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Studies on the Alimentary Tract of Merino Sheep in South Africa IX.—The H-ion Concentration in the Forestomachs of Fistula Sheep under Different Experimental Conditions.

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Introduction.

Considerable attention has been paid by various workers to the pH in the forestomachs of ruminants. Most of the determinations were made on material collected after slaughtering. Thus Schwarz and Gabriel (1924) found the ruminal ingesta in cattle constantly alkaline with an average value of pH 8·89. In contrast, Knoth (1928) using a different sampling technique whereby the necessary precautions were taken to exclude aeration, found the ruminal ingesta weakly acid, with an average pH of 6·9. He stressed the point that, on stirring the samples to allow aeration and liberation of CO_2 to take place, alkaline values were obtained for the same material with an average pH of 7·9. Other workers, employing the stomach tube to aspirate ingesta from the rumen of sheep, found it to be alkaline. Ferber (1928) using a similar technique, reported an average pH value of 7·9, with a range of pH 7·6-8·5.

Recently, Monroe *et al* (1939) determined the pH of bovine rumen ingesta, where various rations were fed. On a diet of hay plus grain this was found to average pH 7·01, while on hay, grain plus silage it was pH 6·95, maize plus grain pH 6·87, blue grass pasture pH 6·47, and on lucerne pH 6·66. These determinations were made by the use of the Quinhydrone Electrode method. Olsen (1941) in a study on slaughtered bovines records an average pH value of 6·86. Recent work indicates that the reaction of the forestomachs in most cases is weakly acid, and that the type of feed consumed determines a definite pH range as shown by Monroe *et al.*

Samples obtained under abattoir conditions are far from satisfactory however, in comparison to those from fistula animals under experimental conditions in which both the composition of the diet and the methods of feeding are well known. Moreover, sampling in such cases is easy, and can be repeated whenever necessary, both before and after feeding.

Aspiration of ingesta by stomach tube on the other hand, distresses the animals, besides leading to excessive salivation and hence to undue dilution and contamination of the ruminal samples with a viscid saliva. In this report data are presented on the hydrogen-ion concentration of fresh ingesta aspirated through permanent fistulae from the rumen of Merino sheep. The animals were kept on a variety of controlled diets which in several instances included the administration of acid directly into the rumen. A further series of tests on ruminal material was carried out "*in vitro*" with the object primarily of studying the adequacy of its buffer system as well as the influence of carbohydrate fermentation on pH.

Sampling of the Ingesta.

Since the method of sampling may affect the results, it will be necessary to give a brief description of the technique which was followed. By inserting a glass tube, fitted at one end with a short length of rubber tubing, into the fistula of the sheep, small quantities of rumen ingesta could be obtained through gentle aspiration by mouth at the rubber end. Immediately afterwards small glass cylinders (10 c.c. capacity) were completely filled and suitably stoppered so as to prevent undue exchange of gas.

Measurements of pH were regularly carried out within a few minutes of sampling. Unless otherwise stated, all samples were taken in the early morning before feeding.

In order to ascertain the influence of exposure to air on the pH of rumen ingesta, some of the samples were covered with a thin layer of liquid paraffin immediately after collection, to compare with our routine method, as well as with samples of the ingesta left uncovered in open containers. The samples were taken after the animals had been fed and during a period of active digestion.

Subsequent determinations of pH yielded the following results:—

Sheep No.	Routine Method.	Covered with Liquid Paraffin.	Routine Method.	Exposed 10 mins.	Exposed 20 mins.	Exposed 30 mins.
32.....	6·28	6·28	6·25	6·28	6·50	6·58
43.....	5·90	5·95	6·68	6·88	7·10	7·15
58.....	5·92	6·00	5·62	5·68	5·90	5·98
59.....	6·10	6·15	6·60	6·92	6·98	7·02
63.....	6·29	6·22	6·41	6·52	6·68	6·68
66.....	6·58	6·50	5·98	5·98	6·12	6·28

Measurements of the pH of Ruminal Ingesta.

