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Studies on the Alimentary Tract of Merino Sheep in South Africa. XV.—The Influence of Different Factors on the Rate of Cellulose Digestion (a) in the Rumen and (b) in Ruminal Ingesta as studied *in vitro*.

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THE breakdown of cellulose and other related compounds constituting so-called " crude fibre " is generally regarded as the most important in a series of chemical changes undergone by plant food stuffs within the forestomachs of ruminant animals. In the absence of any cellulose-splitting enzyme secreted from the glands of the digestive tract itself, this disintegration of cellulose is largely, if not exclusively, dependent upon the presence and activity of a specific microflora within the forestomachs and to some extent in the large intestines.

From data presented by the Cambridge workers, Barcroft, McAnally, Phillipson, Elsden and others, it is evident that a considerable proportion of the organic acids thus formed from cellulose and other carbohydrates in the rumen is largely absorbed into the blood stream through the walls of the forestomachs themselves. Great difficulties, however, are encountered in studying the speed with which different food constituents are broken down and also the nature and exact quantities of the different products liberated in this process. This is due to a variety of causes of which the following may be cited:—

(1) Due to the close proximity to each of the entrance and exit of the rumen as well as the voluminous sac-like nature, this organ does not readily empty itself even after prolonged fasting. This results in the accumulation and constant mixing of the food consumed over an extended period and therefore in different stages of disintegration.

(2) The rate of passage of ingesta from the rumen to the rest of the digestive tract depends upon its physical condition. This in turn is determined by the fibre content and bulk of the food, the efficacy of mastication, ruminal motility and the activity of the cellulose splitting organisms.

(3) While part of the breakdown products are passed through to the abomasum, some may be directly absorbed from the forestomachs as mentioned above. Yet a third portion may either be completely oxidised by the organisms into carbon dioxide and water,

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with the liberation of a considerable amount of heat, or else resynthesised to glycogen and other products to constitute part of their own protoplasm. Subsequently these organisms pass on with the ingesta through the abomasum and intestines where they are exposed to the action of the ordinary digestive enzymes.

(4) In view of the variety of organisms normally inhabiting the forestomachs and the fact that they are influenced by both the diet and the physiological state of the host animal, an intricate dynamic balance must at all times be maintained between the different species. This is essential in preventing outright putrefaction in the forestomachs and the consequent dangers of toxaemia.

From these considerations it is obvious that a complete chemical study of all the concurrent changes undergone by the food in the forestomachs is a physical impossibility. Not only is aliquot sampling rendered difficult but also no true estimate can be made of the total ruminal contents at any given time.

As described in previous publications by Quin and Van der Wath, some of these difficulties could, however, be overcome by the use of animals carrying permanent ruminal fistula tubes through which various test materials could be suspended in the ruminal mass itself. By the periodic withdrawal of these materials some information could be gained as to the type of organisms attracted to them and also the rate at which such test substances were broken down. For this purpose small cylindrical bags were made from a very thinly woven natural silk. These were then filled either with a few cereal grains, pieces of straw taken during different stages of plant growth or pure cellulose in the form of paper pulp. Although the material was constantly exposed both to the mechanical and the bacterial action in the rumen, experience showed that where materials such as paper pulp were at all inclined to become clumped in the bags, no sharp end-point was reached and hence the total time taken for complete disintegration could not be reliably ascertained. Where whole cereal grains of different types were exposed, satisfactory readings were usually obtained as to the times taken for the starchy portion to disappear. The same did not, however, apply to the cellulose husks which required long periods for complete disintegration. This delayed reaction especially in cellulose digestion might possibly be associated with some degree of stagnation in the bags and an accumulation of various breakdown products which were not being removed rapidly enough.

Improved Technique for Measuring Rate of Cellulose Disintegration.

In view of the great importance attached to the actual rate of cellulose breakdown in the forestomachs under different feeding conditions and its effect on appetite and daily food consumption, further efforts were made to devise a more reliable method of measuring this factor. It was realised, however, that a true picture concerning the rate of breakdown of the different components normally comprising crude fibre in the various feeds could not be hoped for seeing that the degree of incrustation of plant tissues by highly indigestible lignin and other related compounds was bound to vary with such factors as species, freshness, stage of growth, soil, water and climatic conditions. Consequently it was considered necessary to rely on pure cellulose in some suitable form and free from any lignin as the most desirable test material. This was achieved by the use of short strands of ordinary sewing cotton loosely tied to the end of a thin metal rod covered by a close fitting

rubber tube. By inserting this rod through the fistula tube, the end carrying the cotton threads could be kept deeply plunged in the ruminal mass while the other end remained firmly fixed to a cork stopper inserted in the neck of the fistula tube. This ensured continual exposure of the threads to the full effects of ruminal activity at constant depth. Moreover, by limiting the use to only one grade of cotton (No. 40) of a well-known manufacture, reliable comparative data could be obtained as to the rate of change in the breaking strength of individual cotton threads, and the periods required for complete disintegration. In comparison with the results obtained by suspending the materials in silk bags, this simple technique afforded a far better opportunity for following the rate of cellulose disintegration, from its onset to the stage of final completion. As will be noted from the following results, differences could be recorded not only between individual animals but also as a result of changes in the diet.

