STUDIES ON THE ALIMENTARY TRACT OF THE MERINO SHEEP WITH SPECIAL REFERENCE TO THE ROLE OF THE RUMINAL MICROFAUNA AND FLORA.

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INTRODUCTION.

The process of digestion in the ruminant animal entails the combined effects of mechanical, physical and chemical factors. The mechanical aspect has received due attention by numerous workers, e.g. Bergman and Dukes (1925), Wester (1926), Alvarez (1928), Schalk and Amadon (1928), Trautmann and Schmitt (1933), Trautmann (1933), Brüggemann (1935), Krzywanek and Quast (1936), Quin and van der Wath (1938). Several others have described the physiology of the ruminant stomach and the motility of the rumen in great detail.

The chemical and physical aspects of digestion in the forestomachs however, have not yet fully been elucidated, nor are the processes of digestion and absorption in the abomasum and intestines clearly understood. Assumptions are freely based on what is known in regard to the human being and animals with simple stomachs, e.g. the dog and the rat, but in the ruminant differences in anatomical structure and in the type of diet may modify or even completely alter the mode of digestion.

The character of the predigested food passing from the rumen to the abomasum and intestines is unknown. The processes whereby cellulose is broken down and the nature of its products still form subjects for speculation. The most important structural changes of the rumen ingesta are due to the fermentative action of enzymes present both in plants ingested and especially also in the rich microfauna and flora in the rumen. Fermentation in the true sense of the word, is a strictly anaerobic process associated with gas production.

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However, not all the processes in the rumen e.g. the breakdown of cellulose may take place according to this conception. A certain amount of air is swallowed by the animal during feeding and rumination, so that the O_2 tension within the rumen may show definite fluctations, ranging from aerobiosis to almost complete anaerobiosis. This creates favourable conditions in the rumen for the proliferation of a great variety of micro-organisms, including many species of bacteria, protozoa, yeasts, fungi and even spirochaetes.

Although the combined action of all these organisms is generally accepted to be responsible for the disintegration of the food substances within the rumen, the role played by each type or group in the breakdown of specific food substances still requires explanation. Thus the role of infusoria in starch and cellulose digestion, although intensively studied by Becker, Schulz and Emmerson (1930), Westphal (1934), and Trier (1936), has not yet been clearly ascertained. With a view to obtaining more information on the contribution of infusoria to the digestion of starch and cellulose, the author planned several experiments in which merino sheep with closed permanent ruminal fistulae were used. The first object was to determine whether any correlation existed between the types and number of infusoria and the diet of the animal. For this purpose fistula sheep were fed different basal rations, and by a system of supplementation with starch, the effects of various changes in the diet on the infusoria, were closely followed. Secondly, to assess the influence of infusoria on the digestion of starch, the mode and rate of starch disintegration by infusoria was studied. This was effected by observing the fate of starch granules ingested by infusoria in the rumen. Chemical determinations were also made to ascertain the rate of

digestion/.....

digestion of starch in the rumen of sheep with their normal infusorial population, as compared with the rate of digestion in the same animals without infusoria. A similar experiment was conducted to determine the influence of infusoria on the digestion of cellulose in the rumen.

A study of infusoria in domesticated animals confined to paddocks or stables may not be comparable to that of animals under natural conditions.

Moreover, as it is known that infection with infusoria occurs from mouth to mouth, the species and number of infusoria in ruminants may possibly vary with different habits of grazing. Consequently a study of the infusoria in sheep on natural pasture as compared with stable fed conditions and also of wild antelopes in their natural habitat, domorised a portion of the work undertaken by the author. Differential counts of the infusoria were made, as well as total counts, to determine the density of the infusorial populations under the above conditions.

Due to the large proportion of cellulose normally present in the diet of ruminants, and also to the fact that, as far as is known, no cellulose splitting enzyme is present, in any of the digestive secretions, these animals have to rely largely on the symbiotic action of micro-organisms specifically capable of disintegrating cellulose and other related fibrous materials. This disintegration of food materials assists in the reduction of bulk in the rumen, exposes encrustated food materials, like proteins and minerals, to digestion, and accelerates the passage of ingesta through the gastro-intestinal tract. This in turn stimulates the appetite and increases consumption. Although Meyer (1927) and Baker and Martin (1938) have shown that

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ruminal bacteria penetrate into and rupture the cellulose walls of plant tissues, the influence of the ruminal flora on the degree of cellulose digestion in the rumen has not yet been defined. As cellulose, particularly that in mature grass veld hay, is only partially digested by the ruminant, some factor or factors influencing the digestion of cellulose are probably in operation. Of these may be mentioned (1) the influence of lignification of the plant tissues as indicated by Louw (1942), and (2) the absence of sufficient ruminal micro-organisms to ensure maximal disintegration of the cellulose in the rumen. The importance of the lastnamed factor was investigated in the course of this study in the following way:- The degree of cellulose digestion in sheep on a mature veld hay diet was determined by means of cellulose metabolism studies. After supplementation of the veld hay diet with variable amounts of protein and starch, to augment the bacterial population, the degree of cellulose digestion was again determined, so as to ascertain the influence of the increased number of ruminal bacteria on the cellulose digestion.

Apart from the digestion of cellulose, it is logical to expect that other food substances in the rumen, e.g. sugars, starches, proteins and fats, would also be attacked and utilised by the ruminal micro-organisms, which, as living cells, are in constant need of various elements essential for tissue building. Moreover, some of these substances, e.g. sugars and starches, may even be used in preference to cellulose, seeing that cellulose is of a more complex structure and much more indigestable (Hamilton, 1942). In view of the fact therefore, that normal digestion in the ruminant is so closely associated with the maintenance of

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certain desirable qualities in its ruminal flora and fauna, a more comprehensive study of these organisms and their requirements was considered essential. This is particularly necessary in Southern Africa where seasonal fluctuations in the nutritive value of the veld are very marked and where deficiencies both in the quantity and quality of the food may be either seasonal or permanent. A great deal of work has been done in regard to the requirements of pathogenic bacteria (Bainbridge, 1911 and Kendall, 1922), but little or no information is obtainable from the literature concerning ruminal bacteria. In order to obtain information on the normal nutritional requirements of the ruminal flora, various feeding trials were carried out during which bacterial counts were made. Different ratios of carbohydrate and nitrogen were used to determine the effect on the bacterial population. Similarly, also, the influence of minerals on the ruminal flora was determined to some extent. Furthermore, a study was made of the types of organisms responsible for the disintegration and degradation of starch in sheep.

Another aspect of digestion in the ruminant which should not be overlooked, is the effect of predigestion in the rumen on the later stages of digestion in the abomasum (or true stomach) and intestines. In humans and in carnivores, food is swallowed directly into the true stomach where it is exposed immediately to peptic-hydrochloric acid digestion, simultaneously with salivary (ptyalin) digestion. The food is prepared in the stomach for further digestion in the intestines. As no enzymes are secreted either in the saliva of the ruminant (Scheunert and Trautmann, 1921) or from the walls of its forestomachs, digestion of food

entering/.....

entering these compartments can proceed only by means of enzymes contained either within the plant tissues themselves or those elaborated by the ruminal micro-organisms. Only after thorough rumination, maceration and hydration do the finest food particles pass through from the rumen to the abomasum. Meanwhile the foodmass in the rumen is exposed to the action of various micro-organisms in preparation for digestion further down the tract, where only very fine food particles are found. Should the larger coarse material be allowed to pass through onto the surfaces of the abomasum and intestines covered by a very delicate mucous membrane, the digestive processes in these regions would probably be distubed. The significance of this predigestion of food in the rumen only becomes apparent when one compares the physical state of the ingesta in the different compartments of the digestive tract. As a result of this predigestion, the food entering the abomasum is in a more advanced state of digestion than is the case in animals with simple stomachs. In these species the food, although insalivated, is as yet completely undigested when it reaches the stomach. However, the concentration of hydrochloric acid in the gastric juice of ruminants appears to be less than in omnivorous and carnivorous animals, (Grosser, 1905, Rosemann, 1907, and Belgowski, 1912), suggesting that digestion in the abomasum itself is less efficient than in the stomach of the carnivore or omnivore. This, however, may be compensated for again by predigestion in the rumen.

Observations recently made by the author have indicated that starch and cellulose introduced into the abomasum of the sheep are digested much more slowly than in the rumen. Thus it was found that finely crushed maize particles suspended in silk bags in the rumen are digested within 48 hours, and cellulose (maize bran) within five days.

Similarly/.....

Similarly, maize particles suspended directly in the abomasum through an abomasal fistula was found to be completely digested only after a period of five days, whilst bran showed no visible signs of digestion after 21 days. It seems therefore as if predigestion in the rumen significantly accelerates the digestion of foodsubstances, probably through the agency of ruminal micro-organisms. This aspect of digestion seems to have considerable importance and to be worthy of further investigation. Unfortunately, for the purposes of the present study, investigation of the chemical processes occurring within the rumen could not be carried further.

Apart from the process of food disintegration in the forestomachs, McElroy and Goss (1939) and Wegner et al (1940), have shown that bacteria, in the rumen of cattle and sheep are capable of synthesising the vitamin B complex. These investigators proved, therefore, that the ruminal micro-organisms could supply their host with vitamin B should this be deficient in the diet. The ability of rumen micro-organisms to synthesise vitamins, prompted the author to investigate the possible synthesis of an important amino acid such as cystine, which is indispensable for growth (Abderhalden, E. 1922) and of significance in the production of wool and hair.

The utilisation of non-protein-nitrogen by ruminants has been established by several workers (Hart et al, 1939, and Owen, Smith and Wright, 1941), but the mechanism of conversion of non-protein-nitrogen, as contained in urea, into protein, is still unexplained. In view of the fact that urea is an inexpensive source of nitrogen which can be used as a substitute for proteins in times of scarcity, an attempt was made to ascertain the influence of the ruminal bacteria on urea introduced into the rumen. For

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this purpose, experiments were first conducted in vitro to study the chemical effects following the introduction of urea into fresh ruminal ingesta incubated and kept in continuous to-and-fro movement. At the same time bacterial counts were made to determine whether there was an absolute increase in bacteria due to the addition of non-protein-nitrogen. On the results of these preliminary experiments further experimentation with sheep was planned. Metabolism experiments were carried out to determine whether the observed increase in ruminal bacteria as a result of amide-feeding could be correlated with the utilisation of the amide by the animals or not.

This brief reference to some of the known and unknown aspects of ruminal biology and biochemistry, suggests an important form of symbiosis between host and micro-organisms. The implications of this problem, particularly in regard to practical economic feeding of domestic stock have stimulated the author to attempt an elucidation of some of the digestive and synthetic functions of these micro-organisms and their relationship to the host animal.

The chemical data was obtained with the co-operation of colleagues from the Department of Chemistry.

The literature referred to in these studies is not discussed collectively, but separately with each section of the work as dealt with. This arrangement, it is hoped, will assist the reader to correlate the relevant literature with the researches actually carried out.

II. TECHNIQUE AND METHODS EMPLOYED.

(1) Collection of materials.

(a) Ruminal samples for the counting of

(i) Infusoria,

(ii) Bacteria.

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For the purposes of this study merino sheep with closed permanent ruminal fistulae were used, as described by Quin, van der Walt and Myburgh (1938). In some cases material was withdrawn by stomach tube or collected at post-mortem, immediately after killing. Previous workers on this subject apparently have employed one of the four methods below:

1. Material withdrawn from the rumen by stomach tube.
2. Material obtained at abattoirs.
3. Cultures in vitro (Westphal, 1934).
4. Digestion trials (Becker, Schulz and Emmerson, 1930).

Our methods of administration of materials and the collection of samples through the fistulae greatly facilitated the work and allowed some experiments to be carried out in a novel way.

Repeated collection of ingesta from the rumen was carried out by the insertion of a glass tube of half inch diameter through the fistula opening into the depth of the ruminal mass, and aspirating by mouth the required amount of ingesta. To the outer end of the glass tube was attached a piece of rubber tubing of slightly smaller calibre, to facilitate aspiration. Where sheep had to be kept on natural pasturage under conditions unfavourable for animals carrying a ruminal fistula, the sampling was done by means of a stomach tube. The tube was lightly oiled and the end inserted into the rumen plugged with a small pledget of cotton wool to exclude the entry of any mucus or saliva encountered in passing down. When well into the rumen mass, the pledget was expelled by blowing it out. By subsequent aspiration, ruminal contents could then be obtained without difficulty.

Material collected at post mortem was usually taken immediately after slaughter and treated without delay so as

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to prevent further undesirable post mortem changes.

ADMINISTRATION OF FOODSTUFFS.

Apart from allowing for the easy collection of rumen contents, the fistula method was also extensively used for the administration, directly into the rumen, of various substances either in powder or liquid form. The method of administering material per os has the disadvantage that, instead of passing into the rumen, some or all of it may pass into the abomasum (Watson, 1941). Thus in the experiments carried out, materials such as starch or maize meal were shaken up with enough water to make it flow easily and then poured with a funnel through the fistula tube. Furthermore, small samples of solid material, such as maize kernels, could be exposed to ruminal digestion and withdrawn at will. These were suspended in the rumen in thin natural silk bags, approximately one centimeter in width and three to five centimeters in length. Several of these bags could be linked up to each other in a row. The bags were attached to a surgical silk thread and after inserting them through the fistula opening, the strand of silk was fixed to the outer part of the tube, and the cork replacedin the usual manner. In this way the material was actually suspended in the ruminal mass and subjected to digestion and ruminal movements but not to rumination. The smallest mesh size of the natural silk bag was found to be 74.5μ x 149μ whilst the largest was 283.1μ x 298μ. The average being 149μ x 20μ. Thus micro-organisms in the rumen had free access to the contents in the bags. It is noteworthy that bags made of cotton, artificial silk etc., could not be used as these were soon digested in the rumen.

(1) COUNTING OF INFUSORIA.

All sheep were dosed with 2.5 litres of tapwater through the fistula daily at 2 p.m. so as to keep the intake

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of water constant. Material for infusorial counts were always withdrawn at 9 a.m. into 50 cc. glass tubes and placed in ice water to stop fermentation and break the froth. When this was achieved the material was shaken vigorously for a moment to mix it properly. One cubic centimeter was then sucked up into a wide-mouthed 2 cc. pipette. This ingesta was then added to 7 or 8 cc. of corrosive-sublimate-alcohol fixative and, after washing with alcohol-iodine and 70% alcohol, it was stained with borax-carmine. The stained material was suspended in 3 cc. oil of cloves in which it can be kept for years. After diluting the total amount of stained infusoria 10 or 100 times in oil of cloves, a drop of known volume from a capillary pipette was placed on a glass slide and covered with a coverslip. The total number of infusoria (i.e. under the coverslip) was counted, from which the number of infusoria per cubic millimetre of ingesta was then calculated. Duplicate counts were made. The mean error was 5.45% which could not affect the results significantly.

Material from antelopes was always collected immediately after the animal was shot. Excepting in the case of the browsers, there was very little or no froth as a rule, so that the one cc. sample was fixed immediately. There being no ice available under field conditions, the ingesta could not be chilled, and for this reason, collection of material from browsers with frothy rumen ingesta, was always deferred for half an hour or so after the rumen was opened to allow the froth to subside.

(ii) COUNTING OF BACTERIA.

In counting the ruminal bacteria, total counts were made throughout and always at the same time of the day in relation to feeding and watering, so as to eliminate dilution factors as much as possible. Water was removed as

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a rule from the metabolism boxes or crates of sheep at 6.15 a.m. daily, and material for counts withdrawn at 10 a.m. before feeding. Sheep used in these experiments were fed at 10 a.m. and 2 p.m. and invariably had consumed the food given to them early during the same evening, so that the material for counts was withdrawn usually approximately 12 hours after completion of feeding.

These counts are of course not necessarily true total counts, since an unknown percentage of organisms penetrate into or become absorbed to food particles. The total count as given must, therefore, be considered always as being less than the true count would be. These organisms which cannot be counted can, however, be considered to remain more or less constant in number under uniform conditions, i.e. the animals were always fed and material withdrawn for counting at the same times of the day. It can reasonably be assumed therefore, that the error would remain approximately the same from day to day on the same ration. In examining food particles and ruminal fluid microscopically after fixation and staining for bacteria, it is seen that only a very small proportion of the organisms is not counted, due to intimate relationship with food particles.

In attempting to evolve a technique for the counting of ruminal bacteria, several difficulties had to be overcome, firstly, the method to be employed, secondly, the most effective fixative and stain, and thirdly, the best time to make the counts so as to obtain most uniform results. The last mentioned point has been discussed above.

It was realised at the outset that the plating and Wright's methods could not be employed. In plating

rumen/.....

rumen contents the medium usually becomes so overgrown by various bacteria and fungi, that it becomes impossible to distinguish colonies. Selective media were also tried, but without success.

Wright's method, in its simplified form, consists in mixing measured quantities (usually equal volumes) of the fluid to be examined with normal human blood. Film preparations of this mixture are made and suitably stained. By ascertaining the ratio of red cells to bacteria in a number of microscopic fields an estimation of the bacterial count is obtained. According to Leishman (1910) results with this method are not consistent, and errors of 50-100 per cent. may occur.

The enumeration of bacteria (for the purposes of standardising bacterial emulsions for vaccines) in a haemocytometer chamber, was suggested by Mallory and Homer Wright (1908). They employed a well 0.02 mm. deep, an optically plane coverslip, a 1/16 inch dry lens and no staining fluid. In 1908 Leith Murray likewise used an ordinary Thoma-Zeiss haemocytometer with a well 0.1 mm. deep, an ordinary coverslip, a 1/12 oil immersion lens and a staining solution of weak Giemsa.

For the purposes of this work, the author selected a Petroff-Hauser bacterial counting chamber, which has a well 0.02 mm. deep. This chamber has a reinforced precision coverslip, optically plane and 0.18 mm. thick. The apparatus can be used with dark and bright field illumination and is suitable for all achromatic oil immersion lenses as well as dry lenses. Further advantages are (Glynn, Powell et al, 1913) that almost all the bacteria settle to the bottom of the chamber in five to ten minutes, when accurate counts can be made. Bacteria adhering to the under surface

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of the coverslip, and any still floating in the chamber, are easily enumerated. The optical definition of the bacteria is good owing to the small quantity of fluid.

After trying out several stains and methods of killing the bacteria suspended in the ruminal fluid, a five per cent. carbolic solution, or 30 per cent hydrogen peroxide was found to be most suitable, although not entirely effective, probably due to the great variety of organisms present.

Fresh rumen ingesta has a definite discolorising action on stains, and unless the concentration is increased, several times beyond that required for ordinary bacterial emulsions, the stain is reduced to such an extent that it loses its staining power almost completely. A further point that had to be considered was the fact that staining is most effective when a stain is so used that its hydrogen ion concentration is changed as little as possible so that the stain is allowed to act at the pH where it stains best. Thus a stain which would be effective at a pH of approximately 6.8 to 7.2 had to be sought. For this purpose nileblue-sulphate in a one per cent concentration was found to be most suitable. If a stronger concentration is used the organisms are inclined to clump. After considerable experimentation, the following routine method was adopted in preparing material for counting:-

Ruminal ingesta is withdrawn into a sterilised glass tube and chilled for fifteen to thirty minutes in ice cold water, so as to stop fermentation and break the froth. After this the tube is shaken for a few seconds to mix the material, and one cubic centimeter of it is drawn into a wide-mouthed 2 cc. pipette. This is run into a small 3 cc.

glass/.....

glass tube with stopper. To this 0.25 cc. of a 5% carbolic acid solution, or 30% hydrogen peroxide is added. The mixture is stirred with a thin glass rod and then allowed to stand for one hour. After this 0.3 cc. of nilebluesulphate is added and the mixture well stirred. After staining for an hour 1 cc. of the stained material is pipetted off and mixed with 257 cc. of sterilised distilled water, the pipette being washed out well in this water. In this way a dilution of 1 in 400 is obtained (Neser, 1923), which was found to be the most suitable dilution for ordinary counts. When bacterial populations were low the dilution was reduced to 1 in 200. Unstained bacteria can be counted in a similar way, but it was found that by staining, eyestrain was much reduced and accuracy increased.

After the final dilution is made the suspension is allowed to stand for two minutes so as to allow the coarser material to settle. With a Thoma-Zeiss pipette a drop of the emulsion is allowed to pass under the coverslip by capillary attraction. The counting chamber is then placed in position on a level microscopic stage and allowed to stand for five minutes in order to allow the bacteria to settle. Ten blocks of nine small squares are then counted, giving the total number of bacteria in 90 squares. The volume of each square being .0008 cubic mm., the total volume is .072 cubic mm. If N = total number of organisms in .072 cubic mm., then with a dilution of 1 in 400 there would be 400 N organisms in .072 cubic mm. Thus in one cubic mm. there would be $\frac{400\ N}{.072}$ organisms. If the number of bacteria in 90 small squares is known, the total number per cubic mm. could thus be calculated by multiplying with $\frac{400}{.072}$ for a dilution of 1 in 400 or $\frac{200}{.072}$ for a dilution of 1 in 200 and

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so forth.

The experimental error involved in this technique does not exceed 4.8 per cent. as calculated statistically from a number of observations.

Throughout the investigations Zeiss Huygens Oculars 7 X and a Zeiss Achromate 40 X dry lens were used, with excellent results.

(b) COLLECTION OF FAECES AND URINE FOR CHEMICAL ANALYSIS.

(i) Sheep.

(ii) Rats.

(i) Sheep: Forbes' metabolism crates were used for the collection of faeces and urine in the sheep experiments. Some of the features of these crates, constructed locally, may be briefly mentioned. The inside area in which the animal can move about is 5 2/3 x 4 feet. One end opens out into a detachable galvanised iron manger. Water is supplied in a trough attached on the inside away from the manger. There are two false bottoms to the crate. An upper wire screen of half inch mesh on which the animal stands permits the excreta to pass through. About four inches below this is a second wire screen of 1/16 inch mesh which catches the faeces and allows the urine to pass through on to the funnel shaped base constructed of galvanised flat iron, and thence into a receptacle below the crate. When collections of faeces and urine have to be made the upper movable part with the animal in it, are moved over on to a cleaning table hooked to the crate and corresponding with it in dimensions. This table is a duplicate of the lower half of the crate.

During the preliminary periods the sheep were kept in separate feeding pens measuring about 15 x 15 feet. These open up into an outdoor exercising camp free of

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vegetable matter.

COLLECTION AND SAMPLING OF THE EXCRETA.

The sheep were weighed at the beginning and again at the termination of each experimental period. With the exception of period II. in the urea utilisation experiment, the experimental periods were preceded by preliminary periods of such a length that the excreta could be presumed to represent that of the experimental ration. For this purpose periods of 8 to 10 days are generally accepted to be of sufficient length in the case of the ruminant. The period of collection usually continued for 8 to 10 days.

Before starting a collection period the crates were thoroughly cleaned, care being taken to remove any faecal matter adhering to the wool and hoofs of the animals before entering the crates. The exact time of the day when the animals were put into their cleaned crates was noted, and taken to be the beginning of the collection period which was concluded at the same hour at the termination of the period. Excreta was collected in the usual way daily at 9 a.m. except for the final collection which depended on the time the animals were put into the crates at the commencement of the period. Urine was collected in 10% sulphuric acid for preservation.

The mixture of acid, urine and washings are filtered through glass wool, all containers being rinsed with a small volume of distilled water. The total volume of filtered urine and water is then made up to 2 litres or more as required, and the final volume noted. Of this, ten per cent was taken as an aliquot after thorough shaking, and stored in a clean flask in which the daily aliquots were pooled. In the urea experiments an extra sample of urine of approximately 50 cc. was taken daily at the same time as the aliquot for the nitrogen determination.

