optimal in the pH range 6.5—7.0.

In the studies of ANNISON (1956) variations of pH in the range 6.0—8.0 had relatively little effect on the casein degradation by washed rumen bacteria. Breakdown of peptides and deamination of amino acids was optimal at pH 6.9.

In the work of SIROTNAK *et al.* (1953) deamination of aspartic acid by washed rumen bacteria had a pH optimum of 6.9.

LEWIS (1955) found a pH optimum of 6.5 for aspartic acid deamination by washed rumen bacteria. In an earlier study (1954) he found the same pH optimum for the reduction of sulphates. The pH optima for the reduction of nitrate, nitrite and hydroxylamine were 6.2, 5.6, and 6.2—6.5, respectively (LEWIS 1951).

In the work of HOLTENIUS (1957) the pH optimum for the reduction of both nitrate and nitrite was about 6.5.

The optimum pH range for urease activity in the rumen content was found by PEARSON and SMITH (1943 a) to be 7—9.

JURTSHUK *et al.* (1958) found that, with one exception, the decomposition of purines by washed rumen bacteria was most rapid under neutral or alkaline conditions.

CONCHIE (1954) isolated from the rumen contents of sheep a β-glucosidase preparation which had two pH optima, one at pH 5.4 and the other 5.8, when tested using o-nitrophenyl β-D-glucoside as the substrate. The preparation was essentially microbial in origin.

KITTS and UNDERKOFLER (1954) isolated from rumen bacteria an enzyme preparation which hydrolyzed cellulose with a pH optimum of 5.5.

GILL and KING (1957) obtained from rumen fluid a preparation containing free cellulases; its effect in degrading carboxymethylcellulose was most active at a pH of about 6.5.

FESTENSTEIN (1958) extracted rumen bacteria with butanol and obtained a carboxymethylcellulose-degrading preparation with an optimum pH at about 6.4.

The cellulolytic preparations isolated by STANLEY and KESLER (1959) from rumen liquid had pH optima in the range 5.5—6.0.

MOULD and THOMAS (1958) isolated a specific α-amylase from *Isotricha* as well as *Dasytricha* species. The first-mentioned had its amylase activity optimum at pH 4.8, while the amylase activity curve of *Dasytricha* showed two peaks, at pH 5.0 and 6.0.

A glucuronidase preparation isolated from rumen bacteria by KARUNAIRATNAM and LEVVY (1951) was found to have a pH optimum of 6.1 for the decomposition of phenolphtalein glucuronide.

RAUN *et al.* (1956) observed that the phytase activity of rumen bacteria had a maximum at about pH 5.5.

JOHNS (1951 a) isolated from sheep rumen the lactate-fermenting micrococcus *Veillonella gazogenes*, which caused the decarboxylation of succinic acid to propionic acid. The reaction was found to occur most rapidly in the pH range 5.9—6.2. In a subsequent work (JOHNS 1951 b) the pH optimum for the decomposition of pyruvate was about 6.2.

PRESENT INVESTIGATION

I. Rumen pH, volatile fatty acids and neutralizing capacity

A. Experimental animals and their feeding

The experimental animals were two Ayrshire cows, Tupu and Ulpu, both in good condition, whose weights were 559 and 564 kg, respectively. Rumen fistulas had been made according to the procedure of STODDARD *et al.* (1951). During the periods when no measurements or samplings were made, the tube inserted into the aperture was plugged by an ordinary large-sized cork. The fistulation of the cow Ulpu was highly successful, with practically no escape of fluid. The fistula in the cow Tupu leaked a little, but the escape of liquid was slight enough to be considered insignificant.

The animals were fed two times daily at intervals of 12 hours. Because of the time required for measurements and samplings, feeding of one of the animals was begun ½ hour later than that of the other. The rations were given in the order: concentrates, fresh fodder (mangolds), and hay. The concentrates were given in the form of mixtures, into which the mineral supplement was added. The animals always had free access to water.

The diets used in this investigation are shown in Table 1. Endeavours were made to arrange the feeding so that the energy content of the rations corresponded to the requirements of the animals varying in accordance to their milk production. It was not possible, however, to follow this principle precisely. The concentration of the rations was limited by the appetite of the animals. A preliminary test served to make sure that the rations would be eaten up completely. As the period of the study was not limited to one year, variations occurred in the quality of the hay, since it was not considered advisable to use hay from the previous season.

Prior to pH measurements and rumen sampling the animals were fed on the diet in question for a minimum of seven days.

Table 1. Composition of the experimental diets (kg/day).

Ingredients	Diet no.								
	1	2	3	4	5	6	7	8	9
Hay (mainly timothy)	9.5	9.5	7.5	5.0	7.5	5.0	5.0	5.0	5.0
Protein concentrate mixture (Hankkija I) [1]		1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3
Maize			4.0	7.0	2.0	4.0	4.0		
Mangolds					15.0	15.0	30.0		
Wheat bran								4.0	8.0
Mineral mixture (Hankkija I) [2]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

[1] Composition: dry matter 91.8 %, crude fat 3.8 %, crude protein 45.0 %, crude fiber 10.5 %, nitrogen-free extract 25.0 %, ash 7.5 %.
[2] Contents: Ca 27.0 %, P 8.8 %, NaCl 4.0 %, plus trace elements of Cu, Co and J.

B. pH measurements of the rumen contents

The pH measurements were made with a Beckman pH meter Model G, with a glass electrode and a saturated calomel electrode, both mounted in a special *in vivo* measuring probe described in a previous paper (LAMPILA 1955). However, the curved arm of the instrument was replaced by a straight member. The instrument was standardized prior to each measurement, using two buffer solutions (pH 7.0 and 4.6) at a temperature of 39° C.

The measurements on the upper part of the rumen contents were partly made *in vitro*, since their compact consistency and low water content occasionally rendered it difficult to establish electrical contact between the electrodes. Such a condition was usually encountered in the measurements made 3 and 6 hours after the beginning of feeding, at which times there was little free fluid in the rumen. In these cases, *in vitro* pH determinations were made on the upper part of the contents, the point being located about 10—20 cm cranially from the fistula and 5—15 cm below the surface of the food mass. Forceps of special design were used to extract a quantity of food matter, from which the liquid was squeezed by hand into a small beaker placed in a 39° C water bath. The pH of the liquid was then immediately determined, using the *in vivo* measuring probe.

pH measurements were made on four different parts of the rumen contents in the following order: upper, central, lower, and lower forward part. The purpose of this particular sequence was to avoid errors which otherwise might have occurred, since occasionally liquid from the lower part of the rumen would rise to the upper part by the channel the measuring probe made in the food mass.

The point representing the central part of the rumen was located in the ventral region of this organ in the central longitudinal plane of the animal's body and approximately half-way between the surface of the food mass and the bottom of the rumen. Viewing the animal from the side, this point was approximately at the site of the lower end of the 13th rib (see SISSON and GROSSMAN 1956, p. 461, Fig. 390). Satisfactory contact between the contents and the glass electrode as well as between the two electrodes — or failure to obtain contact — was judged by the sensitivity by which the pointer of the pH meter responded when the adjusting dial of the meter was moved. Satisfactory contact was achieved without difficulty as a rule.

The measuring point representing the lower part of the rumen was located on the floor of the ventral part of the organ in the central longitudinal plane of the animal and in the same transverse plane as the "central" measuring point described above.

The fourth point of measurement, i.e., that representing the lower forward part of the rumen, was located on the floor of the dorsal sac between the reticulum and the anterior pillar (*Pila cranialis*; see SISSON and GROSSMAN,

loc. cit., p. 460, Fig. 389 a); this point was located by probing with the instrument. When inserting the probe to this point, and also when samples were taken from it, care was taken not to enter the reticulum.

When measurements were made on the central and lower parts, care was exercised to avoid transportation of more strongly acidic matter with the probe, by which the pH measurements might have been affected. With this in mind, slight reciprocating movements of the probe were made at the measuring point until the reading remained approximately constant. Slight fluctuations in the reading caused by normal mixing of the rumen contents were often observed, especially when measurements were made at the lower points. In such a case, several consecutive readings were made, and the one which remained stable the longest or which occurred most often was recorded.

C. *Analyses and determinations*

1. Total volatile fatty acids. — Samples for these determinations were taken from the upper, lower, and lower forward parts of the rumen. The sampling points were the same as mentioned above for the pH measurements.

The samples from the upper part were obtained in the same manner as with the *in vitro* pH measurements, i.e., liquid was pressed from solid food material obtained from the sampling point. Samples from the lower points were obtained by means of a brass tube ($^7/_8$″ dia.) having two one-centimetre holes about 5 cm from its lower end. The tube had a rubber plunger with a rod, by which it could be moved to open and close the holes. The holes were opened after the tube had been kept at the sampling point for a moment.

The samples were cooled immediately after collection by immersing the beakers in cold water. If necessary, the samples were stored in a refrigerator at $+4^\circ$ C. Storage in excess of 1—2 days was avoided.

The samples were clarified either by allowing them to stand or by centrifuging. They were then analyzed for total amount of volatile fatty acids by FRIEDEMANN's (1938) steam distillation method.

FRIEDEMANN states that from 1 to 5 per cent of the lactic acid and from 5 to 25 per cent of the pyruvic acid present in the sample come over in the first distillate. For this reason he considers it necessary to re-distill the distillate with HgO or $HgSO_4$. In this work, however, only the first distillation was performed since re-distillation did not essentially change the results when the method was tested with rumen fluid.

2. Neutralizing capacity of the rumen fluid. — This determination was made by means of two steam distillations. One of the distillations was to determine the total amount of volatile fatty acids, as explained above. A second identical sample was also distilled in the same manner except that a known equivalent amount of dilute sulphuric acid was

added before distillation. The amount of acid was such that only part of the acids were released from their salts. No other additions were made. The dilute sulphuric acid was necessary because, particularly in the samples from the lower parts of the rumen, the neutralizing capacity of the fluid exceeds the amount of volatile acids and the result of distillation would therefore be zero if no acid were added. In such a case, the neutralizing capacity in excess of that required for neutralizing the volatile acids present would not be elucidated. Since 10 ml samples of rumen liquid were consistently used in the distillations, the addition of 5 ml of 0.1 N sulphuric acid was always sufficient to liberate a part of the volatile acids from their salts.

In calculating the results, the amount of added sulphuric acid was subtracted from the value showing the equivalent amount of volatile fatty acids which was obtained in the second distillation. This difference was then subtracted considering its plus or minus sign from the figure showing the total amount of volatile acids, obtained by the first distillation. This final difference was then taken to represent the equivalent amount of alkalies in the sample, or its neutralizing capacity.

It is evident from the preceding that the results obtained by this method only express the neutralizing capacity of the rumen liquid available for volatile fatty acids and other volatile acids, e.g. carbonic acid. The neutralizing capacity for non-distillable acids is not revealed by the method. On the strength of previous studies, however, the accumulation of lactic acid at the pH values occurring in this study would seem to be so small that particular elucidation of its potential effect on the neutralizing capacity was not considered to be of significance.

D. Results and discussion

The results of the pH measurements on different diets are shown in Figures 1, 2, 4—8, 10, 11, 13, 14, 16 and 17. Three series of measurements were usually made, and thus each pH value shown in the figures represents the average from three determinations. Exceptions to this were Diet 1 with TUPU (Fig. 1) and Diet 4 with ULPU (Fig. 8), in which four determinations were made, and Diet 8 with TUPU (Fig. 16) in which only two measurements were made.

The concentration of volatile fatty acids and the neutralizing capacity of the rumen fluid was determined for Diets 1, 4, 6, 7, 8 and 9. The results of these analyses are shown in Figures 3, 9, 12, 15, 18 and 19, respectively. Each was derived from one series of samples.

Measurements at 0 and 12 hours, as well as the sampling at these times, were made immediately before the beginning of feeding. Because the day and night periods were of equal length, the results at these times might theoretically

Table 2. Statistical analysis of the effect of differences concerning point of measurement, repetition of the measurements and trial animal upon the variation in pH values of the rumen contents.

Diet No.	Cause of variation in pH	Degrees of freedom	F values and significance level[1]) at different hours after commencement of feeding				
			0	3	6	9	12
1	Point of measurement	3,6	0.44 —	9.31 *	1.31 —	13.18 **	1.56 —
	Repetition [2])	2,6	0.36 —	0.33 —	4.96 —	9.86 *	3.32 —
	Animal	1,6	0.02 —	9.96 *	4.20 —	0.07 —	1.25 —
2	Point of measurement	3,6	8.39 *	23.18 **	6.61 *	10.84 **	13.05 **
	Repetition	2,6	3.92 —	0.81 —	1.00 —	0.87 —	10.04 *
3	Point of measurement	3,6	—	17.89 **	26.40 ***	134.63 ***	3.51 —
	Repetition	2,6	—	3.93 —	0.06 —	5.22 *	0.86 —
	Animal	1,6	—	6.42 *	3.24 —	3.66 —	4.37 —
4	Point of measurement	3,6	90.42 ***	91.03 ***	45.59 ***	94.12 ***	27.75 ***
	Repetition	2,6	1.72 —	1.96 —	0.96 —	0.21 —	5.35 *
	Animal	1,6	6.39 *	0.48 —	0.36 —	4.79 —	2.69 —
5	Point of measurement	3,6	51.33 ***	14.40 **	25.59 ***	14.36 **	19.60 **
	Repetition	2,6	4.96 —	1.37 —	10.47 *	0.46 —	5.78 *
6	Point of measurement	3,6	1.06 —	40.91 ***	17.31 **	27.16 ***	5.58 *
	Repetition	2,6	3.04 —	4.56 —	2.28 —	2.89 —	0.00 —
7	Point of measurement	3,6	4.16 —	62.08 ***	11.28 **	38.46 ***	2.98 —
	Repetition	2,6	4.63 —	4.27 —	0.01 —	0.18 —	0.86 —
	Animal	1,6	2.19 —	7.08 *	2.09 —	4.48 —	5.12 —
8	Point of measurement	3,3	0.33 —	14.74 *	7.82 —	14.12 *	17.34 *
	Repetition	1,3	0.39 —	14.86 *	6.47 —	4.52 —	1.85 —
9	Point of measurement	3,6	2.91 —	23.96 ***	12.73 **	3.50 —	4.88 *
	Repetition	2,6	2.42 —	0.32 —	1.79 —	0.26 —	6.35 *

[1]) *** = significant at $P < 0.001$
** = » » $P < 0.01$
* = » » $P < 0.05$
— = not significant

[2]) The measurements were repeated on different days.