The use of cotton threads was extended also to investigations *in vitro*. For this purpose the fluid portion of expressed ruminal ingesta drawn under different conditions was incubated in test tubes with lengths of the same cotton suspended in it. Again the threads could be withdrawn at set intervals and the rate and extent of disintegration compared with that noted in the animal body itself. This afforded an opportunity not only of comparing the *in vivo* with the *in vitro* breakdown of cellulose, but especially also of ascertaining to what extent the results obtained *in vitro* could be regarded as falling within normal physiological limits.

In addition to the tests on cotton cellulose, attempts were also made to ascertain the rate of protein breakdown in the rumen. For this purpose strands of surgical gut as well as fibres manufactured commercially from casein and peanut protein were likewise suspended in the rumen. From the unduly long periods taken for any of these materials to disappear from the rods, it was evident, however, that they were refractory to the action of the ruminal organisms. At first this was explained as being due possibly to the extensive changes undergone by the protein in the process of manufacture. Further tests were then carried out in which thin strips of fresh muscle obtained from cuts of beef or from the hind legs of recently killed rats were similarly exposed to ruminal action. Seeing that these strips did not disappear until the fourth or fifth day after exposure, it was concluded from the various tests made that the disintegration of these animal proteins, both in the fresh and in the denatured condition was by no means comparable to the more prompt digestion of protein normally found in the stomach and intestines. Further investigations in regard to protein digestion in ruminants are, however, being undertaken.

The Interpretation of the *in vivo* Tests on Cellulose Digestion.

Standard six cord white sewing machine cotton, gauge 40 (J. & P. Coats) was used throughout. This was attached to rods as illustrated.

It was found that the free ends or tails (B) were usually digested before the portions lying against the rods (A). The threads were examined manually for breaking strength at set intervals and the following annotation used to record the results:—

O No change.
(+) "Tails" break with a snap.
\+ "Tails" pull apart.
\+ + "Tails" absent, threads pull apart.
\+ + + "Tails" and "Threads" absent.

As it was found quite impossible to record small variations in the amount of digestion of the threads after they had been exposed for a fixed period, the time factor was also introduced in the interpretation of the data. The threads were examined twice daily until complete digestion had been reached.

In order to allow of graphic representation of the speed of cellulose digestion, the following arbitrary scale was devised. (Table 1.)

TABLE 1.

Scale of Rate of Digestion of Cotton Threads.

Rate or Digestion.	Hours within the Rumen.						
	24	30	48	64	72	96	120
14	+++						
13	++	+++					
12	+	+++					
11	(+)	++	+++				
10	0	+	+++				
9	0	(+)	+++				
8	0	0	+++				
7	0	0	++	+++			
6	0	0	+	++	+++		
5	0	0	0	+	+++		
4	0	0	0	0	++	+++	
3	0	0	0	0	+	+++	
2	0	0	0	0	0	++	+++
1	0	0	0	0	0	+	++
0	0	0	0	0	0	0	+

The graphs depicting the rate of cellulose digestion *in vivo* are all drawn to this scale.

Comparison between *in vivo* and *in vitro* Tests.

The *in vitro* tests were conducted by placing threads of the standard cotton in ruminal ingesta and incubating at 38° C. At first 2 per cent. calcium carbonate was added to the ingesta to act as a buffer, but it was found that this made very little difference to the rate of cellulose digestion. In general the cellulose in the *in vitro* tests was digested at a speed very similar to that occurring in the rumen itself. The following results from simultaneous tests illustrate this point. (Table 2.)

Table 2.

The Comparison between the Rate of Cotton Digestion in vivo and in vitro (Simultaneous Trials).