Some of the values were obtained by measuring the samples potentiometrically by the use of the saturated KCl-Calomel halfcell-Quinhydrone method and calculating the results as indicated in tables 1-7. Where Quinhydrone was added to the samples, the necessary precautions were taken in recording the readings. According to Yoshimura (1936), readings may be considered accurate when recorded within 1-5 minutes on aqueous buffered solutions in the vicinity of pH 8·0. The Quinhydrone electrode applied to alkaline buffers, amino-acids, proteins and plasma causes a drift, with the

result that readings are inaccurate after a 5 minute period. On some samples (Tables 8 and 9), the pH was measured by means of the Beckman Glass-electrode apparatus, which was found to yield very accurate data when compared with the results obtained by the use of the hydrogen-electrode.

Experimental Results Obtained with Fresh Ruminal Ingesta.

In order to study the influence of diet on the pH of the ruminal ingesta, a total of 23 fistula sheep were used at various times while being kept on one of the following daily diets:—

(*a*) Lucerne Hay	300 grams.
Crushed Maize	360 grams.
(*b*) Lucerne Hay	1 kilogram.
(*c*) Crushed Maize	500 grams.
Lucerne Hay	100 grams.
(*d*) Green Lucerne (fresh)	1 kilogram.
(*e*) Crushed Maize	600 grams.
(*f*) Mature Grass Hay	400 grams.

Table 1.

pH of ruminal ingesta of fistula sheep.

Diet: Lucerne Hay (C) ...300 grams daily
Crushed Maize ... 360 grams daily

Sheep No.	Number of Samples.	pH Variations.	Average pH Variation for 5 Sheep.
36	6	6·4–7·1 (av. 6·8)	6·4–7·1
37	7	6·4–6·8 (av. 6·6)	
43	4	6·7–7·0 (av. 6·9)	
49	4	6·8–6·8 (av. 6·8)	
50	4	6·6–6·8 (av. 6·7)	

Table 2.

pH of ruminal ingesta of fistula sheep.

Diet: Fresh Green Lucerne ... 1 kilogram daily.

Sheep No.	Number of Samples.	pH Variations.	Average pH Variation for 2 Sheep.
32	7	7·4–7·6 (av. 7·5)	7·3–7·7
35	5	7·3–7·7 (av. 7·5)	

TABLE 3.

pH of ruminal ingesta of fistula sheep.

Diet: Crushed Maize ... ... 600 grams daily.

Sheep No.	Number of Samples.	pH Variations.	Average pH Variation for 2 Sheep.
37	8	5·3–6·2 (av. 5·8)	5·3–6·2
45	8	5·4–5·9 (av. 5·7)	

TABLE 4.

pH of ruminal ingesta of fistula sheep.

Diet: Crushed Maize ... ... 500 grams daily.
Lucerne Hay (B) ... ... 100 grams daily.

Sheep No.	Number of Samples.	pH Variations.	Average pH Variation for 2 Sheep.
14	11	5·6–6·5 (av. 6·1)	5·5–6·8
17	11	5·5–6·8 (av. 6·2)	

TABLE 5.

pH of ruminal ingesta of fistula sheep.

Diet: Mature Grass Hay (poor quality) ... ... 400 grams daily.

Sheep No.	Number of Samples.	pH Variations.	Average pH Variation for 8 Sheep.
20	6	6·8–7·2 (av. 7·2)	6·8–7·6
21	6	6·9–7·5 (av. 7·2)	
23	6	6·8–7·3 (av. 7·1)	
25	6	6·8–7·6 (av. 7·2)	
25	6	6·8–7·6 (av. 7·2)	
26	6	6·8–7·6 (av. 7·2)	
27	6	6·8–7·6 (av. 7·2)	
28	6	6·9–7·5 (av. 7·2)	
29	6	6·8–7·6 (av. 7·2)	

TABLE 6.

pH of ruminal ingesta of fistula sheep.