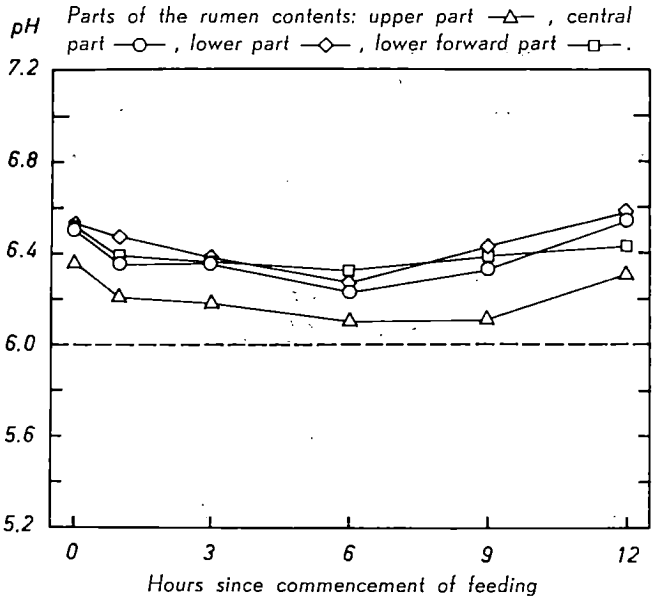


Fig. 1. pH of the rumen contents of cow TUPU on diet 1.

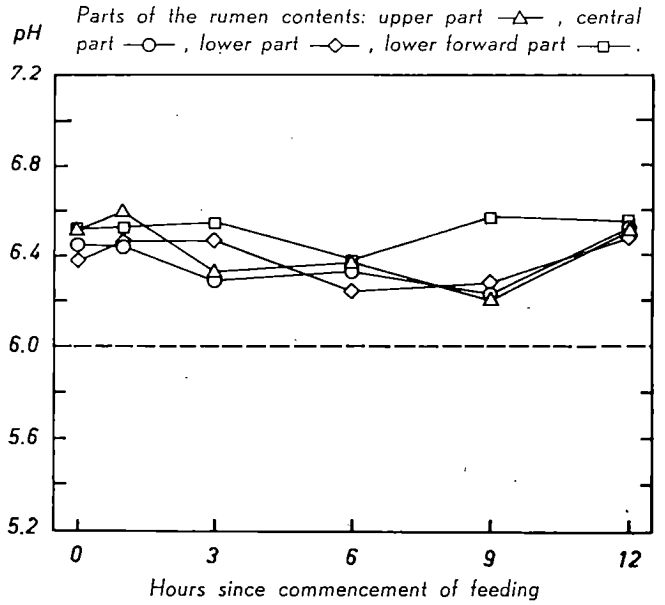


Fig. 2. pH of the rumen contents of cow ULPU on diet 1.

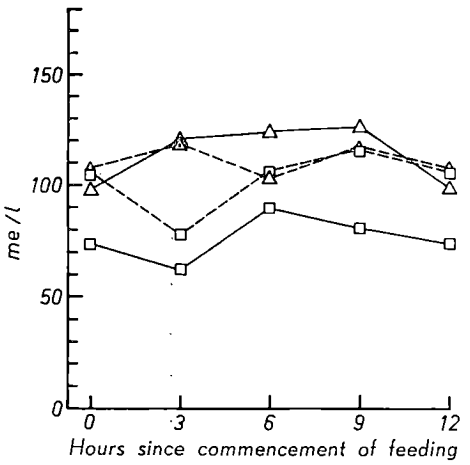


Fig. 3. VFA concentrations ——— and neutralizing capacity ------ of the rumen fluid of cow ULPU on diet 1. The symbols refer to the same parts of the contents as with the pH curves. 0 hour results are presented at the 12 hour position for a second time.

have been indentical. Although the results were not ideal in this respect, 12 hour sampling was sometimes omitted. In order to clarify the periodical changes in the volatile acid concentrations and neutralizing capacity, or their failure, the 0 hour results are presented at the 12 hour position for a second time, as will appear in the text of figures in question.

The results of the pH measurements were statistically treated by variance analysis with the aim of determining the effect of the measuring point, repetition of measurement and experimental animal on the pH values. The results obtained 1 or 1 ½ hours after the beginning of feeding were omitted from this statistical analysis. Concurrent measurements with both cows were made only on four of the diets, as can be seen in Table 2, in which the results of statistical analysis have been stated. Four series of pH determinations were made on Diet 1 with TUPU and on Diet 4 with ULPU. Only three values, however, were used in the variance analysis since the number of determinations had to be the same for either animal in the statistical procedure that was employed. The value omitted in each case was the one closest to the mean of all four. Since the difference in pH between different parts of the rumen on Diet 4 was the sole highly significant factor affecting the variation, the above-described method of selection evidently did not unfavourable increase the relative amount of variation appearing in the repetition of measurements. In either diet (1 and 4), the repetition of measurements was statistically significant in one case only. Also in these instances, the difference between various parts of the rumen was even more pronounced.

In Diet 1, consisting entirely of hay, the fluctuations in pH between feedings were relatively slight (Figs. 1, 2), and the average pH values remained steadily above 6.0. The same observation was made in Diet 2, which contained 1.3 kg protein concentrate mixture in addition to hay and minerals. Although

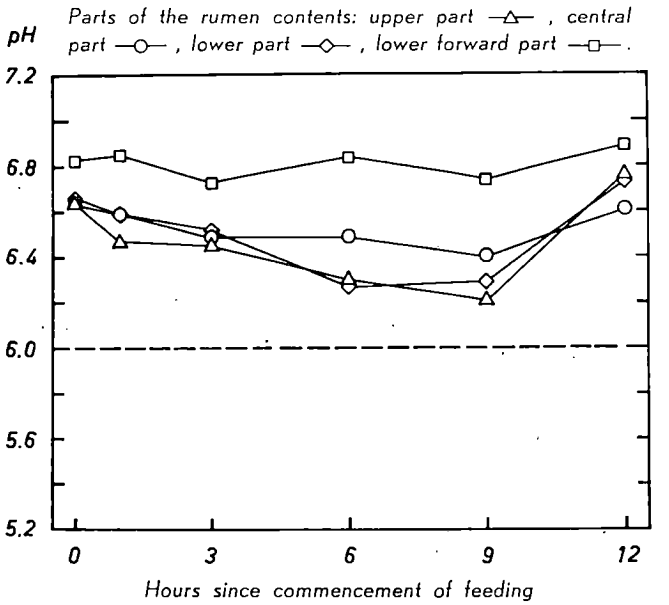


Fig. 4. pH of the rumen contents of cow TUPU on diet 2.

the effect of the point of measurement on the variation in results (Table 2) was sometimes statistically significant ($P < 0.05$) and even highly significant ($P < 0.01$), there are hardly any consistent differences in pH between the different parts of the rumen, which would indicate stratification of the contents. Under these conditions it is especially noteworthy that the concentration of volatile fatty acids in the upper part of the rumen of ULPU on Diet 1 (Fig. 3) was nevertheless distinctly and constantly higher than in the lower part. Since the neutralizing capacity [1]) of the liquid in the lower part always exceeded the concentration of volatile acids, it is evident that the buffering action of the bicarbonate system on the pH was apparently of fundamental importance. The pH in this same part of the rumen is seen to vary approximately within the range 6.4—6.6 (Fig. 2). The pH of the upper part ranged from 6.2 to 6.5, or only slightly lower than that of the lower part, even though the concentration of volatile fatty acids appears to have been approximately equal to the neutralizing capacity and sometimes even greater. It seems, therefore, that when the neutralizing capacity exceeds the amount of volatile acids, the pH is no longer sensitive to the magnitude of their equivalent difference. Unfortunately no VFA determinations were made on Diets 2 and 3, in which the pH values of the lower forward part (Figs. 4—6) were quite high, within maximum mean values as high as 6.9—7.0.

[1]) As has already explained, the term "neutralizing capacity" refers here to the total equivalent alkalinity available for neutralization of the acids volatilized in the steam distillation.

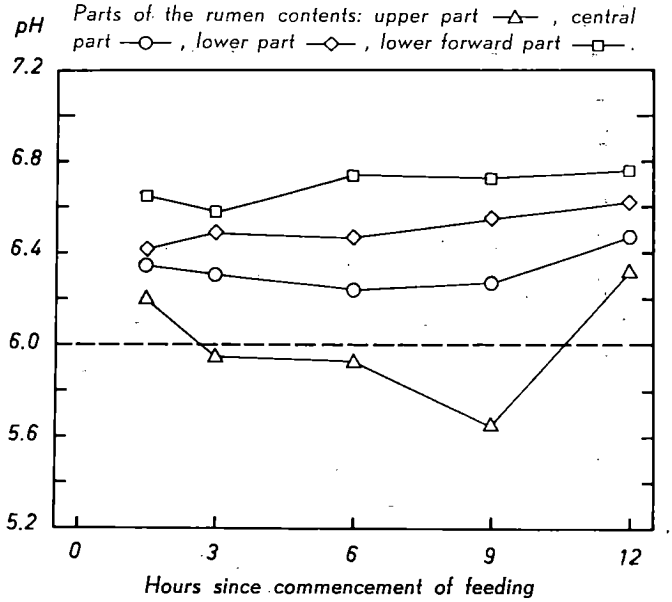


Fig. 5. pH of the rumen contents of cow TUPU on diet 3.

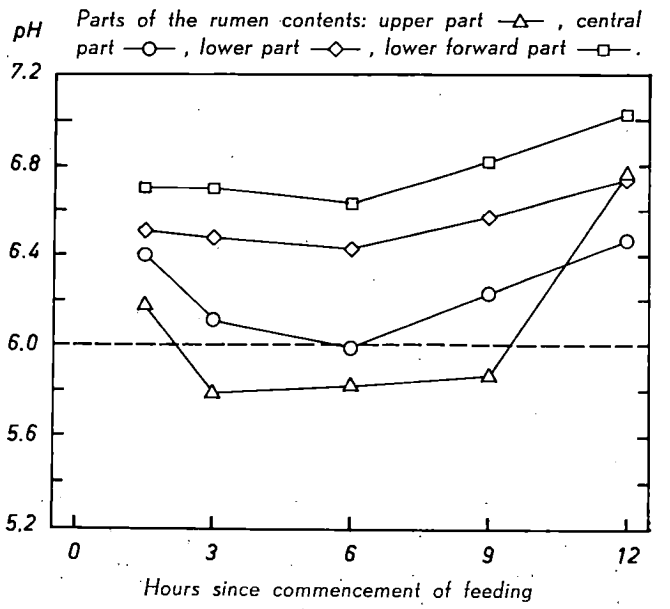


Fig. 6. pH of the rumen contents of cow ULPU on diet 3.

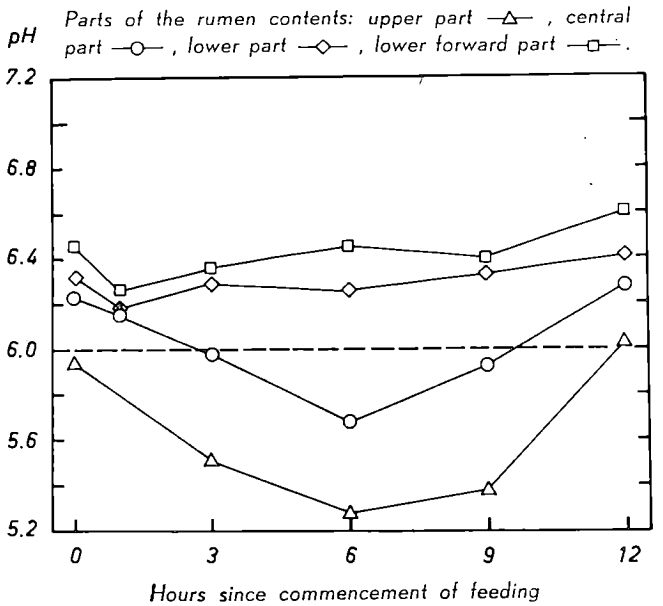


Fig. 7. pH of the rumen contents of cow TUPU on diet 4.

In Diet 3, in which the ratio between concentrates and hay was about 0.7, the pH difference between the upper and lower parts of the rumen contents was distinctly evident (Figs. 5, 6). The influence of the point of measurement on the variation in pH (Table 2) was highly significant ($P < 0.01$ or $P < 0.001$) in the measurements made 3, 6 and 9 hours after the beginning of feeding. The pH values in the upper part were below 6.0, but only slightly. Comparison between Diets 3 and 5 (Fig. 10) shows that maize (in the former) activated the fermentation less strongly than did mangolds (in the latter), which replaced part of the maize without appreciable change in amount of dry matter (Table 1). Since mangolds contain sugars in abundance and maize contains great quantities of starch, which two different groups of carbohydrates differ in their rate of fermentation (PHILLIPSON and MCANALLY 1942), the fairly distinct difference in pH level noted for the two diets appears quite logical. However, the pH differences between Diets 3 and 8 (Fig. 16) are not explainable on the same basis. Diets 3 and 8 both had the same amount of concentrates, but the amount of hay was less in Diet 8 than in Diet 3 (5 kg as opposed to 7.5 kg). The difference can hardly be significant with regard to the pH of the rumen, seeing that in the studies of GORDON (1958 a), for example, lowering the hay ration from 2 lbs. to 1 lb. per day did not affect the rumination time in sheep. The mineral composition in wheat bran, which is generally known to be acidic in nature, is a likely reason for the lower pH values in Diet 8. This explanation is supported by the relatively low figures found for the neutralizing capacity of

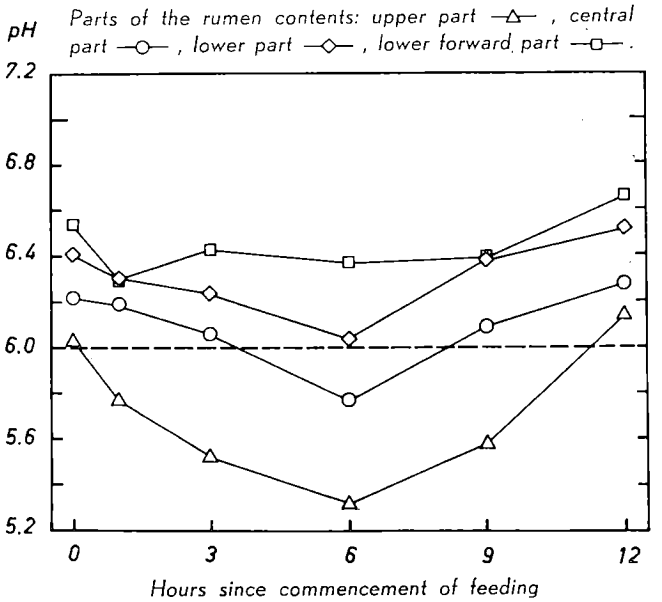


Fig. 8. pH of the rumen contents of cow ULPU on diet 4.

the rumen fluid (Fig. 18) and by the relatively high phosphate concentration (Fig. 34 B, p. 59).