Sheep No.	Method *a = in vivo.* b = *in vitro.*	Time in Hours.				
		24	30	48	54	72
4	a	0	0	0	0	+
	b	0	0	0	0	0
6	a	0	(+)	++	+++	
	b	0	0	++	+++	
9	a	(+)	+	+++		
	b	0	(+)	+++		
10	a	(+)	+	++	+++	
	b	0	0	++	+++	
12	a	(+)	+	+++		
	b	0	0	+++		
13	a	(+)	+	+++		
	b	0	0	+++		
17	a	0	(+)	++	+++	
	b	0	0	+++		

The basic diet used throughout the first series of experiments consisted of poor quality grass hay. This feed is high in fibre and low in easily assimilable carbohydrate, protein and phosphate. Alone it does not form a maintenance diet for sheep. Previous experiments have shown that sheep on this hay lose weight steadily but no gross clinical disturbances in digestion are noted, even after extreme emaciation has been reached.

The Effect of Sugar on the *in vitro* Digestion of Cellulose.

Numerous trials were made to test the effect of various concentrations of glucose on the *in vitro* digestion of cellulose. Table 3 illustrates the results obtained.

TABLE 3.

Cellulose Digestion.

Glucose Percentage.....	0	·08	·16	·40
Sheep 1.....	(+)	++	+	(+)
Sheep 2.....	(+)	++	++	(+)
Bovine 1.....	++	+++	+++	0
Bovine 2.....	++	+++	+++	0

All the above animals were on a diet of poor hay and the readings on their ruminal ingesta taken after 36 hours incubation. As will be seen from the above table, cellulose digestion was accelerated by the addition of 0·08 and 0·16 per cent. glucose but was depressed by 0·4 per cent. It was subsequently found that the concentration of glucose required for optimal cellulose digestion *in vitro* varied between the narrow limits of 0·1 and 0·2 per cent.

THE EFFECT OF FEEDING SUGAR WITH HAY.

In order to test the above finding, four sheep were placed on a diet of grass hay only for a period of six weeks. They were then fed the same hay but with the addition of sugar. A strong solution of sucrose was mixed into the hay so as to give the desired percentage of sugar and the hay rapidly sun dried to prevent fermentation. The average daily hay consumption and rate of cellulose digestion through the experiment are depicted in Graph 1.

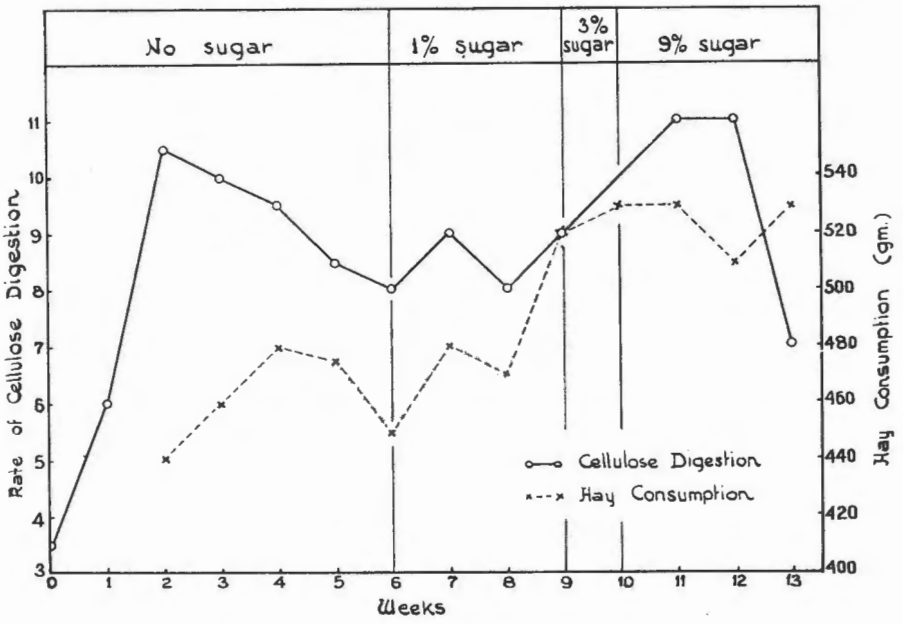
Prior to the actual experiment the animals were receiving lucerne hay and maize. The rate of cellulose digestion improved rapidly when the ration was changed to one of grass hay only and reached a peak after two weeks. After this it showed a tendency to decline but after six weeks was still much more rapid than it had been when lucerne and maize were fed. During this period the average weight of the four sheep dropped from 69 to 59 lb. The hay consumption showed the same trend as the rate of cellulose digestion after a time lag of approximately two weeks.

After the sixth week hay containing 1 per cent. of sucrose was fed. This had the effect of stabilising the rate of cellulose digestion and increasing the hay consumption. During the 10th week of the experiment 3 per cent. sugar was added to the hay and after that it was raised to 9 per cent. As will be seen the extra sugar caused an improvement in the cellulose digestion for the first two weeks but subsequently a decided drop took place. The hay consumption was definitely improved and maintained at a high level till the end of the experiment. It is probable that the retardation of cellulose digestion noted during the last week would have depressed the hay consumption had the experiment been continued longer.

