PRELIMINARY STUDIES ON THE VARIATIONS OF pH AND VOLATILE FATTY ACID CONCENTRATION OF THE RUMEN CONTENTS OF THE COW

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Several authors investigating the reaction of the rumen contents (e.g., 3, 4, 8, 9, 14) have observed that, in spite of the abundant formation of organic acids, the mechanism regulating the reaction is capable of stabilizing the pH of the contents in the vicinity of the neutral point. According to these investigators, acidity never seems to hinder the activity of the microorganisms, and hence the digestion of fodder, in the rumen. However, investigations and observations indicating the existence of the opposite possibility have also been made (e.g., 6, 7, 10, 13, 15, 19). In these investigations rather low pH values of the rumen contents have been established at times, both with the cow and with the sheep as experimental animals. Even though our knowledge of the effect of acidity upon the vital processes, of the microorganisms in the rumen is still rather incomplete, it seems justifiable to assume that the processes of decomposition are slowed down by such acidities. Various kinds of fodder have been found to have different effects upon the degree of acidity of the rumen contents. The amount of food consumed likewise seems to have an influence. It has also been shown that the measuring technique employed in various cases may introduce factors which tend to render the observed pH values obviously too high in several instances. The commonest source of error is the escape of carbon dioxide before the measurement. The reaction may also differ considerably according to the part of the rumen of the cow from which the sample is taken (12). It has been shown that lower pH values are obtained by the *in vivo* technique than on measurement of samples taken from the rumen of the cow (19).

The established errors caused by the measuring method have consistently been of such a nature that they have resulted in an increase of the pH. Since, as far as I am aware, only one investigation (19) has been carried out by the *in vivo* technique, it is conceivable that opinions on the reaction of the rumen contents of cattle may be

based mainly on erroneously high pH values. It should also be noted that microbiological investigations throwing light on the influence of the degree of acidity upon the fermentation processes of the bacterial flora in the rumen are almost non-existent. In view of the facts reviewed above, the Department of Animal Husbandry of the Agricultural Research Centre has deemed it necessary to subject the entire acidity question to closer investigation. The preliminary results of this work will be presented below.

Experimental animal and methods

The experimental animal was an Ayrshire cow of about 470 kg live weight, which had been provided with a rumen fistula using the technique of STODDARD et al. (20). During the experiments, the plastic tube forming the fistula, and its flanges, were replaced by a rubber capsule of such design that it could be inflated to close the opening of the fistula tightly.

The *in vivo* pH measurements were performed with the Beckman pH meter, model G, with a glass electrode and a saturated calomel electrode. The glass electrode, of standard model, was detached from its mounting arm and fitted with a shielded lead of about 2.5 m length. The electrode was attached by means of a rubber stopper to the measuring probe shown in fig. 1. At measurement it was inserted in the rumen together with this probe. The calomel electrode was connected with the solution to be measured by means of a saturated KCl-agar bridge, which is seen in fig. 1.