In comparison with Diet 3, Diet 4 was concentrated by replacing 2 ½ kg of hay with 3 kg of maize (Table 1), with the result that its total amount of concentrates was 8.3 kg and the ratio of concentrates to hay was about 1.7. This change in diet composition is clearly reflected in the pH curves for the upper part of the contents (Figs. 7, 8) their lowest points (pH 5.25 and 5.30)

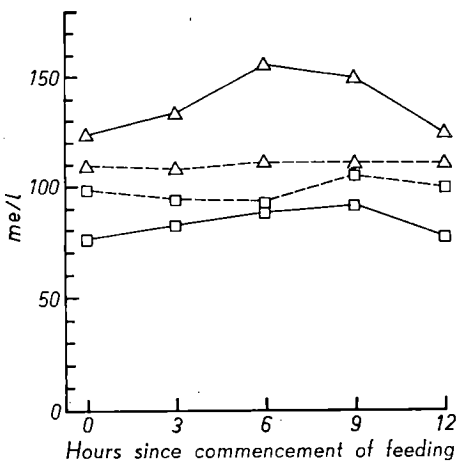


Fig. 9. VFA concentrations ——— and neutralizing capacity ------ of the rumen fluid of cow TUPU on diet 4. The symbols refer to the same parts of the contents as with the pH curves. 0 hour results are presented at the 12 hour position for a second time.

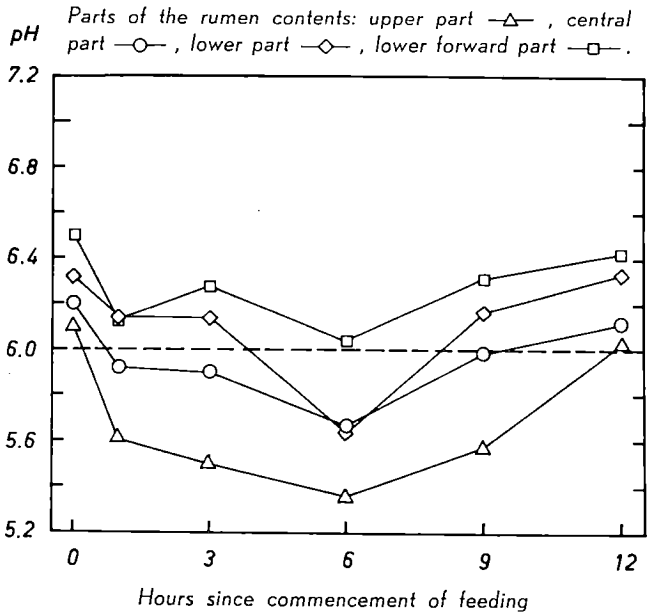


Fig. 10. pH of the rumen contents of cow ULPU on diet 5.

touching on the limiting level; in this study the means did not fall below this figure. Likewise, the maximum value of VFA recorded here (150—160 me./l) was obtained in the upper rumen of TUPU, 6 hours after the beginning of feeding (Fig. 9). Simultaneously, the pH difference between lower forward and the upper part of the rumen contents exceeded one pH unit. Further more, the VFA concentration in the upper part was about 52 % higher than in the lower forward part and about 40 % higher than the corresponding neutralizing capacity.

When calculations are made with this latter percentage figure, a pH value of 5.2 is obtained for the buffer system consisting of the volatile fatty acids and their salts (assuming pK = 4.8 for the acid mixture). This value agrees closely with that determined by measurements in the upper part of the rumen after 6 hours. According to the results of TURNER and HODGETTS (1955 b), hydrogen ion concentration of the rumen fluid is almost entirely dependent upon the phosphate-acetate (= -VFA) buffers when the pH is below 5.5 and when there are no appreciable amounts of lactic acid. Owing to their small concentrations, phosphates are of little importance in the opinion of the above-mentioned investigators. By calculations based on the results shown in Fig. 8 and 9 the same conclusion can be drawn, namely that at the lowest pH levels the hydrogen ion concentration is almost entirely dependent upon the buffer system formed by the volatile fatty acids and their salts.

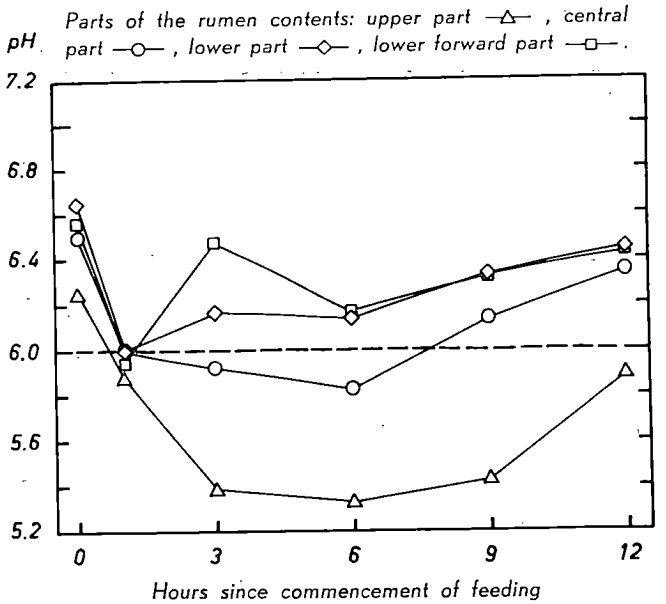


Fig. 11. pH of the rumen contents of cow TUPU on diet 6.

The differences in composition between Diets 5, 6 and 7 as well as those between Diets 8 and 9 apparently had no appreciable effect on the variations in pH of the rumen contents (Figs. 10—14; 16, 17). Likewise, no noteworthy differences in VFA concentration were observed between Diets 6 and 7 (Figs. 12, 15), nor between Diets 8 and 9 (Figs. 18, 19). However, in the latter two diets the concentration of volatile acids in the upper part of the rumen appeared

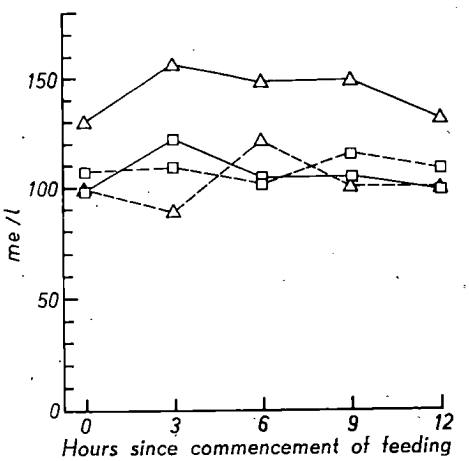


Fig. 12. VFA concentrations ——— and neutralizing capacity ------ of the rumen fluid of cow TUPU on diet 6. The symbols refer to the same parts of the contents as with the pH curves. 0 hour results are presented at the 12 hour position for a second time.

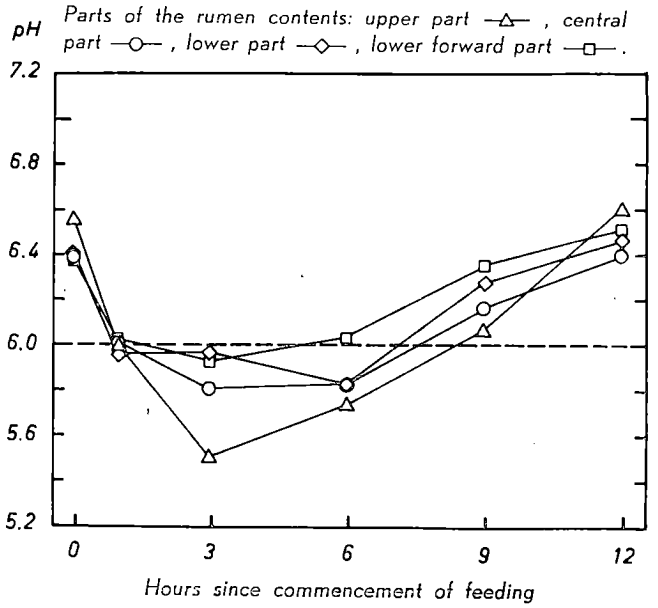


Fig. 13. pH of the rumen contents of cow TUPU on diet 7.

to be slightly less than in Diets 6 and 7, as far as can be deduced from one single series of samples. The relatively low average values of the neutralizing capacity as well as their rather pronounced fluctuations in the diets containing wheat bran (8 and 9) indicate that this component, which is characterized by its acidic mineral matter, tended to reduce the neutralizing capacity. Under such conditions, the smaller acid concentrations may have produced the same minimum pH values as the higher concentrations in Diets 6 and 7. It should be noted, however, that greater water intake in the trial with Diets 8 and 9, if such has occurred, could also be responsible for the differences in neutralizing capasity.

The fact that the pH of the upper part of the rumen contents became stationary at nearly the same minimum level regardless of differences in diet may partly be due to the efficient buffering capasity of the rumen fluid in the range of the acetate buffering system (e.g. TURNER and HODGETTS 1955 b). Thus small variations in acid concentration have no appreciable effect on the pH. Another factor, however, is the fact that lowering of the pH retards the fermentative formation of acids, as is evident from the results shown in Fig. 20 (page 46). Thus, as far as can be concluded from these results, the state of equilibrium established between the formation and removal of acids is not readily altered by changes in the diet. As far as this stabilization is due to fermentative retardation, it implies a limitation in the digestive capacity of the rumen. The presence of this unfavourable effect and its influence in the

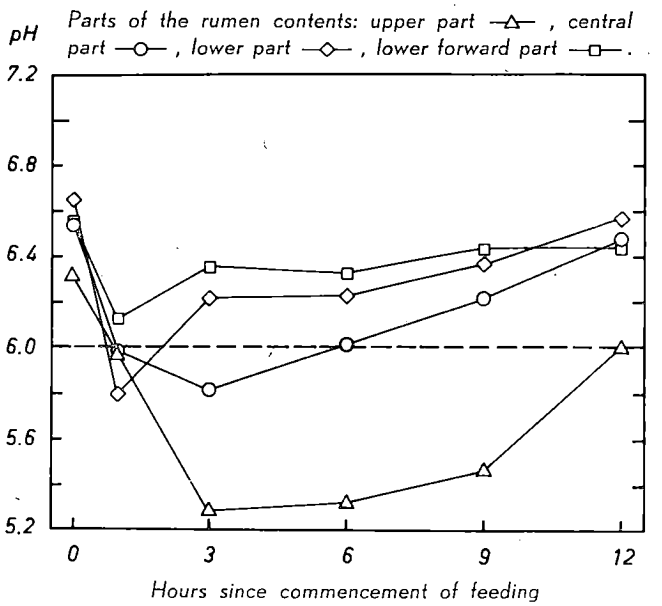


Fig. 14. pH of the rumen contents of cow ULPU on diet 7.

upper parts of the rumen, where the concentration of food material is greater than in the lower parts (BALCH 1950), seems to be clear and pronounced in Diets 4—9, as judged by the results shown in Fig. 20.

The distinct differences in VFA and hydrogen ion concentrations between the upper and lower parts of the rumen, which have been noted in altogether three cows (including the animal concerned in the preliminary study of LAM-

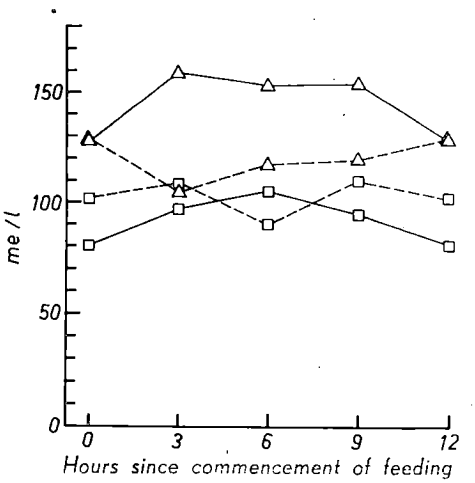


Fig. 15. VFA concentrations ——— and neutralizing capacity ------ of the rumen fluid of cow ULPU on diet 7. The symbols refer to the same parts of the contents as with the pH curves. 0 hour results are presented at the 12 hour position for a second time.

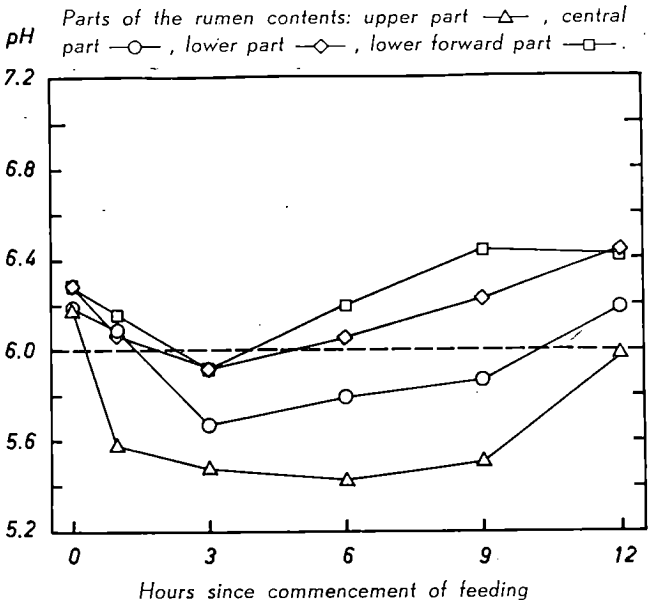


Fig. 16. pH of the rumen contents of cow TUPU on diet 8.

PILA 1955) appears to be a normal phenomenon, at least in fistulated animals. Since SMITH *et al.* (1956) found such differences also in a normal cow, by sampling the rumen fluid immediately after a fistula had been made, it seems highly probable that these differences are also common in normal cows. Since the differences in acidity occurred at a pH level where they exert a marked influence on the fermentative activity, such an effect is very noteworthy from the standpoint of microbial digestion in the rumen.

Owing to the unequal, stratified distribution of the food matter in the rumen, the stating of pH as an average value would seem inadequate as a basis for suggestions concerning its effects. This is also evident in the results of BALCH and JOHNSON (1950) and MILES (1951), in which there were definite differences in the degradation of cellulose and other structural carbohydrates between the upper and lower parts of the rumen.