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On the final day of collection, both wire screens and the flat iron bottom of the crates were thoroughly scrubbed with a clean hard brush and rinsed with distilled water to remove urinary matter which might have become adherent to the screens during the experimental period in spite of the daily washing.

The faeces from a daily collection were spread out on an enamel tray and left exposed in the metabolism room for 24 hours after which they were found to contain no more than about 12 per cent. moisture. Aliquots equal to one-tenth of the total weight were then taken and stored in "Bail" jars. This process was repeated each day during the collection period and the daily aliquots mixed in the same jar. This composite sample was weighed two or three days after the termination of the period and then finely ground, thoroughly mixed and a representative sample taken for chemical analysis.

Feed refused by an animal in the course of a collection period was left in the manger until the end of the trial when it was collected, weighed and stored for chemical analysis when required.

(ii) Rats.

For the collection of faeces and urine of rats specially constructed earthenware metabolism cages as illustrated and described by Marais and Smuts (194 0) were used. The rats stand on a horizontal wire screen with a 3/8 inch mesh which allows the faeces to drop through on to a second wire gauze of 1/16 inch mesh, on which it is then collected.

The urine and faeces were collected daily. The daily faeces collections, after careful removal of adherent hair, were digested according to the usual Kjeldahl method.

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The week's digests were analysed for nitrogen. For the urine collections, the metabolism cages were washed out daily with 0.5 per cent. tartaric acid solution. The daily urine collections were kept in dilute H_2SO_4 and at the end of the collection period a suitable aliquot digested for nitrogen determination.

To distinguish between faeces of the preliminary and collection periods, Fe_2O_3 was used as a marker. The collection periods were of 7 days duration. To prevent food wastage a special food basin, also described by Marais and Smuts (1940), was used. The rations were stored in an ice chest to prevent deterioration. The composition of the rations are given in Table 1(d).

(2) DESCRIPTION OF RATIONS.

During the course of these studies numerous different rations were fed to the experimental animals. These rations are, however, not fully described in their respective experiments; the actual quantities fed are given but it was thought necessary to tabulate the various rations and present their composition on a percentage basis. In tables 1(A) to 1(D) the rations of the various experiments are grouped together. In table 17 the rations fed to the different sheep in the cellulose digestion trials, are tabulated.

Table 1(A). Sheep rations - Infusoria Experiment.

	Ration Number.							
Ingredient	1.	2	3	4	5	6	7	8
Green lucerne						98.0		
Lucerne hay	45.2				98.0			
Maize	52.8	98.0	52.8					3.5
Wheat straw			45.2	98.0				
Teff hay							98.0	
Veld hay								94.5
NaCl	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Table 1 B/.....

TABLE 1 B. SHEEP RATIONS - BACTERIAL EXPERIMENTS INCLUDING UTILISATION OF UREA.

Ingredient	Ration Number. 9	10	11	12	13	14	15	16	17	18	19
Wheat straw		70.0	69.6	69.0	69.0	68.0	49.0	64.7	70.7	72.5	96.5
Lucerne hay	63.0										
Maize	30.0					7.7	30.0	25.0	22.0		
Dextrinised starch		22.7	22.5	21.7	20.3					24	
Meatmeal (Sefco 80%)						17.0	13.7	5.0	2.0		
NaCl	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Mineral mixture	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0		
Yeast		0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Urea			0.6	0.3							
Bone meal										1.2	1.2
White fish meal				1.7	3.4						
TOTAL	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Ration No. 9 - Standard lucerne-maize ration.
" " 10 - N-low plus dextrinised starch.
" " 11 - N-low " " " plus urea supplement.
" " 12 - N-low " " " " " " plus fish meal.
" " 13 - N-low " " " " fish meal.
" " 14 - N-high " carbohydrate low.
" " 15 - N-high " " normal.
" " 16 - N - 2 X normal plus carbohydrate normal.
" " 17 - Balanced wheat straw ration.
" " 18 - Wheat straw plus dextrinised starch plus bone meal supplement.
" " 19 - " " " " bone meal supplement.

TABLE 1 C. CYSTINE EXPERIMENT: SHEEP:

Ingredients	Ration Number 20	21	22
Lucerne hay	58.0	56.0	
Cystine		.20	
Cod Liver Oil	2.0	2.0	2.0
Salt	2.0	2.0	2.0
Bone ash	3.0	3.0	3.0
Dextrinised starch	35.0	36.8	73.0
Agar	-	-	20.0
TOTAL	100.0	100.0	100.0

Table 1(D). Cystine Experiment - Rats.

Ingredients.	Ration Number. 23	24	25	26	27
Lucerne hay.	36.8	-	-	-	-
Ruminal ingesta.	-	41.1	39.7	-	-
Incubated lucerne.	-	-	-	-	41.4
Dextrinised starch.	37.2	32.9	34.1	40.0	32.6
Sucrose	10.0	10.0	10.0	10.0	10.0
Butterfat	8.0	8.0	8.0	8.0	8.0
Cod liver oil	2.0	2.0	2.0	2.0	2.0
Harris yeast	2.0	2.0	2.0	2.0	2.0
Salt mixture x	3.0	3.0	3.0	3.0	3.0
NaCl	1.0	1.0	1.0	1.0	1.0
Agar	-	-	-	2.0	-
Cystine	-	-	0.2	-	-
Egg white.	-	-	-	2.0	-
	100.0	100.0	100.0	100.0	100.0
Per cent. Nitrogen	1.12	1.13	1.03	0.47	

x New salt mixture of Hubbel R., Mendel J.B., and Wakeman A.J. (Jnl. Nutr. 14, 273-285, 1937).

(3) ANALYTICAL METHODS EMPLOYED.

(1) Starch: For the determination of starch the extraction procedure described by Hanes, C.S. (1936), and the chemical methods of Edwards et al (1938) were followed.

(2) Cellulose: For cellulose determinations the method described by Crompton and Maynard (1938) was followed, in the investigations concerned with the infusoria. In all the other cellulose digestion trials Norman and Jenkins' method described in 1933 was used.

(3) Nitrogen: For nitrogen determinations the usual Kjeldahl method was employed.

III./.....

III. EXPERIMENTAL DATA.

A. RUMINAL INFUSORIA IN SHEEP.

(1) Literature.

(2) Infusorial populations in relation to diet:

(a) stable fed animals.

(b) sheep on natural pasture.

(3) Rumen infusoria in sheep as compared with those of wild antelopes under natural conditions.

(4) Morphological studies on the digestion of maize starch by infusoria in vivo and its relation to glycogen formation.

(5) Chemical data on the influence of infusoria on starch digestion within the rumen.

(6) Observations on the effect of infusoria on cellulose digestion.

(1) LITERATURE:

Ever since the discovery of ruminal infusoria by Gruby and Delafond in 1843, these organisms have interested research workers in a twofold way, firstly, as a source of comprehensive morphological and evolutionary study (Dogiel, 1927), and secondly, as a biological problem.

Gruby and Delafond (1843), Stein (1858), Fiorentini (1889 and 1890), Eberlein (1895), Crawly (1923), Dogiel (1921-1927), and many others, have laboured incessantly to describe and classify the family Ophryoscolecidae. Dogiel, in his monograph (1927), gave a final classification and description of all known species and forms. Gruby and Delafond, as well as Eberlein and Schuberg (1888), expressed the opinion that the Ophryoscolecidae may have some biological significance. Ferber (1925, 1929) held that these organisms convert plant proteins into easily digestible animal protein, in the form of their own body protoplasm, and that they served as

an/.....

an important source of animal protein to their herbivorous hosts. According to Ferber, a sheep with 3 Kg. rumen ingesta, and an infusorial population of 900 per cubic millimetre, received 0.327 gram of protein daily from its infusoria. If one considers that approximately half of this protein is of bacterial origin, it implies that a sheep receives only about 0.16 grams of infusorial protein over a period of 24 hours. This is insignificant, as the maintenance requirements of adult sheep of 45 Kg. body weight, are approximately 19.0 grams digestible protein daily (Smuts and Marais, 1938).

Further possible rôles attributed to the ruminal infusoria deserve mention.

(a) They are harmless commensals.

Biedermann (1911), Scheunert (1924), Scheunert and Schieblich (1927), and Becker, Schulz and Emmerson (1929), maintain that these organisms do neither harm nor good to their hosts and that they are merely found in the rumen because it provides a favourable habitat for them to live in.

(b) They assist in the digestion of cellulose.

Since their discovery it had been thought that infusoria might play a part in the digestion of cellulose in the rumen. Schuberg and Eberlein noted the ingestion of plant material by infusoria and concluded that the organisms could digest cellulose. Eberlein also noted the disintegration of plant material within the organisms and the expulsion of detritus from the anus. Dogiel and Federowa-Winogradowa (1925), observed that cellulose particles did not always leave the anus as detritus, but that large particles were sometimes extruded morphologically unchanged, suggesting that these particles may have undergone a preliminary chemical digestion.

Mangold/.....

Mangold (1927) believes that cellulose digestion within the infusoria occurs by means of similarly ingested cellulose splitting bacteria.

In 1930 Becker, Schulz and Emmerson conducted digestion trials by using goats with and without infusoria, and concluded that cellulose digestion in the host is neither due to, nor materially assisted by the infusoria. Unfortunately the diet of these goats contained a high proportion of grain which strongly attracts infusoria, so that the cellulose in the diet could be considered as having received very little attention from the infusoria. From observations made by the author these organisms definitely prefer starch to cellulose. Appreciable quantities of cellulose being ingested by the infusoria only when grain is not present in the rumen.

(c) They assist in the digestion of starch.

Trier (1926), and Westphal (1934), drew attention to the relationship between infusoria and the digestion of starch. Westphal did not assign any significance to the digestion of starch by infusoria, and from the results of his work conducted in vitro, he concluded that they were only commensals particularly dependant on starch.

(d) They are of mechanical and physical aid in digestion. Bundle (1895), Braune (1913), Scheunert (1924), and Scheunert and Schieblich (1927) considered that these micro-organisms were purely of a mechanical and physical importance to their host in that they assisted in the soaking, macerating and mixing of the rumen contents.

(e) Infusoria are injurious parasites.

Apart from the view held by Zürn (1887) that infusoria are injurious parasites which, through their presence may lead to a catarrh of the alimentary tract, no other workers have suggested a similar role. The

possibility/.....

possibility still exists that these organisms may rob the host of part of its food, although this point has as yet not been experimentally established.

(2) INFUSORIAL POPULATIONS IN RELATION TO DIET.

(a) Stable fed sheep.

Technique: All sheep were dosed with 2.5 litres of tapwater through the fistula daily at 2 p.m. so as to keep the intake of water constant. Material for infusorial counts was always withdrawn at 9 a.m. After shaking vigorously 1 cc. was sucked up into a wide mouthed pipette. This was added to 7 or 8 cc. of corrosive-sublimate-alcohol fixative, and after washing with alcohol-iodine and 70% alcohol, it was suspended in 2 cc. oil of cloves in which it can be kept for a long time. After diluting to 1/10 or 1/100 in oil of cloves a drop of known volume from a capillary pipette was placed on a glass slide and covered with a coverslip. The total number of infusoria per drop was counted, from which the number per cubic millimetre ingesta was then calculated. Duplicate counts were made.

Experiment 1. (a) Maize and lucerne ration - (Ration No. 1).
(b) Maize ration - (" No. 2).

Sheep No. 45 and No. 37 on a mixed ration of 360 grams of crushed yellow maize plus 300 grams of lucerne hay were used in an experiment to follow up the influence of a change from the above ration to a pure maize ration containing approximately 75% carbohydrate, 10% protein and 2% fibre.

Infusorial counts were made weekly, and when necessary, also at shorter intervals. Differential counts were made to correlate adaptive changes in these organisms with changes in the diet. A record of the food consumption of the animals was kept, and is reflected in graphs 1 and 2

opposite/.....

opposite the infusorial counts.

Discussion and Conclusions.

1. Maize and Lucerne Ration.

(a) Infusorial population fluctuated between 1000 and 3500 per cubic millimetre in sheep No. 45, and between 1200 and 3300 in sheep No. 37.

The differential counts disclosed that in both sheep the genus Entodinium comprised 95% of the total infusorial population. The rest was made up of the genera Diplodinium. Bütohlia and Isotricha.

(b) The hydrogen ion concentration of the rumen ingesta of these sheep varied between pH 6.6 and 7.2.

2. Maize ration.

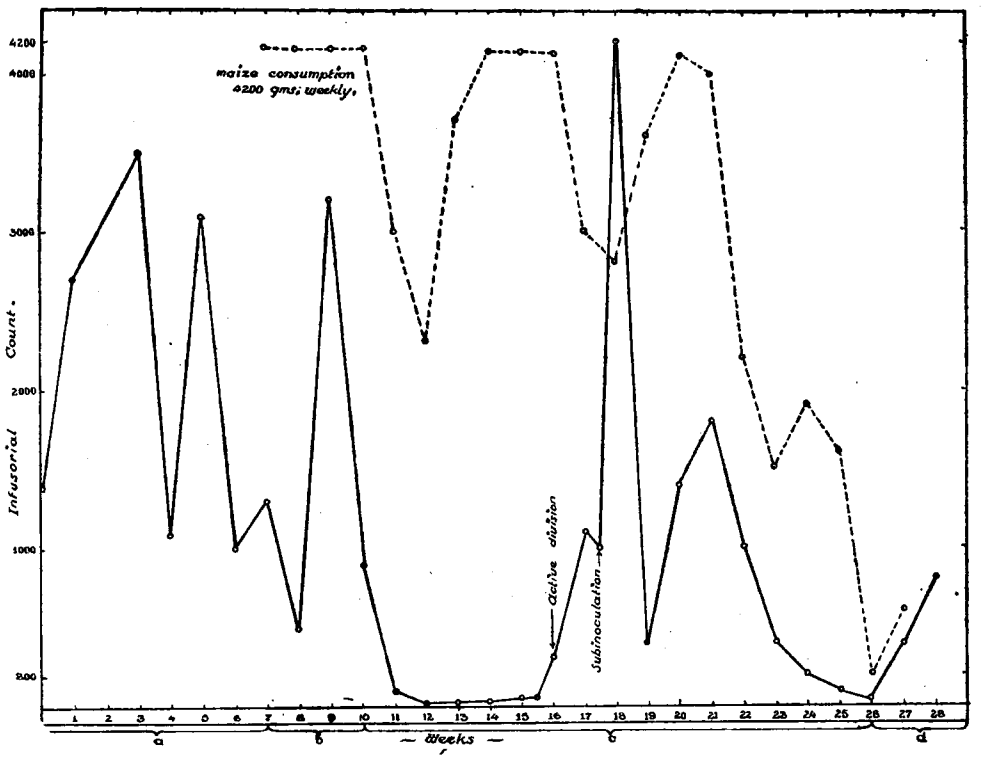
(a) With an increase in maize and reduction of lucerne, the larger types of infusoria diminished until finally on maize exclusively, they became extinct. An attempt to start a fresh culture in sheep No. 45 by subinoculation with 50 cc. rumen ingesta from another sheep whose infusorial population was known, was unsuccessful. The conditions in the rumen had thus become unfavourable for infusoria other than the genus Entodinium, the most primitive of all.

(b) A critical stage was rapidly reached when only maize was fed. Sheep Nos. 45 and 37 reacted differently.

In the case of sheep No. 45 the amount of maize consumed dropped in two weeks from 4200 grams per week to 2300 grams, and then rose rapidly again to 4200 grams. This was followed by a second drop to 2800 grams and a recovery. Finally it was depressed to 200 grams, at which stage the ration was supplemented by lucerne hay. The rumen ingesta during this critical stage had a very sour and rancid smell with a cheesy appearance.

The infusoria reacted sharply to the first fall when only/.....

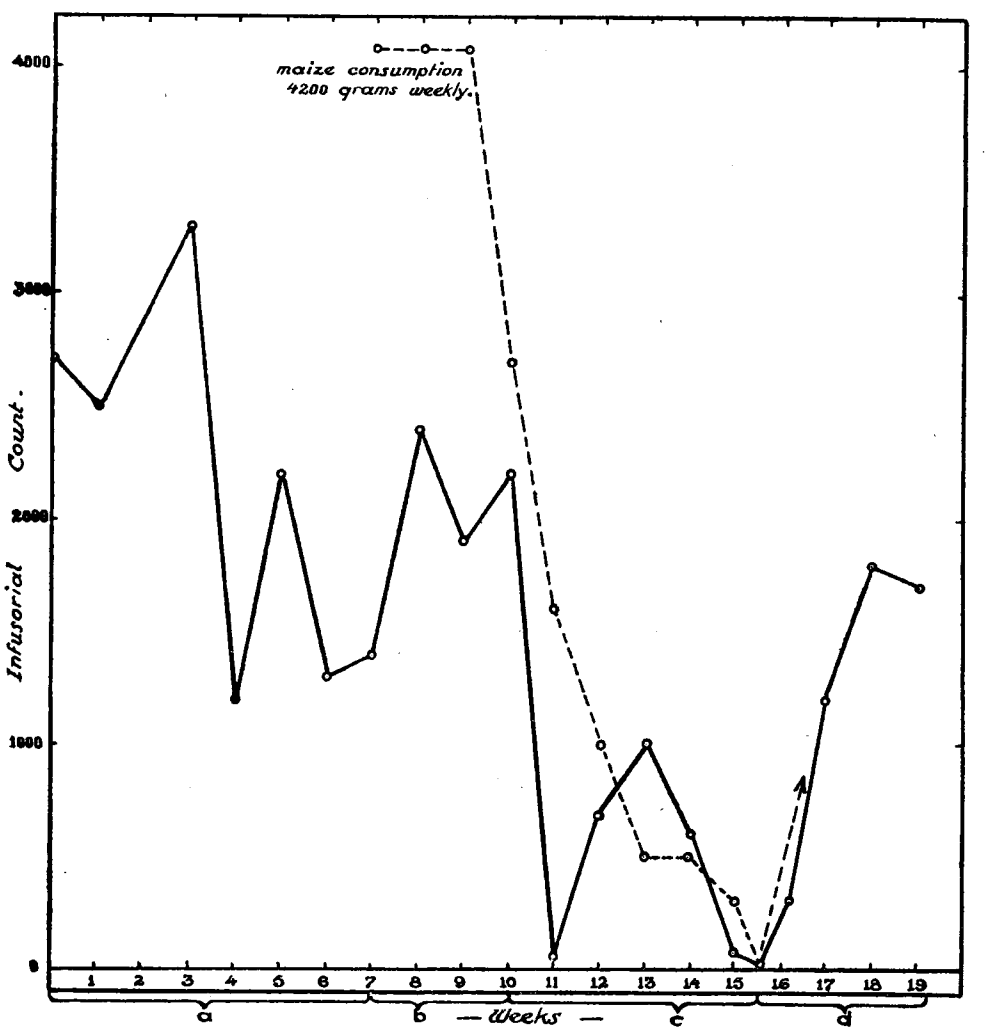
Graph I: Sheep 45.



Study of Infusoria on diet of :-

(a) Maize 360 grams + L. Hay 300 grams. (c) Maize 600 grams.
(b) " 600 " " " 150 " (d) " " " + L. Hay 300 grams.

Graph II : Sheep 37.



Study of Infusoria on diet of :-

(a) 360 grams y. maize + 300 grams Lucerne Hay.
(b) 600 " " " 150 " " " .
(c) " " yellow maize.
(d) " " " " + 300 grams Lucerne Hay.

only maize was fed. The count dropped from 900 to 0.5 per cubic millimetre. After remaining at this low level for 5 weeks, conditions in the rumen became more favourable as shown by an improvement in appetite and chemical analysis. This provoked an immediate response by the infusoria. Active multiplication was seen, and the count rose from 0.5 to 1100 organisms per c.mm. within 10 days. At this stage the larger types of infusoria had all become extinct. An unsuccessful attempt was made to re-establish them by subinoculation. The host finally consumed less and less maize, which led to starvation after 16 weeks of pure maize diet. The infusorial population fell to 30 per c.mm.

Sheep No. 37 adapted itself very poorly to the maize diet. Immediately lucerne was withdrawn inappetance set in, leading to starvation within 7 weeks. The infusoria maintained themselves well for a period of 5 weeks until the animal stopped ruminating. In vitro tests at this stage proved that the organisms were hungry, due to the absence of small starch particles. The symbiotic harmony between host and infusorium was thus disturbed by failure to ruminate. Supplementation with lucerne hay also induced rapid multiplication.

(c) Of the Entodinium species E.nanellum was the most resistant, and thrived better under conditions adverse to other species. A small number of E.furca, E.simplex, E.elongatum and E. dubardi survived, as seen in Table 1. below, showing average percentage of Entodinium species per cubic millimetre.

Table 1.

Sheep	Period	E.nanellum.	E.furca	E.simplex	E.elongatum.	E.dubardi
45	Before maize.	32	21.5	5.5	26.5	10
	After maize.	66	12	5	11	6
37	Before maize.	42	17	8.5	21.5	6
	After maize.	62	9	7	17	5

(d)/.....

TABLE 2.

DISTILLATION OF RUMEN INGESTA.

Sheep No.	Date.	Diet.	Acetic Acid	Butyric acid	Non-Volatile acid.	Total organic acids.	Total Volatile acids.	pH of ingesta.	Remarks.
42	23/9/38	300 grams lucerne hay + 360 grams yellow crushed maize.	17.4	13.32	4.62	35.34	30.72	6.8	
43	10/11/38	do.	17.52	6.44	7.70	31.66	23.96	6.8	
35	12/11/38	Green lucerne.	13.42	4.76	1.90	20.08	18.18	6.9	
32	17/11/38	do.	12.05	6.46	5.50	24.01	18.51	7.0	
45	30/9/38	600 grams yellow	16.6	9.61	18.20	44.31	26.11	5.5	
	5/10/38	crushed maize.	7.96	3.78	8.00	19.74	11.74	5.4	
	13/10/38		26.90	4.68	5.60	37.18	21.58	5.4	
	14/10/38		21.90	2.52	5.72	30.12	24.42	5.4	
	18/10/38		26.70	10.85	5.74	43.29	37.55	5.2	
	19/10/38		27.80	9.41	15.70	52.91	37.21	5.9	
	9/11/38		21.00	4.55	2.50	28.05	25.55	5.6	
	29/ 9/38	600 grams yellow	15.60	4.58	6.50	26.68	20.18	5.4	
	1/10/38	crushed maize.	18.50	8.68	14.65	41.83	27.18	5.4	
	6/10/38		14.05	4.45	25.60	44.10	18.50	5.4	
	13/10/38		9.90	3.83	7.00	20.73	13.73	5.3	
37	14/10/38		9.24	3.82	21.50	34.56	13.06	5.6	
	18/10/38		7.64	3.28	8.00	18.92	10.92	5.4	
	19/10/38		14.40	3.88	14.00	32.28	18.28	5.4	
	6/11/38	Starvation.	1.62	0.34	1.50	2.46	1.96	5.4	
40	19/11/38	Straw.	14.51	3.91	5.70	24.12	18.42	-	Both 40 and 39 ate poorly on 22/11/38.
	24/11/38	do. ½ ration.	5.90	2.20	5.20	13.30	8.10	-	
39	15/11/38	Straw	12.40	4.82	4.98	22.20	17.22	-	and 24/11/38.
	22/11/38	do. ½ ration.	4.24	1.16	5.00	10.40	5.40	-	

All acid values are expressed as ml. $^{N}/_{10}$ NaOH per 100 gms. ingesta.

(d) Chemical analysis of the rumen ingesta is reflected in Table 2. After acidifying, distillation was carried out according to Wiegner's process as reported by Smith (1938). Material was withdrawn through the fistula daily at 9 a.m. before feeding.

Discussion and Conclusions.