These results showed that supplementing sugar to the poor hay to an extent of less than 9 per cent. had the effect of accelerating cellulose digestion and increasing hay consumption. Despite this the sheep continued to lose weight as they had done on the hay only. The average body weight of the four animals fell from 59 to 52 lb. during the seven weeks that they were fed the sugared hay. This indicates that the addition of sugar only, to a diet otherwise deficient can stimulate both appetite and cellulose digestion in sheep without, however, beneficially affecting body weight.

GRAPH 1

THE EFFECT OF SUGAR SUPPLEMENTATION TO A DIET OF HAY.



THE INFLUENCE OF PROTEIN ON THE OPTIMAL CONCENTRATION OF GLUCOSE FOR CELLULOSE DIGESTION.

During the *in vitro* trials on the effect of glucose on cellulose digestion it was noted that this process was inhibited by excess glucose. The concentration of glucose required to exert this effect was higher in ingesta taken from sheep on a high protein diet than in that from sheep on hay. The following results illustrate this point. (Table 4.)

In the experiment 2 per cent. calcium carbonate was added to each sample and the readings taken after 42 hours incubation at 38° C.

It will be noted that inhibition of cellulose digestion took place in lower concentration of glucose in the case of the sheep on hay. The ingetsa from the sheep on a high protein diet showed full digestion in maximal concentrations of 1·2, 0·80 and 0·56 per cent. glucose respectively, whereas in the sample from hay-fed sheep, cellulose digestion was retarded by concentrations of sugar above 0·4 per cent.

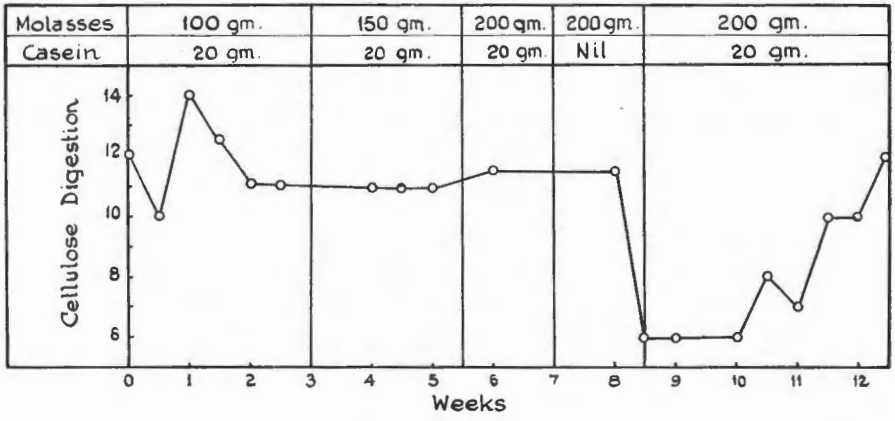
It will be noted also that the pH decreased with increasing glucose concentrations but that this effect was not so marked in the samples containing a higher level of protein. This is probably due to the buffering action of the protein in the ingesta. The facilitation of cellulose digestion cannot, however, be ascribed to this buffering action alone. In sheep No. 3 (high protein) complete cellulose digestion took place at pH 6·52 whereas inhibition was noted at levels of pH 6·82 and 6·98 in sheep 8 and 9 respectively.

TABLE 4.

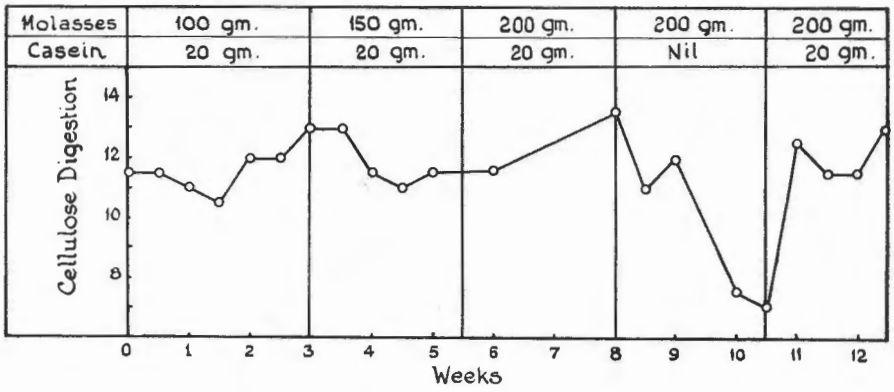
Diet.	Sheep No.		Glucose (Percentage).					
			·08	·24	·40	·56	·80	1·2
Lucerne casein	1	Digestion pH	+++ 7·80	+++ 7·80	+++ 7·46	+++ 7·12	+++ 6·98	+++ 6·62
Lucerne casein	2	Digestion pH	+++ 7·30	+++ 7·30	+++ 7·10	+++ 7·16	+++ 6·90	(+) 6·60
Lucerne casein	3	Digestion pH	+++ 6·82	+++ 6·88	+++ 6·58	+++ 6·52	+ (+) 6·61	(+) 6·28
Hay.....	8	Digestion pH	+++ 7·42	+++ 7·20	+ 6·81	0 6·52	0 6·25	0 5·75
Hay.....	9	Digestion pH	+++ 7·32	+++ 7·20	+++ 7·12	+ 6·98	(+) 6·60	0 6·58