Diet: Changed from Mature Grass Hay to
Lucerne Hay (B) ... ... 1 kilogram daily.

Sheep No.	Number of Samples.	pH Variations.	Average pH Variation for 2 Sheep.
28	12	5·8–7·5 (av. 6·7)	5·8–7·5

TABLE 7.

pH of ruminal ingesta of fistula sheep.

Diet: Changed from Mature Grass Hay to:—

Lucerne Hay (B) ... ... 300 grams daily.
Crushed Maize ... ... 360 grams daily.

Sheep No.	Number of Samples.	pH Variations.	Average pH Variation for 6 Sheep.
20	14	5·8–7·1 (av. 6·5)	5·8–7·3
21	14	6·2–7·1 (av. 6·7)	
23	14	5·8–7·1 (av. 6·5)	
25	14	5·8–7·0 (av. 6·4)	
26	14	6·2–7·3 (av. 6·8)	
29	14	6·4–7·3 (av. 6·7)	

TABLE 8.

pH of ruminal ingesta of fistula sheep, sampled at various periods of digestion.

Diet: Lucerne Hay (C) ... ... 300 grams daily.
Crushed Maize ... ... 360 grams daily.

B.F. = Before feeding. A.F. = After feeding. Hr. = Hour.

Sheep No.	Average pH Variations on Consecutive Days.								
	B.F.	¼ Hr. A.F.	½ Hr. A.F.	1 Hr. A.F.	1½ Hrs. A.F.	2¼ Hrs. A.F.	4¾ Hrs. A.F.	6 Hrs. A.F.	24 Hrs. A.F.
43	6·12–6·50	—	—	6·00–6·60	—	—	—	6·42–6·90	6·12–6·50
59	6·32–7·05	—	—	6·50–7·18	—	—	—	6·20–7·40	6·32–7·05
43 (4 days)	6·15–6·65	5·85–6·12	5·70–6·08	6·00–6·15	6·00–6·22	5·75–6·02	6·12–6·12	5·80–6·40	6·20–6·72
59 (4 days)	6·00–6·72	5·80–6·28	5·65–5·98	6·00–6·15	5·95–6·02	5·96–6·00	6·05–6·60	6·52–6·62	6·10–6·72

TABLE 9.

pH of ruminal ingesta of fistula sheep, sampled at various periods of digestion.

Diet: Lucerne Hay (C) ... ... 1 kilogram daily.

B.F. = Before Feeding. A.F. = After Feeding. Hr. = Hour.

Sheep Nos.	Average pH Variations for 5 Sheep on Consecutive Days.						
	B.F.	¼ Hr. A.F.	1 Hr. A.F.	2¼ Hrs. A.F.	4¾ Hrs. A.F.	6 Hrs. A.F.	24 Hrs. A.F.
39, 58, 59, 63, 66	6·45–6·72	—	6·12–6·32	6·11–6·36	5·90–6·36	6·21–6·45	6·63–6·95
39, 59, 59, 63, 66	6·63–6·95	6·38–6·58	6·20–6·51	6·00–6·42	6·04–6·35	6·45–6·59	—

TABLE 10.

Composition of Feeds.

Expressed as grams per cent. on absolute dry basis.

Type of Feed.	Moisture.	Protein.	Fibre.	Ether Sol. Ext.	Total Ash.	N.-free Ext.	P.	Ca.	Mg.
Lucerne Hay (A).....	7·4	14·1	36·0	2·1	8·9	38·9	—	—	—
Lucerne Hay (B).....	6·1	16·3	31·5	2·2	8·1	41·9	·16	1·93	·60
Lucerne Hay (C).....	9·6	19·2	25·0	2·2	9·6	44·0	·25	1·46	·52
Green Lucerne.....	75·3	16·3	31·0	3·0	8·0	41·7	—	—	—
Yellow Maize.....	9·9	10·9	1·9	3·1	3·1	72·8	·24	·025	·12
Mature Grass Hay.....	5·4	4·6	41·6	1·5	6·1	46·1	·06	·27	·12