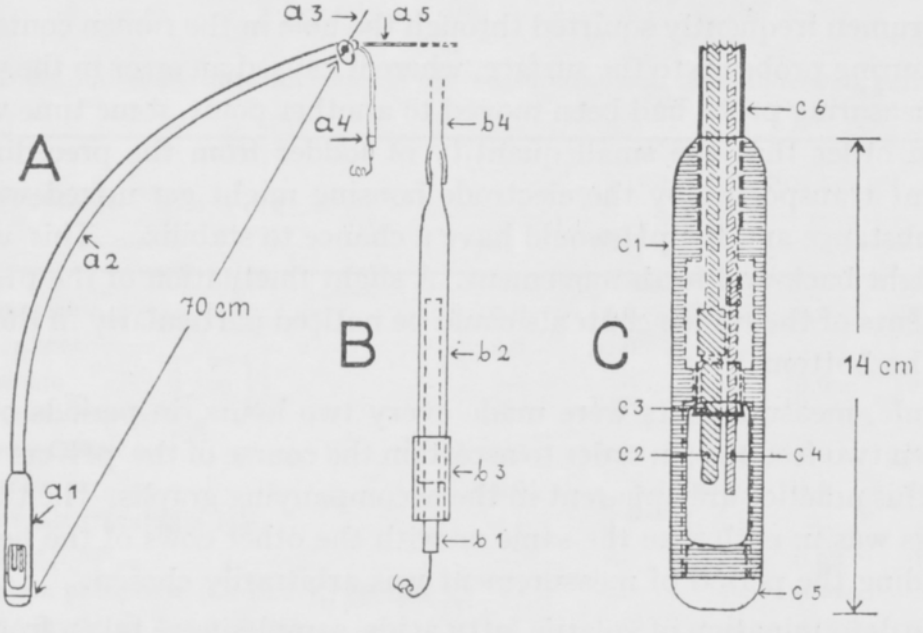

Fig. 1. A. The entire measuring probe: a1, electrode housing; a2, shaft (curved brass tube); a3, pointer, clamped to shaft aligned to point at tip of measuring probe; a4, calomel electrode; a5, lead from glass electrode. — B. Arrangement of calomel electrode: b1, small calomel electrode; b2, glass tube filled with KCl — agar; b3, rubber tube attachment; b4, thin plastic tube filled with KCl — agar. — C. Longitudinal section of electrode housing: c1, Perspex body; c2, glass electrode, c3, rubber stopper; c4, tip of KCl — agar bridge (glass tube); c5, stopper; c6, shaft of measuring probe.

It was evident even at the initial stage of the work that the hydrogen ion concentration is different in different parts of the rumen. Therefore measurements were made at four different points, which were chosen in such a way as to reveal the limits of variation of the pH and the direction of its change.

The pH values characterizing the acidity of the upper part of the rumen contents were obtained from the surface region of the ingesta in the dorsal sac at a point about 10—20 cm cranially from the fistula opening and, on an average, 5 cm below the surface of the ingesta. During a few hours after feeding, however, the surface of the ingesta was frequently too dry to establish satisfactory electrical contact between the electrodes. To eliminate this difficulty, it was sufficient to place a tight cotton plug in the electrode housing of the measuring probe, in which the ends of the glass electrode and of the KCl-agar bridge were embedded. The liquid drawn from the ingesta into the cotton plug would then establish the connection. This plug was removed before the measuring probe was moved to the next point of measurement. The pH values characterizing the acidity of the central part were measured in the ventral sac of the rumen on the centre line of the animal at a point approximately equidistant from the bottom of the rumen and the surface of the ingesta. In the curves representing the results of the pH measurements, the third point of measurement below this central point, at about 5 cm from the bottom of the rumen, has been termed the »lower part». The fourth point, the »lower forward part», was similarly on the bottom of the rumen in its dorsal sac, near the opening of the reticulum.

The measurements at the different points were carried out in the order mentioned above, starting at the upper part, since it was observed that fluid from the lower part of the rumen frequently squirted through the hole in the rumen contents pierced by the measuring probe up to the surface, where it caused an error in the pH reading. After the measuring probe had been moved to another point, some time was allowed to elapse in order that the small quantity of fodder from the preceding point of measurement transported by the electrode housing might get mixed with the surrounding substance and the pH would have a chance to stabilize. This was accelerated by a slight back-and-forth movement. A slight fluctuation of the pH caused by the movements of the rumen contents could be noticed particularly in the measuring points on the bottom.

As a rule, measurements were made every two hours, in periods covering the time between two feedings, in order to ascertain the course of the pH curves. Deviations from this practice are apparent in the accompanying graphs. The time between two feedings was in each case the same as with the other cows of the herd. During pasture feeding the period of measurement was arbitrarily chosen.

For the determination of volatile fatty acids, samples were taken from the upper part of the rumen with forceps with trough-like jaws. The samples from the lower part were taken with the aid of a metal tube fitted with a rubber piston to lay open or close the holes in the lower side of the tube, through which the fluid had access to the tube. The pH of the sampling point was measured immediately before the taking of the sample. The total volatile fatty acids were determined from the liquid separat-

ed with the centrifuge, according to the method of FRIEDEMANN (5), the distillation being performed only once.