In order to test the significance of the protein : sugar ratio on cellulose digestion, two sheep (4 and 9) were fed a basic ration of grass hay only. Simultaneously casein and molasses were administered daily through the fistula. The results are depicted in Graphs 2 and 3.

GRAPH 2 : SHEEP 4



GRAPH 3 : SHEEP 9



The amount of molasses given was raised from 100 gm. to 150 gm. after a period of three weeks and then to 200 after a further period of 2½ weeks, while the casein was kept constant at 20 gm. daily. As will be seen from the graphs the rate of cellulose digestion was maintained at a high level throughout the first eight weeks of the experiments.

As indicated in the test with sugared hay already described, the addition of 9 per cent. sugar to the hay caused a marked drop in the rate of cellulose digestion after the second week. As the average daily intake of hay was approximately 520 gm., this represented about 47 gm. of sugar daily. The molasses used in this experiment contained 40 per cent. sugar, the daily dose of 200 gm. thus representing 80 gm. of sugar. Despite this higher sugar intake the rate of cellulose digestion was not effected.

In order to ascertain whether this was due to the casein supplement, the latter was stopped at the end of the seventh week but the dosing of molasses continued as before. It is clearly evident from Graphs 2 and 3 that this was followed by a marked drop in the rate of cellulose digestion in both these sheep. Subsequent restoration of the casein supplement caused the rate of cellulose digestion to revert to its former level.

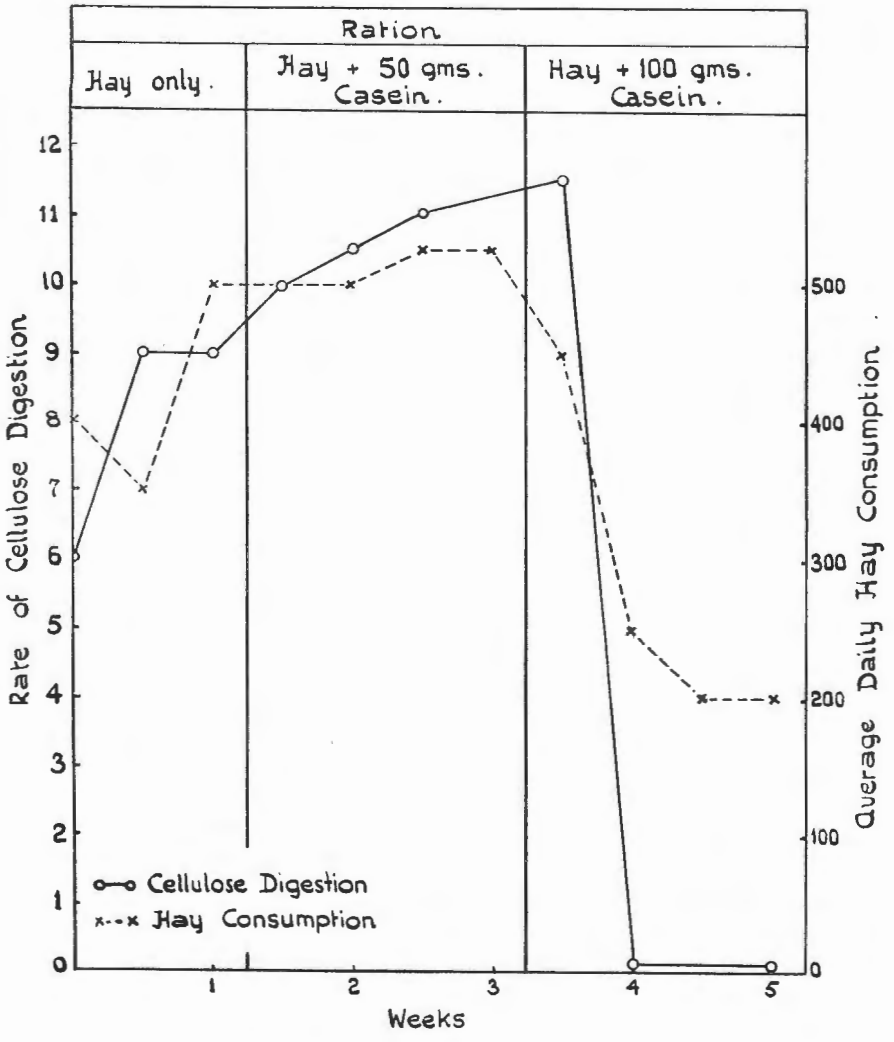
The effect of protein on the tolerance to a high sugar intake was further shown on two other sheep (Nos. 2 and 3). These animals had been kept on a high protein diet comprised of dry lucerne leaves and casein continuously for a period of three months. The administration of 200 gm. molasses per day for two weeks had no depressing effect on the rate of cellulose digestion nor did it cause any clinical digestive disturbances.