The former workers attribute the more rapid digestion in the lower part to its higher water content. But since it is hardly appropriate to speak of scarcity of water in the upper part either, this explanation is not very satisfactory. Higher VFA concentration (assuming that the pH would be the same) might be a factor contributing to the observed result, provided that it would exert, in the range of its normal fluctuation, a detrimental effect on the microbial population. Judging from the trial results reported later in section II B (Fig. 21), however, this does not seem likely. Since, according to P. H.

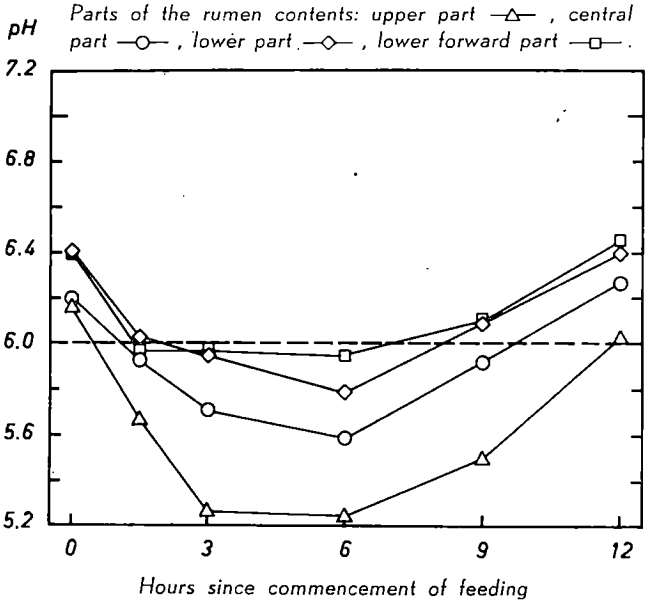
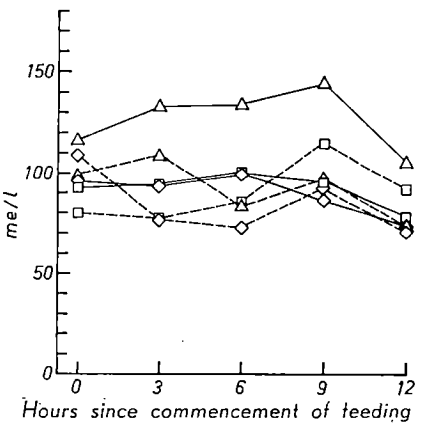


Fig. 17. pH of the rumen contents of cow ULPU on diet 9.



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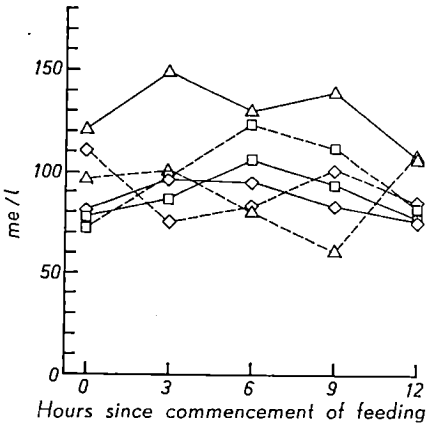

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Figs. 18 and 19. VFA concentrations ——— and neutralizing capacity ------ of the rumen fluid of cow TUPU on diet 8 (Fig. 18) and cow ULPU on diet 9 (Fig. 19). The symbols refer to the same parts of the contents as with the pH curves.

SMITH *et al.* (1956), the nutrient supply of the microorganisms is at least as good in the upper part of the rumen as in the lower part, the only logical conclusion to be made from the present data is that the higher hydrogen ion concentration in the upper part is the principal cause of the observed lower fibre digestion rates.

BALCH and ROWLAND (1957) state that the greatest difference in VFA concentration between samples from different parts of the rumen contents was only 0.1 mM %. Since their sampling method has not been described, no conjectures are possible as to what reason has caused the divergence between their results and those emerging from the present study. Difficulty in obtaining representative samples from different parts of the rumen, caused by mixing up during sampling was encountered in the work of P. H. SMITH *et al.* (*loc. cit.*).

The determination of the neutralizing capacity made in the present study partly constituted a try-out of a new method in view of its practicability in investigations on the reasons responsible for the variations of pH in different parts of the rumen contents. It is evident that further investigations are needed concerning the influence of the ammonia content and its variations in the results obtained by this method. However, it can be said on the strength of the determinations that have been made that there were no marked differences in neutralizing capacity between the upper and lower part which could have been important causes for the established pH differences. The lower pH values in the upper part of the rumen appear thus to be mainly a consequence of the higher concentrations of volatile fatty acids in this part.

II. The effect of pH and volatile fatty acid concentration on the fermentation activity in culture

A. Experimental methods

1. In vitro cultures. — The cultures were made from ingesta taken from the rumen during different diets. The material was collected 4—5 hours after the commencement of feeding. Food material containing little or no free liquid was first taken from the upper part of the contents, and liquid obtained from the lower part with a pump was added until the consistency of the mixture was that of a thick sludge.

Preliminary tests showed that it was quite easy to adjust the dry matter content of the mixture to 6—8 % simply by visual assessment. If the dry matter content was higher, mixing was difficult, and endeavours were therefore made to keep the consistency in every instance within the above-mentioned

limits. The samples were initially made somewhat thicker, leaving a margin of 10 % of the ultimate volume in reserve for later addition of liquid by which the pH of the mixture could be adjusted. The dry matter content of the cultures at the start of incubation was assumed in the subsequent calculations to be 7 %.

The material from the rumen was collected into a large glass jar containing carbon dioxide instead of air. During sampling and transportation to the laboratory, the jar was kept in a water bath at about 40° C.

Incubation was carried out in 500 ml glass jars, into which 360 ml of the rumen contents were measured. The original pH of the culture was adjusted to various levels by means of saturated sodium bicarbonate and 1 N HCl, and the volume was brought to 400 ml with distilled water.

The cultures were prepared and incubated in a thermostatically controlled room at a temperature of 39—40° C. In all stages of culture, a carbon dioxide stream was supplied in order to avoid contact with the air. The incubation flasks had covers permitting the escape of fermentation gasses but preventing the entrance of air.

During the period of incubation the flasks were shaken, when needed, at 30 minute intervals to keep the material homogenous. At two-hour intervals the pH was measured and adjusted to the original level with sodium bicarbonate. The time of incubation was 6 hours.

For many of the pH-adjusted cultures there were two or three replications; these were purposely made in order to investigate the effect of the concentration of volatile fatty acids. The method of their preparation was the same as that described above, with the exception that a concentrated, neutral sodium salt solution of volatile fatty acids was added to the cultures. This solution was obtained from rumen liquid by means of steam distillation. Each of the solutions came from the same cow with the same diet from which the rumen material under incubation was derived.

2. Determination of fermentation activity. — The rate of VFA formation was used as a measure of fermentative activity. The changes in acid concentration during the period of incubation were determined by steam distillation (according to FRIEDEMANN 1938) of samples taken at the beginning and end of incubation. In calculating the increase in total amount of acid, the concentration change was multiplied by a factor to correct for the dilution caused by the addition of liquid in adjusting the pH. The original volume of liquid was estimated to be 93 % of the total volume of the culture.

The average pH of each culture during the period of incubation was calculated as the weighed mean of the averages in the intervals between the different measurements.

The averages of the initial and final VFA concentrations are those stated in the results for the average concentration of volatile fatty acids.

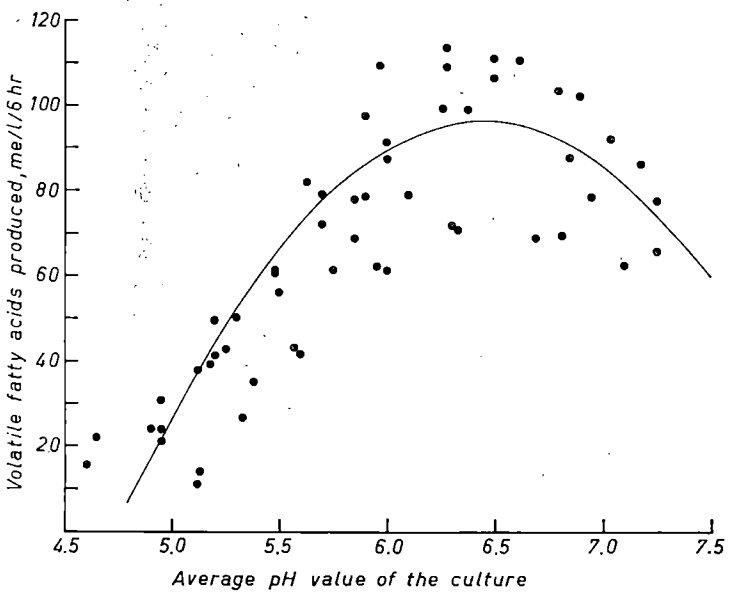


Fig. 20. VFA production at different pH levels after 6 hr. *in vitro* incubation of the rumen contents.

B. Results and discussion

Figure 20 shows the formation of volatile fatty acids in the cultures at different pH levels during the 6-hour incubation period. Figure 21 shows how this formation was affected by the concentration of total volatile acids. The latter figure also indicates the limit values of the ranges containing the average pH values of each culture.

The formula for the second-degree regression curve shown in Fig. 20, calculated on the basis of the experimental results, is

$$y = -1266 + 422\ x - 32.7\ x^2$$

where y denotes the formation of volatile fatty acids in milliequivalents per litre of solution in 6 hours and x denotes the average pH of the culture during the period of incubation. The second-degree regression is highly significant ($P < 0.001$) and better, on a highly significant level, than the linear regression calculated from the same results. According to this formula, the most rapid formation of volatile fatty acids (95.5 me./l/6 hours) occurred at pH 6.45.

On the basis of the results shown in Fig. 21, calculations were made on the linear regression between the VFA formation and the average VFA concentration. These calculations were made for the entire experimental results

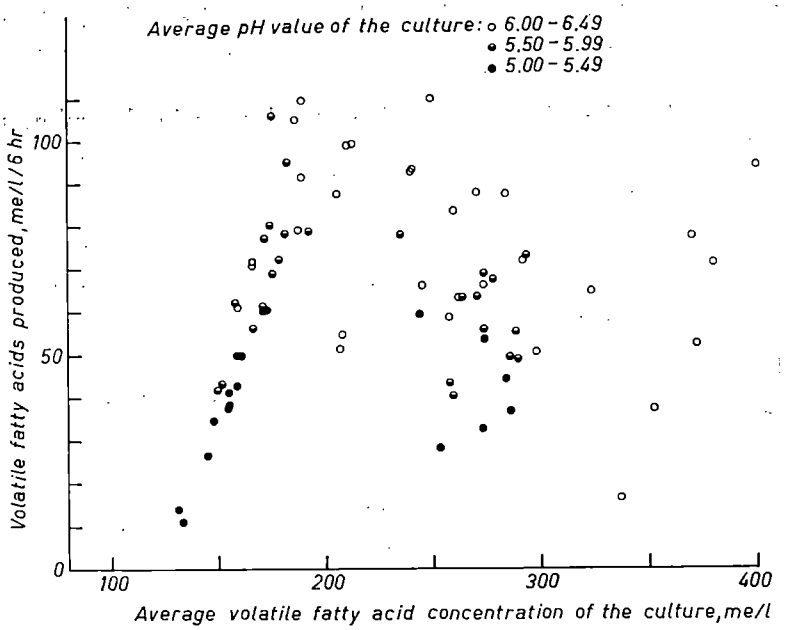


Fig. 21. VFA production at different VFA and pH levels after 6 hr. *in vitro* incubation of the rumen contents.

as well as separately for the two highest pH levels and the lowest level. In no case was the deviation of the regression coefficient from zero significant ($P > 0.05$).

The rate of VFA formation was the only practicable basis for determining the fermentation activity in the *in vitro* method used. Since according to the trials of MARSTON (1948) an average of about 73 % of the degraded cellulose appeared in the form of $C_1 - C_4$ acids, their relative amounts among the fermentation products can be considered a sufficiently reliable indication of the extent of, and variations in, the fermentation rate occurring in stabilized culture conditions. In view of the type of substrate used in the present study, it is improbable that there was any noteworthy accumulation of lactic acid in the pH ranges of the cultures.

As has been described previously, rumen contents for the incubations were collected 4—5 hours after the beginning of feeding. Transportation of the samples to the laboratory and preparation of the cultures took about 1—1 ½ hours, during which time fermentation continued actively. The composition of the unfermented rumen material at the beginning of incubation can thus be estimated to have been similar to that in the normal rumen about half-way between the two daily feedings. The acid formation occurring during the 6-hour

incubation period thus corresponds to the fermentation taking place in the rumen during the latter half of the interval between feedings, a period during which fermentation is considerably less active than the average. Another factor to be considered is that the concentration of substrate in the cultures (about 6—8 % dry matter) was clearly lower than that which has been observed in the rumen (PALOHEIMO and MÄKELÄ 1959, Table 3 a; MÄKELÄ 1960, Table 3 a). When acid formation is used as the indicator of fermentative activity, this latter factor may have an appreciable effect on the total production.

The maximum rate of VFA formation in the cultures varied somewhat, depending on the diet of the animal. In all of the trials, however, acid formation was invariably retarded also above the optimum pH level.

The maximum mean VFA concentrations shown in Fig. 21 are approximately twice the maximum values observed *in vivo* (BALCH and ROWLAND 1957). It is evident from this that the osmotic pressure in these cultures was also much higher than normal. Since, however, no statistically significant effects were found in the results, it can be concluded that apparently the rumen microflora and -fauna were not harmed much by either factor.

The above observation is noteworthy when judgements are to be made on the suitability of the *in vitro* procedure employed in these experiments, as well as similar *in vitro* methods in general. Criticism of such methods has usually been based on the assumption that the accumulation of fermentation products, principally of volatile fatty acids, acts as an impediment to the normal course of this process.

In the experiments concerning the effect of pH the average acid concentration rarely exceeded 200 me./l. Thus the accumulation of acids apparently did not hinder the manifestation of an effect due to pH.

The pH range investigated did not rise much above 7. The reason for this was that the abundant production of carbon dioxide in the cultures would have hampered the use of carbonate for keeping the pH at high levels. The carbonate would rapidly have been converted to bicarbonate with a consequent decrease in pH, and the end result would have been only an apparent increase in average pH readings, while the actual mean pH between measurements would have been lower.