It is evident from the table that in sheep No. 45 acetic and butyric acid rose very high at the time when the infusorial population diminished significantly. On the other hand, the acetic and butyric acid values kept within normal limits (see sheep 42, 43 and 35) in the case of sheep No. 37 with a pH of 5.4. Under these conditions the infusoria thrived until they were starved. Excessive amounts of acetic and butyric acid in the rumen are therefore harmful to the infusoria. In vitro tests confirmed this. The exceptionally large quantity of maize consumed by sheep No.45 was the cause of the acidity of its rumen.

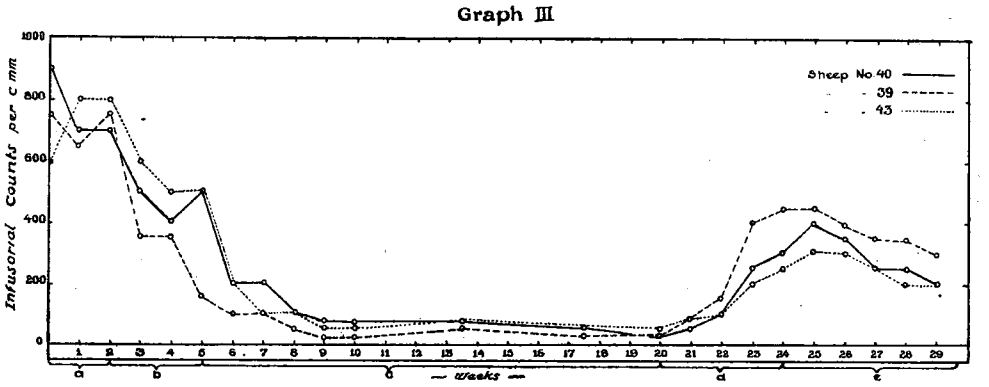
Experiment No. 2.

Effect of wheat straw diet on infusoria.

Three sheep on a diet of 300 grams wheat straw and 360 grams of crushed yellow maize (Ration No. 3) were used for infusorial counts. The amount of maize was first reduced to 100 grams and then omitted, with a corresponding increase in wheat straw to 600 grams (Ration No. 4). Counts were made every second day for 28 days. At this stage the effect on the infusoria of a supplementation of 100 grams yellow mealie meal, introduced directly through the fistula, was tested for a period of eight days. Mealie meal was then substituted by 100 grams of maize starch to eliminate the protein contained in the mealie meal. Differential counts were made as in Experiment No. 1. See graph III and Table 3 below.

Discussion/.....

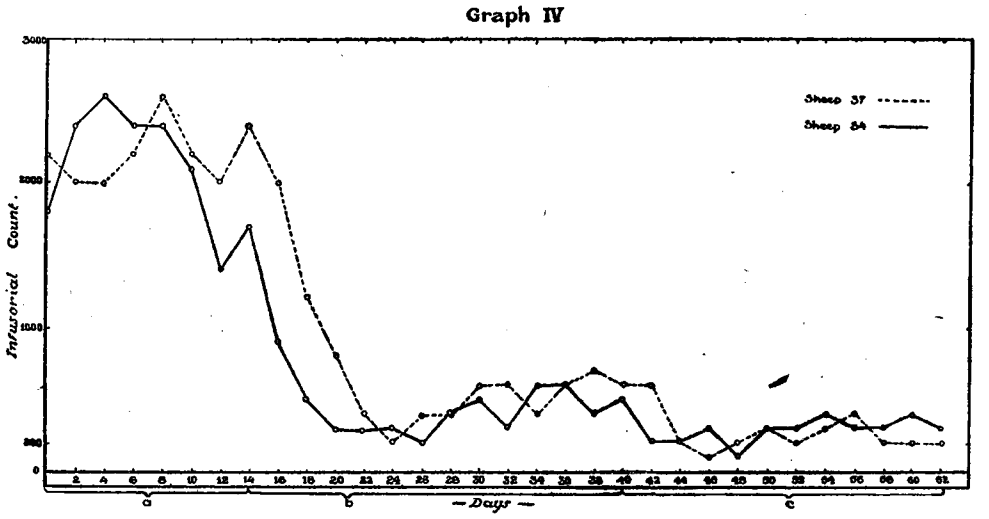
Graph III



Study of Infusoria on diet of :-

(a) 360 grams crushed y maize + 300 grams wheat straw
(b) 100 " " " " + 600 " " "
(c) 600 " wheat straw.
(d) 600 gms. wheat straw + 100 gms. y. mealie meal.
(e) " " " " " " maize starch.

Graph IV



Study of Infusoria on diet of :-

(a) 360 grams crushed maize + 300 grams L.Hay.
(b) 1 k.gram dry Lucerne Hay.
(c) 3 K.grams green Lucerne Hay.

(a) On a wheat straw and maize diet the infusorial population fluctuated between 700 and 900. The genus Entodinium comprised 99% of the total. Upon reduction and later omission of maize, there was a significant fall in numbers to an average of 50 organisms per cubic mm. as a result of starvation. On the addition of maize again, the infusoria doubled their numbers within 24 hours, and after a week the count had risen to 50 per c.mm. On replacing the mealie meal by starch, multiplication was retarded.

(b) Of the Entodinium species, Entodinium simplex adapted itself best to the wheat straw ration. It increased from 4% to 35%, whereas E.furca diminished from 50% to 18%. The genus Diplodinium increased from .5 to 4%.

Table 3.

Period	Sheep No.	E.nanel-lum.	E.furca.	E.sim-plex.	E.elon-gatum.	Uniden-tified Entodi-nium species.	Diplodi-nium species.
Maize	37	27	48	4.0	3	4	1
+ wheat	40	49	40	3.0	1.5	6	.5
straw.	43	21.5	58.5	5.5	-	13	.5
Wheat	37	33	12	38	7	6	4
straw	40	21	18	40	12	5	4
only.	43	36	20	30	-	12	3.5

Experiment No. 3.

Comparison of infusorial counts in sheep on a diet of:

(1) 360 grams of maize and 300 grams dry lucerne hay (Ration No. 1).

(2) 1 Kg. of dry lucerne hay (Ration No. 5).

(3) 3 Kg. of green lucerne (Ration No. 6).

Infusorial counts were made every second day for periods of three weeks successively on sheep which were on the above diets. The results are recorded graphically in graph IV.

Conclusions/.....

Conclusions.

(a) On maize + lucerne hay the population fluctuated between 1400 and 2600 per cubic mm. With omission of maize the number of infusoria decreased rapidly and established a new level fluctuating between 200 and 700 per cubic mm.

(b) On feeding green lucerne only, a lower level was reached, the numbers varying from 100 to 400 per cubic mm.

(c) This decrease is attributed to the corresponding decrease in the starch content of the different diets.

Experiment No. 4.

Effect of teff hay diet on the infusorial population.

Five sheep on a Kilogram of teff grass hay daily were used for counts. The number of infusoria per cubic mm. was found to vary between 215 and 485. This corresponds with the population of a sheep on green lucerne diet.

(2) b. SHEEP ON NATURAL PASTURE, NOOITGEDACHT EXPERIMENTAL FARM, ERMELO, TRANSVAAL.

Experiment No. 5.

Seasonal fluctuations of infusoria.

Experimental: A group of 40 healthy merino wethers were allowed to graze freely in a camp of 100 morgen with a typical Transvaal highveld pasture and running water. These sheep were gathered twice daily in a small paddock for a change of faeces bags and withdrawal of rumen ingesta by stomach tube. This was done every day at 7 a.m. The faeces bags were used to collect faeces for a concurrent experiment on grass consumption by Smuts and Marais of this Institute. The sheep were closely watched when grazing so as to determine the grasses selected by them. Supplies of these grasses were collected for analyses and feeding trials.

The months of July, October, January and April represent/.....

represent the critical times of the four seasons, so that observations were confined to these months on twenty out of the forty wethers selected at random. The sheep were weighed weekly so as to reflect their condition during the seasons concerned.

Results:

(a) The infusorial and bacterial counts are reflected in Table 4.

It will be noted that in all but one sheep (which suffered from bluetongue) there is an increase in the infusorial and bacterial populations from July to January, and a decrease from January to April, the April counts corresponding with those of October.

There was an average of 100 infusoria per cubic millimetre, in July, 277 in October, 455 in January and 278 in April.

In July 20.6% of the infusoria consisted of the larger types, i.e. excluding the genus Entodinium; in January this figure had fallen to 15.7%. This difference is due to the fact that the large types thrive relatively better than the Entodinium species on lignified diets. The Entodinium species flourish when diets are rich in starch and nitrogen. As they are the most primitive genus of infusorium they probably multiply more rapidly than the other more developed genera.

(b) Comparison of counts and feeding conditions.

In Table 5 is given a complete summary of the nutritional quality of the grazing during the different seasons of the year as well as the average infusorial and bacterial counts of sheep subsisting on it. An interesting and significant feature in this respect is the marked and prominent fluctuation in nutritional conditions during the year. From October to March there is a super abundance of a fairly

good/.....

good quality grazing as a result of which both the maintenance and the growth requirements can be met. However, this condition is completely reversed during April to September. In this interval grazing is extremely poor. The protein drops from an average of 9% to approximately 3%. (Smuts and Marais, 1940). Together with this rapid decline in protein there is a tremendous increase in fibre. These factors are closely linked with the stage of maturity of the grazing. The grazing conditions under which this experiment was carried out are therefore very variable in nature. Thus, in certain seasons there was an abundance of nutrients available while a deficiency existed during the rest of the year. Such fluctuating nutritional conditions, as can be readily appreciated, must tax the digestive system, the process of utilisation, as well as the general health and vitality of the animal in a severe manner. Physiologically one must assume that the normal digestive powers and reactions, together with the intricate functions of utilisation of nutrients cannot be best accomplished below a certain level of nutrition. Consequently, it appears that while the processes of assimilation and utilisation of feed form an inseparable physiological unit, the reactions experienced by one function will automatically be reflected by the other.

In others words, there should exist a close relationship between quality and utilisability of feed and the physiological factors concerned in the digestion of such feed. That such a relationship actually appears to exist is evident from a comparison between the protein content of the grazing, the digestibility and the infusorial and bacterial populations. Whether such a relationship is connected primarily with the protein in the grazing or with the absence or presence of other nutrients or finally with the vitality of the animal is difficult to assess at present. It is

nevertheless/.....

nevertheless remarkable that a low protein content, or a deficient nutritional state, which invariably affects the vitality as well as the health of the animal, markedly reduces the infusorial and bacterial populations. From these observations it would appear that the multiplication and normal influence of infusoria and bacteria in digestion depends largely on a suitable substratum in the rumen and reticulum. Such a suitable substratum, the composition of which is as yet not fully appreciated, is dependent on the nutritional condition of the animal. That protein must play an important part as a necessary component of such a substratum is obvious from Table 5, where the infusoria and bacteria decrease almost proportionately with the decrease in protein content.

This fact is furthermore substantiated by the observation previously indicated in Graph III that the infusorial population decreases on a nitrogen low diet composed of maize starch and a roughage.

TABLE 4.

Seasonal fluctuations in infusoria of sheep on pasture. Nooitgedacht, Ermelo (organisms per cubic millimetre).

Sheep No.	July	October	January	April
52100	80.8	359.0	359.6	351.0
51840	57.8	189.0	497.8	224.0
52079	50.3	150.1	455.4	333.3
52082	42.3	250.9	427.1	298.7
51652	170.1	240.5	315.4	228.0
51904	20.2	175.5	379.2	256.6
51765	144.2	162.2	451.3	372.4
51741	117.0	224.0	162.4	125.4(blue tongue).
51833	132.1	152.7	780.2	376.2
51738	119.8	270.5	345.8	171.0
51885	77.6	369.4	530.7	294.4
52030	138.6	420.6	451.8	301.8
51812	96.4	319.6	485.0	281.0
51693	150.8	427.7	510.6	326.8
51757	59.6	315.0	420.7	317.8
52010	29.6	315.0	570.0	209.0
52025	30.5	425.6	470.9	260.5
51694	128.9	330.4	451.0	279.6
51689	183.7	166.8	430.0	288.8
51738	160.1	288.0	560.5	171.0
Av. per c.mm.	100	277	455	278

Table 5/.

TABLE 5.

	July	October	January	April
Average weight of sheep.	28 Kg.	33	40	39
Dry grass consumed	649 Gm.	785	907	861
% Nitrogen	0.49	1.44	1.17	0.73
Biological value	83	62	74	82
Digestibility of nitrogen.	2%	60%	51%	12%
Digestibility of dry matter.	43	58	60	46
Nitrogen balance	-2.40	0.46	0.53	-1.40
Number of infusoria per cubic mm.	98	277	455	273
Number of bacteria per cubic centimetre	$1067X10^6$	$1889x10^6$	$1944x10^6$	$1756x10^6$

(3) RUMEN INFUSORIA IN SHEEP AS COMPARED WITH THOSE OF WILD ANTELOPES UNDER NATURAL CONDITIONS.

Buisson (1923) published an account of the various species of ciliates found in the African rhinoceros and elephant. In 1924 he described the ciliates present in African antelopes from the Belgian Congo. Two species of the genus Entodinium, and two of genus Diplodinium described by Buisson in these antelopes have been found by Fantham (1925) and Schuurman (1926) to occur in South African sheep and cattle.

Dogiel (1925) obtained his material in 1914 from Lake Naivasha and Kilimanjaro in East Africa. He examined material from six different species, which are also indigenous to South Africa. This led to the description of a number of new species of ciliates. Most of the species described by Dogiel, excepting the species Diplodinium costatum and genus Opistotrichum have been observed by Fantham and Schuurman in their studies on sheep and cattle.

The genus Ophryoscolex seems to have been well

represented/.....

represented in South African sheep and cattle as well as in East African antelopes. Schuurman expressed the opinion that our cattle and sheep became infected from antelopes on the same veld. Apart from the above systematic work on South African sheep and cattle and on East African antelopes, no work has been done to ascertain firstly, the relationship between the infusorial populations of antelopes on different natural diets and, secondly, between antelopes and domesticated animals on comparable diets.

To gain information on these points the writer made use of an opportunity to accompany Dr. A.D. Thomas of this Institute on a zoological survey collection tour into the Transvaal lowveld. During this expedition ten species of antelopes were shot. Ruminal material was collected immediately afterwards and treated as outlined before. Duplicates from some species were collected at different localities.

Table 6 shows the species, its natural diet, the differential count between small and large types of infusoria, and the total infusoria per cubic millimetre.

Table 7 shows the dominant ciliate(s) in the various antelopes.

Discussion and conclusions:

(a) Antelopes may be divided roughly into two classes according to their natural diet. Under normal conditions the Klipspringer, Duiker, Impala and Kudu feed almost exclusively on legumes, leaves of certain trees and shrubs, and berries. They are also very fond of young and tender grass and green cereals, e.g. oats and wheat. The Duiker also digs up roots and tubers. On the other hand the Steenbuck, Reedbuck, Waterbuck, Sassaby, Sable Antelope and Blue Wildebeest feed almost exclusively on grass and reeds. In times of scarcity they also feed on leaves and legumes. The

Steenbuck/.....

Steenbuck is a very delicate feeder and selects only the finest and tenderest grasses.

The difference between the two types of diet is significant; camel thorn pods and kopani leaves contain 12.5% and 12.1% protein respectively. Other legumes and leaves were not analysed but could be considered to correspond closely to the above figures. Berries, roots and tubers contain a high percentage of carbohydrate. The protein and carbohydrate contents of this diet are therefore considerably higher than a diet of grass which contains approximately 4% protein and very little starch in the lowveld during the month of July. Antelopes feeding on a diet rich in starch and protein have a very frothy ruminal ingesta with large amounts of gas escaping, whereas those grazing on grass do not develop such an active ruminal fermentation.

Table 6 shows a significant difference in the total infusorial counts as well as in the proportions of the various types of infusoria present. This could be very closely correlated with the diet. The group with the richer protein diet harbours more than five times the number of infusoria than the group of the low protein diet does. The former group (browsers) shows a ratio of 1 : 2.8 between large and small infusoria (genus Entodinium), whereas in the latter group (grazers) the ratio is 2 : 1. In antelopes, as in sheep, the larger types of infusoria thrive better than the smaller types on diets containing a high proportion of cellulose, probably because they are able to ingest the larger cellulose particles better. In diets rich in protein and carbohydrates, the genus Entodinium can maintain itself better and thus proportionately outnumbers the larger types.

In sheep grazing freely on the veld in the month of

July/.....

July when the protein was at its lowest for that year (2.9 - 3%), the larger infusoria comprised 20.6% of the total population. In January when the protein rose to its highest level (9%) there was a drop to 15.7% in the number of large infusoria.

The average number of infusoria per antelope of the grazer group in July was 313 per cubic millimetre. This is comparable to the average of 278 and 277 in sheep during April and October when the condition of highveld pastures approximates that of some midwinter lowveld pastures.

(b) The dominant species of infusoria in antelopes of the same species do not differ. In the different Blue Wildebeest the dominants were Eudiplodinium neglectium Dog (1925) forma gigantium Dog (1925), and Entodinium simplex Dog (1925). In the Sable Antelope the dominants were Eudiplodinium maggii Fior (1889), and Entodinium caudatum Stein (1859). In Impala Epidinium ecaudatum Fior. (1889) and E.simplex Dog. (1925). dominated. In the Duiker the dominants were an undescribed Eudiplodinium species and Entodinium nanellum.

(c) Different species of antelopes grazing on the same veld do not harbour the same dominant infusoria excepting in the case of the Blue Wildebeest and the Sassaby. The dominant organisms in these did not appear in the Sable Antelope, Waterbuck or Reedbuck found in the same veld, nor in any of the browsers of the same locality. The dominant organism in the Impala, namely Ent. simplex Dog. (1925), did appear in the Wildebeest as the dominant of the smaller type of infusoria, but only in very small numbers.

(d) Impala, from an area where, owing to lack of grazing, more browsing is done, showed up to eight times more infusoria than Impala in areas where young green grass and young shoots are abundant.

(e) The genus Ophryoscolex was not seen in sheep or antelopes.

(f)/.....

(f) The sub-genera Eudiplodinium neglectium Dog. (1925), Ostracodinium and Opistotrichum were not seen in any of the sheep examined.

(g) The genus Epidinium, although present both in antelopes and sheep, occurs more frequently in the former.

The genus Diplodinium, on the other hand, occurs with greater regularity in sheep. The genus Entodinium is commonly seen in both, and is invariably the dominant organism in animals on diets rich in protein and carbohydrates.

(h) Several undescribed species were seen in the material examined. These will be described in due course.

(4) MORPHOLOGICAL STUDIES ON THE DIGESTION OF MAIZE STARCH BY INFUSORIA IN VIVO AND ITS RELATION TO GLYCOGEN FORMATION.

As the results of the experiments described under section (1) indicated a probable significant rôle of infusoria in so far as the digestion of starch is concerned, it was decided to investigate this possibility by studying the actual digestion of starch, firstly, within the organism itself and secondly, within the rumen of the sheep in the ordinary process of digestion.

Experiment No. 1.

Digestion of starch within an infusorium from material in vivo, i.e. material withdrawn from the rumen of sheep through the fistula.

For this experiment sheep were fed wheat hay free of starch granules. This approximates starvation of the infusoria and enables one to follow up closely the intake and digestion of starch granules administered into the rumen.

Sheep Nos. 39, 40, 43 and 45 were used. Their infusoria were examined daily by staining fresh drops of rumen ingesta on a slide with Gram's iodine to differentiate starch and glycogen. As soon as the infusoria were found to contain no more traces of starch or glycogen, 2 grams of

finely/.....

TABLE 6.

No.	Species	Natural diet.	Differential count G.entodinium	G.diplodinium	Total infusoria per c. mm.
1	Transvaal Klipspringer: Oreotragus oreotragus Roberts.	Browser.	122	94	1161
2	Transvaal Duiker: Sylvicapra grimmi Roberts.	do.	275	5	1260
3	do. do.	do.	502	178	3024
4	"Rooibok", Impala, Typical Impala: Aepyceros melampus Loht.	do.	143	82	1012
5	do. do.	do.	1347 (E.R.)	511	8271
6	do. do.	do.	203	86	1296
7	Zambesi Kudu: Strepsiceros strepsiceros Lorenz.	do.	108	59	751
8.	Lowveld Steenbuck: Raphiceros rufescens Thos.& Schiz. W.	Grazer	110	63	376
9	Reedbuck : Rietbok. Redunca arundinum Bodd.	do½	7	59	297
10	Waterbuck : Waterbok. Cobus ellipisiprymnus Ogilby	do.	32	102	303
11	Sassaby, Basterhartbees: Damaliscus lunatus Burch.	do.	13	62	337
12	Swartwitpens, Transvaal Sable antelope. Ozanna nigra Harris.	do.	14	27	184
13	do. do.	do.	27	42	310
14	Blue Wildebeest, Blou Wildebees: Gorgon taurinus Burch.	do.	18	32	224
15	do. do.	do.	39	66	472

TABLE 7.

Antelope	Dominant Ciliate(s)	
	Large types	Small type (Entod.)
(1) Blue Wildebeest	(1) Eudiplodinium neglectum forma GIGANTIUM Dog. (1925) (2) Anoplodinium bubalidis forma consors Dog. (1925)	Entodinium simplex Dog. (1925).
(2) Blue Wildebeest	(1) Eudiplodinium neglectum forma gigantium Dog. (1925) (2) Anoplodinium bubalidis forma bubalidis Dog. (1925).	Entodinium simplex Dog. (1925)
(3) Sable antelope	Eudiplodinium maggii Fior. (1889)	Entodinium caudatum Stein (1859).
(4) Sable Antelope	Eudiplodinium magii Fior. (1889)	Entodinium caudatum Stein (1859)
(5) Sassaby	Eudiplodinium neglectum forma gigantium Dog. (1925).	Entodinium nanellum
(6) Waterbuck	(1) Eudiplodinium maggii (2) Ostracodinium gracili. forma gracili Dog. (1925).	Entodinium dubardi gracilicaudatum Buisson (1923).
(7) Reedbuck	(1) Ostracodinium gracili forma Dog. (1925). (2) Anoplodinium costatum forma minor Dog. (1925). (3) Eudiplodinium maggii.	E.caudatum Stein (1859)
(8) Steenbuck	(1) Epidinium caudatum forma Fior. 1889 quadricandatum Sharpe (1914) (2) Anaplodinium costatum major Dog. (1925).	E.triacum Buis. (1923) forma triacum Dog.
(9) Impala	Epidinium ecaudatum Fior. (1889) forma caudatum Fior. (1889)	E.simplex Dog. (1925).
(10) Impala	do. do.	do.
(11) Impala (E.R.)	do. do.	do.
(12) Kudu	(1) Eudiplodinium neglectum Dog (1925). forma impalae Dog. (1925). (2) Epid. ecaudatum Fior. (1889) form undescribed.	do.
(13) Duiker	Eudiplodinium species undescribed	Entodinium nanellum.
(14) Duiker	Same as for No. (13)	Entodinium nanellum.
(15) Klipspringer	Eudiplodinium species undescribed different to Nos. (13) and (14)	Entodinium triacum Buis. 1923 forma triacum Dog.

finely sifted yellow maize meal were given through the fistula tube of each sheep at the desired time. One minute afterwards material was withdrawn and immediately examined microscopically. Further samples were collected at thirty minute intervals, and later at hourly, and longer intervals, and examined without delay. In this way a complete picture was obtained of the process of digestion of starch. The amount of starch dosed was small and quickly ingested by the infusoria so that uningested starch grains were only occasionally found after an hour or more.

It could therefore be safely assumed that the infusoria studied by periodic withdrawal from the rumen had ingested the starch at or soon after the time of dosing. With doses of 10 and 20 grams of mealie meal, free partially digested starch granules can be found in the rumen 18 hours later.