These results show that the rate of cellulose digestion is markedly depressed by giving over 50 gm. of sugar per day to sheep on a diet low in protein. With a high protein intake on the other hand, over 80 gm. of sugar per day failed to produce any similar effect on cellulose digestion.

The Influence of Protein on Cellulose Digestion.

In order to test the effect on cellulose digestion of a protein supplement to the basic diet of poor quality grass hay, one shepp (No. 1) was kept on hay only and casein dosed daily through the fistula. The results are shown in Graph 4.

GRAPH 4 · THE EFFECT OF EXCESS PROTEIN ON CELLULOSE DIGESTION AND HAY CONSUMPTION.



It will be noted from the above graph that the administration of 50 gm. of casein per day for two weeks did not affect the rate of cellulose digestion nor the hay consumption. When the casein was raised to 100 gm. per day however, there was a marked drop in both these factors.

This result indicates that the sudden introduction of a high protein supplement on a diet of grass hay may cause complete inhibition of cellulose digestion.

The Effect of Excess Protein.

Two sheep (Nos. 5 and 6) which had previously been on a low protein ration continuously for three months, were suddenly given lucerne hay leaves *ad lib* together with 100 gm. of casein a day.

One of these animals (No. 5) immediately started to consume more than 1 Kg. of the lucerne hay leaves a day. On the morning of the fourth day it was found *in extremis*. The animal was in coma and showed muscular spasms. On post mortem examination no definite lesions were discovered but the ruminal contents smelt strongly of ammonia. It would appear that the ruminal flora was not adapted to such a high protein diet and consequently a large amount of ammonia was suddenly liberated thus resulting in the death of the animal. This sudden death from excess protein following a poor diet is being investigated further.

The second sheep (No. 6) was not so severely affected but the rate of cellulose digestion dropped rapidly from 11 to 5 with subsequent recovery.

Natural Adaptation to High Protein.

In order to obtain a feed relatively high in protein and low in fibre, the leaves of the lucerne hay were separated from the stems by means of a sieve. Two sheep (Nos. 2 and 3) which had previously been on a ration of lucerne hay and maize, were then offered the lucerne leaves *ad lib*. In addition each animal was dosed with 50 gm. casein daily through the fistula tube. The reaction of sheep No. 3 is shown in Graph 5.