In order to establish a comparison between the *in vivo* and *in vitro* pH measurements, the pH of the samples taken in the way described above was measured in the laboratory with a Beckman, model H-2, pH meter. The samples, which had been cooled in ice water immediately upon taking, were carefully heated to room temperature (20°C). The measurement was made about 45 minutes after the sample was taken.

Results and discussion

First test period. The diets employed during this and the succeeding test periods are seen from Table 1. The same feeding was maintained for 4 days at least before the commencement of measurements. As it was assumed that the length of the time between two feedings might affect the course of the pH curves, measurements were made in the beginning both during the day and the night period. However, the results of these measurements, presented in fig. 2, show that on the diet in question no remarkable differences in pH are observable, although the day period was shorter than the night period by 2 hours. The changes in reaction of the rumen contents were relatively slow.

In addition to the points shown in the figure, measurements were made on the right side of the rumen at the height of the measuring point representing the central part of the rumen contents, and at the same height in the rear of the dorsal sac. The

Table 1. Diets during the different series of pH measurements in the stall feeding period.

Fodder, kg	Diet No.				
	1	2	3	4	5
Concentrates[1]	2.6	4.0	2.6	5.0	5.0
Fodder beet	8.0	30.0	—	—	—
AIV silage	—	—	—	10.0	10.0
Sugar beet leaves	—	—	30.0	—	—
Timothy-Clover hay	4.0	4.0	4.0	5.0	5.0
Oat straw	4.0	ad lib.		1.0	1.0
Fodder salt (Hankkija II)[2]	0.1	0.1	0.1	—	—
AIV salt[3]	—	—	—	0.03	0.03
Dicalcium phosphate (Ca 26.2 %) (P 19.1 %)	—	—	—	0.135	—
Ground limestone (Ca 37.0 %)	—	—	—	0.015	0.085
Disodium phosphate (P 8.7 %)	—	—	—	—	0.33

[1] Concentrates: Diets 1, 4 and 5: oats; diet 2: 30 % of concentrate mixture Hankkija II, 30 % wheat bran, 30 % oats, 10 % rye; diet 3: mixture of 2/3 oats, 1/3 wheat.

[2] Containing: P_2O_5 20.0 %, CaO 33.0 %, NaCl 4.0 % plus trace elements Cu, Co, Fe, I.

[3] Containing: P_2O_5 12.5 %, CaO 36.0 %, Na_2CO_3 12.0 % plus trace elements Cu, Co and I.

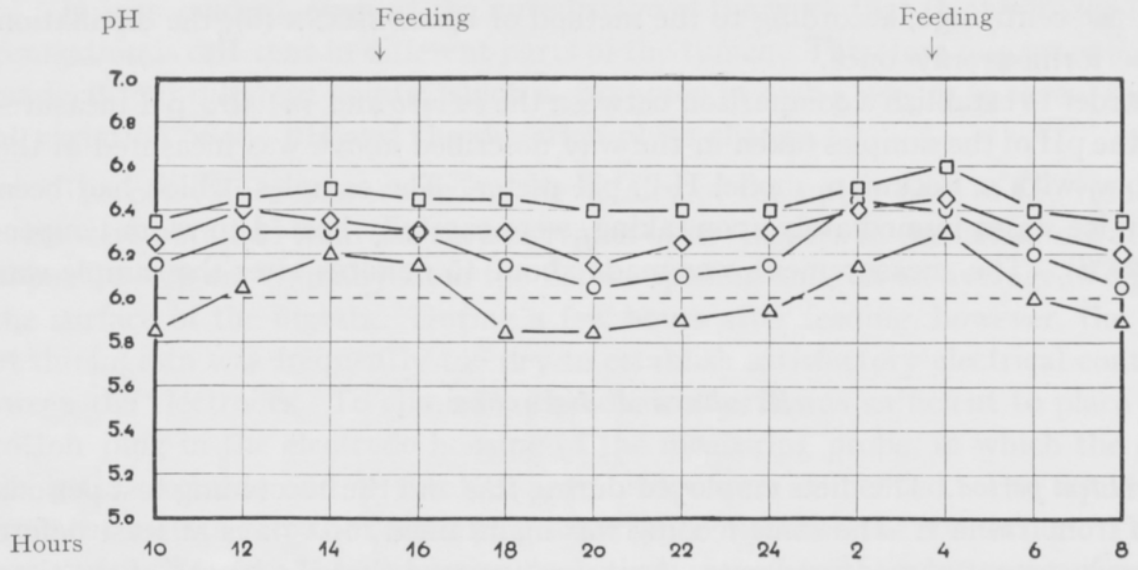

Fig. 2. pH of the rumen contents on diet 1. The pH curves are averages of three series of measurements. Parts of the rumen contents: —△— upper part, —○— central part, —◇— lower part, —□— lower forward part. (Detailed description in the text.)