As the results of the periodic examinations at different times and with different sheep were all in very close agreement, only one such report will be given here. The average rate of digestion is illustrated better by the photomicrographs submitted in plates 1 and 2, and the drawings in plate 3.

Rate of digestion of 2 grams of fine yellow mealie meal.

9.10 a.m. Ruminal ingesta withdrawn and examined by staining with Gram's iodine. Both Entodinium species and Diplodinium species appeared hungry. Some of the Diplodinium contained cellulose material.

9.15 a.m. Dosed 2 grams yellow mealie meal.

9.16 a.m. (1 minute). Material withdrawn and examined. Most organisms had already ingested starch grains, particularly the Entodinium, some containing up to 7 grains.

9.45 a.m. (30 minutes). Practically every organism contained one or more starch grains, some being completely engorged and distended. No evidence of glycogen.

10.15 a.m. /

10.15 a.m. (1 hour). Brownish-red granules are appearing in most infusoria.

10.45 a.m. (1½ hours). Many more brownish-red granules are present now.

11.45 a.m. (2½ hours). Organisms previously engorged with starch grains are now packed with glycogen-like granules.

12.45 (3½ hours). Starch grains are now becoming obscured by brown granules.

2.45 (5½ hours). In engorged organisms no change observed. Those with one or two grains only, show signs of disintegration of the starch comparable to karyolysis, with many brown granules around the disintegrating grain.

4.45 p.m. (7½ hours). The processes are much more advanced.

9.45 p.m. (12½ hours). Disintegration of the starch grain is now taking place as in simple diastatic digestion of starch. There is complete loss of its original globular form and staining affinity. The deep violet changes to a pale blue and then to bluish-brown. Masses of brown granules are now present in ecto- and endoplasm giving the organism a deep dark-brown granular appearance (see plate No. 3).

9 a.m. On following day (24 hours). Disintegration of the starch granule was completed. Brown granules are markedly reduced in the organisms. Colourless, transparent granules are now seen in increasing numbers in the regions previously occupied by brown granules.

3 p.m. (30 hours). Still fewer brown granules.

9 p.m. (36 hours). Glycogen granules clearing up rapidly.

9 a.m. next day (48 hours). All brown granules have disappeared and numerous colourless transparent granules have taken their place.

Discussion and Conclusions:

(1) Within 48 hours after ingestion the maize starch is completely digested and utilised within an infusorium.

This/.....

This confirms the results obtained in vitro by Trier (1926). Mangold (1929) quotes Trier extensively to explain the ways in which starch granules are ingested by infusoria. Trier found that as a result of intracellular digestion within the organism glycogen granules appear in their ectoplasm. He thus assumed that there was an intracellular synthesis of glycogen by the infusorium which is utilised by the organism itself. The infusorium is said to perform this breakdown of starch by an endogenous diastatic enzyme. It was however found in the course of this study, that free starch grains within the rumen, when not ingested by infusoria, are digested at the same rate by being directly attacked by bacteria. It should be noted that according to Scheunert and Trautmann (1921) the saliva of the ruminant does not contain a diastatic enzyme as suggested by Westphal (1934).

Furthermore, by adding 1% glycogen or maltose to ruminal juice containing starved infusoria and incubating at 39°C for one or two hours with periodic shaking, it was found that infusoria do take in fluid material from their surrounding medium, as in less than an hour after adding the sugar, glycogen granules began to form within the organism. After three or four hours the organisms were packed with brown granules similar to those seen after a heavy starch meal.

By staining fresh preparations of rumen ingesta intravitally with Janus Green, it could be established beyond doubt, that the so-called glycogen granules as well as the colourless transparent granules were actually bacteria situated within the foodsack and plasma of the infusoria. Most of these bacteria show typical Brownian movement and change their position within the ectoplasmic cavity. Large numbers of bacteria mixed with debris are present in the foodsack or body cavity and are rotated by the energetic movements

of/.....

of the membranelles. With ingestion of starches or sugars by the infusorium or food material containing starch or sugar, these substances are digested by enzymes secreted by the bacteria present there. Such bacteria as are able to synthesise glycogen within their own bodies utilise the products of digestion of the food material present and react to glycogen stains. The infusorium thus has within its body a process of digestion from which it derives definite benefits without any digestive contribution of its own excepting for its capacity as host. Ample proof of the advantages gained by the infusorium is afforded by the fact that rapid multiplication follows whenever the so-called "glycogen granules" appear in some measure after a feed. The fact that uningested starch grains are digested by free ruminal bacteria at the same rate as starch grains within an infusorium, proves that no enzymatic contribution is made by the infusorium itself towards the digestion of starch, and that it is wholly dependent upon ruminal bacteria and bacterial action for its own nourishment and the synthesis of glycogen within its body. It is probably for this reason that Westphal could not keep or promote multiplication of ruminal infusoria in cultures formany length of time without the daily addition of fresh ruminal juice.

The question as to how these bacteria gain entrance from the foodsack to the ectoplasm is still to be investigated. The fermentation products of starches, cellulose and sugars bathe the organisms in the foodsack and probably reach those in the ectoplasm by simple diffusion out of the foodsack.

The synthesis of glycogen by bacteria, once the necessary substrata are available, is a common occurrence in the rumen as numerous bacteria and moulds in the ruminal juice show typical glycogen staining after a meal of glucose,

maltose/.....

maltose or starch. After a time, if no more of these substances are available, the glycogen containing bacteria and fungi lose their staining affinity, and become colourless, the larger ones becoming transparent, as in the case of those trapped by the infusoria.

(5) CHEMICAL DATA ON THE INFLUENCE OF INFUSORIA ON STARCH DIGESTION WITHIN THE RUMEN.

That infusoria are intimately linked up with the digestion of starch within the rumen is the natural conclusion drawn at first sight considering the large numbers of these organisms consuming a considerable amount of starch when available. However, the results of the previous experiment nullify any significance that infusoria may have been believed to have in starch digestion. The following experiment was planned in order to confirm this. It was decided to compare the rate of disappearance of a given quantity of finely sifted yellow mealie meal dosed into the rumen of a sheep containing its normal infusorial population, with the rate of disappearance from the same animal after sterilising its ruminal fauna of infusoria.

For this purpose two sheep were selected and placed on a grass hay diet (No. 8) containing no chemically detectable starch. An amount of 20 grams of mealie meal was then dosed daily through the fistula tube in order to establish an infusorial population of 800-1000 per cubic millimetre. When this was reached samples of ruminal contents were withdrawn for quantitative chemical determination of starch 10 minutes, 5 hours, 9 hours, 12 and 15 hours after dosing. The extraction procedure described by C.S. Hanes (1936) and the chemical methods of Edwards et al (1938) were followed.

After/.....

After a series of analyses with satisfactory results the animals were sterilised of their infusoria by dosing each with 2 grams of copper sulphate in 2% solution on three consecutive days following 24 hours starvation and allowing water ad libitum. Microscopic examinations over a period of ten days were all negative. Not only were all the infusoria exterminated but also the starch attacking cocci previously present, and of which pure cultures had been obtained. These organisms were cultivated again in the rumen of both sheep by inoculating each of them with fairly heavy cultures from six plates. Mealie meal (20 grams) was dosed daily as before, to encourage the bacterial culture to develop. When these organisms could be seen attacking starch grains as before, sampling was commenced and continued until conclusive results were obtained. See Table 8.

Discussion and Conclusions:

(a) Owing to the anatomical structure of the forestomachs it is impossible to collect for any period of time, material passing from the rumen and reticulum to the omasum and abomasum through the omasal groove. Hence the quantity and composition of ingesta passing through the omasal groove is unknown. For this reason it was impossible to determine the amount of starch which passed out of the rumen undigested, so that the estimations were confined not to the rate of digestion of starch but to the rate of disappearance from the rumen. This includes the amount of starch digested in the rumen as well as that passed out of the rumen. Under controlled conditions of feeding and watering the latter could be taken as constant over periods of 12 or 24 hours.

(b) In both sheep 20 grams of yellow mealie meal had completely disappeared by the 15th hour when the rumen had an infusorial population of 800-1000 organisms per cubic millimetre. After sterilising the rumen of both sheep from their

infusoria/.....

TABLE 8,

RATE OF DISAPPEARANCE OF STARCH FROM RUMEN.

Date.	Amount of starch at different periods after dosing.					Remarks.
	10 minutes	5 hours	9 hours	12 hours	15 hours	
I. Sheep 49.						**Note:** The amount of starch is expressed in grams per 100 grams ingesta.
27/11/39	0.130			0.018	0.10	An iodophilic coccus was noticed to be present
29/11/39	0.117			0.054	0.32	in fairly large numbers and competing with
1/12/39	0.156			0.016	Negative.	the infusoria in the breakdown of starch.
15/12/39	0.120			0.037	0.012	
17/12/39	0.083			0.033	Negative	
19/12/39	0.122			0.026	"	
21/12/39	0.148			0.014	"	
	After sterilisation with $CuSO_4$ and inoculation with iodophilic bacteria.					On 30/12 the sheep was inoculated intra-
						ruminally with 6 plates of pure culture of the
2/ 1/40	0.073			Trace	Negative	iodophilic coccus which was destroyed in the
4/ 1/40	0.053			"	"	rumen by $CuSO_4$.
6/1/40	0.074			0.014	"	
II. Sheep 43.						
23/ 2/40	0.083	0.059	0.020	-	Trace	An iodophilic coccus similar to the type seen
26/ 2/40	0.087	0.017	0.008	-	"	in sheep No. 49 as well as an iodophilic
28/ 2/40	0.069	0.036	0.016	-	"	bacillus were present in fairly large numbers
1/ 3/ 40	0.058	0.022	Trace	-	Negative	attacking starch grains.
3/ 3/40	0.059	0.039	0.025	-	Trace.	
	After sterilisation with $CuSO_4$ and inoculation with iodophilic bacteria.					On 10/3/40, sheep was inoculated with 6 plates
						of pure culture of iodophilic organisms as in
13/ 3/40	0.076	0.012	Negative	-	Negative.	the case of sheep 49. No culture being
15/ 3/40	0.076	0.041	0.011	-	Trace.	available the bacillus could not be inoculated
17/ 3/40	0.055	0.025	Trace	-	Negative	in the rumen, it having also been destroyed by
19/ 3/40	0.089	0.054	0.014	-	Trace.	the $CuSO_4$.
21/ 3/40	0.085	0.062	Trace	-	Negative.	

infusoria and inoculating the rumen with cultures of starch splitting cocci usually present, but destroyed or inhibited by $CuSO_4$ dosage, there was no decrease in the rate of disappearance of the same quantity of mealie meal.

If infusoria did play a rôle in the digestion of starch one would have expected to find significant undigested starch residues after 15 hours owing to the absence of infusoria to digest it. It is, however, clear that the function of these organisms is taken over entirely by the starch splitting bacteria and moulds of the rumen.

(6) OBSERVATIONS ON THE EFFECT OF INFUSORIA ON CELLULOSE DIGESTION.

The rate of digestion of crushed lucerne stalks was determined first in sheep harbouring the normal infusorial population and subsequently in the same sheep freed of infusoria. For this purpose silk bags, as described by Quin, van der Wath and Myburgh (1938) were used. Known weights of a sample of crushed lucerne stalks were suspended through the fistula tube by means of a silk thread and exposed to ruminal digestion for periods of 24 and 48 hours. Duplicate bags were suspended each time, one being withdrawn at 24 and the other at 48 hours. The sheep were kept on an adequate dry lucerne hay diet during the experiment. Analysis for average percentage cellulose in residues were made and compared with the percentage cellulose present in the homogenous stock from which the samples were taken.

The experiment was done first in two sheep, the paunches of which were sterilised of infusoria with $CuSO_4$ three weeks before. Subsequently these sheep were infected with infusoria and when the fauna was well established the experiment was repeated.

Table 9 shows the results of the analyses.

Discussion/.....

Discussion and Conclusions:

As seen from the analyses the results tend to show an increase in the amount of cellulose digested within the first 24 hours when infusoria were present in the rumen. It was, however, found in the course of another experiment on the same and other sheep with normal unsterilised fauna, that the rate of cellulose digestion may vary by more than 5% in 24 hour periods under controlled conditions of feeding and watering. The results therefore do not justify at this stage the conclusion that infusoria have a beneficial effect on cellulose digestion. For statistical purposes the experiment should be extended to include several more animals.

From the table it appears that the rate of cellulose digestion is uneven over a period of 48 hours. An average of 13.2% cellulose was digested within the first 24 hours, whereas an average of 15.6% was digested over a period of 48 hours. Thus during the second 24 hour period, 2.4% only was digested. This decrease in rate of digestion is probably due to the fact that the portion of cellulose more exposed to attack by bacteria and enzymes is broken down first, and that the more incrustated and deeper seated cellulose is only gradually reached by digesting influences. It was clearly demonstrated by Baker and Martin (1937) that in the caecum of the horse and the rabbit, and in the rumen of the sheep (1938), cellulose particles are attacked by specific organisms which either adhere to the surface or penetrate into the substance of these particles. In addition there is the possibility that ruminal fluid may be rich in enzymes secreted by cellulose digesting bacteria and which bathe the cellulose particles.

The mechanisms employed in ruminal digestion of cellulose is therefore on a par with ruminal digestion of

starch/.....

starch. The digestion of cellulose within the body of the infusorium is also considered to be primarily due to the enzymes of cellulose digesting bacteria ingested by the infusorium. On this basis it is believed by the author that the ciliates contribute nothing towards the ruminal digestion of cellulose. This supports Mangold's theory that cellulose breakdown does occur within the infusoria but by means of similarly ingested cellulose digesting bacteria.

TABLE 9.

RUMINAL DIGESTION OF CELLULOSE.

Sheep No.	Condition of rumen.	Period of exposure to digestion.	Number of periods exposed.	Average % total loss	Average % loss due to leaching.	% Cellulose in sample. a.	Average % cellulose in residues calc. on original weight of sample. b.	Average % cellulose digested. (a - b).
58	Free from	24 hours	4	41.2	25.5	40.9	30.0	10.9
59	infusoria	24 "	4	41.0	25.5	40.9	28.3	12.6
58	do.	48 "	3	46.8	25.5	40.9	26.1	14.8
59	do.	48 "	3	49.9	25.5	40.9	23.8	17.1
58	Infected with	24 hours	3	40.3	25.5	40.9	27.0	13.9
59	infusoria	24 "	3	43.6	25.5	40.9	25.7	15.2
58	do.	48 hours	3	43.3	25.5	40.9	26.9	14.0
59	do.	48 "	3	48.5	25.5	40.9	24.1	16.8

PLATE I. Infusorial digestion of starch: Gen. entodinium.

1. Hungry Entodinium X 500.
2. Freshly ingested starch granule. X 220.
3. Brown granules gathering within infusorium after 1 hour. X 220.
4. Increased brown granules after 2 hours.
5. Complete obliteration of structure by brown granules - 6 hours.
6. Granules losing their iodophilic reaction - 18 hours.
7. Further advanced stage after 24 hours. At 48 hours the granules are usually translucent again.

PLATE II. Infusorial digestion of starch: Gen. Diplodinium.

1. Hungry Diplodinium. X 270.
2. Freshly ingested starch granule. X 270.
3. Brown granules gathering 1 hour after ingestion of granule. X 270.
4. Brown granules increased 2 hours after feeding.
5. Some freshly ingested starch granules 6 hours after initial feed.
6. Brown granules masking infusorial structure after 18 hours.
7. Granules clearing up after 24 hours.

PLATE III. Photo of coloured plate showing digestion of starch granule within an infusorium. Gen. Entodinium.

a = anus.

s = starch granule.

n = nucleus.

m = mouth.

d = glycogen containing bacteria.

e = discharge through anus.

Digestion cycle:

1. Hungry infusorium containing some transparent bacteria.

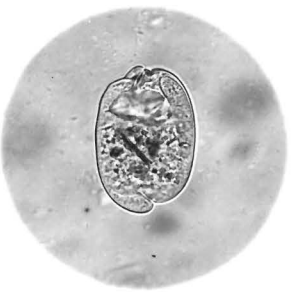
2./.....

2. Infusorium with freshly ingested starch granule.
3. Engorged infusorium showing commencement of bacterial synthesis of glycogen.
4. Synthesised glycogen staining brown in the bacteria contained within the infusorium.
5. Disintegration of starch granule commenced.
6. Accumulated masses of glycogen containing bacteria with expulsion of some from anus.
7. Complete disintegration of starch granule leaving only a dark mass of iodophilic bacteria.
8. Almost complete metabolism of glycogen by bacteria.

PLATE I.

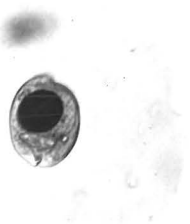
INFUSORIAL DIGESTION OF STARCH : GENUS ENTODINIUM.

1



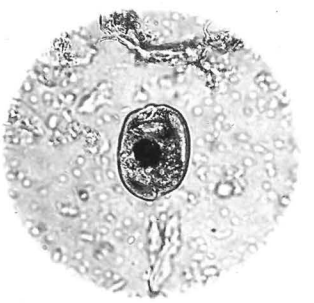
X 270

2



X 220

7



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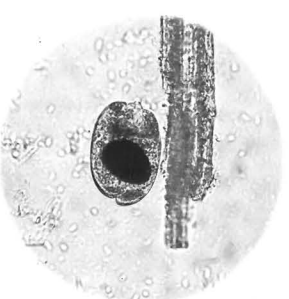

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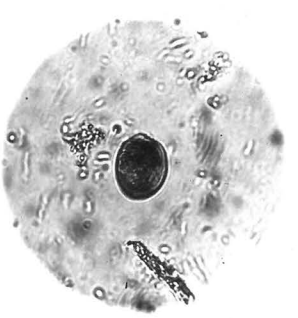

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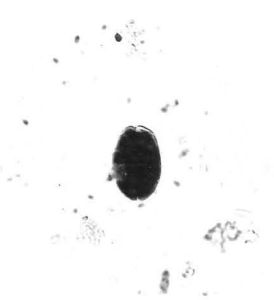
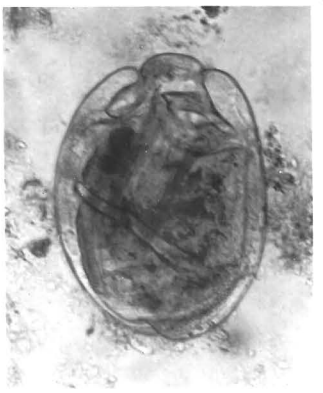


PLATE 2

Infusorial Digestion of Starch: Genus Diplodinium.



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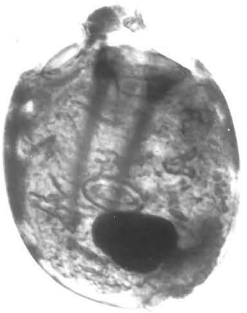

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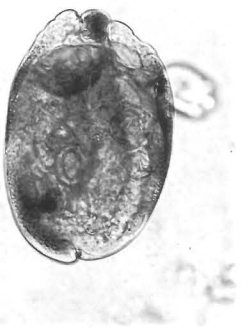

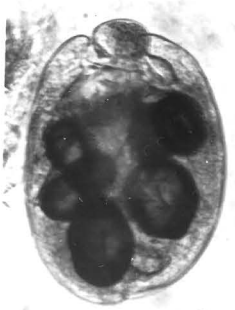

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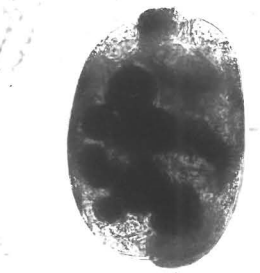

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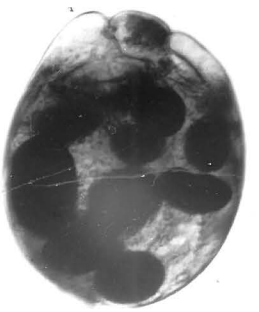
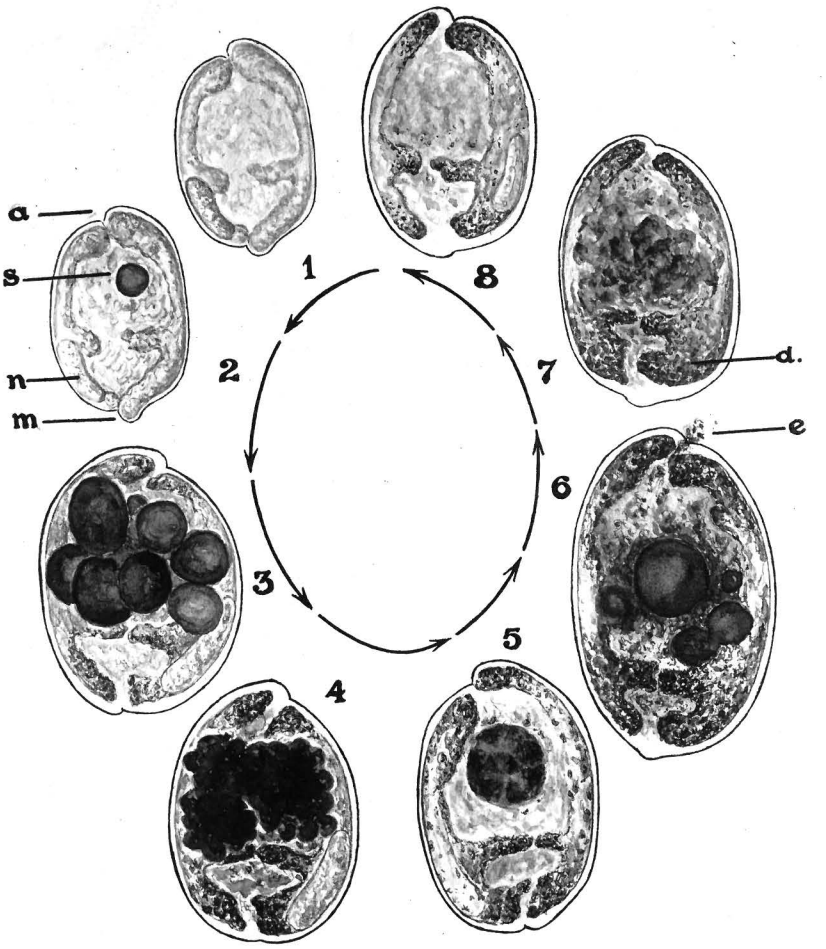


PLATE 3.

PHOTO OF COLOURED PLATE SHOWING DIGESTION OF STARCH GRANULES WITHIN AN INFUSORIUM.

X 220



III. B. (1)

BACTERIAL POPULATIONS IN RELATION TO DIET WITH PARTICULAR REFERENCE TO THE NITROGEN, CARBOHYDRATE AND MINERAL LEVELS OF THE FEED.

1. Literature.
2. Experimental.
3. Discussion of experimental results.
   (a) the influence of the nitrogen and carbohydrate content of the diet on the number of ruminal bacteria present.
   (b) the influence of minerals on the ruminal microflora.
4. Conclusions.

1. Literature.

Hart et al (1939) studied the effects of the substituion of urea for the protein portion of the food, and attempted to draw conclusions in regard to the number of ruminal bacteria in their experimental animals by making bacterial cultures from ruminal contents taken after death of the animal. They concluded "that no essential qualitative or quantitative differences were revealed". The culture technique used by these authors was not described. This is the only reference traced in the literature relevant to the determination of the number of bacteria in the rumen of animals. Reference to the ruminal bacteria has been made freely by several research workers; the amount of digestible protein derived from rumen bacteria has even been calculated by Schwarz (1925). Notwithstanding all this interest and activity no technique for the counting of these micro-organisms has as yet been evolved, and consequently no information is available in regard to the variety and density of the ruminal bacteria.