As will be noted from the above tables Nos. 1-7, the pH values recorded for rumen ingesta on the different diets are on the acid side in the majority of cases. Where the ration was comprised of excessive amounts of starchy material e.g. crushed maize, the pH was more definitely acid with a range of pH 5·5-6·8. In a few animals kept exclusively on crushed maize, the pH showed a further decrease to pH 5·3-6·2, the ingesta developing an excessively sour, cheesy odour. This was followed ultimately by a complete loss of appetite and other digestive disturbances.

Where this maize diet was supplemented with lucerne hay, the pH ranged from 6·4-7·1. On a ration of fresh green lucerne only, the values were slightly alkaline with a pH range of 7·3-7·7.

Exclusive feeding of dry lucerne hay, however, resulted in a slightly acid ingesta with the pH ranging from 6·45-6·95.

Well matured grass hay cut from the open veld and of low nutritive value, yielded ruminal ingesta, verging on the alkaline side with a pH range of 6·8-7·6. When the sheep which had subsisted on this poor grass hay for a considerable time, were placed on lucerne hay to improve their condition, the pH of the ingesta, after the animals had been established on the lucerne diet, was found to fluctuate between 5·8-7·5. Similarly, some of the above animals when placed on a mixed diet of lucerne hay and maize, yielded ingesta varying in pH from 5·8-7·3.

From the results presented in tables 8 and 9 it will be noted that the pH of the rumen ingesta is subjected to a very limited fluctuation during the course of digestion of a single meal, the tendency being towards greater acidity within the first 4-6 hours. After this it steadily returns to its previous level. This acidification of the ingesta is more evident after the consumption of a mixed ration containing both lucerne and maize than a meal composed of lucerne hay only.

The Buffer System of Ruminal Ingesta.

In order to maintain optimal conditions under which the normal density and the physiological activity of the various micro-organisms within the forestomachs can be safeguarded, it is essential to limit pH fluctuation as far as possible. This can be achieved only through the presence of an efficient buffer system which, as in the case of blood, is capable of ensuring relatively stable conditions despite variations in its acid and alkali content. Through constant production of organic acids mainly as a result of bacterial action on carbohydrates there is a distinct tendency towards undue acidification in the forestomachs. Normally, however, this is effectively counteracted by the free flow of an alkaline saliva containing a significant concentration of sodium bicarbonate. Together with the food proteins, the $NaHCO_3$ and phosphates of the saliva on being mixed with the food, exert the desirable buffering effect. Consequently, the type and amount of the food as indicated in the previous section normally provoke relatively small fluctuations in pH of a temporary nature.

With the object of studying the efficiency of this buffer system a series of experiments were conducted in which the pH of ruminal ingesta was correlated with its power to ferment glucose. In these tests, attempts were made to alter the pH in a variety of ways, e.g., by constant shaking, standing of ingesta at room temperature, the addition of either acid or alkali, or of carbohydrates such as molasses and glucose.

The Influence of Standing on the pH of Rumen Ingesta and on its Ability to Ferment Glucose.

After withdrawal from the same animal immediately prior to feeding, aliquot amounts of rumen ingesta of 50 c.c. each were kept standing at room temperature in open Erlenmeyer flasks. As indicated below various materials were added to these flasks. This was followed by the determination of the pH immediately afterwards and again 24 hours later.

Material Added.	Initial pH.	pH after 24 Hours Standing.
1. None	7·45	8·40
2. 0·2 gm. Glucose	7·22	7·36
3. 1·0 gm. Glucose	7·28	5·30
4. 0·5 gm. Molasses	6·96	6·91
5. 2·5 gm. Molasses	6·80	5·28
6. 5 c.c. N/10 HCl	6·66	8·16
7. 10 c.c. N/10 HCl	6·39	7·76
8. 15 c.c. N/10 HCl	6·16	7·52

After standing 24 hours at room temperature (18°-25° C), test fermentation of glucose was carried out on all the above flasks. Gas production was found to be normal only in flasks Nos. 2, 4, 7 and 8, showing a range from pH 6·91-7·76, whereas in the remaining flasks in which the H-ion concentration was either above pH 8·0 or below pH 6·0, no gas at all was produced.