Living protozoa were regularly observed in the cultures. Changes in their fermentative activity due to pH variations have thus contributed to the results. In the studies of CHRISTIANSEN *et al.* (1962) the optimum pH for protozoic fermentation was about 7.0, or approximately one-half unit higher than the optimum in the present experiments for a mixed culture of protozoa and bacteria. At a pH level of 5.5 and lower, protozoa were observed to accumulate on the walls of the incubation flasks and their motility decreased or even ceased. This phenomenon was not further investigated, nor were potential differences in pH susceptibility or resistance of the different species determined.

III. The effect of pH on the equilibrium between ammonia-producing and ammonia-consuming reactions

A. Experimental methods

1. In vitro cultures. — The cultures were established in 500 ml glass jars by the same procedure as described in the previous section. The method of culture preparation, however, was different.

In all the cultures, the primary substrate consisted of hay (composed mainly of timothy) which was ground in a Wiley mill to pass through a 1 mm sieve. An amount of 30 g of ground hay was used in each culture. The liquid component in each culture at the beginning of incubation totalled 400 ml, comprising 200 ml rumen fluid and 160 ml salt solution. The remaining 40 ml were reserved for the addition of pH-adjusting solution, and distilled water was added to bring the total volume to 400 ml. The salt solution contained 5.25 g/l sodium bicarbonate and 0.69 g/l $NH_4H_2PO_4$. In the trials with glucose in the substrate in addition to hay, 1.926 g/l of ammonium chloride was added to the salt solution in order to increase the ammonia content of the culture.

The rumen liquid, which constituted both the inoculum and part of the culture fluid, was obtained from the bottom of the ventral part of the rumen by pumping, 4—5 hours after the beginning of feeding. (The diet consisted of hay and concentrates.) In order to separate most of the solid food material from the liquid, the mixture was allowed to ferment for about ½—1 hour at 39° C. During this period part of the solid matter settled to the bottom and another part rose to the surface. The intermediate, relatively clear liquid was removed with a siphon and used in the preparation of the cultures.

In one trial the C:N ratio of the substrate was lowered by adding 3 g of casein (HAMMARSTEN) to the culture. In another trial this ratio was increased by the addition of 8 g of glucose.

The initial pH of the cultures was adjusted by means of 1 N HCl and saturated sodium bicarbonate. During incubation the pH was measured and adjusted at intervals of one hour. At these times samples were taken and the material was mixed; mixing was also performed in the intervals when needed. In other respects the cultures were treated according to the same procedure as described above under section II A 1. Since it had previously been observed in similar trials (LAMPILA 1959) that no ammonia escapes from the cultures in the pH range investigated, no special control measures in this regard were considered necessary.

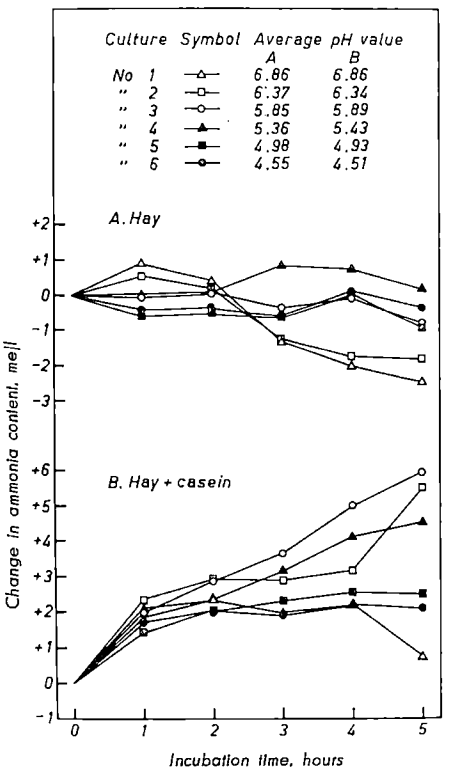
2. Analyses and calculation of results. — The ammonia contents of the cultures were determined according to the method of CONWAY as described earlier (LAMPILA 1959). The solution samples were clarified either by allowing them to stand or by centrifugation. The amount

of ammonia formed from nitrogenous compounds during the time when the analysis dishes were standing was considered to be the same as the increase in ammonia occurring during a further standing period of equal length.

In two of the trials, determinations were made not only of the ammonia but also of the total VFA according to the distillation method of FRIEDEMANN.

In calculating the changes in amount of ammonia and volatile fatty acids occurring during the period of incubation, consideration was made of both the decrease in liquid quantity due to sampling and also its increase and dilution by the addition of pH-adjusting liquid. The changes were calculated to have occurred during the interval between samplings. In the results they are stated as milliequivalents per litre of solution, summed in consecutive order of time.

The average pH of the cultures was calculated as the weighed mean of the average values between determinations, in the same way as in the previously described trial (Section II).



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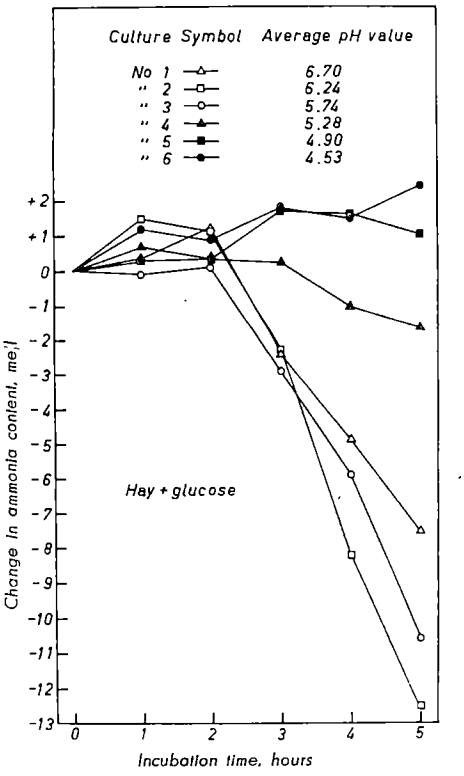

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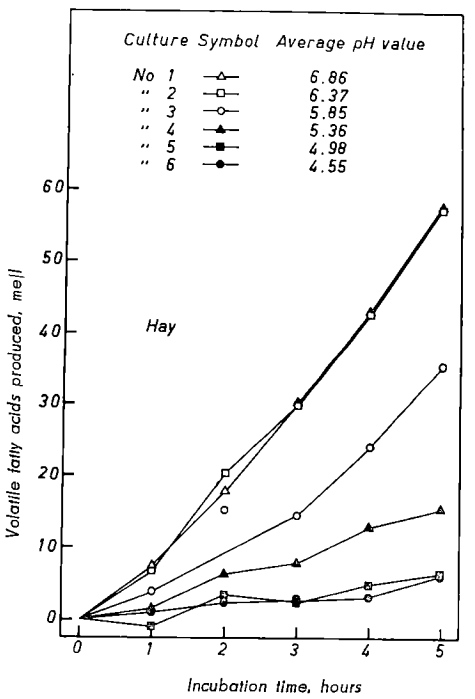
Figs. 22 and 23. Change in ammonia content of the *in vitro* cultures at different pH levels. Substrate: Hay (Fig. 22 A), hay + casein (Fig. 22 B), hay + glucose (Fig. 23).

B. Results and discussion

The changes in ammonia content of the cultures with hay or hay plus casein substrate are shown in Fig. 22. The changes in the cultures with hay plus glucose are shown in Fig. 23. The average pH values in each group of cultures are seen in the figures. The formation of volatile fatty acids occurring in the cultures with substrates of hay or hay plus casein are presented in Figures 24 and 25, respectively.

When hay was the only substrate, the changes in ammonia concentration in the cultures (Fig. 22 A) were quite small. At the upper pH levels (6.86 and 6.37), however, a decrease in concentration can be seen. This can be interpreted as indicating that the favourable pH level promoted the utilization of ammonia in microbial cell synthesis more than its formation by decomposition of nitrogenous compounds. The production of volatile fatty acids was equal in magnitude at both pH levels and clearly greater than at lower pH levels (Fig. 24). A definite correlation is thus evident between energy-yielding fermentative reactions and energy-utilizing synthetic reactions.



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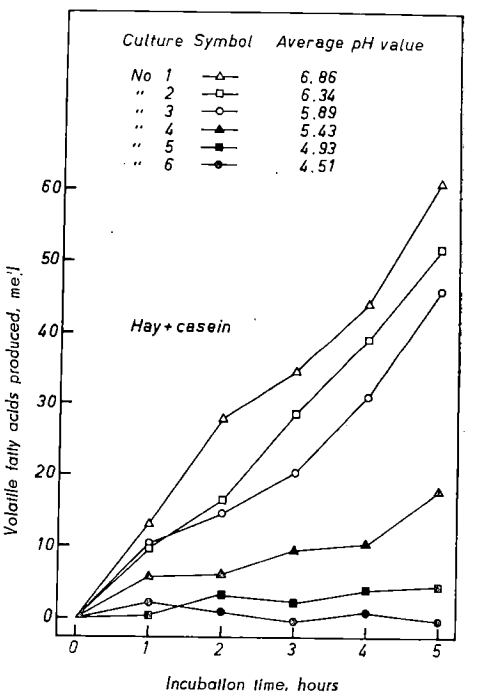

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Figs. 24 and 25. VFA production at different pH levels in the cultures shown in Fig. 22 where changes in ammonia content have occurred.

The net consumption of ammonia in this trial may have been limited by the low ammonia concentration, which varied in the different cultures within the limits 8.7—9.7 me./l at the beginning of incubation.

The addition of casein (Fig. 22 B) resulted in an increase in ammonia in all of the cultures. The increase was smallest at the highest pH (6.86), and the concentration did not rise any more after the first hour of incubation. During the last hour actually a decrease in the ammonia concentration was noticed. The reactions producing and consuming ammonia in the culture were thus approximately balanced. A similar tendency is also seen at the next lower pH level (6.34), while at pH 5.89 the ammonia production was distinctly greater than the consumption. Since the formation of volatile acids after the first incubation hour was approximately equally abundant in both cultures as in culture no. 1, fermentative and synthetic activity did not seem to be directly related to each other. The changes in ammonia concentration, however, were rather small considering the fact that casein is known to be one of the most easily degraded proteins.

The results shown in Fig. 23 demonstrate the marked effect of sugar in increasing the consumption of ammonia. This could be clearly seen because the initial concentration of ammonia in the cultures had been increased by greater additions of ammonium salt to the solution than in the previous two trials. However, the initial concentration, varying from 18.2 to 20.6 me./l in the different cultures, was perhaps not high enough to enable the maximum possible net consumption of ammonia to take place. In an unpublished experiment, using similar cultures, a considerably higher net consumption was observed when the initial concentration of ammonia was about twice that in the present trial.

VFA formation was not determined in this trial, but judging from the total amount of sodium bicarbonate added to adjust the pH, acid production was appreciably greater at the two highest pH levels than at the same corresponding levels in the previous trials.

The optimum pH for the net consumption of ammonia nitrogen in these trials appeared to be within the range 6.24—5.74, mainly closer to the upper limit. A rather sudden decrease in consumption occurred when the pH dropped from 5.74 to 5.28 and also when it increased from 6.24 to 6.70. Below pH 5 the amount of ammonia increased slightly.

In comparing the results from these three trials, the observation can be made that as the C:N ratio of the substrate increased (as a result of the additions made), the optimum pH for ammonia utilization appeared to shift toward lower values. However, because of the small number of trials performed, this picture is not certain and requires further investigation. It is possible, for example, that the types of carbohydrates and proteins used are more significant than the actual C:N ratio itself.

In two previously published trials (LAMPILA 1959) with rumen contents as substrate, the net consumption of ammonia was greatest at the highest pH levels (about 6.6 and 6.7). Results of SYM (1938) and WARNER (1956) concerning the pH optimum of rumen microbial proteolytic activity, as well as the results of SIROTNAK *et al.* (1953), LEWIS (1955) and ANNISON (1956) on the pH optimum of amino acid deamination, indicate clearly that conditions for the total production of ammonia are improved as the pH increases toward the neutral point. Since, however, the equilibrium between the ammonia-forming and ammonia-consuming reactions shifts at the same time in favour of the latter, it can be concluded that the processes involving ammonia consumption increase relatively more strongly as the pH rises. According to the results presented in this section, the nature of the substrate appears to modify to some extent the relationship of these processes.

IV. Concentrations of certain minerals in the rumen fluid

A. Material and methods

1. Sampling and preparation of samples. — The samples of rumen liquid for these determinations were obtained by the same method as for the volatile fatty acid studies, which was described previously in section I C 1. Likewise, the samples representing different parts of the rumen contents were taken from the same parts. The liquid samples were centrifuged with a Wifug H centrifuge for 20 minutes at the highest speed (ca. 4000 rpm) and the supernatant was then used for the analyses.

2. Analyses. — The liquid samples (usually 50 or 100 ml) were evaporated to dryness in the same dishes in which the residues were ashed. After the evaporation residues had been weighed, they were incinerated in an electric furnace, raising the temperature at first slowly to 300—350° C and, after the emission of smoke had ceased, to 450°, which was maintained until the ash became light-coloured. The ash was weighed and then treated with 5 ml of 4 N HCl, which was evaporated to dryness on a steam bath. The soluble material was dissolved on the steam bath with a small amount of water containing 2 me. HCl, filtered, and transferred by washing with hot water into a 100-ml volumetric flask which was filled to the mark after cooling. [1])

Phosphorus: 10 ml of this solution and 1 ml of 1 N H_2SO_4 were put into a 50-ml volumetric flask, which was kept for one hour on a steam bath. The flask was filled to the mark and an aliquot of the solution was taken

[1]) This procedure, as well as the analyses described below, follow the method of plant material analysis (SALONEN *et al.* 1962) which is in routine use in the Department of Agricultural Chemistry and Physics of the Agricultural Research Centre. The literature citations to which reference is made can be found in the above-mentioned paper.

for analysis of phosphate by the vanadate method of GERICKE and KURMIES (1952).

P o t a s s i u m, s o d i u m, c a l c i u m, m a g n e s i u m: For the determination of these ions, the phosphate was first removed from the solution by running it through an anion exchanger (IR-4 B) in a Tompkins apparatus. Potassium and sodium were then determined by the flame photometric method; the solution was first diluted when necessary. Water solutions of KCl and NaCl were used as standards.

Calcium and magnesium were both determined by the versenate titration procedure of CHENG and BRAY (1951), with the exception that naphthol green B was added to the calcium indicator (KNIGHT 1951). Control determinations of magnesium were also made colorimetrically using titan yellow (SCHACHTSCHABEL and ISERMEYER 1953).