2. Experimental.

In order to ascertain the relationship existing between the number of bacteria in the rumen and their

nutritional/.....

III. B. RUMINAL BACTERIA.

(1) Bacterial populations in relation to diet with particular reference to the nitrogen, carbohydrate and mineral levels of the feed.

(2) The digestion of starch by ruminal bacteria.

(3) Cellulose digestion as influenced by

(a) the bacterial population of the rumen.
(b) the lignification of plant tissues.
(c) the nitrogen and carbohydrate content of the feed.

(4) The possibility of cystine synthesis in the rumen and its relationship to nitrogen metabolism.

(5) The utilisation of urea by ruminal micro-organisms.

nutritional requirements, it was deemed advisable to tabulate the different rations that were fed to the sheep over extended periods during which counts of the ruminal bacteria were regularly undertaken (table 10). Counts were made according to the technique described above. Groups comprising five to twenty sheep at a time were used and the average bacterial counts for the groups taken during the different periods. As a rule there were very small individual variations in the bacterial counts, excepting when a sheep went off its feed, or if, for some reason or another it developed unfavourable conditions in the rumen.

It will be observed from the data given below that a number of the rations were actually used as experimental rations in other sections of this work and will be discussed again, though from another point of view.

Three different basal rations consisting of wheat straw, mature veld hay and lucerne hay were employed throughout. These were fed as roughage, firstly, without supplementation and afterwards were supplemented by various feeds, e.g. urea (a non-protein-nitrogen compound), minerals in the form of bone meal, an amino acid viz cystine, dextrinised starch, yellow crushed maize, meat meal and white fish meal. Observations made on sheep running on natural pasture during different seasons of the year are also included.

3. Discussion of Experimental results.

(a) The influence of the nitrogen and carbohydrate content of the diet on the number of ruminal bacteria present.

According to a statistical analysis of the bacterial populations present in the rumen under different experimental

condition/.....

conditions, the difference between any two counts must be of the order of 255 x 10^6 per cubic centimetre to be of significance. This will be borne in mind in the discussion of these results. From table 10 it appears that sheep on a daily diet of 600 grams of wheat straw only, had a bacterial population of 580 million per cc. Supplementation of this diet, which contained approximately 0.3 per cent. nitrogen (protein = N x 6.25), with 200 grams of dextrinised starch, which is practically free of nitrogen, produced no significant increase in the bacterial population. When, however, 5 grams of urea (= 2.33 grams of non-protein-nitrogen) was added to the wheat-straw-dextrinised-starch ration, there was a significant response and the bacterial flora increased by 74 per cent. On substituting white fish meal containing a corresponding amount of nitrogen, for the urea, the bacterial population increased by 206 per cent.

As the diet composed of wheat straw was very low in nitrogen as well as in starch, it could not maintain the bacterial population at the normal level. The bacterial population varies with the diet, but as will be seen from the table, a wheat straw diet correctly supplemented (e.g. trial No. 4) should maintain a flora of approximately 1800 million per cc. Consequently there appears to be little doubt that with sufficient carbohydrate present, nitrogen was the limiting factor to bacterial proliferation. This is further borne out by the counts observed in feeding trials Nos. 7, 8, 9 and 10. In trial 7, only 7.7 per cent. of maize was added to the ration compared to 17.0 per cent. of meat meal containing 80 per cent. of protein. This constituted a ration deficient in starch, with an excess of nitrogen. A bacterial count of 1200 million, which is well below the

normal/.....

normal count expected was observed. On increasing the percentage of maize to 30, that is, to a more or less normal level, and maintaining an excess of meat meal, the bacterial growth was still depressed. An excess of nitrogen, therefore, exercised an inhibitory influence on bacterial proliferation, not quite to the same extent as in the case of a nitrogen deficiency.

A further trial (No. 9) was undertaken to observe the effects of a diet containing the amount of carbohydrate normally required and supplemented with twice the necessary amount of nitrogen. In this instance the bacterial population increased by 46 per cent and reached the same level as was obtained on a balanced diet of the same components in trial No. 10. It is interesting to note that the experimental animals actually lost weight (on an average 2 lb. per sheep) during feeding trials number 7 and 8, although difficient nutrients were ingested by them at least to maintain their body weight. An excess of nitrogen in the form of meat meal therefore proved harmful not only to the ruminal flora but also to the host animal itself.

With regard to the influence of carbohydrates on the bacterial population, it was found that the addition of 200 grams of dextrinised starch to a diet of 600 grams of veld hay did not improve the bacterial population significantly (trials 12 and 13). With the addition of 100 grams of maize to a veld hay diet and sufficient meat meal to keep the sheep in nitrogen equilibrium, the bacterial flora doubled itself. With a further increase in carbohydrate to 200 grams and a reduction in meat meal (trial 18), to keep the nitrogen level constant, the bacterial population increased slightly from 1785 to 1964 million per cc. On feeding at a 300 gram level of maize, which now in itself

provided/.....

provided sufficient nitrogen for maintenance, a decreased bacterial proliferation was brought about. The population was lowered to 1567 million, i.e. by 24 per cent. Sheep which continued receiving a small amount of meat meal together with a large maize supplement showed a similar depression. It appears therefore that of the different levels at which maize was supplemented, the amount of 200 grams had the most favourable influence on bacterial growth, provided adequate nitrogen was present. As is the case with nitrogen, both a deficiency and an excess of carbohydrate had an inhibitory influence on bacterial proliferation. Optimal conditions for bacterial growth in the rumen are therefore created when the animal receives a balanced ration as in period 6, table 15.

TABLE 10.

Bacterial populations in relation to different diets.

I. Wheat straw.

Feeding trial No.	Diet	Ration No.	Bacterial count per cubic centimetre. X 10^6
1	Wheat straw (600 grams)		580
2	" + dext. starch (200 grams)		612 and 837
3	" + dext. starch + urea	11	1068
4	" + dext. starch + white fish meal	13	1875
5	" + dext. starch + bone meal.	18	1106
6	" + bone meal	19	1094
7	" + maize + meat meal	14	1200
8	" + " + meat meal	15	1244
9	" + " + " "	16	1818
10	" + " + " "	17	1890

II. Veld Hay/.....

II. Veld Hay.

Feeding trial No.	Diet	Ration No.	Bacterial count per cubic centimetre. X 10^6
11.	Natural veld ad lib. (table No. 5).		
	(a) July (midwinter)		1067
	(b) January (summer)		1869
12	Veld hay (600 grams)		980
13	" " + dext. starch (200 grams)		991
14	" " + dext. starch + bone meal.		1254
15	" " + bone meal		1102
16	" " + meat meal	Period 2, table 15.	1557
17	" " + maize (100 grams) + meat meal.	Period 4 table 15.	1785
18	" " + maize (200 grams) + meat meal.	Period 6, table 15.	1964
19	" " + maize (300 grams) in excess.	Period 7, table 15.	1567

III. Lucerne.

20	Dry lucerne hay (500 grams)					1956
21	" " + maize (300 grams)	--	9	--		1874
22	" " + cystine (1 gram).					2330
23	Green lucerne (800 gm.)					1571.

Sheep on a dry lucerne hay diet showed no significant changes in the number of their ruminal bacteria when the ration was supplemented with maize. This is probably due partly to the fact that the composition of a good lucerne hay approaches that of a balanced ration for mature sheep. Lucerne is, however, known to be low in cystine (Haag, 1931); supplementation of lucerne hay with one gram of cystine daily stimulated bacterial proliferation from 1956 million to 2330 million, i.e. an increase of 20 per cent. This indicates

that/.....

that a cystine deficiency had a limiting influence on bacterial multiplication.

(b) <u>The influence of minerals on the ruminal microflora.</u>

While some organisms multiply on a comparatively simple medium, it is usually necessary to supply protein, carbohydrates, and mineral salts if a profuse growth of pathogenic bacteria is required. For the optimal growth of ruminal bacteria, the majority of which are probably non-pathogenic, one would also expect that at least these three main constituents of protoplasm would be required in the medium. A deficiency of either nitrogen or carbohydrate has been shown above to have an inhibitory influence on the ruminal organisms. The influence of the third factor viz. minerals, will now be discussed on the basis of the results tabulated above.

On a diet of veld hay supplemented with dextrinised starch (trial 13), the average bacterial count was found to be 991 million. Both the hay and the starch was deficient in phosphates. After feeding 10 grams of bone meal daily (P_2O_5 content = 20 per cent.), to the sheep for 10 days, the average counts were found to have increased to 1254 from 991 million, i.e. by 26 per cent. over a period of several weeks. On repetition of the experiment, using wheat straw instead of veld hay (trial 5), the bacterial population increased from 837 to 1106 million per cc., that is by 32 per cent. The increase in both trials was definitely significant. The ration of mature veld hay is comparable to that received by the majority of farm animals in large areas of the Union during autumn and winter, so that, although there would be no improvement in the amount of cellulose digested by the

augmented/.....

augmented bacterial flora (vide section III B (3)), the animal would at least benefit to the extent of an increased amount of bacterial protein in the rumen. If the same basis of calculation is used as in Section III.B (4), a sheep would derive approximately 2.55 grams more protein per day from its ruminal bacteria, should it be supplemented with 10 grams of bone meal daily. The increased bacterial protein must, however, be derived in part at least from other proteins already present in the rumen ingesta and bone meal, so that the effect of bone meal on the rumen flora would be to provide an increased amount of easily digestible protein at the expense of other less easily digestible proteins. Whether this is of significance to the animal in such small amounts is doubtful.

Results of experiments conducted by McAnally and Maclean (1935) indicated that yeasts could synthesise much more glycogen and other polysaccharides from available sugar when phosphates were added to the cultural medium. It is probable therefore, that the addition of phosphates to the diet of ruminants would also increase the capacity of the ruminal microflora to synthesise such polysaccharides. This would be beneficial to the host animal in so far as it would lead to the conservation of an increased amount of carbohydrate which would otherwise undergo complete oxidation in the rumen. This would apply particularly to a feed like lucerne in which carbohydrates are present mainly as sugars which are easily fermentable and rapidly oxidised. With this loss of energy the ruminant probably does not derive the full benefits of the sugar stored in lucerne. Attempts to produce fat lambs in the Union on lucerne have failed so far, notwithstanding the fact that lucerne theoretically contains sufficient nitrogen and soluble carbohydrates to produce fat lambs. The cause of this failure has however, not yet been elucidated.

III./.....

III. B. (2).

BACTERIAL DIGESTION AND SYNTHESIS OF STARCH.

1. Literature.
2. Bacterial disintegration of starch granules within the rumen.
3. Rate of disintegration of maize starch
   (a) in rumen with developed flora,
   (b) in rumen with undeveloped flora.
4. Rate of disintegration of starch granules of different cereals.
5. The digestion of starch in the rumen.
6. Types of ruminal iodophilic micro-organisms and their reaction to alcohols and carbohydrates.
7. Isolation of an iodophilic streptococcus and some of its characteristics.

1. Literature.

A low-power microscopic examination of rumen ingesta shows that infusoria are the most conspicuous organisms. A more detailed examination, however, also reveals the presence of large numbers of non-pathogenic bacteria, yeasts, fungi and spirochaetes constituting the microflora of the rumen. Non-pathogenic bacteria are of great significance in biology. The fertility of the soil, production of silage, and the digestion of masses of fibre in the rumen and large intestines of herbivorous animals are processes singularly dependant upon a microflora and its products. Thus the soil carries its own microflora, through which life and fertility are either promoted or alternatively depressed by the production of toxins (Neilson-Jones, W., 1941).

Similarly the production of good silage is dependant upon the definite reaction which is determined by the microflora present and which in turn is controlled by

the /.....

the composition of the materials (Smith, 1938). The rumen and the large intestines of herbivores are large storage organs in which micro-organisms digest cellulosic structures and make available to the host animal previously encrustated food materials such as pectins, proteins and carbohydrates (Meyer, W., 1927). Meyer has shown that in this process the cell membranes first become swollen, then they appear lighter in colour, and with progressive digestion the cellulose lamellae become torn, cavities are formed and disintegration sets in. The bacteria are seen surrounding the cells and later penetrating into the interior where the digestive process is continued.

Baker and Martin (1938) also investigated the disintegration of cell-wall substances in the gastro-intestinal tract of herbivora. According to them disintegration is evidenced by the formation of clearly defined zones of erosion, with changes in microchemical and staining reactions associated with micellar disorganisation. Cellulose, hemicelluloses and pectic substances are dissolved, while cutin and heavily lignified structures entirely resist attack. In the caecum of the guinea pig, the rabbit and the horse, as well as in the rumen of the sheep, the primary agents of this disintegration were found to be iodophilic micro-organisms, giving a blue (amylodextrin) reaction with iodine. There is very little information to be found in the literature on the digestion in the rumen of the remaining foodstuffs, namely protein, fats and starch. Mangold and Schmitt-Krahmer (1927) demonstrated bacterial digestion of fats in the rumen. Ferber (1928) showed that infusoria readily ingested fat droplets, and that these fats were digested within the organism by bacteria.

Baker and Martin (1933) observed that in the

caecum/.....

caecum of the horse there was a deposition of polysaccharides in the indigenous micro-organisms. The indication is therefore, that these organisms had the ability to form starch-like polysaccharides within their own bodies, from the surrounding medium containing cellulose and other encrustated foodmaterials, or other fermentation products.

Since no researches on bacterial digestion of starches in the rumen are recorded, this study was undertaken in an attempt to elucidate some aspects of this problem.

2. BACTERIAL DISINTEGRATION OF STARCH GRANULES WITHIN THE RUMEN.

Eight fistula sheep were placed on a wheat-straw diet without any chemically detectable starch. On this diet the multiplication of starch digesting bacteria in the rumen could be controlled either by the addition or the reduction of starches or sugars.

The sheep were also freed from infusoria by copper sulphate administration some weeks before, so as to have all the starch administered into the rumen available for undisturbed bacterial digestion only.

Smears were made daily from rumen ingesta, withdrawn through the fistule, by adding a drop of Lugol's iodine to a drop of ruminal ingesta on a slide and covered by a coverslip. These preparations were then examined for starch granules. When no more granules could be seen, 5 grams of maize starch was administered into the rumen of each of four sheep at a time. After this, small samples (2 cc.) of rumen ingesta were withdrawn hourly to follow up the process of disintegration of the starch granules. The preparation of smears was usually continued until the starch was completely disintegrated.

The mode of attack and disintegration of the

starch/.....

starch granules is clearly illustrated in plate IV. Fig. 2 shows a few slightly mauve cocci adjacent to the starch granule, one hour after administration of the starch into the rumen. Fig. 3 shows a starch granule with double the number of mauve cocci around it, photographed one hour later. Fig. 4, taken three hours later again, shows an uncountable number of mauve and dark mauve organisms surrounding the starch granule.

Fig. 5, taken seven hours later illustrates another granule with almost a pure culture of iodophilic streptococci surrounding it. The typical disintegration of a starch granule is well illustrated. It is excavated from the sides and there are bacteria filling these craters and enlarging them until the granule breaks up into fragments.

In Fig. 6 the remnants of such a granule are seen after having been in the rumen for 24 hours. Only a mass of dark mauve and brown cocci is left. Some of these cocci are already losing their iodophilic reaction. Three or four hours later most of them will have become translucent again unless fresh starch granules or sugars are introduced.

Although in most instances there were numerous iodophilic organisms of various types present in the rumen, the disintegration of starch granules was mainly brought about by an iodophilic streptococcus usually present in large numbers. The disintegration followed a definite pattern.

By incubating sterilised starch granules with bacteria-free ruminal fluid obtained both by centrifugation and filtration (Zeitz filter and collodion membranes), disintegration of starch granules could not be produced. On the other hand, when incubated in a synthetic medium, similar to that used by Woodman and Stewart (1928), in their researches on cellulose digestion, with cultures of the

iodophilic/.....

iodophilic streptococcus isolated from the rumen, starch was attacked as in the rumen, without noticeable evolution of gas.

Furthermore, disintegration of starch granules was never observed in the rumen if the granules were not attacked by iodophilic bacteria, mainly streptococci.

It seems, therefore, that in the absence of diastatic enzymes in the saliva of the sheep, other diastatic enzymes were either not present in the ruminal fluid, or otherwise too dilute to have any perceptible effect. Consequantly, the only diastases present in the rumen seem to be those secreted by ruminal bacteria, which would then be responsible for the entire starch disintegration seen here.

3. RATE OF DISINTEGRATION OF MAIZE STARCH GRANULES. IN THE RUMEN.

This is influenced by the presence in the rumen of:-

(a) A developed iodophilic flora.

(b) An undeveloped flora.

(a) Developed flora:

To promote the development of a starch digesting flora 100 grams of maize starch was administered daily for 10 days through the fistula tube of a sheep on a hay diet. After this the rumen was prepared for starch digestion trials by omitting the administration of starch 48 hours before commencement of the trial. After this period the rumen had become free of starch and the organisms had all lost their iodophilic reaction. Under these circumstances the rumen thus contains a rich iodophilic flora ready to attack any starch that may be administered.

On the day of the trial 100 grams of maize starch was introduced through the fistula of each of three sheep,

using/.....

using different sheep in subsequent trials. A small sample (2-3 cc.) of rumen content was withdrawn for microscopic examination at regular intervals of 30 minutes after administration of the starch. Microscopic preparations were made as in the previous experiment. The procedure was continued until neither starch nor iodophilic organisms could be seen in the preparations.

The results were briefly as follows:-

(1) After 30 minutes a few iodophilic streptococci were present around most starch grains, some of the organisms staining light mauve, whilst others were still translucent. Pseudo-yeasts (Fig. 5 (b), Plate V), dispersed in the medium, stained a pale-greenish-yellow with Lugol's iodine.

(2) After one hour the number of organisms were notably increased and more of them staining mauve.

(3) After 2 hours most starch grains were completely surrounded by iodophilic streptococci. A type of large iodophilic streptococcus forming rather long chains was usually visible from this stage onwards.

(4) After 3 hours layers of iodophilic streptococci were packed around practically all the starch grains with streptococcal chains and groups of tetracocci (Fig. 3, (b), Plate V) dispersed throughout the medium.

(5) After 5 hours disintegration of starch granules commenced.

(6) After 6½ hours disintegration of some starch granules was completed, and iodophilic bacteria were dispersed throughout the ruminal fluid in large numbers. (Plate V, fig. 3, (a), (b) and (c)). Both pseudo-yeasts and the cigar-shaped bacilli (Fig. 5(a), Plate V), commenced to show iodophilic reactions at this stage.

(7) After 18 hours only remnants of starch granules were

present/.....

were present. These were surrounded by masses of streptococci, most of which had become translucent again. The iodophilic micro-organisms that were dispersed throughout the ruminal fluid were rare because they had completely metabolised the polysaccharides responsible for their previous iodophilic reactions.

(8) After 20 hours there were neither starch residues nor iodophilic reacting bacteria. Starch disintegration was therefore completed in from 18 to 20 hours.

(b) Undeveloped flora.

For the purpose of these observations 8 fistula sheep on a hay diet without starch supplementation were used. Microscopic examination of their rumen ingesta revealed no iodophilic micro-organisms. The trials were carried out as in (a).

(1) After 1½ hours, compared with 30 minutes in (a), translucent bacteria were seen in contiguity with the starch granules.

(2) After 2½ hours the organisms reacted to iodine, i.e. 1½ hours later than in (a).

(3) Disintegration of starch granules commenced after 7 hours, i.e. 2 hours later than in (a).

(4) Disintegration was completed after 26-28 hours. In one sheep complete disintegration took 36 hours.

CONCLUSIONS.

In the rumen of sheep receiving a regular supply of starch in the diet the bacterial disintegration of starch granules commenced after 5 hours and was completed within 18-20 hours, whilst in the case of sheep not receiving starch in the diet disintegration commenced after 7 hours; it was much slower and took 8-10 hours longer. In the sheep receiving starch regularly, the bacterial flora concerned in the disintegration of starch is well developed and consequently more organisms are available to attack the starch

granules/.....

granules.

In the case of starch disintegrated by bacteria within infusoria, it was shown above that disintegration commenced after 5½ hours and was completed within 24 hours.

4. RATE OF DISINTEGRATION OF STARCH GRANULES OF DIFFERENT CEREALS.

Using the same technique as in the previous experiment, a series of experiments were conducted to determine the rate of disintegration of the various cereals that may be incorporated in the feeds of ruminants. According to measurements taken by the author in the course of this investigation, starch granules of the various cereals were found to differ in size and shape, vide Table 11, below. Taylor and Iddles (1926) have found that there is also a difference in composition of the various starches. The sizes and shapes of the granules in a cereal also vary, e.g. potato starch granules may vary in length from 9 to 72 microns. Their shape is either spherical or oblong. The granules of maize, wheat, oats, rice and peanuts have a much more uniform spherical shape.

In the table given below it is shown that starches with large granules are digested more slowly than those with small granules. Potato starch which has the largest granules, require 11 hours more than oats which has the smallest granules. The average maize starch granule has 1/3 the size of the average potato starch granule and is digested 10 hours sooner than the latter.

Table 11./.....

TABLE 11.

Comparative diameters of starch granules in microns and rate of digestion.

Starch	Largest granule.	Smallest granule.	Average granule	Time required for complete digestion.
Potato	72	9	35	28-30 hours:
Wheat	39	6	24	24-26 "
Maize	18	3	12	18-22 "
Peanut	15	3	9	18-22 "
Rice	15	3	6	17-20 "
Oats	12	3	6	17-20 "

From a statical analysis of the diameters of the different types of starch granules and the rate of digestion, a linear relationship was found to exist between the log. of the time and the diameter of the granule. The diameter of starch granules therefore definitely influences their rate of disintegration in the rumen.

5. THE DIGESTION OF STARCH WITHIN THE RUMEN.

Degradation of starch usually results in the formation of soluble carbohydrates like dextrins and sugars (Blake, 1916, Starke, 1942). It has been shown above that some of the products of starch degradation are resynthesised into glycogen and starch-like polysaccharides in certain ruminal micro-organisms. The polysaccharides thus formed are utilised by the organisms for their own metabolism as evidenced by the active division seen in the pseudo-yeasts during these stages.

As weak organic (fatty) acids, e.g. acetic and lactic acid have been found to be usually present in the

rumen/.....

rumen (Woodman, 1927), it is possible that these acids are fermentation products of some of the soluble carbohydrates formed during the process of starch hydrolysis. These acids are in turn changed into salts, e.g. acetates and lactates owing to the presence of large amounts of carbonates in the rumen.

The soluble carbohydrates are therefore partly resynthesised into glycogen and starch-like polysaccharides and probably also partly changed into weak organic acids and their salts. The percentage of starch formed into organic acids is unknown. According to Woodman only about 8% of the total digestible carbohydrate of the food in the rumen is broken down to the gas and organic acid stage. He assumes consequently, that approximately 8% of the sugar present in the rumen will undergo further bacterial breakdown, and that the rest of the sugar will be absorbed in the alimentary tract of the animal. Woodman advanced these arguments to support his theory that cellulose is broken down by bacterial digestion to cellobiose and thence to dextrose. He considers this comparable to the digestion of starch which is first hydrolysed to maltose, and then to dextrose, by enzyme action. Woodman's theory is therefore, that all carbohydrates in the rumen are ultimately broken down to dextrose and absorbed as such excepting for a possible 8% which are broken down by bacterial action to organic acids and gases.

Mangold (1929) opposes Woodman's theory and suggests that in the rumen, substances such as sugars, starches, and cellulose, ferment to form gases, organic fatty acids and alcohols. Neither Woodman nor Mangold, however, considered the possibility that the microflora may be able to reform starch-like polysaccharides and glycogen from the breakdown products of starches introduced into the rumen. They regarded the micro-organisms only as secretors

of/.....

hydrolytic enzymes assisting in the breakdown of organic matter in the rumen.