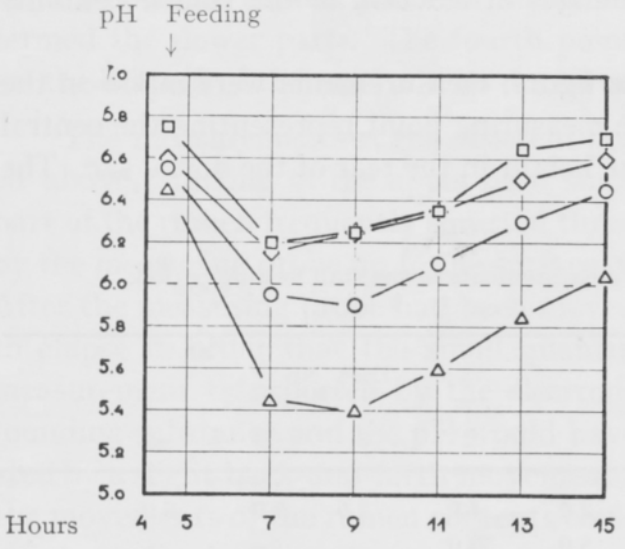

Fig. 3. pH of the rumen contents on diet 2. The pH curves are averages of three series of measurements.Parts of the rumen contents: —△— upper part, —○— central part, —◇— lower part, —□— lower forward part.

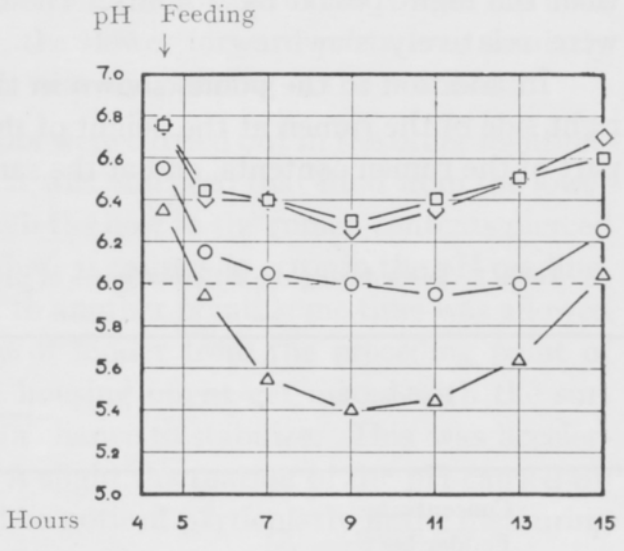

Fig. 4. pH of the rumen contents on diet 2. The pH curves are averages of three series of measurements. Part of the rumen contents: —△— upper part, —○— central part, —◇— lower part, —□— lower forward part.

pH curves obtained at these points were on an average 0.05 pH units higher than the curve representing the acidity of the central part.

Second test period. It can be seen from the curves in fig. 3 that an increase in the amount of fodder units and in the concentration of the ration (cf. Table 1) resulted in a steeper post cenam decline of the pH curve. On the basis of the investigations of

PHILLIPSON and MCANALLY (16) this can be attributed to the increased ratio of more easily fermented carbohydrates. The increase in the difference in pH between the different parts of the rumen contents, as compared with the first test period, indicates a change in the ratio of the rates of formation and disappearance of the acids.