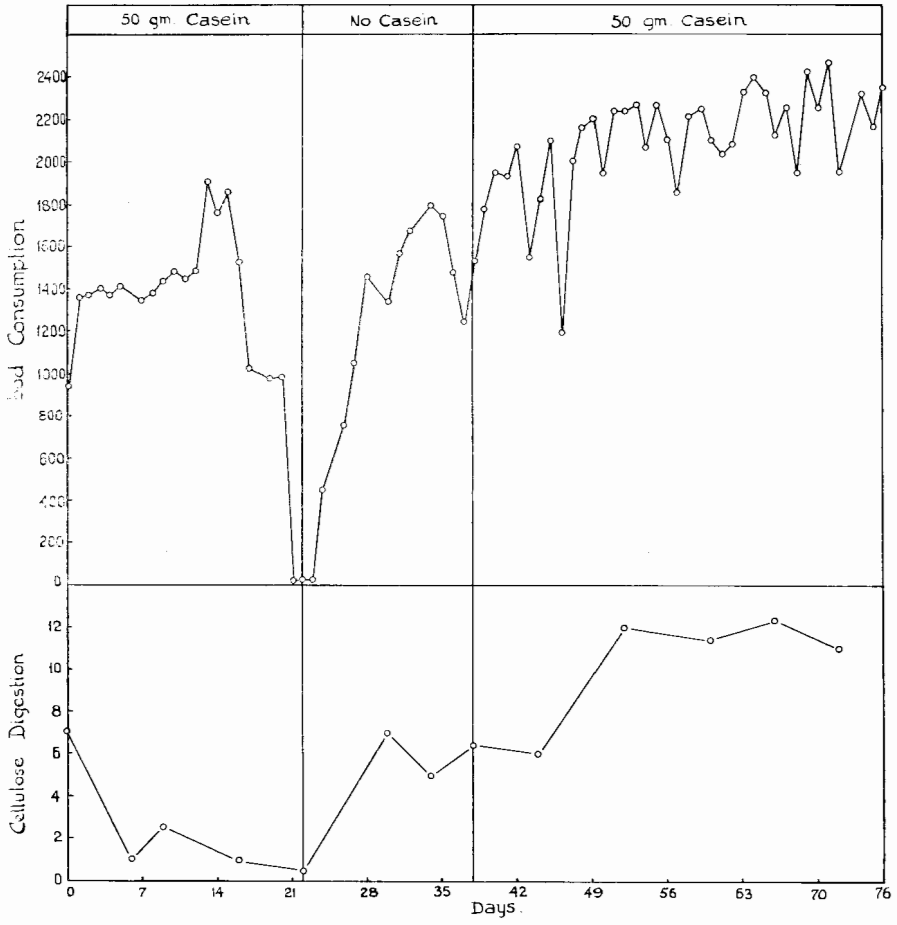
As shown in the graph the animal took to the lucerne leaves readily and by the fifteenth day was consuming over 1,800 gm. in 24 hours. The rate of cellulose digestion, however, was markedly suppressed. This was followed by a rapid decrease in appetite so that for a period of three days the daily intake was only 20 gm. of lucerne leaves. At this point the administration of casein was discontinued but the lucerne leaves offered as before. This resulted in a progressive return of the appetite with the daily consumption rising from an average of 20 grams to 1,340 grams over a period of 17 days. Simultaneously the cellulose digestion improved. After 17 days on lucerne leaves only, supplementation with 50 grams casein daily was resumed. On this occasion there were no ill-effects and both the food consumption and rate of cellulose digestion rose rapidly to very high levels.

The reaction of the second sheep was almost identical.

Natural Adaptation of Sheep to a Change of Diet.

In the foregoing experiments it was shown that there was a direct correlation between the rate of cellulose digestion and the food intake when these factors were studied simultaneously under the influence of various diets (see Graphs 1, 4 and 5). It is logical to assume that retardation of cellulose digestion necessitates a longer sojourn of the food in the rumen and that this in turn would decrease the appetite. Furthermore, it has been shown that a high rate of cellulose digestion and food consumption can be maintained on widely differing diets, such as lucerne leaves and casein on the one hand and poor quality grass hay on the other, provided that the ruminal floral has become adapted to the particular diet. It can, therefore, be assumed that the voluntary intake of fibrous food by sheep serves as an indication of the ability of the ruminal flora to break down cellulose.

GRAPH No. 5.



In order to study the natural adaptation of sheep to changes of diet as reflected in appetite, four non-fistula sheep were used in the following study. For a preliminary period of two and a half weeks two of the animals were given dried lucerne leaves and the other two grass hay only. All four sheep were then offered both these feeds *ad lib.* Throughout the experiment the amounts of food consumed were recorded daily.

It was found that the sheep which had been conditioned to the diet of lucerne leaves took only about 300 gm. of grass hay per day when it was offered. For the first three days the consumption of lucerne dropped by approximately the same amount. Subsequently the intake of lucerne gradually increased to above what it had been before the hay was offered. By the end of the fourth week the animals were consuming 1,400 to 1,500 gm. of lucerne leaves in addition to 200 to 300 gm. of grass hay. As they had only taken between 1,000 and 1,100 gm. of lucerne leaves daily during the preliminary period, the addition of small amounts of grass hay to their diet definitely stimulated their appetite for lucerne leaves, due possibly in part to the low fibre content of the latter.

When the sheep that had been conditioned to grass hay were offered the alternative of lucerne leaves, they took only between 600 and 700 gm. lucerne daily for the first three days. This amount increased steadily until at the end of the fourth week the daily intake had reached over 1,700 gm. In addition they also continued to consume 200 to 300 gm. of grass hay daily.

A similar study in food selection was also undertaken on four other sheep. These were placed on a basic ration of grass hay supplemented by 300 gm. of peanut-meal daily. After two weeks the peanut-meal was offered *ad lib*, and the amounts of both feeds eaten daily were recorded. It was found that the daily consumption of peanut-meal fluctuated markedly in each sheep. At times over 700 gm. were consumed for short periods of one or two days, after which it was halved for equally short periods. The consumption of hay fluctuated inversely with that of the concentrate. In this way the sheep selected what appeared to be a satisfactory diet as judged from general health and weekly increase in body weight.