From the above data it appeared that the fermentation of glucose by rumen ingesta was definitely influenced by the pH of the material, which in turn was found to show considerable fluctuation either on standing at room temperature or after the addition of various acid-forming materials.

The Influence of Glucose Fermentation "in vitro" on the pH of Ruminal Fluid.

While the fistula sheep were being kept on a standard diet of lucerne hay, aliquot samples of ruminal fluid were regularly withdrawn before feeding for the purpose of studying differences in glucose fermentation. The procedure adopted was the same as that described by Quin in article seven of this series, except that in addition to the measurement of the gas volumes, the pH of the material was also studied during the course of fermentation. For this purpose the Beckman Glass Electrode apparatus was used throughout.

In Table 10 results are presented indicating the average range in pH on two successive days taken both before and after the fermentation of glucose (1 c.c. of 20 per cent. solution) by 50 c.c. rumen ingesta under constant movement in a water bath at 39° C.

Table 11.

pH of Rumen Fluid Fermented with Glucose.

Sheep No.	Initial pH.		pH, 10 Minutes after Glucose Fermentation.		pH, 20 Minutes after Glucose Fermentation.		Total Gas Production in 20 Minutes.	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
58.....	6·91	6·91	7·36	7·30	6·60	6·71	27·5	24·6
32.....	6·92	6·89	7·42	7·20	6·69	6·68	20·4	19·5
39.....	6·88	6·89	7·26	7·11	6·28	6·70	21·6	26·5
59.....	6·90	6·70	7·31	6·99	6·35	6·25	25·0	20·6
63.....	6·98	6·82	7·31	7·30	6·81	6·72	24·5	23·5
66.....	7·02	6·65	7·48	7·28	6·92	6·69	15·8	20·2
65.....	7·10	6·80	7·52	7·39	6·98	6·80	25·6	24·0
43.....	7·15	6·81	7·57	7·40	6·99	6·82	25·2	25·0

After fermentation with glucose the ruminal fluid was found to retain its normal light green turbid appearance although the odour was more definitely acid than before. As will be noted from the above table the fermentation of glucose was associated with a primary elevation in the pH of the fluid during the first 10 minutes when all values were found to change over from the slight acidity to slight alkalinity. After a period of 20 minutes, however, the pH was definitely depressed below that of the unfermented material, thus affording evidence of considerable acid formation during the second period of ten minutes.

The Significance of pH in Ruminal Fluid as Related to Glucose Fermentation.

With the object of studying the influence of additional acid on the pH of rumen ingesta, fresh samples of material were repeatedly withdrawn from a single sheep (No. 58). To a series of Erlenmeyer flasks each of which

contained 50 c.c. of freshly strained rumen fluid N. HCl was added in amounts ranging from 1·0 c.c. to 3·5 c.c. and increasing by 0·25 c.c. from flask to flask. After acidification all the samples were fermented with the usual concentration of glucose (0·2 grams) while both the pH and the gas were being recorded at set intervals.

Similarly ruminal fluid was alkalinized by the addition of N. NaOH and the significance of this on pH and on the fermentability of glucose studied in a series of tests, the results of which are presented in Table 12 and the accompanying graph.

TABLE 12.

The Effect of pH Change on Fermentation of Glucose.