Third test period. The course of the pH curves (fig. 4) was changed as a result of replacement of the fodder beets with the same weight of sugar beet leaves, at the same time as the quantity of concentrates was slightly diminished. The initial formation of acids seemed to be somewhat slower immediately after the feeding than in the preceding case. In the upper and particularly the central part of the rumen contents, on the other hand, the pH has remained lower during the latter part of the period of measurement. In addition to the continued formation of acids at a higher rate, this may have been also partly due to a slower escape of the acids from the inner parts of the ingesta. A fact which tends to support this view is the great difference in acidity between the points of measurement in the lower part of the rumen, close to the rumen wall, and in the centre of the ingesta. In part, also, this view rests on the observation that the rumen contents were more compact when sugar beet leaves were fed than on feeding fodder beets; this condition could be noticed when the measuring probe was pushed through the ingesta.

As a consequence of the short time between feedings, the pH of the rumen contents has not recovered its initial level in all parts before the next feeding. This phenomenon was observable in the preceding case as well.

Fourth and fifth test period. The change in the mineral diet has resulted in the difference seen between the pH curves of figs. 5 and 6. Since the amount of ground limestone, and consequently also the buffer action of the mineral ration, was greater in the latter case, it remains uncertain whether the substitution of an approximately equivalent quantity of disodium phosphate by dicalcium phosphate has lowered the hydrogen ion concentration. This question will be studied in later investigations.

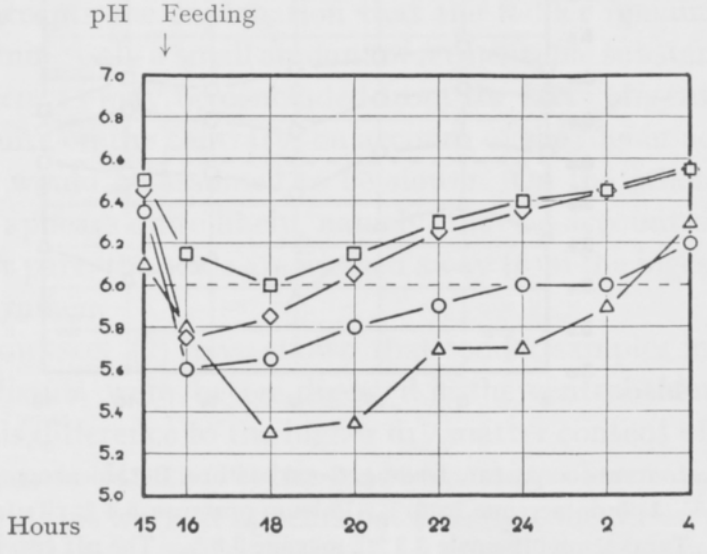

Fig. 5. pH of the rumen contents on diet 4. The pH curves are averages of four series of measurements. Parts of the rumen contents: —△— upper part, —○— central part, —◇— lower part, —□— lower forward part.

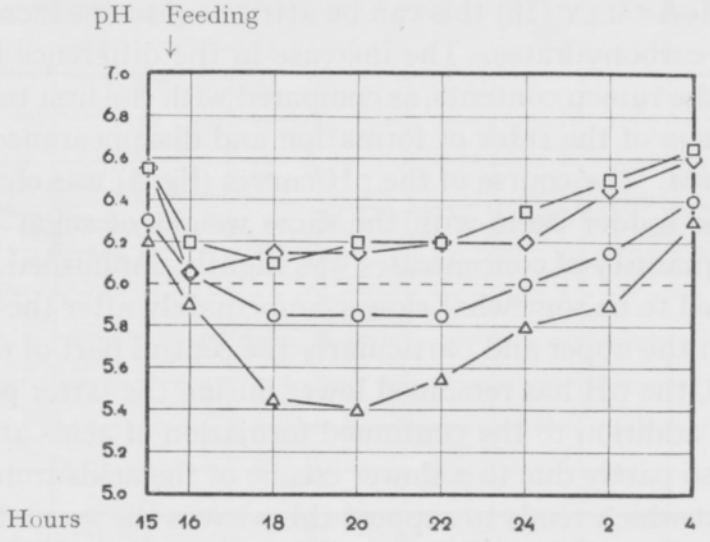

Fig. 6. pH of the rumen contents on diet 5. The pH curves are averages of four series of measurements. Parts of the rumen contents: —△— upper part, —○— central part, —◇— lower part, —□— lower forward part.