The Effect of Aeration on Cellulose Digestion.

The necessity for the maintenance of a strict anaerobic state for the cultivation of cellulose splitting bacteria from the rumen has recently been stressed by Hungate (1946) and Gall (1946). Although the oxygen tension in the rumen must at all times be relatively low, complete anaerobic conditions cannot always prevail as aeration of the food takes place in the mouth during feeding and rumination. According to Washburn and Brody (1937) the percentage of oxygen in ruminal gases (from a cow) varied from 0 to over 5 per cent.

During the course of these studies some observations were made on the effect of aeration on cellulose digestion.

By means of *in vitro* trials it was found that cotton threads emersed in ruminal ingesta were digested equally rapidly when the tubes were placed in anaerobic jars or left standing open. Cellulose disintegration was completely inhibited, however, by the continuous bubbling of air through the medium.

This indicates that cellulose splitting can take place both under conditions of strict anaerobiasis and of low oxygen tension as normally prevailing within the rumen. The aerobic organisms such as yeasts, normally present in the rumen, may play an important role in maintaining a low oxygen tension, thus aiding the action of the cellulose splitting bacteria.

In order to confirm the results obtained *in vitro*, a sheep was suitably controlled and oxygen continuously insufflated into the ruminal mass for a period of ten days. An oxygen cylinder was used and the gas first passed through a wash bottle and then through the fistula to be introduced deep into the contents of the rumen. Some difficulty was experienced in maintaining a constant flow of oxygen and on three occasions it was found to have stopped during the night. For the greater part of the experimental period however, an inflow of approximately 20 bubbles of oxygen per minute was maintained. The oxygen insufflation was subsequently stopped but the animal kept under conditions otherwise exactly similar for a further period of 10 days. The ration consisted of lucerne hay and maize throughout.

During the period of oxygen insufflation cotton threads introduced into the rumen were digested only after 120 hours. When the flow of oxygen was stopped the time required for disintegration of the threads was reduced to 72 hours. While oxygen was being administered the average daily consumption of hay amounted to 575 grams which increased to 725 grams after stopping the inflow of oxygen.

Cellulose digestion and appetite were therefore retarded, but not completely inhibited, by a high degree of oxygenation of the ruminal contents.

Discussion.

Over a great part of South Africa the natural winter grazing consists of dry frosted grass, either standing as such on the veld or after being cut as hay. Owing to the intense sunlight and very rapid growth during the summer, this mature dry grass contains a high proportion of cellulose while the protein, phosphorus and carotene contents are usually deficient. In addition it is often highly lignified and encrusted with silicates so that the average digestibility of the cellulose is only 40 to 50 per cent. One of the great problems in stock feeding is therefore to supply minimal amounts of the essential supplements in order to balance this winter grazing without adversely affecting the utilisation of the cellulose.

The present experiments have shown that where the diet is high in crude fibre but deficient in both protein and carbohydrates (starches and sugars) the addition of either of these alone in amounts sufficient to benefit the animal causes a definite depression in cellulose digestion. This indicates that any supplement to a highly fibrous diet must contain balanced amounts of both protein and carbohydrate. Thus it was found that cellulose digestion could be well maintained on a diet containing as much as 200 gm. of molasses a day provided that at least 20 gm. of protein (casein) was given simultaneously.

In individual cases death may follow an abrupt change from a low to a high protein diet. It would appear that this is associated with excessive liberation of ammonia in the rumen and its subsequent absorption into the blood stream. The majority of animals however, regulate their intake until fully adapted to the new diet. In this connection attention should be directed to the fact that animals with an increased appetite, e.g. during pregnancy and lactation, are usually more subject to digestive and metabolic disturbances following sudden changes in diet.

Summary.

(1) The rate of cellulose digestion in ruminal ingesta was studied both *in vivo* and *in vitro* by a new technique in which cotton threads were used as test material.

(2) It was found that the rate of cellulose digestion was markedly influenced by the diet.

(3) The appetite for either lucerne or grass hay was directly affected by the rate of cellulose digestion.

(4) On a basic diet of poor quality grass hay both cellulose digestion and appetite were stimulated by small amounts of sugar but markedly depressed by excessive amounts. More sugar could be toleratаed if protein was also given. Optimal cellulose digestion necessitated a balance between readily available carbohydrate and protein.

(5) Excess protein suddenly introduced after a period on a diet low in both protein and carbohydrate caused marked inhibition of cellulose digestion and inappetence. One case of sudden death was possibly associated with ammonia poisoning.

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