The amount of digestible carbohydrates formed into acids and gases in the rumen is difficult, if not impossible, to assess, because material is constantly passing out of the rumen to the omasum and abomasum, particularly during feeding (Quin and van der Wath, 1938). It is thus impossible to calculate at any one moment, how much of a substance placed in the rumen at a given time is still left in it at a later stage. Under these experimental difficulties it is therefore impossible to evaluate the amount of starch digested in the various ways in which it may be dealt with in the rumen.

A schematic presentation is given below of ruminal digestion of starch, based on the data obtained in this study as well as on some known facts about starch hydrolysis.

Starch administered into the rumen.

(a) Passed directly from rumen to abomasum and intestines - hydrolysis by digestive acids and enzymes. (Observations made on sheep with abomasal fistula).

(b) Formation of soluble carbohydrates in rumen by the action of bacterial diastatic enzymes and possibly also maltase of the endosperm.

(c) Starch and glycogen are synthesised in iodophilic organisms.

(d) Portion of sol. carbohydrate formed into weak organic acids (chiefly fatty acids e.g. acetic and lactic acid and gas (Woodman).

(e) Passed to abomasum and intestines and digested by proteolytic and other digestive enzymes.

(f) Production of heat, energy and gas in bacterial metabolism.

(g) Formed into the salts of these acids.

Passed to abomasum

6. TYPES/ .....

6. TYPES OF RUMINAL IODOPHILIC MICRO-ORGANISMS AND THEIR REACTION TO ALCOHOLS, AND CARBOHYDRATES.

In studying the digestion of starch in the rumen several types of iodophilic micro-organisms were observed when sheep were fed either starches or sugars. Baker and Martin (1937) described the iodophilic organisms encountered in a study of cellulose digestion in the caecum of the rabbit. They found similar organisms in the caecum of the horse and guinea pig and subsequently (1938) also in the rumen of the sheep. They concluded that the primary agents in the disintegration of cell wall substances in the rumen were iodophilic micro-organisms, very similar to those observed in the horse, guinea pig and rabbit. By using special methods these investigators cliam to have observed iodophilic bacteria in the interior of lacunae formed in the cellulose particles. The iodophilic bacteria and pseudo-yeasts concerned in the digestion of starch in the rumen can be demonstrated without difficulty by using Gram's or Lugol's iodine. To a drop of ruminal fluid on aclean slide a drop of Gram's iodine is added and covered with a thin coverslip. Any iodophilic organisms, whether brown or blue, are immediately discerned.

The bacteria and pseudo-yeasts enumerated below (table 12), were observed inthe rumen of the sheep used in these experiments. Apart from their size and physiological action, detailed descriptions of these organisms will not be given. They are clearly illustrated in phot-micrographs Nos. 1 to 5, Plate V.

TABLE No. 12/.....

TABLE NO. 12.

Micro-organisms.	Size (microns)	Synthetic Produts Glycogen	Starch
Streptococcus (Photo 1, Plate V).	.65		X
Streptococcus, large type. (Photo 2, Plate V).	1.35		X
Tetracoccus (Photo 3(b), Plate V).	5 x 5		X
Small bacilli - various types (Photos 3(c) and 5, Plate V.)	(.5 x 3 ((.5 x 2	X	X
Large iodoph. cigar-shaped bac. (Photo 5(a), Plate V)	(2.5 x 16 (4 x 13		X
Pseudo-yeasts, dividing by binary fission (Photos 4 and 5(b), Plate V.)	4 x 8	X	

In the course of this study it was observed that these iodophilic organisms reacted differently to different carbohydrates, e.g. starch and glucose. If glucose was administered into the rumen, the pseudo-yeasts and some bacteria, especially the cocci, soon reacted iodophilically by staining dark brown and blue, respectively. When starch was administered these organisms reacted similarly, but the reaction was much delayed and not as strong. It was therefore decided to test the reaction of the iodophilic flora to the introduction of various alcohols, sugars and other carbohydrates, into small quantities (10 cc.) of ruminal ingesta incubated at 39°C. immediately after withdrawal from the rumen. The test material was used in concentrations of two and five per cent in the ruminal ingesta. Microscopic preparations stained with Lugol's iodinewere made and examined after 15 minutes, 45 minutes, 3 hours and in some instances 20 hours after the commencement of incubation. The observations on starch were carried out <u>in vivo</u> at the same time and on the same sheep.

The/.....

The results are tabulated below (Table 13).

It will be observed that:

(1) The three pentoses used namely, arabinose, xylose and rhamnose were not assimilated by the micro-organisms to form iodophilic polysaccharides. The other monosaccharides used were hexoses. Of these dextrose (glucose) and laevulose (fructose) were very readily assimilated and condensed into iodophilic polysaccharides. Galactose, however, produced an iodophilic reaction in the cigar-shaped bacillus only.

(2) The disaccharides maltose and saccharose were assimilated by all types of iodophilic micro-organisms, but not as fast as laevulose and dextrose. Lactose and cellobiose produced delayed iodophilic reactions in the bacteria only.

(3) The trisaccharide, raffinose, produced an early iodophilic reaction in all types of iodophilic organisms.

(4) Amongst the polysaccharides, starch and pectine showed delayed reactions in the bacteria and to a lesser extent in the pseudo-yeasts as well. The readily soluble polysaccharides inulin and soluble starch were assimilated within 15 and 45 minutes respectively.

(5) Of the alcohols, isodulcite produced no iodophilic reaction, whilst minnite and sorbite were assimilated soon, although the last named was apparently poorly assimilated by bacteria.

CONCLUSIONS.

(1) Of all the substrates used, laevulose (fructose) and dextrose (glucose) were most readily assimilated by the iodophilic flora, as judged bytheir intense iodophilic reaction.

(2) Starch was metabolised, but very slowly, probably owing

to/.....

RATION OF RUMINAL IODOPHILIC MICRO-ORGANISMS TO ALCOHOLS, SUGARS AND OTHER SOL. CARBOHYDRATES.

Substrate	Nature of substrate	Period of incubation and reaction of orgs. 15 minutes	45 minutes.	3 hours.	20 hours.
Galactose	Monosacch.	Cigar shaped bac. light blue	As at 15 minutes.	As at 15 minutes.	
Arabinose	"	neg.	neg.	neg.	
Xylose	"	neg.	neg.	neg.	
Rhamnose	"	neg.	neg.	neg.	
Glucose (Dextrose)	"	Pseudo-yeasts - dark brown and swollen. Cigar.bac.: dark blue bands. Bacteria: blue	As at 15 minutes.	As at 15 minutes. Many more blue bact.	
Laevulose (Fructose)	"	As for glucose, but more blue bacteria.	As at 15 minutes	As at 45 minutes. but numerous blue bacteria.	
Lactose	Disacch.	neg.	neg.	Cigar. bac.: blue.	
Maltose	"	As for glucose.	As at 15 minutes.	As at 45 minutes.	
Saccharose	"	As for glucose, but Pseudo-yeasts only showing brown patches.	As at 15 minutes.	Pseudo-yeasts: dark brown and swollen.	
Cellobiose	"	neg.	Some small bact. brown.	Fairly numerous brown and blue bact.	
Raffinose	Trisacch.	Pseudo-yeasts: light brown Cigar. bac.: blue bands Bact.: some are light blue.	- deeper brown - deep blue - light blue	As at 45 minutes.	
Mannite	Alcohol	Ps.-yeasts: brown poles Cigar. Bac.:blue bands. Bact.: light blue	-brown extending from poles. -blue throughout -light blue	- dark brown - dark blue - blue,	
Sorbite	"	Ps.-yeasts: light brown Cigar, bact.: blue bands Bact.: neg.	As at 15 minutes.	- brown poles. - blue - blue bact. rare.	
Isodulcite	"	neg.	neg.	neg.	
Inosite	Benzol	neg.	neg.	neg.	
Salicin	Glucoside	Ps.-yeasts:brown poles Cigar. bac.: blue Bact.: neg.	-brown -dark blue -small no. blue.	As at 45 minutes.	
Inulin	Polysacch.	Ps.-yeasts:brown spots Cigar.bac.:light blue Bact.: neg.	Spots. -larger -light blue -neg.	Brown spots. - and poles - blue - small no. blue	- brown - blue - fair no. blue
Pectin	"	neg.	neg.	neg.	(Ps.-yeasts-some are brown. (Cigar.bac.- neg. (Bact.: Fairly numerous blue ones.
Untreated Maize starch	"	neg.	neg.	neg.	(Ps.-yeasts:some brown (Cigar. bac.: neg. (Bact.:fair no. blue.
Soluble Starch	"	neg.	Ps.-yeasts: light brown Cigar. bac.: neg. Bact.: Fairly numerous blue As at 45 minutes	As at 3 hours.	

to its insolubility and complexmolecular structure.

(3) The pentoses did not produce iodophilic reactions in any of the ruminal micro-organisms. These substances have been shown not to be fermented by yeasts (Plimmer, 1940).

(4) Cellobiose, derived from cellulose, produced a delayed iodophilic reaction.

(5) Some alcohols are assimilable by the ruminal microflora.

It appears therefore that the microflora of the rumen can assimilate a wide range of carbohydrates. The rate and extent of assimilation is in some measure determined by the complexity of the molecule. This ability of the ruminal microflora is of great advantage to the host animal as the great variety of complex carbohydrates naturally occurring in its diet can be hydrolysed to less complicated structures, and made available to the host animal in a more assimilable form.

7. ISOLATION OF AN IODOPHILIC STREPTOCOCCUS AND SOME OF ITS CHARACTERISTICS.

A small coccus was observed to be the dominant iodophilic bacterium, always closely associated with starch granules in the rumen and present in every sheep examined. An attempt was therefore made to isolate this organism and study some of its characteristics in pure culture.

A small silk bag of natural silk as described by Quin, van der Wath and Myburgh (1938), was filled with crushed and shelled sterilised maize kernels. This was suspended in the rumen through the fistula. After 48 hours of ruminal digestion the bag was withdrawn and transferred into a sterile petri dish. It was then opened and a few partly digested kernels dropped into sterile saline. From this suspension surface cultures were made on dextrose-agar or starch-agar slants and incubated at 39° C for 24 hours.

Two/.....

Two or three types of colonies were usually present after incubation. Of these, the smallest dew drop type of colony almost invariably proved to be the streptococcus associated with the maize kernels in great numbers. Subcultures were made on dextrose-agar. Smears were usually made of some of the kernels and stained with Lugol's iodine and with Gram's stain. Clusters of Gram-iodophilic cocci were observed in most instances around the starch particles. (Photo 1, Plate VI) Although some other organisms were usually also present, they occurred only in small numbers and were not iodophilic. In a similar way, chemically pure cellulose suspended in a separate compartment simultaneously with maize, was found to encourage various types of Gram negative bacteria (Photo 3, Plate VI). A few Gram positive bacilli , coccobacilli and large cocci were also present. Casein (79 per cent. protein) encourages predominantly Gram positive organisms, comprised also of large cocci, cocco-bacilli and bacilli (Photo 2, Plate VI).

The indications are therefore, that materials submitted to digestion in the rumen encourage specific types of bacteria to multiply in its immediate surroundings. In the case of starch the organisms concerned was found to play a significant rôle in its disintegration within the rumen. The organisms associated with the cellulose and casein probably also assist in their disintegration.

SOME CHARACTERISTICS OF ISOLATED STREPTOCOCCUS.

1. Size : .65 microns (First subcultures).
2. Strongly Gram positive.
3. Non-sporulating ovoid cocci usually in pairs or short chains.
4. Growth:

   (1) Grows easily in dextrose broth with final pH = 4.0

(2)/.....

(2) Dextrose- and starch-agar - in 24 hours at 37°C discrete semi-transparent colonies.

(3) Does not grow in peptone water pH = 7.5

(4) Serum agar - chains up to 10 organisms. Mostly appears in pairs, longitudinally, or in chains of 4 to 5.

(5) Serum broth - short chains of 4 to 5. Longer chains of 12-15 infrequent.

(6) No liquefaction of gelatin.

(7) Good growth in gelatin at room temperature (25°C).

(8) Methylene blue in milk not reduced.

(9) Acid formed in milk but no coagulation.

(10) Growth on ordinary agar feeble. More profuse on dextrose-agar, soluble starch-agar and serum-agar. No growth on dextrose-agar at room temperature in 24 hours but minute colonies are present after 48 hours.

(11) Broth - Uniform granular turbidity in broth after 24 hours and even earlier. Heavy deposit. Chains short.

(12) Starch - In synthetic medium (Woodman and Stewart, 1928), the organism ferments starch with the production of acid but no gas.

(13) After three months in stabculture (dextrose-agar) sealed with wax and kept at room temperature, growth was produced on dextrose-agar at 37°C within 24 hours.

5. Fermentation of Sugars:

(1) Ferments : Glucose, Lactose, Saccharose, Maltose, Laevulose, Raffinose, Arabinose, and Inulin to produce acid but no gas.

(2) Does not ferment : Mannite, Galactose, Salicin, Sorbite, Rhamnose, Inosite and Dulcite.

It also ferments Aesculin in bile medium producing acid.

6./.....

6. Resistance to heat:

Withstands heating in broth at 60°C for 20 minutes but is killed after 30 minutes.

7. Pathogenicity for laboratory animals:

Fresh (24 hour old) broth cultures were found non-pathogenic to rabbits and mice on intravenous and intro-peritoneal injection of .5 cc., 1 cc. and 1.5 cc.

PLATE IV. BACTERIAL DIGESTION OF STARCH.

Photo 1. Potato starch granules X 220.

" 2. One hour after administration of starch into rumen a few iodophilic cocci are seen attacking it.

" 3. After 2 hours the iodophilic organisms have increased.

" 4. After 4 hours a cluster of iodophilic bacteria are surrounding the starch granule.

" 5. After 8 hours excavation of the surface of a starch granule is well advanced.

" 6. 26 Hours only remnants of a granule are left. They are surrounded by iodophilic bacteria.

PLATE V. RUMINAL IODOPHILIC MICRO-ORGANISMS X 500.

Photo 1. Pure culture of Gram + iodophilic streptococcus.

" 2. Iodophilic streptococcus (Large type).

" 3. X 220 (a) Iodophilic streptococci photographed in the last stages of their iodophilic reaction and grouped around the remnants of a starch granule.

(b) Iodophilic tetracoccus.

(c), Small iodophilic bacillus.

" 4. Showing numerous pseudo-yeast cells filled with glycogen. Several are showing a central constriction and partition in the act of dividing.

" 5. (a) Large cigar-shaped bacilli. These bacilli represent various stages of starch synthesis within them. Some have blue discs, others blue rings, and those farther advanced have broad dark blue confluent discs.

(b) Pseudo-yeast cells.

PLATE VI./.....

PLATE VI. INTRARUMINAL DIGESTION OF SPECIFIC FOODSUBSTANCES. X 1000.

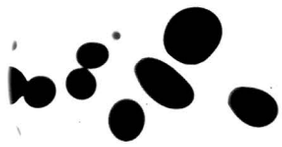
Photo 6. A starch granule surrounded by an almost pure culture of the Gram + streptococcus.

" 7. Casein taken from a silk bag suspended in the rumen for 36 hours. A rather mixed flora is seen, consisting mainly of large Gram + cocci, cocco-bacilli and bacilli. A small number of Gram - organisms are present.

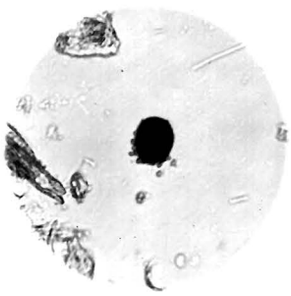
" 8. Chemically pure cellulose suspended in the rumen for 36 hours. A predominantly Gram - flora is present. Relatively few Gram + organisms are present.

PLATE 4.

BACTERIAL DIGESTION OF STARCH IN THE RUMEN. X 220.

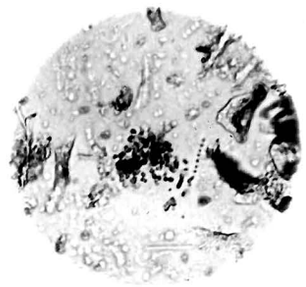


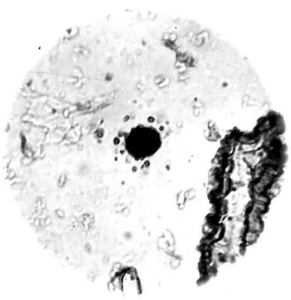
1



2

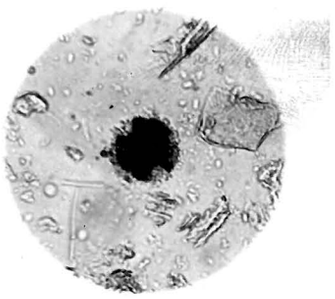
6





3

5



4

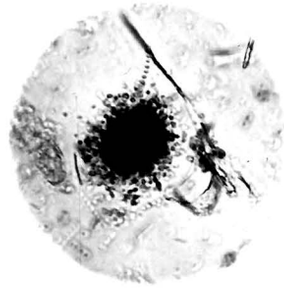
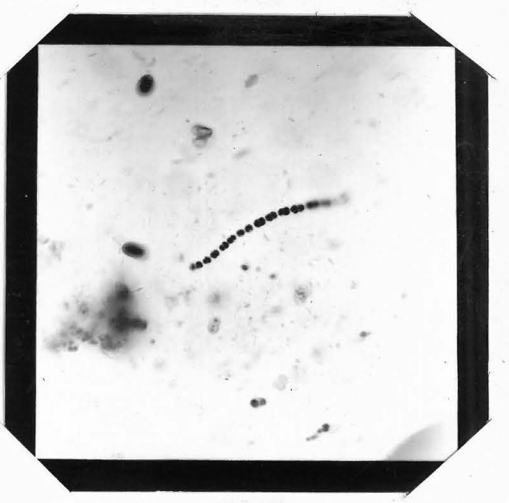


PLATE 5.

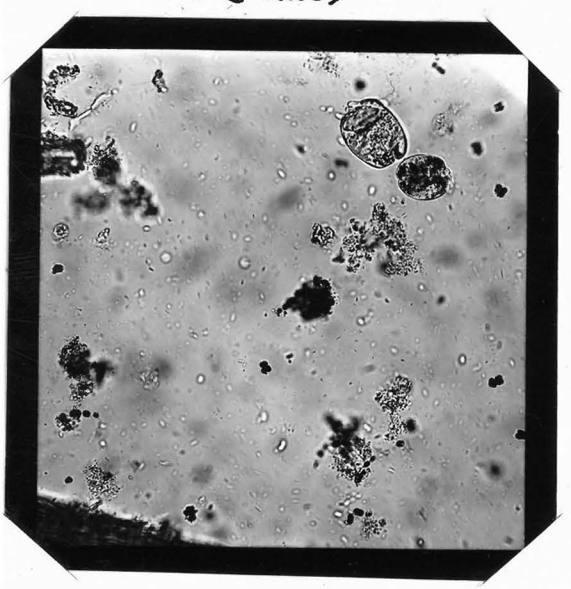
Ruminal Iodophilic Micro-organisms. x500.
Stained with Lugol's Iodine.

1 (X 500)

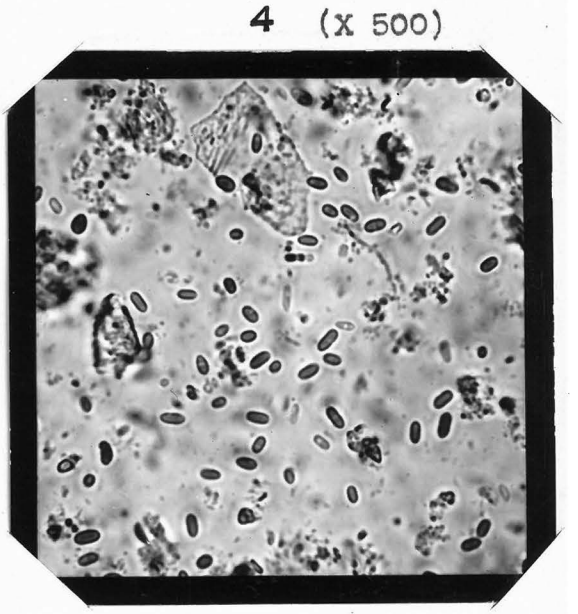
2 (X 500)



3 (x220)



4 (X 500)



5 (X 500)

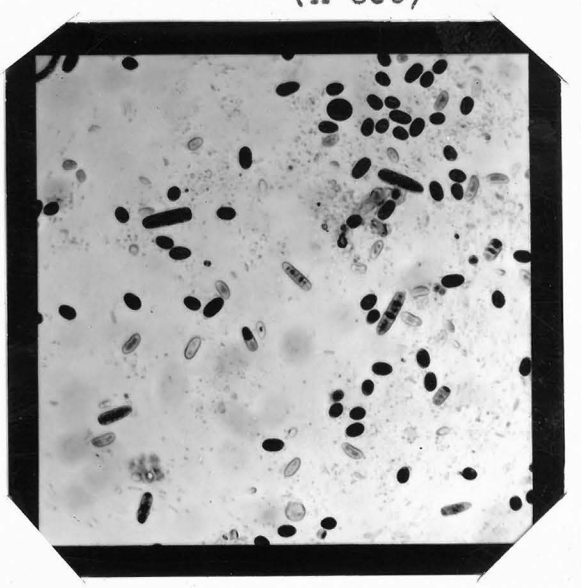
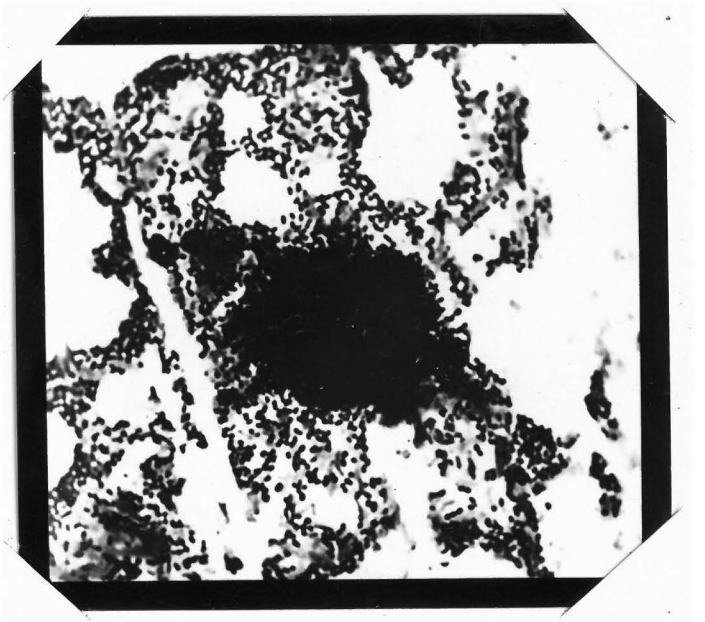


PLATE 6.