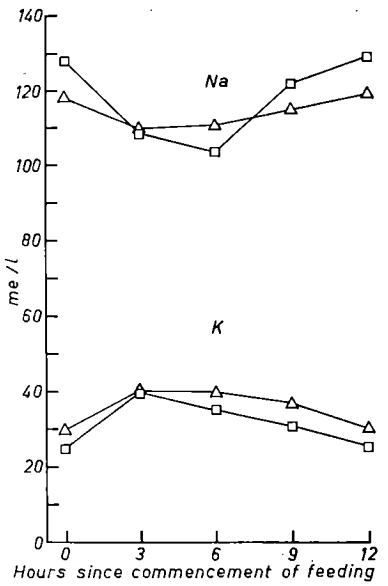
B. Results and discussion

The determinations of mineral concentrations were made on Diets 6 and 8 with TUPU and on Diets 7 and 9 with ULPU. The rumen fluid samples were taken at different times after the beginning of feeding in order to study the 12-hour changes in concentration depending upon feeding. For testing of the hypothetical differences between various parts of the rumen, samples were taken from both the upper and lower part. The results representing each diet were derived from one sampling series. The concentrations of sodium and potassium are presented in Figs. 26—29, those of calcium and magnesium in Figs. 30—33, and those of phosphorus in Figs. 34 and 35. The approximate amounts of minerals consumed in each diet are shown in Table 3.

For Diets 6 and 7, the mineral quantities in the fodder were based on fodder analysis results and those in the mineral supplements upon the values stated in the guarantee certificate. The amounts of minerals in the mineral supplements given in Diets 8 and 9 were also calculated on the basis of the certificate. The mineral contents of the hay and of the protein concentrate with these latter diets were not analyzed and previous analysis results were

Table 3. Approximate consumption of five mineral elements on four diets (g/day).

Diet No.	Cow	K	Na	Ca	Mg	P
6	TUPU	164	17	56	20	42
7	ULPU	220	20	61	24	48
8	TUPU	136	13	55	29	76
9	ULPU	180	15	61	47	125



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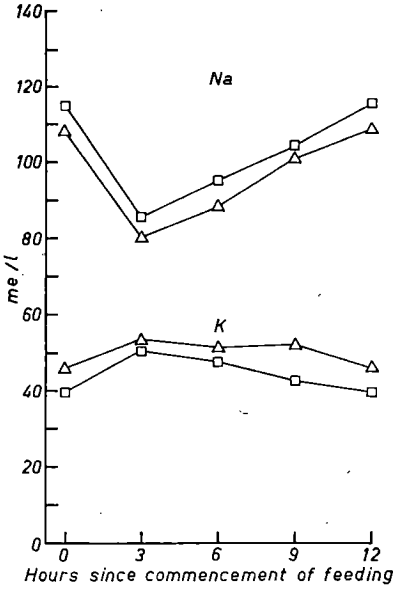

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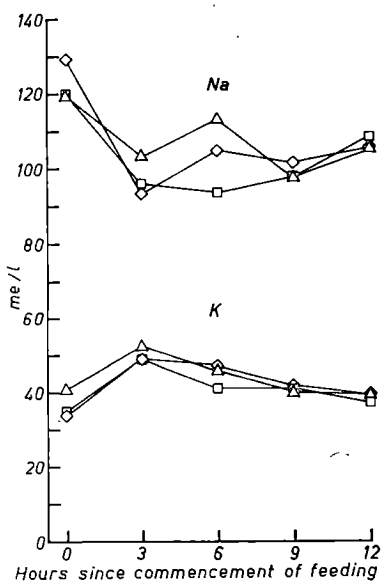

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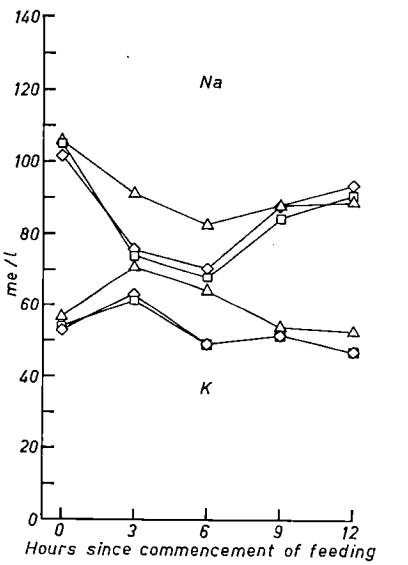

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Figs. 26—29. Concentrations of sodium and potassium in the centrifuged rumen fluid of cow TUPU on diet 6 (Fig. 26) and 8 (Fig. 28), and cow ULPU on diet 7 (Fig. 27) and 9 (Fig. 29). Parts of the rumen contents: upper —△—, lower —◇—, lower forward —□—. In Figs. 26 and 27 the 0 hour results are presented at the 12 hour position for a second time.

used instead. Because of possible variations in dry matter content as well as in the mineral content of the hay, the values given in Table 3 are not completely accurate. Since, however, the consumed quantities of minerals in wheat bran were determined by actual analysis, this possible source of error can be assumed to have no notable effect on the magnitude of the figures nor on their relative proportions.

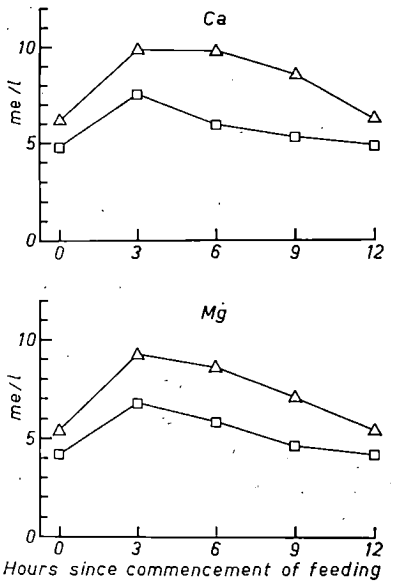
In examining the concentrations of the various cations with regard to their significance in neutralizing the rumen liquid, the primary rôle of sodium is clearly evident. Since the amounts of sodium consumed were very small in comparison to the concentrations of this ion in the rumen at any particular moment, it seems obvious that the immediate effect of the ingested sodium had no significance. Thus, the salivary secretion of this ion is left as practically the only regulatory mechanism of its concentration in the rumen, provided that sodium removal from the rumen is at a constant rate. The relatively high concentration of this ion as compared to the other cations confirms the very great importance of salivary secretion for buffering the rumen contents, a fact which conforms with the results of BAILEY (1961 b).

The decreases in sodium concentration occurring after the beginning of feeding, which were not observed in the experiments of BAILEY (*loc. cit.*), appear to be consistent, even though each concentration curve was obtained from only one sampling series. They were evidently due to dilution by the water intake of the animals. In the said studies of BAILEY the sodium concentration of the rumen liquid remained at an average 17 % lower than that in the saliva at the same time. He thinks that this may have been due to absorption of sodium into the blood. The comparatively minimal magnitude of the difference, however, would seem to indicate that the drinking water together with the water content of the fodder could be responsible for such a dilution.

The concentration difference indicating the possible indirect effect of the consumed sodium quantity seems to be separately observable in both test animals. On the other hand, on the same basis it does not seem possible to account for the difference in concentration between the two animals.

The equivalent concentration of potassium was second in significance after that of sodium. The increases in its concentration in the rumen after feeding show, contrary to the case of sodium, that potassium also had a direct effect on the concentration of this ion in the rumen fluid. However, the correlation between ingested quantity and rumen concentration appears to be reversed in the two cows, a divergence which could possibly be due to differences in salivary secretion.

BAILEY (1961 b) observed a positive correlation in potassium concentrations between mixed saliva and rumen fluid. In the experiments of BAILEY and BALCH (1961 b, Fig. 4) a definite and close negative correlation was noted between the sodium and potassium concentrations of the saliva. The same seems to be observ-



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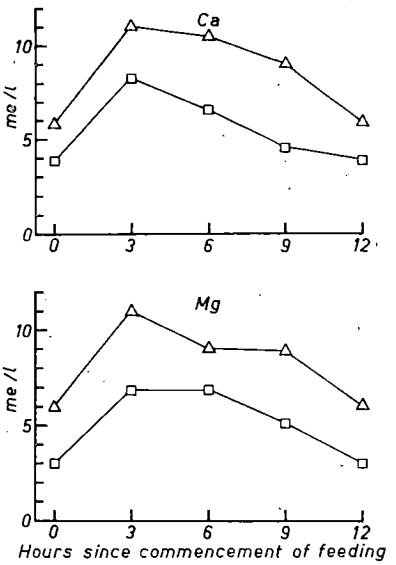

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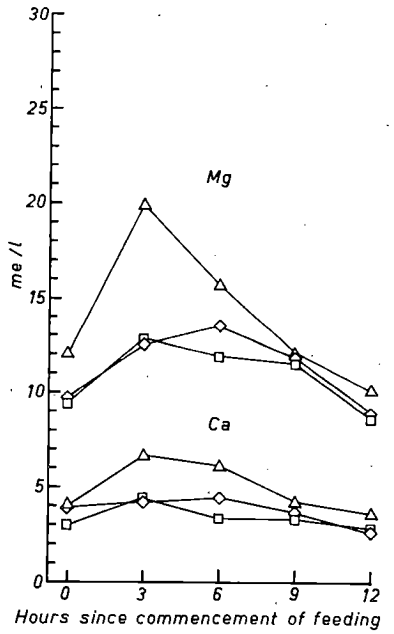

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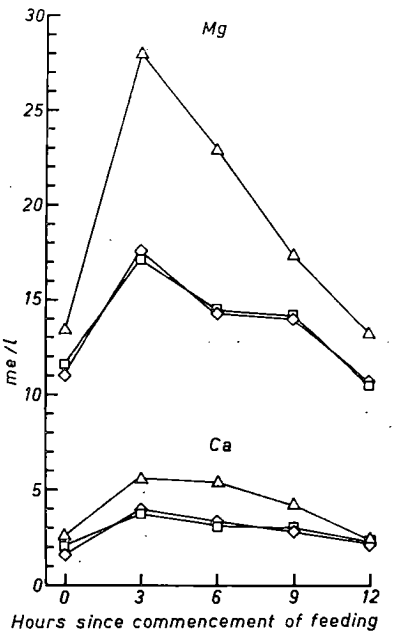

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Figs. 30—33. Concentrations of calcium and magnesium in the centrifuged rumen fluid of cow TUPU on diet 6 (Fig. 30) and 8 (Fig. 32), and cow ULPU on diet 7 (Fig. 31) and 9 (Fig. 33). Parts of the rumen contents: upper —△—, lower —◇—, lower forward —□—. In Figs. 30 and 31 the 0 hour results are presented at the 12 hour position for a second time.

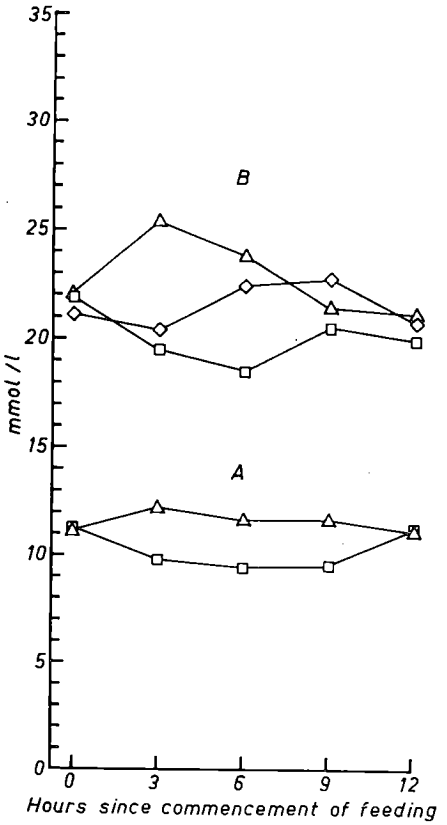
able also in Figs. 26—29 concerning the concentrations of the rumen fluid, both between animals and between diets. Since the correlation between consumed potassium and its concentration in the rumen with the same cow is negative, the effect of the consumed amount appears unexpectedly small. The reason for the above-mentioned divergence in concentrations is thus apparently attributable to the negative effect exerted by sodium on the concentration of potassium in the saliva, and this effect seems to be dominant, judging from the evidence presented in the foregoing.

The low calcium contents of the rumen liquid indicate that its share in buffering the rumen contents has been small. According to the analyses of McDougall (1948), sheep parotid saliva contained an average of 0.4 me./l calcium and mixed saliva about 0.8—1.5 me./l. These low figures show that salivary secretion of calcium has only a slight influence on the concentration of the rumen liquid. The results of Phillipson and Mangan (1959), on the other hand, indicate an opposite possibility, since they found the calcium contents of sheep parotid and residual saliva to be approximately 3—7 me./l.

In the present studies the calcium concentrations were nearly on the same level in Diets 6 and 7 and also Diets 8 and 9 when each pair is considered separately. The level in the latter pair of diets, however, was distinctly lower than in the first-mentioned, although there was no similar difference in calcium consumption (Table 3). Since in all cases the rise in concentration following feeding as well as the differences between the upper and lower parts of the rumen point to the direct effect of feeding, the said difference in calcium level may have been due to variation in solubility of the calcium salts. Another explanation could be that there were differences in salivary secretion of calcium; this appears possible on the basis of the divergent results stated by the above-mentioned investigators.

The ratio of magnesium concentration in the rumen fluid to the quantities consumed was definitely higher than in the corresponding ratio for calcium. The reason for this difference was obviously the greater solubility of the magnesium salts, since according to McDougall (1948) the concentrations of magnesium in the saliva do not differ essentially from those of calcium. This idea is also supported by the quantitatively great increases in concentration occurring in the upper part of the rumen after feeding, especially in Diets 8 and 9. The shape of the concentration curves on different diets indicates consistently that the contents of both magnesium and calcium in the rumen fluid were primarily dependent upon a direct effect of feeding.