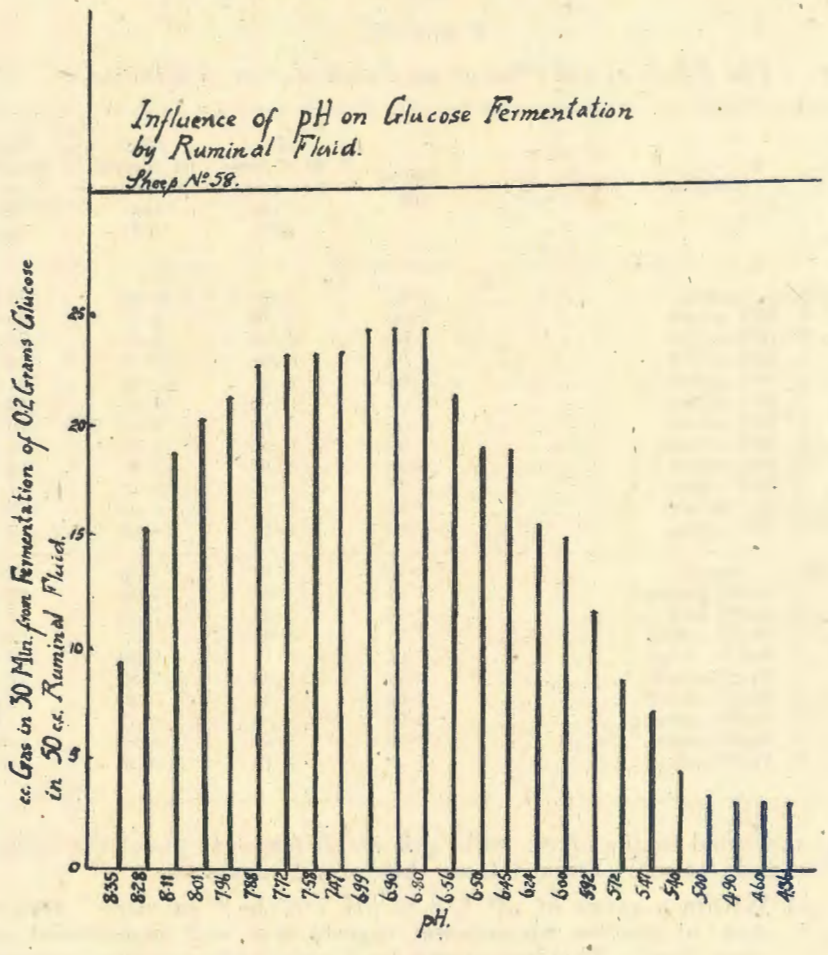
Material.	Initial pH.	After 20 Minutes Fermentation pH.	After 30 Minutes Fermentation pH.	Gas Evolved in 30 Minutes c.c.
Control (50 c.c. ingesta)	7·02	7·47	6·86	23·0
1·00 c.c. N. HCl added	7·02	6·99	6·51	24·5
1·25 c.c. N. HCl added	7·02	6·90	6·31	24·5
1·50 c.c. N. HCl added	7·02	6·80	6·20	24·4
1·75 c.c. N. HCl added	7·00	6·56	5·96	21·5
2·00 c.c. N. HCl added	7·00	6·50	5·83	19·2
2·25 c.c. N. HCl added	7·00	6·45	5·64	19·0
2·50 c.c. N. HCl added	7·00	6·24	5·45	15·5
2·75 c.c. N. HCl added	6·96	6·00	5·29	15·3
3·00 c.c. N. HCl added	6·96	5·92	5·12	11·4
3·25 c.c. N. HCl added	6·96	5·72	5·00	8·6
3·50 c.c. N. HCl added	6·96	5·47	4·90	7·2
Control (50 c.c. ingesta)	7·11	7·72	7·30	23·2
0·15 c.c. N. NaOH added	7·11	7·88	7·39	22·8
0·25 c.c. N. NaOH added	7·11	7·96	7·46	21·2
0·35 c.c. N. NaOH added	7·11	8·01	7·51	20·5
0·80 c.c. N. NaOH added	7·01	8·11	7·30	18·9
0·90 c.c. N. NaOH added	7·01	8·28	7·30	15·3
1·00 c.c. N. NaOH added	7·01	8·35	7·39	8·9
1·50 c.c. N. NaOH added	6·78	8·59	8·57	0
2·00 c.c. N. NaOH added	6·78	8·89	8·83	0
2·50 c.c. N. NaOH added	6·78	9·18	9·20	0