The exceptionally steep and strong decrease in pH which is seen in fig. 5 may be due, at least in part, to the acidity of the AIV silage. The pH of the AIV silage was determined in connection with one of the four separate series of measurements. In another case the pH had been determined the day before. These pH values were 4.0 and 3.6 respectively. Since the fodder was taken from one and the same silo throughout the tests, there is no reason to assume that in the other two instances its

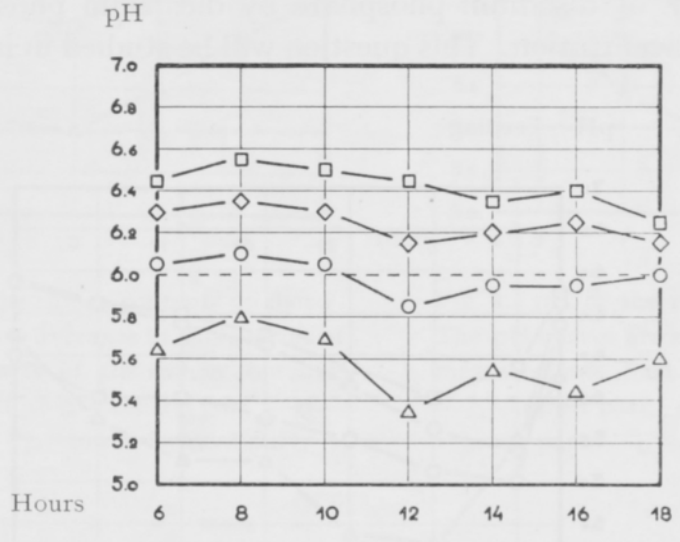

Fig. 7. pH of the rumen contents on pasture feeding. Good pasture. Botanical composition of the pasture: Festuca pratensis 62,3 %, Lolium perenne 12.6 %, Phleum pratense 5.4 %, Trifolium pratensis 3.3 %, Trifolium repens 3.3 %, Taraxacum officinale 3.3 %, foggage 9.8 %. The pH curves are averages of three series of measurements. Parts of the rumen contents: —△— upper part, —○— central part, —◇— lower part, —□— lower forward part. — Botanical analysis was made at the Department of Plant Husbandry of the Agricultural Research Centre.

pH differed from this level, which is considered normal, particularly as the pH curves in these two cases were quite similar, on the average, to those obtained in experiments where the pH of the silage was measured. Therefore, it seems indicated to study the question of whether it might be possible to improve the digestibility of the fodder during abundant feeding of AIV silage by means of a more efficient neutralization of the acids in this fodder than before.

Sixth test period. The changes in the reaction of the rumen contents during pasture feeding were relatively insignificant (fig. 7) and no distinct periodicity is noticeable, as was to be expected, since the cow grazed at short intervals throughout the period of measurement. Between the upper region and the lower forward region of the rumen contents there was consistently a great difference in acidity, at the maximum 1.1 pH units. Here, as in fig. 2, the two curves representing the acidity of the lower part are on distinctly different levels all the time. The coincidence of these curves in the other cases presented above may be indirectly caused by the fact that, on account of the large fodder rations, the rumen has at times been fuller than during the scanty stall feeding or on the pasture.

The distinct, and at times quite remarkable, difference in acidity, especially between the upper part and the lower parts of the rumen contents, which can be seen in all the pH graphs presented above, indicates that the ratio between the rates of formation and vanishing of the acids is different in different parts of the ingesta. Since the measuring points in the lower part were closer to the walls of the rumen than the other points, the escape of the acids by way of resorption has taken place at a higher rate than in the parts of the ingesta more distant from the walls. However, the acidity of the ingesta increases upwards, even though the distance from the resorption area does not increase further from the measuring point in the centre of the rumen contents. It cannot be assumed, at least not in all circumstances, that the formation of acids is relatively more abundant in the upper part than in the lower part, even if one accepts the explanation that the fodder remaining from previous feedings and containing only a small amount of fermentable substances remains at the bottom of the rumen, as may be concluded from the facts presented by SISSON and GROSSMAN (18). Quite on the contrary, on account of the higher acidity in the upper part, fermentation would be assumed to be slower. On the other hand, there is an explanation which appears quite likely, namely, that on account of the higher water content in the lower part, the acids are washed away from the ingesta at a higher rate in this part of the rumen.