The changes in phosphate content of the rumen fluid, shown in Fig. 34 and 35, appear to follow a different course than those of the cations. The decrease in concentration in the lower part of the rumen after feeding gives reason to suppose that salivary secretion is the principal regulator of the rumen phosphate content, since this decrease is similar to that occurring in the case of sodium. On the other hand, the definite increase occurring in the upper part after feeding



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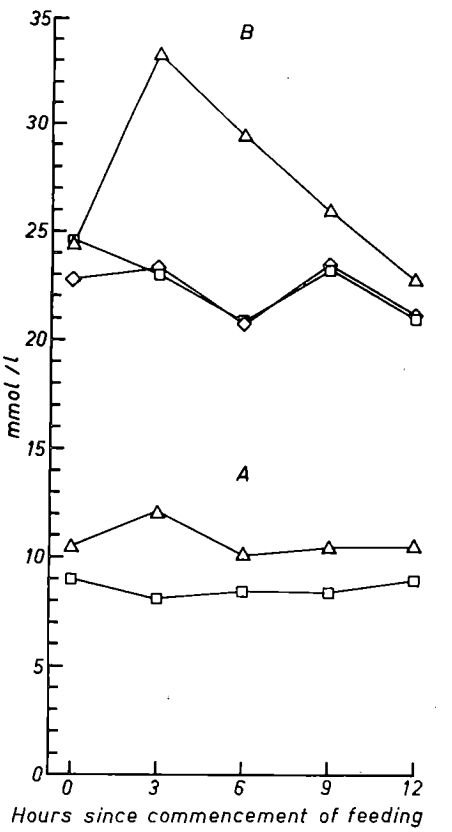

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Figs. 34 and 35. Phosphate concentration in the centrifuged rumen fluid of cow TUPU on diet 6 (Fig. 34 A) and 8 (Fig. 34 B), and cow ULPU on diet 7 (Fig. 35 A) and 9 (Fig. 35 B). Parts of the rumen contents: upper —△—, lower —◇—, lower forward —□—.

on Diets 8 and 9 indicates a direct influence due to feeding. Since the phosphorous intake on these diets (particularly on the latter) was exceptionally large, the changes occurring in direct response to the ingestion of fodder can also be considered to be exceptional. The absence of such concentration changes in the lower part of the rumen, together with the uniformity of the phosphate level between feedings on the other diets, can be considered as the best evidence of the generally small direct effect of this ion.

On the basis of the studies carried out by A. M. SMITH *et al.* (1956) with radioactive phosphorous, it appears that the secretion of phosphate from the blood to the rumen of cattle occurs almost entirely by means of the saliva. According to the above-described results, the variations in this secretion obviously caused the differences in concentration levels in the present experiments, which are clearly evident when comparisons are made between the various diets.

GENERAL DISCUSSION

Investigations on the pH and the volatile fatty acid (VFA) concentration of the rumen contents have previously consistently been made using methods which are based on the assumption that the contents are approximately homogenous. In general, determinations are made on samples taken from the lower part of the rumen. However, the results of the present study show, in agreement with those of earlier experiments (LAMPILA 1955, LAMPILA and POIJÄRVI 1959), that such methods yield pH values higher than the average, and correspondingly too low VFA concentrations. The work of BALCH *et al.* (1955) and BALCH and ROWLAND (1957) was exceptional in that they took samples from the upper part of the rumen, presenting their results as being representative of the entire rumen contents. In this case, the idea gained from the situation concerning the pH and VFA values differs in the opposite direction from the average conditions. It is true that either sampling method can be used for comparisons between different diets and even to find statistically significant differences between them, but a correct overall picture of the situation is not obtained. If, on the other hand, samples are taken from random points in the rumen, as can be considered appropriate when the contents are assumed to be sufficiently homogenous, rather heterogenous and illogical results may be forthcoming. These last-mentioned circumstances in particular instigated the writer to undertake investigations of such differences as exist between different parts of the rumen contents.

The differences in question complicate the study of the pH of the rumen and the evaluation of its significance. However, seeing that such differences did occur consistently and on a statistically significant level (Table 2, p. 31), thus affecting the scattering of the results, simple use of the mean values, which obscures the differences, does not seem to be justified. In addition, the differences in rate of fermentation of structural carbohydrates which has been observed between the upper and lower parts (BALCH and JOHNSON 1950, MILES 1951) is great enough to endow its cause to a physiological significance and to deserve detailed investigation. As has been concluded earlier in section I D. (p. 44) and also in a previous paper (LAMPILA 1959), the difference in rate of fermentation between different parts of the rumen is mostly or entirely due to a corresponding difference in hydrogen ion concentration.

In evaluating the effect of the rumen pH, the main difficulty caused by the differences occurring in the different parts is the fact that it is not easy to estimate the partition of the food mass represented by each pH value or range. It might be possible to solve this problem by removing the rumen contents in layers and determining the pH and dry matter content of each layer. This procedure, however, was not used in the present study, since it was impossible to remove the rumen contents by hand through the small fistula opening.

According to measurements made by BALCH (1950), the dry matter content of the ventral part is around 5—6 % and that of the dorsal part about 13—14 %. Such a distinct difference in dry matter content indicates that the zone between the pH curves relating to the central and upper part represents the pH of the bulk of the rumen ingesta. Since in the present investigation in Diets 4—9 this zone had a pH lower than 6.0 during the greater part of the time between feedings, a pH level at which an increase in hydrogen ion concentration has a very pronounced retarding effect on fermentation (Fig. 20, p. 46), this effect must have been quite great also quantitatively. If the above-mentioned diets are characterized by the daily milk production that would have been possible on the energy supplied by them, the output would be between 7 and 21 litres of 4 % fat-corrected milk. These diets thus do not reach the height of those of high-producing dairy cows. Obviously, then, there is hardly any doubt that on a more concentrated diet the effect of the hydrogen ion concentration would be even greater.

Since the pH of the rumen contents differs quite clearly from the optimum level on diets with intermediate fodder unit content, a notable effect on the utilization of fodder in dairy cows is evident. With the exception of cases of strong indigestion caused by overfeeding, this effect on the nutrition of the cows is difficult to evaluate. By retarding fermentative digestion in the reticulo-rumen, it increases the bulk of the fodder as compared to what it would be under optimum conditions. In other words, if fermentation would occur at the maximum rate, an even greater share of the food requirement could be met by cheaper coarse fodders.

The per cent digestion of fodder in the rumen is the product of fermentative activity and retention time of the food in the rumen. If one of these factors decreases, the product will also decrease unless the other factor increases correspondingly. The studies of PALOHEIMO and MÄKELÄ (1959) show that when the dry matter ingested by the cow increases, the time of retention of the dry matter and of its organic nitrogen-free components decreases. If the same change occurs in diets for which the unfavourable effect of pH increases as the amount of dry matter increases, the consequence is obviously a diminishing in rumen digestion due to simultaneous decrease in both of the above-mentioned factors. If the digestion in the lower parts of the digestive tract does not compensate for this, the result is a decrease in the utilization of the fodder.

PALOHEIMO and MÄKELÄ (1960) determined the digestibility of nitrogen- and lignin-free organic matter in rations of different size, both in the proventriculi and in the whole digestive canal. The experimental material was mostly the same as in their previously-mentioned study. An increase in consumed dry matter was observed to have no effect on the digestion in the proventriculi nor in the whole digestive tract. However, the largest quantities of dry matter were still too small to render the unfavourable effects of pH probable on the diets used.

MÄKELÄ (1956, pp. 56—58) has made reference to many studies in which it was often observed that an increase in the amount of rations led to a decrease in digestibility of the fodder. Since this may be caused both by a reduction of the time spent by the fodder in the rumen as well as by a simultaneous decrease in fermentative activity in the reticulo-rumen, it is impossible to evaluate the separate effect of each factor. Due to the general belief that under normal feeding conditions the pH of the rumen remains at optimum level regardless of the rations, the effect of pH was rarely noticed in earlier studies as a factor influencing the digestion.

The results shown in Fig. 21 (p. 47) show that the effect of VFA concentration on the fermentation activity was so slight that it was not statistically significant. This somewhat unexpected result indicates that the accumulation of acids in the rumen produces its effect principally by means of the hydrogen ion concentration. The neutralization of acids thus appears to be sufficient to eliminate at least the greater part of this effect when the concentrations in the rumen are at normal levels.

Microbial cell synthesis and the utilization of ammonia by microbes is related to energy-yielding fermentation processes. Therefore, as far as the activity of such synthesis is concerned, it is evidently dependent upon the pH in the same way as the fermentation processes are. The correlation between pH and ammonia-producing reactions, however, may be essentially different, in which case the net result of the opposing reactions may diverge considerably from what would be expected on the basis of the first-mentioned relation. The well-recognized differences in decomposition rate of various proteins (McDONALD 1954, McDONALD and HALL 1957), combined with the abundance of proteins and other nitrogenous compounds in the rumen contents, affect the total production of ammonia. Consequently the effect of pH on the end result (from the viewpoint of utilization of nitrogenous compounds) may be quite different under different conditions.

In previously published *in vitro* experiments by the writer (LAMPILA 1959) with rumen contents as substrate, the observation was made that an increase in pH in the range 5.0—6.7 accelerated the ammonia-consuming reactions relatively more than the reactions producing ammonia. At a pH of about 5.9 and above, the ammonia-consuming reactions were dominant.

The modifying effect of the substrate on the results in the present trials is seen in Figs. 22 and 23. Hay does not appear to be an especially good substrate, neither for the ammonia-producing nor the ammonia-consuming reactions. At the highest pH levels (6.37, 6.85), however, the latter appear to be dominant. When casein was included, the same tendency was observed only at the uppermost pH level (6.86). When the pH increased from the lowest levels to about 6, the net formation of ammonia was increased, a result which is the opposite to what occurred when rumen contents were used as substrate.

The marked influence of sugar in promoting ammonia utilization (Fig. 23, p. 50) indicates that carbohydrates used as substrate have an essential effect on the utilization of nitrogenous compounds, as has been observed in many investigations concerning this subject (reviewed by REID 1953). It is noteworthy, however, that even at pH 5.28 the decrease in ammonia was already quite small and that at the lowest levels (4.53 and 4.90) the ammonia quantities actually increased. The significant share of the pH in affecting the utilization of nitrogenous compounds thus appears to be very possible also when conditions are favourable for protein synthesis as regards the energy supply available to the microbes.

In connection with the present studies, trials were also made in which the rate of formation of C_2—C_5 fatty acids was determined at different pH levels in cultures with rumen contents as substrate. The results of one trial have been previously published (LAMPILA and POIJÄRVI 1959). The substrate for the cultures in this trial was obtained from the rumen contents of cow Ulpu on Diet 7. The results show that a lowering of pH from 6.85 to 5.30 greatly retarded the formation of acetic acid and also decreased to some extent the share of propionic acid in the mixture that was formed (assuming that the acid mixture at the beginning of incubations remained unchanged in composition). The production of butyric and valeric acids, on the contrary, did not decrease either quantitatively or relatively, with the consequence that their share in the increment of acids became markedly greater. A difference in composition of the acid mixture which could be due to the effect of pH was also observed between the upper and lower part of the rumen contents.

When incubation trials were made using substrate taken from the rumen on other diets, the results were so heterogenous that no conclusions on the possible effect of pH were considered justified. The main reason for these uncertain results was the fact that the total acid production at the lowest pH levels was too small. It is thought, however, that some conclusions concerning the possibility of the pH effect being dependent upon the substrate can be made by comparing the acid compositions of the upper and lower parts of the rumen. These determinations will be discussed in another publication.

EMERY and BROWN (1961) added sodium or potassium bicarbonate to dairy cow rations containing little roughage (2 lbs. of long or pelleted hay per day) with the aim of studying the effect of these additions on the pH and VFA concentrations of the rumen as well as on the fat content of the milk. A reduction in fat content, which was observed on the above-mentioned diet itself, appeared to be partly prevented by bicarbonate addition, and at the same time the pH of the rumen increased. In this regard, the results agree with the effect of pH described by LAMPILA and POIJÄRVI (1959), when the increase in formation of acetic acid is presumed to prevent a decrease in fat content. The relative proportions of the acids in the rumen did not, however, change essentially as a result of the bicarbonate additions.

The assumption presented in the preliminary investigations (LAMPILA 1955) that a pH lower than optimum could considerably reduce the activity of microbial digestion in the rumen on normal diets, was confirmed in the present study. At the same time the differences in physico-chemical conditions between different parts of the rumen became repeatedly apparent. It seems that the presence of such differences should be taken into consideration when attempting to evaluate the possibilities and to find means of expediently regulating the pH of the rumen.

The higher concentration of fermenting food material in the upper part of the contents (BALCH 1950) is apparently a consequence of the decrease in specific gravity of the food particles, which is due to small gas bubbles which are either on the surface or within these particles. This buoyancy mechanism, together with the mixing of the contents, may fractionate the material in such a way that the more completely digested food accumulates in the lower part. The greater concentration of the food material in the upper part and the possible difference in fermentability are perhaps partial reasons for the fact that the VFA concentration is higher and the pH lower than in the lower part of the rumen. At least one contributing reason for these differences, however, is that the free fluid in the rumen is on the floor of this organ, as is clearly seen from the illustrative drawing presented by BALCH (1958 a, p. 35). According to his conception (p. 43) the liquid cannot constantly remain at the cardiac level or above, since then it would interfere with belching. In the lower part of the contents the food matter is submerged in the fluid, which rapidly transports the acidic products of fermentation to the absorbing surface. In contrast, the food in the upper part can be figuratively thought to float on the surface of the liquid. The removal of acids from the upper part of the mass is also by means of the fluid when it rises to the upper layers in response to the contractions of the reticulo-rumen (BALCH *loc. cit.*). Since the mass of the upper part is often very compact, the movement of the fluid in this mass in obviously rather strongly restricted. The essential thing, however, is that the acids in the liquid bound to the food mass are removed only when the concentration in this retained liquid is higher than in the free liquid. This fact in itself already shows that concentration differences are probable when acids are constantly formed in the rumen and are removed from it by absorption through the wall.

Since high hydrogen ion concentration appears to be a problem mainly in the upper layers of the contents, factors which promote the removal of acids from these layers obviously have a primary significance in attempts to eliminate the unfavourable effects. The relatively small pH differences observed between the different parts of the rumen in one of the cows (Tupu) on Diet 7 indicate that there are possibilities of minimizing the differences even when the diet is abundant and concentrated. When the removal of acids from the food mass is

improved, it is to be expected that their absorption into the blood is also accelerated.

The determinations made on the neutralizing capacity and mineral contents of the rumen liquid were partly undertaken with the aim of finding out what are the possibilities of adding alkaline mineral supplements to the diet in order to increase the neutralizing capacity of the liquid. The relatively low neutralizing capacity values noted in the diet containing wheat bran, as well as their fairly wide variations (Figs. 18 and 19), can be interpreted as implying that at least in some cases and within certain limits there are possibilities of increasing the capacity. The same seems to be indicated by the variations in buffering capacity observed by AMMERMAN and THOMAS (1952).

The predominant position of sodium among the cations in the rumen liquid (Figs. 26—29) and the nearly complete dependence of its concentration upon salivary secretion emphasize, however, the great importance of saliva in acid neutralization. Since in addition an appreciable part of the potassium and phosphorus in the rumen liquid is evidently introduced with the saliva, the possibilities of an immediate effect due to mineral ingestion appear to be relatively small.