As indicated in the above table and graph (on next page) the following were the main results obtained from these experiments:—

(*a*) Within a range of pH 6·8 to pH 7·8 the "*in vitro*" fermentation of glucose by ruminal ingesta was well maintained at an even level. This was shown by a practically constant rate of gas production within these limits of pH range.

(*b*) The addition of progressively increasing amounts of N. HCl below pH 6·8, resulted in a sharp decline in pH which coincided with a progressive decrease in gas production following glucose fermentation. The end point of this was reached at pH 5·0 to 5·4. With the decrease in gas yield, the motility of the ruminal micro-organisms became depressed.

(*c*) The addition of N. NaOH to ruminal ingesta beyond pH 7·8 led to a rapid decline in gas production from test fermentations of glucose. Above pH 8·35 no gas at all was produced. This increased alkalinity was accompanied by a darkening of the ruminal fluid which assumed a characteristic musty odour repeatedly noted also in the ingesta withdrawn from animals during starvation. Under these conditions the ruminal organisms likewise displayed decreased motility, the infusoria in particular showing a tendency to die off in large numbers if the medium became too alkaline.



THE EFFECT OF pH WHEN ACID WAS DOSED INTO THE RUMEN OF FISTULA SHEEP.

Following the observation that a change in pH due to acidification of ruminal fluid, affected its fermentative activity, tests were made by dosing equivalent quantities of acid into the rumen.

For this purpose sheep were dosed with dried lucerne leaves suspended in water followed shortly afterwards by 150 c.c. N. HCl through the fistula. This resulted in intense frothing up of the ruminal ingesta. Subsequent to this the pH of freshly drawn samples was recorded at set intervals.

pH of Rumen Ingesta ("in vivo").

Sheep No.	Initial pH.	After Dosing Leaves pH.	pH after Dosing Acid.			
			25 Minutes.	1·5 Hours.	3·75 Hours.	4·7 Hours.
39.....	7·01	6·55	5·90	7·12	7·00	7·00
32.....	7·58	7·60	6·52	7·31	—	—

Although the pH of the rumen was temporarily depressed to about pH 5·9 by the dosing of 150 c.c. N. HCl. this did not adversely affect ruminal activity seeing that the animals readily consumed food which was offered shortly afterwards.

Summary.

1. Depending on the carbohydrate content of the diet, the pH of ruminal ingesta in Merino sheep, immediately after withdrawal through rumen fistulae was found to vary between pH 5·5 and 6·8.

2. Whereas the feeding of lucerne hay yielded slightly acid ingesta (pH 6·45 to 6·95), fresh green lucerne (flowering stage), as well as mature veld grass hay produced slightly alkaline conditions varying from pH 7·3 to 7·7.

3. Normally the pH of rumen ingesta in sheep showed slight fluctuation only during the digestion of a single meal, the tendency being towards increased acidity within the first 4 to 6 hours after which it steadily reverts to its previous level.

4. The "*in vitro*" fermentation of glucose by rumen ingesta resulted in a rapid and definite acid production with pH ranging from 6·25 to 7·00.

5. The addition either of N. HCl or of N. NaOH to rumen material showed that it was relatively well buffered between the pH 6·8 to 7·8, whereas beyond this range both on the alkaline and on the acid side the efficiency of this buffering was distinctly reduced.

6. Dosing of 150 c.c. N. HCl directly into the rumen of sheep caused a distinct though transitory depression of pH to 5·9, which however had no obviously detrimental effects on ruminal function.

7. The colour and odour of rumen ingesta was found to be closely correlated with its H.-ion concentration and its ability to ferment glucose.

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