BALCH and JOHNSON (2) have shown that fodder samples introduced into the rumen through a fistula were better digested in the ventral than in the dorsal sac. They attributed this difference to the higher dry matter content of the ingesta in the dorsal sac of the rumen. MILES (11) has made the same observation. In the light of the facts presented in this work, it appears possible that the difference in efficiency of digestion observed by these authors is due to the different degree of acidity of the rumen contents, which in its turn is a result of the difference in water content. If this assumption is correct, the differences in digestive capacity indicate that the acidity

of the rumen contents has a remarkable influence upon the digestion of fodder in the rumen.

Several investigators, e.g., POIJÄRVI (17) and WATSON and his collaborators (21), have shown that an increase of the fodder ration lessened the digestibility of the fodder substances. In the light of what has been said above, this phenomenon can in part be attributed to the reduced rate of fermentation caused by the increased acidity.

Since the decrease in the pH of the rumen contents is mainly due to an increase in the concentration of fatty acids, as was shown by PHILLIPSON (15) in his investigation on sheep, the direct influence of the concentration of these acids has to be taken into consideration in addition to the pH. Preliminary (unpublished) work bearing on this question has already been done in this laboratory.

AMMERMAN and THOMAS (1) have found in their investigation on sheep that the rumen contents of different animals were of different buffering capacity, even if the feeding was identical. The possible occurrence of similar individual differences in the case of the cow has to be kept in mind in discussing the pH values presented in this paper, which have been obtained with a single experimental animal.

In order to study the correlation between the pH of the rumen contents and the concentration of fatty acids, as shown in fig. 8, samples were taken during the second test period, from which the total volatile fatty acids were determined. Such samples were taken at varying lengths of time after feeding, and simultaneously from the surface layer of the rumen contents, from the dorsal sac of the rumen and from the bottom of the ventral sac, at the same points where the pH values were measured in the test series. The pH of the sample point was always measured immediately before sampling. These results have only been given as a correlation between the pH and

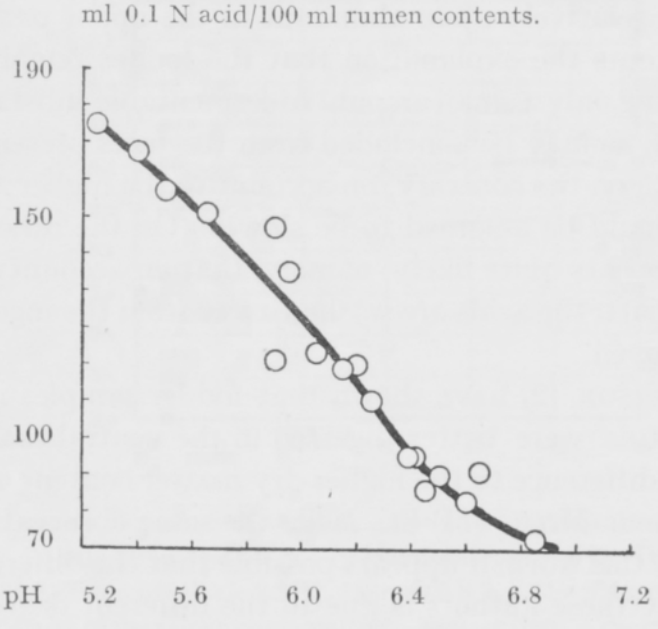

Fig. 8. Correlation between the concentration of volatile fatty acids and pH of the rumen contents *in vivo* on diet 2.

Table 2. pH of the rumen contents as measured *in vivo* and *in vitro*.