The tentative estimates of BAILEY (1961 a) suggest that the daily secretion of saliva may rise to nearly 200 litres. The determinations of LAMPILA and POIJÄRVI (1959) on the flow of liquid through the rumen indicate similar maximum amounts of salivary secretion. In order that the proportion of added alkaline minerals to those in the saliva should have any practical significance, the mineral additions must evidently be quite large.

Several investigations have recently been published on the effect of alkaline mineral supplements on the utilization of fodders when the diet does not contain sufficient roughage to maintain normal rumination (NICHOLSON *et al.* 1963, LASSITER and COOK 1963). The cations have generally been Na and K, either singly or both together. Although the conditions for the appearance of an influence of such supplements are relatively better than on normal diets, the positive effects obtained have been relatively small. The alkalinity of the mineral mixture appears, however, to be indispensable in order that the animal is able to manage at all on such a diet (MATRONE *et al.* 1957, 1959). The hazard of kidney disorders appears to be great when large amounts of alkaline salts are consumed (NICHOLSON *et al. loc. cit.*).

SUMMARY

This paper deals with the accumulation of volatile fatty acids (VFA) in the bovine rumen contents and its possible unfavourable sequelae — either directly or by means of the hydrogen ion concentration — in limiting the physiological activities of the microbe population.

The pH of the rumen contents was measured by the *in vivo* method on two fistulated Ayrshire cows which were given nine different diets. Measurements were made during the 12-hour period between feedings, some of them being performed concurrently on both cows. On six of the diets determinations were made on the total concentration of volatile fatty acids in the rumen liquid as well as the neutralizing capacity of the liquid during the interval between feedings. The effect of VFA concentration and pH on the activity of microbial fermentation was studied in short-term incubation trials in which rumen contents were used as substrate and the rate of formation of volatile acids was considered the measure of fermentative activity. The effect of pH on the equilibrium between ammonia-producing and ammonia-consuming microbiological reactions was investigated in incubation trials using ground hay as the primary substrate and glucose and casein as supplementary additions. On four diets the concentrations of Na, K, Ca, Mg and P and their variations during the interval between feedings were determined on centrifuged rumen liquid. The purpose was to investigate the relative importance of these ions in the neutralization and buffering of the contents.

The following results were obtained:

1. When the diet consisted of hay only (9.5 kg/day + mineral supplement) or if only small amounts of concentrates were included (1.3 kg protein concentrate per day), the average pH values of the rumen contents remained within the limits 6.0—7.0. The twelve-hour fluctuations due to feeding were slight, and there were only small differences in pH between the different parts of the contents. When the amount of concentrates was increased and that of hay simultaneously reduced to 7.5 or 5.0 kg/day, the fluctuations in pH increased. They were greatest in the upper part and smallest at the measuring points in the lower part. As a result of these changes, a distinct divergence in pH values appeared between the upper and lower parts, amounting to as much as over one unit. The pH values of the central part of the contents were generally intermediate between those of the upper and lower measuring points. In most cases the effect of the pH differences between the different parts of the rumen on the scattering of the results at different times and on different diets was significant ($P<0.05$) or highly significant ($P<0.01$). The minimum average pH values in the upper part fell to 5.25. On six diets with energy contents corresponding to a daily milk production of 7—21 kg (4 % fat-corrected), the main part of the zone remaining between the pH curves representing the upper and central parts of the rumen contents was below pH 6 in one or in both cows.

2. The VFA concentration in the upper part of the rumen contents was without exception higher than in the lower part. A distinct difference in concentration was also seen in a diet of hay only, although no corresponding difference in hydrogen ion concentration could be observed. The acid concentration in the upper part was, in the different cases, 21—93 % higher than that recorded at the same time in the lower part.

3. The neutralizing capacity of the rumen fluid for the neutralization of VFA was from 61 to 129 me./l. No differences were noted in the neutralizing capacity between the upper and lower parts of the rumen which could have affected the pH differences between these parts. No 12-hour variation in neutralizing capacity due to feeding could be established.

4. In *in vitro* trials carried out by incubating rumen contents under pH controlled conditions, the regression equation between pH and the formation of volatile fatty acids was:

$$y = -1266 + 422\,x - 32.7\,x^2$$

where y denotes the VFA formation in milliequivalents per litre of liquid in 6 hours and x denotes the mean pH of the culture. This second-degree regression is highly significant ($P < 0.001$). According to the formula, the acid formation was most rapid (95.5 me./l/6 hrs.) at a pH of 6.45. The range of pH investigated was 4.6—7.2.

5. The increase in VFA concentration in the region about 130—400 me./l appeared to have a slight retarding effect on the acid formation in *in vitro* cultures which were carried out in the same way as those mentioned above in point 4. However, no significant linear regression existed between the acid concentration and the rate of acid formation ($P > 0.05$).

6. The effect of pH on the equilibrium between ammonia-producing and ammonia-consuming reactions *in vitro* varied according to the substrate. When the substrate consisted of hay only, the ammonia content of the cultures decreased somewhat at the two upper pH levels (6.37, 6.86) but remained approximately the same at the lower levels (to pH 4.55). The addition of casein resulted in an increase in ammonia content at all pH levels (4.51—6.86). The increase was greatest in the range 5.43—6.34. When glucose was added, the ammonia concentration dropped steeply in the pH range 5.74—6.70 but only slightly when the average pH was 5.28. At the very lowest pH levels (4.53, 4.90) there was a slight increase in ammonia content of the cultures.

7. Among the four mineral cations investigated, the equivalent concentration of sodium in centrifuged rumen liquid was the highest (68—129 me./l). In second place was potassium (25—71 me./l). The concentrations of magnesium (3.0—28.0 me./l) were partially of the same order as those of calcium (1.6—11.0 me./l), but they increased in accordance with the amounts of ingested magnesium on two of the diets, rising on an average to 2—4 times those of calcium.

Phosphate was the only anion determined; its concentration (as H_3PO_4) varied within the range 8.1—33.3 mmol./l.

Twelve-hour variations appeared more or less distinctly at all mineral concentrations. In the case of phosphate, an increase could be observed only in the upper part of the contents and only after the consumption of large phosphate quantities. The concentration of sodium declined after the beginning of feeding and subsequently rose until the following feeding. The other cations behaved in an opposite manner. With the exception of sodium, the concentration differences between the upper and lower parts of the rumen contents appeared to be regular so that at a given time they were higher in the upper than in the lower part.

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SELOSTUS

Haihtuvat rasvahapot, pH ja mikrobiston aktiviteetti lehmän pötsin sisällössä

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Kirjoituksessa on käsitelty haihtuvien rasvahappojen kerääntymistä lehmän pötsin sisältöön ja tästä suoranaisesti tai sisällön vetyionikonsentraation välityksellä mahdollisesti aiheutuvia, mikrobiston elintoimintojen aktiivisuutta rajoittavia seuraamuksia.

Pötsinsisällön pH määritettiin *in vivo* -mittausmenetelmällä yhdeksällä eri dieetillä, käyttäen koe-eläiminä kahta pötsifistelillä varustettua Ayrshire-lehmää. Mittauksia tehtiin 12-tuntisen ruokintavälin käsittävinä jaksoina, osaksi kummallakin koelehmällä rinnakkaisesti. Kuudella dieetillä määritettiin pötsiliuoksen haihtuvien rasvahappojen totaalikonsentraatiot sekä liuoksen neutraloimiskapasiteetin arvot ruokintavälin aikana. Haihtuvien rasvahappojen konsentraation ja pH:n vaikutusta mikrobiston fermentaatioaktiviteettiin tutkittiin lyhytaikaisin viljelmäkokein, joissa pötsinsisältöä käytettiin substraattina ja haihtuvien rasvahappojen muodostumisnopeutta fermentaatioaktiviteetin mittana. pH:n vaikutusta ammoniakkia muodostavien ja kuluttavien mikrobiologisten reaktioiden väliseen tasapainoon tutkittiin viljelykokein käyttäen viljelmissä perussubstraattina jauhettua heinää ja lisäyksinä glukoosia tai kaseiinia. Neljällä dieetillä määritettiin natriumin, kaliumin, kalsiumin, magnesiumin ja fosforin konsentraatiot ja niiden vaihtelu ruokintavälin aikana sentrifugoidusta pötsiliuoksesta. Tarkoituksena oli selvittää täten niiden kunkin suhteellista merkitystä pötsissä muodostuvien happojen neutraloinnissa ja sisällön puskuroinnissa. Tutkimuksissa saatiin seuraavia tuloksia:

1. Ruokittaessa pelkästään heinällä (9.5 kg päivässä ynnä kivennäislisä) tai annettaessa lisäksi vain vähän väkirehua (1.3 kg valkuaisväkirehuseosta) pysyivät pötsinsisällön pH:n keskiarvot rajojen 6.0—7.0 sisällä. Ruokinnasta riippuvat jaksolliset vaihtelut olivat vähäiset, samoin sisällön osien väliset erot. Lisättäessä väkevien rehujen annostelua ja vähennettäessä samalla heinän määrää 7.5 tai 5.0 kiloon päivässä voimistui pH:n jaksollinen vaihtelu. Se oli suurin pötsin yläosassa ja pienin alaosan mittauskohteissa. Tämän muutosten erilaisuuden takia muodostui ylä- ja alaosan pH-arvojen välille selvä tasoero, joka suurimmillaan oli yli 1 pH-yksikön. Sisällön keskiosan pH:t olivat yleensä ylä- ja alaosan arvojen välillä. Sisällön osien välisten pH-erojen vaikutus tulosten hajontaan eri ajankohtina ja eri dieeteillä oli useimmissa tapauksissa merkitsevä ($P < 0.05$) tai erittäin merkitsevä ($P < 0.01$ tai $P < 0.001$). pH:n alimmat keskiarvot sisällön yläosassa laskivat 5.25:een. Kuudella dieetillä, jotka energiasisältönsä puolesta vastasivat koelehmillä noin 7—21 kg:n päivittäisen maitotuotoksen (4-%:sen) vaatimuksia, oli pötsinsisällön keski- ja yläosan pH:ta esittävien käyrien väliin jäävä alue pääosaltaan pH 6:n alapuolella, joko toisella tai kummallakin lehmällä.

2. Haihtuvien rasvahappojen konsentraatio oli sisällön yläosassa poikkeuksetta korkeampi kuin alaosassa. Selvä konsentraation tasoero esiintyi myös käytettäessä pelkkää heinäruokintaa, vaikka vastaavanlaista vetyionikonsentraation eroa ei voitu havaita. Yläosan happokonsentraatio oli eri tapauksissa 21—93 % korkeampi kuin samaan aikaan sisällön alaosassa havaittu.

3. Pötsinesteen neutraloimiskapasiteetti (haihtuvien rasvahappojen neutraloimiseen) oli rajoissa 61—129 me./l. Siinä ei ilmennyt sellaisia sisällön ylä- ja alaosan välisiä eroja, jotka olisivat voineet aiheuttaa näiden osien väliset pH-erot. Neutraloimiskapasiteetin muutoksissa ei ilmennyt ruokinnasta johtuvaa jaksollisuutta.

4. *In vitro* -kokeissa, joissa pötsinsisältöä inkuboitiin pH:n suhteen kontrolloiduissa oloissa, oli pH:n ja haihtuvien rasvahappojen muodostuksen välinen regressio yhtälön

$$y = -1266 + 422\ x - 32.7\ x^2$$

mukainen, kun y:llä merkitään happojen muodostumista milliekvivalentteina liuoslitraa kohti 6 tunnissa ja x:llä viljelmän keskimääräistä pH:ta. Tämä toisen asteen regressio oli erittäin merkitsevä ($P < 0.001$). Yhtälön mukaan oli happojen muodostuminen nopeinta (95.5 me./l 6 tunnissa) pH:n ollessa 6.45. Tutkittu pH-alue oli rajoissa 4.6—7.2.

5. Haihtuvien rasvahappojen konsentraation kohoaminen alueella n. 130—400 me./l näytti hidastavan lievästi niiden muodostumista viljelmissä, joiden valmistus ja käsittely oli samanlainen kuin edellisessä kohdassa mainituissa kokeissa. Happojen konsentraation ja niiden muodostumisnopeuden välinen lineaarinen regressio ei kuitenkaan ollut merkitsevä ($P > 0.05$).

6. pH:n vaikutus ammoniakkia muodostavien ja kuluttavien mikrobiologisten reaktioiden väliseen tasapainoon viljelmissä vaihteli substraatista riippuen. Kun heinä yksin oli substraattina, aleni viljelmien ammoniakkisisältö jonkin verran kahdella ylimmällä pH-tasolla (6.37, 6.86), mutta pysyi alemmilla (alin pH 4.55) suunnilleen ennallaan. Kaseiinin lisääminen aiheutti sen, että ammoniakkimäärä kohosi kaikilla pH-tasoilla (4.51—6.86). Lisäys oli suurin alueella 5.43—6.34. Kun lisättiin glukoosia, aleni ammoniakkimäärä voimakkaasti pH-tasoilla 5.74—6.70, mutta vain vähän pH:n ollessa keskimäärin 5.28. Alimmilla tasoilla (4.53, 4.90) ammoniakkimäärä kohosi lievästi.

7. Tutkituista neljästä kivennäiskationista oli natriumin ekvivalenttinen konsentraatio sentrifugoidussa pötsinesteessä korkein (68—129 me./l). Toisella tilalla oli kalium (25—71 me./l). Magnesiumin konsentraatiot (3.0—28.0 me./l) olivat osittain samaa suuruutta kuin kalsiumin (1.6—11.0 me./l), mutta kohosivat magnesiumin saannin mukana kahdella dieetillä keskimäärin noin 2—4-kertaisiksi kalsiumin konsentraatioihin verrattuna. — Anioneista määritettiin ainoastaan fosfaatti, jonka konsentraatio fosforihappona ilmaistuna vaihteli rajoissa 8.1—33.3 mmol./l. — Ruokintakertojen välinen jaksollinen vaihtelu ilmeni enemmän tai vähemmän selvänä kaikissa konsentraatioissa. Fosfaatin kohdalla se voitiin havaita vain sisällön yläosassa ja siinäkin vain runsaan fosforin saannin aikana. Natriumin konsentraatio aleni ruokinnan aloittamisen jälkeen ja kohosi seuraavaa ruokintaa kohti mentäessä. Muiden kationien kohdalla muutokset tapahtuivat päinvastoin. — Lukuun ottamatta natriumia näyttivät sisällön ylä- ja alaosan väliset konsentraatioerot säännönmukaisilta; konsentraatiot olivat yläosassa korkeampia kuin samaan aikaan alaosassa.

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