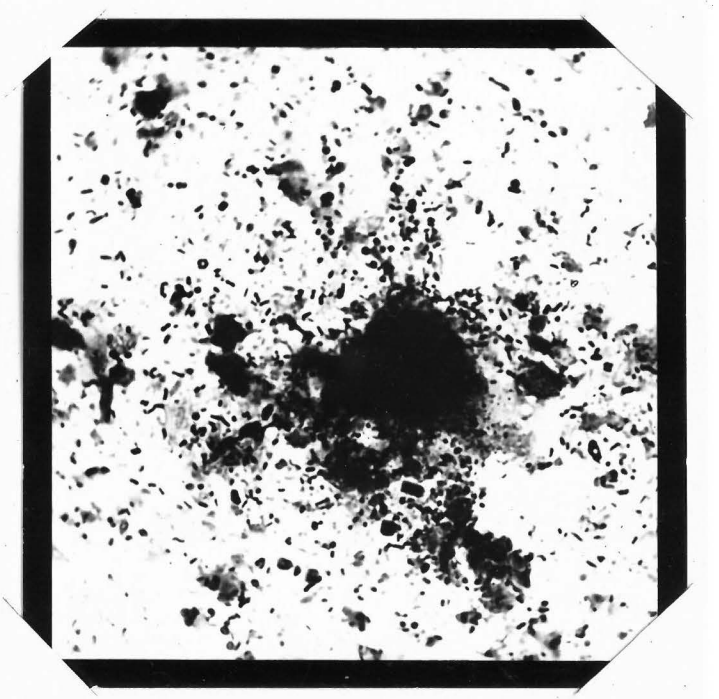
Intraruminal Digestion of Specific Foodsubstances. x 1000.

Gram's stain.

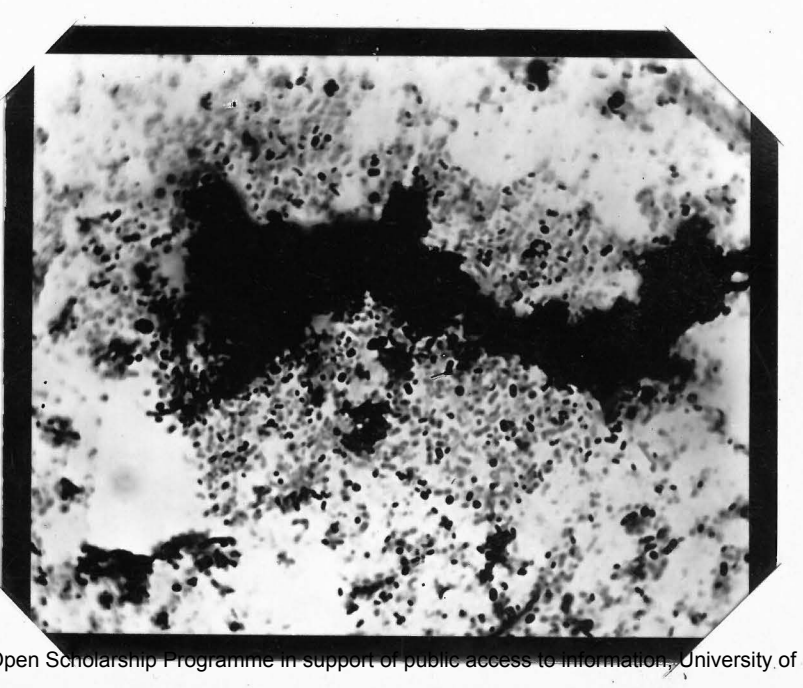
1
Starch



2
Casein



3
Cellulose



III. B. (3). CELLULOSE DIGESTION IN THE RUMEN OF SHEEP AS INFLUENCED BY:

(a) bacterial population of the rumen.

(b) The lignification of plant tissues, and

(c) The nitrogen and carbohydrate content of the feed.

INTRODUCTION:

In a recent publication on the supplementation of winter grazing in the Transvaal, Smuts and Marais (1940) stated that: "it appears fairly definite that there also exists an energy deficiency in the protein deficient grazing". The energy-yielding part of mature grass consists very largely of cellulose and other related polysaccharides, so that an animal subsisting on winter grazing only, would be dependant upon its ability to utilise such carbohydrates for practically all its energy requirements. Consequently, it is a matter of practical importance to determine to what degree cellulose can be utilised in such grazing, and to ascertain to what extent, if at all, this utilisation is enhanced by feeding supplements containing the main deficient constituents, e.g. carbohydrates, protein and phosphates.

The breakdown of cellulose and the hemicelluloses is accomplished not by enzymes secreted from body cells into the digestive tract, but by enzymes derived from symbiotic micro-organisms (Meyer, 1927). The extent of cellulose digestion as a result of bacterial action is, however, suspected to be subject to variations in the type and number of bacteria present, which in turn, are influenced by the composition of the diet (Hamilton, 1942). The degree of disintegration of carbohydrates in the rumen also depends on their chemical and physical nature. It has been demonstrated by Louw (1942) that the complex polysaccharides of

mature/.....

mature plants are not as well digested as those of young, growing plants. The difference is due primarily to the presence of certain encrusting substances, notably lignin, which are deposited in increasing amounts in the cell with advancing maturity. According to Baker and Martin (1938) rumen micro-organisms have little or no action on lignin, particularly that of mature plants. The result is that the cellulose is protected from the action of the organisms by the lignin, or, probably by a lignin-hemicellulose complex. (c.f. Louw, loc. cit.). In a recent publication by McAnally (1942) the percentage cellulose digested in the rumen was determined by suspending known quantities of cellulose in silk bags into the rumen of sheep through fistulae. Extensive experimentation at this Institute has, however, shown that this technique could not be relied upon for quantitative determinations. For qualitative work it proved however, to be more reliable. It is known that pure cellulose is almost completely digestible in the paunch of the ruminant and that lignified plant tissues are relatively indigestible. However, the factors controlling the degree of cellulose digestion are not yet clearly defined, although the following are believed to play a part:-

(1) The number and types of ruminal bacteria.
(2) The degree of lignification of plant tissues.
(3) The amount of sugar or starch present in the diet.
(4) The amount of nitrogen present in the diet.

The experiments described below were planned primarily to obtain information on these points.

The influence of small supplements of protein and carbohydrate rich foodstuffs on the bacterial count in the rumen and the digestibility of cellulose, was at first ascertained. For this purpose a basal ration of mature veld hay containing approximately 3.0 per cent protein was used.

The/.....

The carbohydrate rich supplement was increased to determine the effect on the microbiotic decomposition of cellulose in the hay. In all the trials conducted the nitrogen metabolism was studied.

EXPERIMENTAL.

The experiment consisted of a series of studies on cellulose and nitrogen balance of five full-grown merino wethers ranging in weight from 75-130 lb. and on which closed ruminal fistulae operations had been performed. Mature veld hay containing approximately 3.0 per cent of protein served as a basal ration and was fed during period I., supplemented by mineral ash and yeast only. This ration was therefore deficient in nitrogen as well as in readily available carbohydrates, producing energy. In period III. each animal received daily an additional amount of meat meal sufficient to maintain the nitrogen requirements of the animals. From periods III to VII the ration fed in period II. was further supplemented with increasing amounts of yellow crushed maize. The allowance of meat meal, however, was reduced with each addition of maize in order to maintain a more or less constant intake of digestible protein from period II. onwards. During all the periods each sheep received 3 grams of yeast, 5 grams of bone ash and 5 grams of salt daily. Full details of the rations are given in table 15.

In order to accustom the animals to the diet a preliminary feeding period of 10 days was allowed throughout. The Forbes type of metabolism cage was used. The faeces and the urine were collected daily for periods of 10 days duration. The usual procedures for collecting, preserving and aliquoting of excreta were followed. Feeds, faeces and urine were analysed for total nitrogen by the Kjeldahl method,

The/.....

The method of Norman and Jenkins (1933), was employed to determine the cellulose in the feeds and faeces. For the bacterial counts, samples of ruminal ingesta were withdrawn through the fistulae on at least three consecutive days during a collection period, and the average of the three counts taken. The Petroff-Hauser counting chamber method already described was used. The animals were fed twice daily, at 9 a.m. and 3 p.m., bacterial counts being made from samples withdrawn in the morning immediately before feeding.

RESULTS.

The essential data concerning the collections and the coefficients of digestibility for the dry matter are presented in table 14. The discussion of the results is based on the average values obtained in the seven periods in which five sheep were used. Coefficients of digestibility are presented for both total dry matter consumed and dry matter derived from the hay only. In the latter case the coefficients were calculated on the assumption that the concentrates, maize and meat meal, were completely digested.

The cellulose and protein contents of the feeds appear in table 15. The coefficients of digestibility for cellulose together with the figures for the bacterial counts, representing millions of bacteria per cubic centimetre of ruminal ingesta, are presented in table 16. Data relating to the nitrogen metabolism of the sheep appear in table 17.

DISCUSSION/ .....

DISCUSSION OF RESULTS.

In order to evaluate the significance of the differences between the percentage of cellulose digested (coefficients of digestibility) during the various experimental periods, a statistical analysis was applied. From this it was established that the necessary difference between the mean values of coefficients of digestibility should be 3.30 for P = 0.5 and 4.51 for P = .01, to be of significance (P = probability level). Calculating from table 16 it is found that the difference between the means for periods

1 and 7 = 7.12, i.e. significant at P =.01.
2 and 7 = 3.78, i.e. " " P = .05.
3 and 7 = 5.00, i.e. " " P = .01.
4 and 7 = 3.30, i.e. " " P = .05.
5 and 7 = 3.64, i.e. " " P = .05.
1 and 6 = 5.86, i.e. " " P = .01.
3 and 6 = 3.74, i.e. " " P = .05.
1 and 5 = 3.48, i.e. " " P = .05.
1 and 4 = 3.82, i.e. " " P = .05.

Differences not specified in the above summary were not significant.

The general tendency for the digestibility of the cellulose was to decrease gradually from period 1 to period 7. The digestibility of the hay was therefore, not improved by supplementing it with varying amounts of meat meal and crushed maize in spite of a significant increase in the total number of bacteria in the rumen, the site of cellulose digestion.

One or more of the following possibilities suggest themselves:

(1)/.....

(1) No effective increase in cellulose digesting bacteria was brought about by the enrichment of the medium.

(2) The basal ration of hay created conditions in the rumen of the sheep favourable enough for the existence of a cellulolastic flora, capable of maximal disintegration of the cellulose in the ration.

(3) Lignification of the hay had a limiting influence on the cellulose digesting powers of the bacteria.

(4) The nitrogen and carbohydrate supplements affected the degree of cellulose disintegration.

In regard to the first factor mentioned above, Harris and Mitchell (loc. cit.) proved that the digestion of cellulose was increased considerably (by 21 per cent) when a ration containing only 0.85 per cent protein was supplemented by 2.33 grams urea nitrogen daily. However, they did not study the influence of the urea supplement on the bacterial population of the rumen. It was found by the writer that the addition of 2.33 grams of urea nitrogen to the type of basal ration employed by Harris and Mitchell stimulated the paunch flora to a significant increase of 74 per cent. There are therefore, strong indications that the increased cellulose digestion demonstrated by Harris and Mitchell was due to the augmentation of the bacterial population of the rumen. It may therefore be concluded that the number of bacteria present in the rumen could influence the extent of cellulose disintegration when the ration is very low in protein (0.85 per cent). However, with the percentage of protein normally present in mature veld hay (3.0 per cent), the results of the present investigation have shown that even if the ruminal bacteria were increased in number they did not influence the digestibility of the cellulose favourably. The increases in the number of bacteria from periods 1 to 4 (figures in brackets table 16) were not accompanied by

corresponding/.....

TABLE 14.

SHOWING COLLECTION DATA AND DIGESTIBILITY OF DRY MATTER.

Period	Sheep No.	Weights (lb.)		Daily ration (gm.)			Average daily Residue (gm.dry matter).	Average daily dry matter intake (gm)		Average daily dry matter excreted (gm.)	Coefficient of digestibility for dry matter.			
		Initial	Final	Hay	Maize	Meatmeal		Hay	Total		Total	Average	Hay	Average.
1	3	89	90	600	0	0	107.5	438.5	438.5	232.3	47.0		47.0	
	4	102	104	"	0	0	122.6	423.4	423.4	203.5	51.9		51.9	
	5	117	117	"	0	0	105.5	440.5	440.5	233.1	47.1	47.5	47.1	47.5
	6	81	83	"	0	0	54.3	491.7	491.7	265.1	46.1		46.1	
	7	83	75	"	0	0	37.5	508.5	508.5	278.2	45.3		45.3	
2	3	99	95	"	0	24	26.5	521.3	544.1	288.0	47.0		44.8	
	4	109	106	"	0	25	119.1	428.7	452.4	230.6	49.1		46.2	
	5	128	121	"	0	33	75.6	472.2	503.86	269.4	46.5	45.0	43.0	42.4
	6	87	83	"	0	21	30.8	517.0	537.0	323.8	39.7		37.4	
	7	77	86	"	0	18	28.1	519.7	526.8	308.2	42.6		40.7	
3	3	99	97	"	50	16	20 .2	524.6	585.4	282.9	51.6		46.0	
	4	110	110	"	"	19	109.0	436.8	499.4	221.2	55.7		49.3	
	5	128	127	"	"	27	5.4	539.4	610.6	285.9	53.2	50.7	47.0	44.7
	6	86	86	"	"	13	26.6	519.2	579.0	309.2	46.6		40.5	
	7	78	76	"	"	12	23.5	5.1.3	578.3	309.2	46.6		40.7	
4	3	95	95	"	100	10	3.9	551.1	561.8	306.8	53.0		44.4	
	4	108	109	"	"	16	45.0	510.0	616.4	281.3	54.4		44.8	
	5	125	128	"	"	20	1.0	554.1	664.3	290.8	56.2	51.9	47.6	42.6
	6	92	86	"	"	10	18.5	536.5	637.2	314.0	50.8		41.5	
	7	75	74	"	"	4	59.5	495.5	590.5	324.8	44.9		34.5	
5	3	95	96	"	150	4	13.0	539.0	679.6	306.2	55.0		43.2	
	4	112	110	"	"	7	66.8	485.2	628.6	249.6	60.3		48.6	
	5	132	131	"	"	125	0	552.0	700.7	270.0	61.5	56.0	51.1	43.9
	6	85	87	"	"	4	41.6	510.4	651.0	300.9	53.8		41.1	
	7	76	78	"	"	0	55.0	497.0	633.8	321.3	49.4		35.4	
6	3	96	96	"	200	0	76.0	476.0	658.4	279.7	57.5		41.2	
	4	110	111	"	"	1.5	79.79	472.1	655.9	265.3	59.6		43.8	
	5	131	132	"	"	70	34.7	548.3	737.3	304.6	58.7	56.8	44.4	41.0
	6	88	87	"	"	0	33.1	518.9	701.3	313.9	55.3		39.5	
	7	79	77	"	"	0	39.4	512.6	695.0	328.8	52.7		35.9	
7	3	97	96	"	300	0	166.0	383.0	656.6	239.8	63.5		37.4	
	4	112	113	"	"	0	192.0	357.0	630.6	230.8	63.4		35.4	
	5	135	136	"	"	0	33.6	515.4	789.0	289.0	63.4	60.7	44.0	36.1
	6	91	88	"	"	0	76.0	473.0	746.6	316.5	57.6		33.1	
	7	79	78	"	"	0	60.1	488.9	762.5	340.5	55.4		30.4	

TABLE 15: SHOWING PERCENTAGE COMPOSITION OF FEEDS (DRY MATTER BASIS).

Feed	Constituent	Periods 1	2	3	4	5	6	7
Hay	Cellulose	49.5	48.7	48.7	49.9	48.8	48.6	48.4
	Crude protein	3.00	3.16	3.16	3.55	3.49	3.41	3.42
Maize	Cellulose				1.8			
	Crude protein.				11.0			
Meat Meal	Crude protein.				80.0			
Yeast	Crude protein.				50.0			

TABLE 16: SHOWING COEFFICIENTS OF DIGESTIBILITY FOR CELLULOSE WITH BACTERIAL COUNTS IN BRACKETS.

Period	Sheep 2	Sheep 4	Sheep 5	Sheep 6	Sheep 7	Mean.
1	65.4(1200)	71.4(1189)	65.2(1156)	63.6(1244)	62.1(1356)	65.5(1229)
2	63.2(1511)	66.7(1156)	64.7(1456)	56.6(1307)	59.8(1522)	62.2(1490)
3	65.3(1644)	69.5(1756)	67.7(1722)	57.1(1622)	57.5(1767)	63.4(1702)
4	63.9(1889)	65.3(1789)	57.7(1566)	59.6(1839)	52.4(1844)	61.7(1785)
5	61.9(1785)	67.6(1907)	70.5(1700)	58.2(1781)	52.1(1948)	62.4(1824)
6	59.3(1805)	64.5(1944)	66.4(1750)	57.1(1728)	51.1(1694)	59.7(1782)
7	58.9(1715)	61.2(1622)	67.7(1408)	54.0(1644)	50.3(1641)	58.4(1567)

*Given in millions per cc. ruminal contents.

TABLE 17. NITROGEN METABOLISM OF SHEEP IN PERIODS 1 - 7.

Period			Sheep 3	Sheep 4	Sheep 5	Sheep 6	Sheep 7	Average for 5 sheep.
1	Nitrogen intake:-	(Hay	2.01	2.23	2.02	2.45	2.51	2.24
		(Yeast	.24	.24	.24	.24	.24	.24
		Total	2.25	2.47	2.26	2.69	2.75	2.48
	Nitrogen excreted:-	(Faeces	2.11	2.07	2.18	2.41	2.50	2.25
		(Urine	1.01	1.41	1.41	1.27	1.18	1.26
		Total	3.12	3.48	3.59	3.68	3.68	
	N - balance		- 0.87	- 1.01	- 1.33	- 0.99	- 0.93	- 1.03
	N - (apparently) digested		0.14	0.40	0.08	0.28	0.25	0.23
	% N - (apparently) digested		6.2	16.2	3.5	10.4	9.1	9.1
2	Nitrogen intake:-	(Hay	2.64	2.38	2.39	2.61	2.63	2.53
		(Yeast	.24	.24	.24	.24	.24	.24
		(Meat Meal	3.07	3.20	4.22	2.69	2.30	3.10
		Total	5.95	5.82	6.85	5.54	5.17	5.87
	Nitrogen excreted	(Faeces	3.56	3.17	3.77	3.78	3.50	3.56
		(Urine	2.88	3.60	3.99	2.94	2.80	3.24
		Total	6.44	6.77	7.76	6.72	6.30	
	N - balance		- 0.49	- 0.95	- 0.91	- 1.18	- 1.13	- 0.93
	N - (apparently) digested		2.39	2.65	3.08	1.76	1.67	2.31
	% N - (apparently) digested		40.2	45.6	45.0	31.8	32.3	39.0
3	Nitrogen intake:-	(Hay	2.65	2.39	2.73	2.63	2.64	2.61
		(Yeast	.24	.24	.24	.24	.24	.24
		(Meat Meal	2.05	2.43	3.45	1.92	1.53	2.27
		(Mealies	.81	.81	.81	.81	.81	.81
		Total	5.75	5.87	7.23	5.60	5.22	5.93
	Nitrogen excreted:-	(Faeces	3.30	2.88	3.75	3.37	3.33	3.33
		(Urine	1.88	3.04	3.00	2.22	2.18	2.46
		Total	5.18	5.92	6.75	5.59	5.51	
	N - balance		+ 0.57	- 0.05	+ 0.48	+ 0.01	- 0.29	+ 0.14
	N - (apparently) digested		2.45	2.99	3.48	2.23	1.89	2.61
	% N - (apparently) digested		42.6	50.9	48.2	39.8	36.2	43.5
4	Nitrogen intake:-	(Hay	3.13	3.00	3.15	3.05	2.95	3.06
		(Yeast	.24	.24	.24	.24	.24	.24
		(Meat Meal	1.28	2.05	2.56	1.28	.51	1.53
		(Mealies	1.61	1.61	1.61	1.61	1.61	1.61
		Total	6.26	6.90	7.56	6.18	5.31	6.44
	Nitrogen excreted:-	(Faeces	3.62	3.64	3.77	3.57	3.55	3.63
		(Urine	2.16	3.37	3.09	2.71	2.17	2.70
		Total	5.78	7.01	6.86	6.28	5.72	
	N - balance		+ 0.48	- 0.11	+ 0.70	- 0.10	- 0.41	+ 0.11
	N - (apparently) digested		2.64	3.26	3.79	2.61	1.76	2.81
	% N - (apparently) digested		42.2	47.3	50.2	42.3	33.1	43.0
5	Nitrogen intake:-	(Hay	3.00	2.86	3.08	2.94	2.90	2.96
		(Yeast	.24	.24	.24	.24	.24	.24
		(Meat Meal	.51	.90	1.60	.51	0	.70
		(Mealies	2.42	2.42	2.42	2.42	2.42	2.42
		Total	6.17	6.42	7.34	6.11	5.56	6.32
	Nitrogen excreted:-	(Faeces	3.60	3.24	3.53	3.47	3.52	3.47
		(Urine	1.64	2.17	2.63	2.17	1.68	2.06
		Total	5.24	5.41	6.16	5.64	5.20	
	N - balance		+ 0.93	+ 1.01	+ 1.18	+ 0.47	+ 0.36	+ 0.79
	N - (apparently) digested.		2.57	3.18	3.81	2.64	2.04	2.85
	% N - (apparently) digested.		41.7	49.6	51.9	43.2	36.7	44.6
6	Nitrogen intake:-	(Hay	2.46	2.76	3.00	2.84	2.80	2.77
		(Yeast	.24	.24	.24	.24	.24	.24
		(Meat Meal	0	.19	.90	0	0	.22
		(Mealies	3.22	3.22	3.22	3.22	3.22	3.22
		Total	5.92	6.41	7.36	6.30	6.26	6.45
	Nitrogen excreted:-	(Faeces	3.29	3.51	3.92	3.60	3.68	3.60
		(Urine	1.48	1.99	2.77	1.71	1.54	1.90
		Total	4.77	5.50	6.69	5.31	5.22	
	N - balance		+ 1.15	+ 0.91	+ 0.67	+ 0.99	+ 1.04	+ 0.95
	N - (apparently) digested		2.63	2.90	3.44	2.70	2.58	2.85
	% N - (apparently) digested		44.5	45.2	46.8	42.9	41.2	44.1
7	Nitrogen intake:-	(Hay	2.04	2.37	2.81	2.75	2.80	2.55
		(Yeast	.24	.24	.24	.24	.24	.24
		(Mealies	4.84	4.84	4.84	4.84	4.84	4.84
		Total	7.12	7.45	7.89	7.83	7.88	7.63
	Nitrogen excreted:-	(Faeces	3.58	3.67	3.97	4.23	4.40	3.97
		(Urine	1.55	1.98	2.11	1.74	1.76	1.83
		Total	5.13	5.65	6.08	5.97	6.16	
	N - balance		+ 1.99	+ 1.80	+ 1.81	+ 1.86	+ 1.72	+ 1.84
	N - (apparently) digested		3.54	3.78	3.92	3.60	3.48	3.66
	% N - (apparently) digested		49.7	50.7	49.7	46.0	44.2	48.1

corresponding improvement in the digestibility of the cellulose, as may be deduced from the average values for the digestibility of the cellulose presented in the last column of table 16. It is concluded therefore, that the basal ration as fed in itself contained sufficient nutrients to support a bacterial flora and create conditions favourable enought for it to effect a maximum disintegration of the cellulose present.

In view of the above considerations, the second factor that may possibly have governed the extent of the breakdown of the cellulose in hay containing 3.0 per cent of protein, is the degree of lignification of the plant tissues. This has been shown to influence cellulose digestion in the rumen(Louw, 1942). In the experiments of Harris' and Mitchell supplementation with urea nitrogen did produce a 21 per cent increase in cellulose digestion which was, as previously stated in all probability due to a marked increase in bacteria (74 per cent). In the present investigation, using a basal ration of veld hay containing 3.0 per cent of protein the increase in bacteria on supplementing the ration with protein amounted to 30 per cent only, as compared to 74 per cent when the ration contained 0.85 per cent of protein. The bacterial digestion of cellulose in an almost nitrogen free ration is therefore incomplete owing to the small number of bacteria as compared to the degree of digestion in the veld hay ration which contain 3.0 per cent of protein and is capable of maintaining twice the number of bacteria. Accordingly, one would expect a more complete digestion of cellulose in the nitrogen low ration (employed by Harris and Mitchell) when sufficient nitrogen is supplied to stimulate active proliferation of the bacterial flora. As chemically pure cellulose is completely digested in the rumen, this improved digestion would lead to almost complete

cellulose/.....

cellulose disintegration if the plant tissues were not subject to variable degrees of lignification. With the bacterial flora augmented to the extent of 74 per cent the disintegration of cellulose would therefore, continue until it reached the limitation imposed by the degree of lignification. The indications are, therefore, that the degree of lignification may influence the extent of cellulose digestion in the rumen of sheep subsisting on mature veld hay.