Hours after feeding	Sampling place in rumen	pH		Difference in pH units
		in vivo	*in vitro*	
3	Top	5.25	5.40	+ 0.15
	Bottom	5.95	6.60	+ 0.65
6	Top	5.50	5.70	+ 0.20
	Bottom	6.25	6.80	+ 0.55
9	Top	5.90	6.15	+ 0.25
	Bottom	6.45	7.10	+ 0.65
12	Top	6.15	6.80	+ 0.65
	Bottom	6.85	7.25	+ 0.40

the volatile fatty acid concentration. However, this correlation is strong enough to permit of a conclusion with regard to the difference in fatty acid concentration between the upper and lower parts without any appreciable error. This is achieved by reading from the curve of fig. 8 the fatty acid concentration corresponding to the pH values obtained from the curves in fig. 3. Accordingly, the volatile fatty acid concentration in the upper part was at times about 50 % higher than in the lower part. In some instances the difference was even appreciably higher. In the author's opinion the occurrence of such a difference makes possible a study of the relative rates of formation and resorption of the different acids on the basis of their relative proportions in the mixture of volatile fatty acids in the samples from various points. A work of this kind is under way in this laboratory.

In Table 2, the results of the *in vivo* and in *vitro* pH measurements have been set against each other. The findings are in agreement with those presented by SMITH (19) insofar as the *in vitro* measurements have given higher pH values. With a single exception, the increase of the pH value was greater in the sample from the lower part of the rumen than in the sample taken at the same time from the upper part. Together with the results presented by SMITH, the present results show that measurements in accordance with the *in vivo* technique are essential if reliable data on the reaction of the rumen contents are to be obtained.

Summary.

1. With one fistula cow as experimental animal, measurements have been made of the pH of the rumen contents on various diets, employing an *in vivo* technique.
2. A measuring probe designed for performing the pH measurements is described.

3. The rate and extent of the post cenam decrease in pH is variable, depending on the ration. The least variations in the reaction of the rumen contents were observed on pasture feeding.
4. The different parts of the rumen contents display different reactions. Under normal feeding conditions the upper part of the ingesta is consistently more acid than the lower part. The difference in degree of acidity between the upper and lower parts increases upon feeding simultaneously with the acidity of the rumen contents. The largest difference (1.1 pH units) occurred on pasture feeding.
5. The concentration of volatile fatty acids in the upper part of the rumen contents was in some cases more than 50 % higher than in the lower part.
6. The pH values obtained in *in vitro* measurements were 0.15—0.65 pH units higher than those obtained in parallel *in vivo* measurements.

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SELOSTUS:

pH:N JA HAIHTUVIEN RASVAHAPPOJEN KONSENTRAATION VAIHTELUISTA LEHMÄN PÖTSIN SISÄLLÖSSÄ

Martti Lampila

Maatalouskoelaitos, kotieläinhoito-osasto, Tikkurila.

Yhden pötsifistelillä varustetun ayrshire-lehmän pötsin sisällön pH mitattiin *in vivo*-tekniikkaa käyttäen tavallisesti kahden ruokintakerran välisen ajan käsittävinä mittaussarjoina. 1—2 tunnin väliajoin suoritetut mittaukset osoittivat, että rehuannoksen ry-sisällön lisääminen jyrkentää ruokintaa seuraavaa pH:n laskua ja alentaa pH-käyrien minimikohtaa. Laidunruokinnan aikana ei pötsin sisällön pH:ssa tapahtunut samanlaista jaksollista vaihtelua kuin sisäruokinnan aikana.

Normaalisissa ruokintaolosuhteissa sisällön yläosa on säännöllisesti happamampaa kuin alaosa. Näiden välinen happamuusasteen ero suurenee ruokinnan seurauksena rinnan happamuuden lisääntymisen kanssa. Suurin ero (1,1 pH-yksikköä) todettiin laidunruokinnan aikana.

Haihtuvien rasvahappojen konsentraatio sisällön yläosassa oli joissakin tapauksissa yli 50 % alaosan konsentraatiota suurempi.

In vitro-menetelmää käyttäen suoritetuissa pH-mittauksissa saadut pH-arvot olivat 0,15—0,65 pH-yksikköä korkeampia kuin rinnakkaisesti suoritetuissa *in vivo*-mittauksissa saadut, mikä osoittaa, että ensin mainittua menetelmää käyttäen suoritetuissa tutkimuksissa saadut pH-arvot ovat ilmeisesti virheellisen korkeita.