In the statistical analysis of the results of the cellulose digestion, it has already been indicated that maize supplements exerted a depressing influence on the digestibility of the cellulose in the basal ration. Moreover, the extent of this depression showed a direct correlation with the amount of maize fed. An analysis of the mean values given in the last column of table 14 reveals that the dry matter of the veld hay, which constituted practically the whole of the cellulose in the supplemented rations, was digested in a manner similar to the cellulose in periods 1 to 7. It decreased from 47.5 per cent in period 1 to 36.1 per cent in period 7. Such a reduction in digestibility is in agreement with the findings of several workers (c.f. Armsby, 1917). The effect is most distinct when pure digestible carbohydrates, such as cane sugar and glucose, are added, but manifests itself also when large amounts of feedingstuffs rich in carbohydrates are fed. This is due to the fact that the complex cellulose molecule is not as easily digested as are sugars and starches. Consequently, the bacterial digestion of cellulose is partially diverted to that of the more readily digestible carbohydrates, resulting in a depression of cellulose digestion.

The data relating to the nitrogen metabolism of the sheep are presented in table 17. The mean values on

which/.....

the following brief discussion is based are given in the last column of the table.

In period 1 on the basal ration, the daily nitrogen intake amounted to 2.48 grams, of which only 0.23 gram was apparently digested. The animals were definitely not receiving sufficient nitrogen for maintenance as was evidenced by the pronounced negative balance of 1.03 grams nitrogen per day. In period 2 the total daily nitrogen intake increased to 5.87 grams due to the supplement of meat meal. The nitrogen apparently digested increased ten-fold, to 2.31 grams, but in spite of this the nitrogen balance remained negative at 0.93 grams per day. From period 2 to period 6 the daily nitrogen intake remained practically the same, yet the daily nitrogen balance changed from -0.93 in period 2 to + 0.95 in period 6, i.e. from a relatively strong negative to a relatively strong positive nitrogen balance. The only feasible explanation for these changes in the nitrogen utilisation seems to be that an energy deficiency existed in at least the ration of period 2. The increasing supplements of maize from period 3 onwards, gradually eliminated the energy deficiency with the result that the available nitrogen could more and more be utilised for the replenishment of the nitrogen losses associated with the basal metabolism of the animals,

Similar results were obtained by Smuts and Marais (1940) supplementing basal rations of veld hay and wheat straw with peanut meal and dextrinised starch. The conclusion drawn by these workers that under practical conditions it would be futile to rectify the existing protein deficiency with a minimum quantity of protein, unless the energy requirements are simultaneously satisfied, is strongly supported by the results of the present investigation.

CONCLUSIONS:/.....

CONCLUSIONS.

The experiments discussed above were designed to throw light on cellulose digestion primarily, but necessitates a consideration of the influence of both energy and nitrogen in so far as these three substances are correlated in the process of ruminal digestion.

From a statistical analysis of the figures obtained it appears that the digestibility of the hay was not improved by nitrogen and starch supplementations even though there was a significant increase in the number of ruminal bacteria. The results have shown on the contrary, that the digestibility of the cellulose decreased with increasing amounts of starch in the diet. Furthermore, the animals could not utilise the nitrogen in the diet low in starch because of an energy deficiency. However, the utilisation of the nitrogen improved proportionately to the increase of carbohydrates in the diet. It has, therefore, been shown that the amount of carbohydrate in the diet influences both the percentage of cellulose digested as well as the amount of nitrogen utilised by the animal. These experiments were carried out on a basal ration containing 3.0 per cent of protein. Rations containing 0.85 per cent of protein, i.e. practically on a nitrogen free level, (Protein = N x 6.25), were used by Harris and Mitchell in their study on cellulose digestion. They observed that the addition of nitrogen, sufficient for maintenance of the animals, to a ration containing an adequate amount of starch (energy) increased the digestibility of cellulose significantly. Without the addition of nitrogen this increased cellulose digestion could not be effected. On a diet low in nitrogen cellulose is therefore not digested to its fullest extent. It would, therefore, appear that an optimal level of nitrogen as well as of carbohydrate is required for maximal digestion of

cellulose/.....

cellulose in the rumen. From the data obtained in these experiments, it may be accepted that the maximal amount of cellulose would be digested under normal conditions if maintenance requirements of nitrogen are provided in the diet of the animal and sufficient starch or sugar is simultaneously incorporated so as to allow the animal to utilise its nitrogen.

The percentage of cellulose digested in period 5 amounted to only 62.1 per cent when the conditions created in the rumen of the sheep must have corresponded very closely to the required conditions for maximal digestion formulated above. This incomplete digestion of cellulose may reasonably be accepted to be due to the advanced degree of lignification present in the mature veld hay used in these experiments.

The number of bacteria present in the rumen did not influence the extent of cellulose disintegration in a ration of mature veld hay with a 3.0 per cent protein level. It, however, increased the digestibility of cellulose by 21 per cent when a ration containing only 0.85 per cent protein and sufficient starch was improved by supplementing it with maintenance requirements of urea nitrogen.

III. B (4). THE POSSIBILITY OF CYSTINE SYNTHESIS IN THE RUMEN AND ITS RELATIONSHIP TO NITROGEN METABOLISM.

1. Literature.
2. Experimental.
3. Results and Discussion.
4. Summary and conclusions.

1. LITERATURE:

Apart from the production of starch and glycogen by ruminal micro-organisms, the possible synthesis of amino-acids, and in particular that of cystine, was also considered. Being an essential amino-acid for growth and wool production, cystine has received a fair amount of attention from research workers.

In 1939 it was established by Marais and Smuts that for rats, lucerne is definitely deficient in the sulphur containing amino-acids and that supplementation with either cystine or methionine enhances the biological utilisation of lucerne nitrogen to a significant extent. When, however, the effect of cystine on the nitrogen utilisation of lucerne in mature sheep was investigated (Smuts and Marais, 1939), no beneficial effects could be established. It was concluded tentatively therefore, that either lucerne contained enough cystine or methionine for maintenance, or that cystine could be synthesised by the ruminal flora or, finally, that cystine was not a necessary component for tissue regeneration in sheep. This last assumption by Smuts and Marais, however, falls away, as it was recently shown by Burroughs and Mitchell (1940) that cystine or methionine was indispensable for tissue regeneration in the rat. Consequently, on physiological principles one should accept that this amino-acid would also be indispensable for the sheep. The entire

problem/.....

problem of cystine metabolism in sheep is therefore reduced to a study of the quantitative cystine requirements of these animals and the probability of cystine synthesis in the fore-stomachs. Both these aspects have received a fair amount of attention in the past. Nevertheless, it is apparent that the past disputes on cystine synthesis and cystine requirements of sheep were almost exclusively based on the supposed gross differences between the cystine content of the feed or pasture and that of the fleece. To explain such a difference, Rimington and Bekker (1932) suggested that cystine might be synthesised by a symbiotic action of the intestinal flora.

Fraser and Fraser-Roberts (1932) on the other hand, tried to account for the high cystine content of wool by assuming that the wool follicle possessed a special mechanism whereby cystine formation could be effected. Both these theories are highly speculative and without any experimental evidence to support them.

Although the work of Abdel-Salaam and Leong (1938) and Guerrant, Dutcher and Brown (1937) on rats, McElroy and Goss (1939) on sheep, and Wegner et al (1940) on cattle proved that certain members of the Vitamin B complex could be synthesised by organisms in the colon of rats and by the ruminal microflora of sheep and cattle, it does not follow that cystine could be produced in the same manner.

In the absence of conclusive evidence, the most acceptable view appears to be that of Woodman and Evans (1933) and of Kellermann (1935). According to these authors there is sufficient cystine in the pasture to account for the cystine content of the fleece and that the supposed differences between the cystine content of pasture and wool are to be ascribed to the analytical methods employed. Furthermore,

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it is of the utmost importance, as Burroughs and Mitchell (1940) have pointed out, to bear in mind that the requirements both for cystine and for the other indispensable amino-acids may vary with the characteristic functional activities of the animal as well as with different species.

2. EXPERIMENTAL.

In 1938, Smuts and Marais studied the biological value of lucerne and lucerne supplemented by cystine, in adult sheep. It was found "that the negative effect of cystine in the supplementation experiment was possibly due to the level of protein fed, which provided sufficient cystine for maintenance requirements". For this reason a similar type of experiment was subsequently undertaken by the author on growing sheep, to study the effect of cystine on the nitrogen utilisation of lucerne. Moreover, by feeding separate groups of rats on

(1) ruminal ingesta,

(2) ruminal ingesta plus cystine,

(3) incubated lucerne,

(4) lucerne,

the biological value of these materials was determined. Seeing that the biological value is a reliable measure of the quality and completeness of the amino-acids or protein fed, it was thus possible to determine whether

(a) cystine was indispensable,

(b) whether it supplemented lucerne which is known to be deficient in it, and

(c) whether it is synthesised in the forestomachs of the sheep.

In the experiment on sheep, 5 merino wethers were used after the animals had become accustomed to the metabolism crates. After being fed a standard protein ration of lucerne

(Table/.....

(Table 1(C), Ration 20), they were repeatedly subjected to a diet low in nitrogen (Table 1(C), Ration 22) for the purpose of determining the metabolic faecal nitrogen. From these results an average value was obtained for the metabolic faecal nitrogen of each animal. This was utilised in the calculation of the metabolic faecal nitrogen during the periods when lucerne was fed. The endogenous nitrogen was calculated from the equation $P = .74\ W^{.734}$ (Smuts, 1935).

It was arranged that a period of exclusive lucerne feeding alternated with one during which cystine was supplemented to the basic lucerne diet (Table 1(C), Ration 21), which was kept constant throughout. Each of the three periods during which faeces and urine were collected lasted for 10 days after which representative aliquots were taken for total nitrogen determination.

In the second experiment four groups of six rats each, with an approximate weight of 70 grams per animal, were placed on the following diets: (a) lucerne, (b) incubated lucerne, (c) rumen ingesta, and (d) rumen ingesta plus cystine (Table 1(D)). As in the case of sheep, each group of rats was subjected to periods of low nitrogen intake for the estimation of the metabolic faecal nitrogen and also for the endogenous nitrogen. The rumen ingesta was obtained from sheep 12 hours after they were fed lucerne from the same stock as that on which the biological values were determined. The material was collected from the rumen either through a permanent rumen fistula or after slaughter of the animal at the stipulated time. The ingesta was dried at 60°C, after which it was ground and mixed with the other ingredients of the rations. A quantity of lucerne from the same batch as that fed to the sheep was mixed with water and incubated at

37°C/.....

37°C for 24 hours. It was then washed and dried in the same manner as the rumen ingesta. This product was referred to as incubated lucerne.

The percentage composition of the rations is given in Tables 1(C) and (D).

3. RESULTS AND DISCUSSION.

Complete results on the metabolism studies of rats are presented in table 18. At the 8 per cent protein level usually fed to rats in the determination of biological values, the ration became unpalatable because the proportion of rumen ingesta that had to be incorporated to obtain the desired level of protein, was found to be too high. Consequently, the biological value was determined at a 6 per cent level in order to maintain palatability.

As will be noted in table 18, the average biological value of lucerne was found to be 67 per cent, its apparent and its true digestibilities amount to 50 and 75 per cent respectively. There is only a slight and insignificant variation amongst the biological values of the individual animals which may be attributed partly to the fact that the food intake of each animal was kept constant throughout the collection period.

In compering the results obtained between (a) lucerne, (b) incubated lucerne, and (c) rumen ingesta, it will be noticed that no statistical difference is obtained in the results between (a) and (b), whereas in the case of rumen ingesta a much higher biological value was shown, although the apparent and the true digestibilities are lower than either for lucerne or incubated lucerne. Thus the biological values are as follows: Lucerne, 67, incubated lucerne, 64, and ruminal ingesta, 82. The apparent and true

digestibilities/.....

TABLE 18. METABOLISM DATA AND CALCULATION OF BIOLOGICAL VALUE.

Rat No.	Initail Wgt.	Final Wgt.	Average Wgt.	Daily food intake	Daily N intake	Daily N in faeces	Body N in faeces. per gm. food.	Body N in faeces. per day.	Food N in faeces	Absorbed N	Daily N in urine	Body N in Urine. per 100 gm.wgt.	Body N in Urine. per day	Food N in urine	Retained N	Biological value	Apparent digest.	True digest.
N-low period for lucerne % N = .47																		
1	88	80	84	4.3			2.95					22.7						
2	90	81	86	6.3			2.78					20.0						
3	83	74	79	5.9			2.87					28.5						
4	85	77	81	5.1			2.93					24.2						
5	85	75	80	5.9			2.84					26.8						
6	93	86	90	5.4			2.88					20.1						
Lucerne Period % N = 1.12																		
1.	83	81	82	6.0	67.2	35.0	2.95	17.7	17.3	49.9	32.9	22.7	18.6	14.3	35.6	71	48	74
2	88	82	85	6.0	67.2	33.2	2.78	16.7	16.5	50.7	35.7	20.0	17.0	18.7	32.0	63	51	75
3	76	74	75	6.0	67.2	30.5	2.87	17.2	13.3	53.9	38.0	28.5	21.4	16.8	37.3	69	55	80
4	80	76	78	6.0	67.2	34.5	2.93	17.6	16.9	50.3	34.8	24.2	18.9	15.9	34.4	68	49	75
5	80	76	78	6.0	67.2	36.7	2.84	17.0	19.7	47.5	36.6	26.8	20.9	15.7	31.8	67	45	71
6	91	87	89	6.0	67.2	33.1	2.88	17.3	15.8	51.4	36.1	20.1	17.9	18.2	33.2	65	51	76
															Average	67	50	75
N-low period for rumen ingesta % N = .47																		
7	84	74	81	6.3			3.10					22.8						
8	97	89	93	5.8			2.76					15.7						
9	84	74	79	5.0			3.01					27.4						
10	98	92	95	6.3			2.56					18.4						
11	99	90	95	5.9			3.08					23.3						
12	87	78	83	6.3			2.83					23.0						
Ruminal ingesta period % N = 1.13																		
7	80	76	78	6.0	67.8	41.1	3.10	18.6	22.5	45.3	25.7	22.8	17.8	7.9	37.4	83	39	67
8	89	85	87	6.0	67.8	39.1	2.76	16.6	22.5	45.2	24.3	15.7	13.7	10.6	34.7	78	42	67
9	77	71	74	6.0	67.8	40.9	3.01	18.1	22.8	45.0	26.2	27.4	20.3	5.9	39.1	87	40	66
10	97	89	93	6.0	67.8	40.7	2.56	15.4	25.3	42.5	22.5	18.4	17.1	5.4	37.1	87	40	63
11	90	82	86	6.0	67.8	41.5	3.08	18.5	23.0	44.8	29.6	23.3	20.0	9.6	35.2	79	39	66
12	78	74	76	6.0	67.8	40.4	2.83	17.0	23.4	44.4	25.9	23.0	17.7	8.2	36.2	81	40	65
															Average	82	40	65

TABLE 18 (contd.) METABOLISM DATA AND CALCULATION OF BIOLOGICAL VALUE.

Rat No.	Initial wgt.	Final wgt.	Average wgt.	Daily food intake	Daily N intake	Daily N in faeces	Body N in Faeces. per gm food	Body N in Faeces. per day	Food N in faeces	Absorbed N	Daily N in urine	Body N in Urine per 100 gm. wgt.	Body N in Urine per day	Food N in urine	Retained N.	Biological value	Apparent digest	True digest
N-low period for ruminal ingesta plus .2% cystine % N = 4.7																		
13	113	96	105	6.3			2.98					21.0						
14	91	84	88	6.6			2.81					21.1						
15	81	72	77	5.1			2.85					27.7						
16	99	91	95	6.3			2.70					18.2						
17	83	76	80	5.1			2.64					19.5						
18	88	78	83	5.6			2.97					23.2						
Ruminal Ingesta plus 2% cystine period % N = 1.03																		
13	103	95	99	6.0	61.8	38.2	2.89	17.3	20.9	40.9	22.5	21.0	20.8	1.7	39.1	96	38	66
14	86	82	84	6.0	61.8	36.7	2.81	16.9	19.8	42.0	21.8	21.1	17.7	4.1	37.9	90	41	68
15	79	73	76	6.0	61.8	38.6	2.85	17.1	21.5	40.3	21.0	27.7	21.0	0.0	40.3	100	38	65
16	98	92	95	6.0	61.8	38.9	2.70	16.2	22.7	39.1	20.1	18.2	17.3	2.8	36.3	93	37	62
17	78	75	77	6.0	61.8	37.5	2.64	15.8	21.7	40.1	19.5	19.5	15.0	4.5	35.6	89	39	65
18	86	80	83	6.0	61.8	38.5	2.97	17.8	20.7	41.1	19.3	23.2	19.3	0.0	41.1	100	38	66
															Average	95	39	66
N-low period for predigested lucerne.																		
7	83	79	81	6.5			2.59					25.57						
8	87	81	84	6.3			2.96					19.55						
9	82	74	78	5.8			2.96					21.51						
10	81	74	78	5.7			2.72					22.89						
11	88	78	83	5.4			2.91					24.52						
12	84	75	80	5.5			2.82					24.10						
6% predigested lucerne period N = 1.10%																		
7	79	76	78	6.0	66	35.7	2.59	15.5	20.2	45.8	35.7	25.57	20.0	15.7	30.1	66	46	69
8	82	77	80	6.0	66	34.3	2.96	17.8	16.5	49.5	32.9	19.55	15.7	17.2	32.3	65	48	75
9	74	72	73	6.0	66	35.7	2.96	17.8	17.9	48.1	32.9	21.51	15.7	17.2	30.9	64	46	73
10	77	74	76	6.0	66	33.9	2.72	16.3	17.6	48.4	35.0	22.89	17.4	17.6	30.8	64	49	73
11	79	75	77	6.0	66	33.9	2.91	17.5	16.4	49.6	37.8	24.52	18.9	18.9	30.7	62	49	75
12	77	74	76	6.0	66	33.2	2.82	16.9	16.3	49.7	35.7	24.10	18.2	17.5	32.2	65	50	75
															Average	64	48	73

TABLE 19: THE EFFECT OF CYSTINE ADDITION ON THE BIOLOGICAL VALUE OF LUCERNE IN YOUNG SHEEP.

Animal No.	Average wgt.	Food Consumption Lucerne	Food Consumption Starch	Dry matter intake	N. intake	N. in faeces	Metabolic faecal N	Absorbed N	N in urine	Endogenous N	Food N retained	Biological value	N.balance	Apparent digestibility per cent	True digestibility per cent.
	Kg.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.			
Lucerne Period.															
56408	30	360	200	515	10.04	4.29	2.06	7.81	4.25	1.44	5.00	64	1.54	57	78
56417	23	360	200	515	10.04	4.48	2.06	7.62	3.64	1.16	5.14	67	1.92	55	76
56436	23	360	200	515	10.04	4.40	2.06	7.70	3.49	1.16	5.37	70	2.15	56	77
56386	23	360	200	515	10.04	4.19	2.06	7.91	4.17	1.16	4.90	62	1.38	58	79
56387	24	360	200	515	10.04	4.52	2.06	7.58	3.84	1.22	4.96	65	1.58	55	75
										Average		66		56	77
Lucerne plus cystine (1 gram) period.															
56408	30	360	200	515	10.04	3.20	2.06	8.90	3.58	1.44	6.75	76	3.26	68	89
56417	25	360	200	515	10.04	3.25	2.06	8.85	3.03	1.26	7.08	80	3.76	68	88
56436	23	360	200	515	10.04	3.62	2.06	8.48	3.42	1.16	6.22	73	3.00	64	84
56386	23	360	200	515	10.04	3.08	2.06	9.02	3.47	1.16	6.71	74	3.49	70	90
56387	23	360	200	515	10.04	3.77	2.06	8.33	3.16	1.16	6.33	76	3.11	63	83
										Average		76		67	87
Lucerne period.															
56408	30	360	200	515	10.04	3.13	2.06	8.97	4.39	1.44	6.02	68	1.52	68	89
56417	25	360	200	515	10.04	3.58	2.06	8.52	3.95	1.26	5.83	68	2.61	65	85
56436	23	360	200	515	10.04	3.70	2.06	8.40	3.80	1.16	5.76	69	2.54	63	84
56386	23	360	200	515	10.04	4.21	2.06	7.89	4.20	1.16	4.85	61	1.63	58	79
56387	23	360	200	515	10.04	4.22	2.06	7.88	3.81	1.16	5.23	66	2.11	58	79
										Average		66		62	83

digestibilities respectively, taken in the same order, are 50 and 75, 48 and 73, and 40 and 66 per cent. On the basis of these figures, the higher biological value of rumen ingesta fed at the same level of nitrogen as lucerne, indicates an improvement in the quality of the proteins contained in the ruminal ingesta. This may be interpreted as being either due to the specific synthesis of cystine in the rumen or to the formation by the ruminal organisms of new proteins with a higher biological value. The question of paramount importance is whether the microflora can synthesise its own tissue protein from lucerne which is known to be deficient in cystine? Two possibilities suggest themselves:

(1) The cystine content of lucerne forms a limiting factor as far as the utilisation by these organisms, of other amino-acids is concerned, i.e. unless adequate supplies of cystine are available, at least a portion of the other amino-acids will tend to pass through from the rumen unassimilated by the microflora.

(2) The microflora in the rumen is capable of synthesising additional cystine so as to supplement the natural deficiency of this amino-acid in lucerne. This in turn allows for an increased assimilation also of the other amino-acids in lucerne, and so raises the biological value of the proteins in the rumen ingesta.

In the latter case one would expect a complete protein mixture in the rumen or at least one whose biological value is equivalent to lucerne supplemented by cystine, that is, a value of 95 per cent. This, however, is not the case since a biological value of only 82 was obtained for ruminal ingesta. Nevertheless, this is higher than that obtained for lucerne alone, which has a biological value of 67.

Moreover/.....

Moreover, this value of 67 is further enhanced by the supplementation of 0.2 per cent cystine to lucerne, thereby increasing its biological value to 95 (Marais, 1940). According to the results obtained, the digestive processes in the rumen as such are, therefore, responsible for increasing the biological value of lucerne from 67 to 82. Seeing that the supplementation of cystine to the rumen ingesta further increases its biological value to 95, this affords evidence not so much of the specific synthesis of cystine itself as postulated in the second alternate, but rather of the formation of additional bacterial protein through which the biological value of the rumen ingesta becomes elevated. If cystine synthesis had taken place one would have expected the rumen ingesta to have a biological value at least equal to that of lucerne + cystine, i.e. 95. Consequently, it must be deduced that the first supposition represents the more correct view concerning the rôle of ruminal bacteria in relation to cystine metabolism in sheep kept on a lucerne diet.

On the basis of the results obtained on the rat, the experiments on sheep are more readily explained. As previously shown, the supplementation of cystine to a diet of lucerne was without any effect when fed to adult sheep. This is explained by the fact that lucerne contains sufficient cystine to satisfy that portion of the maintenance requirement which demands a complete assortment of indispensible amino-acids. The role of the microflora in this respect is probably purely symbiotic, by utilising the lucerne nitrogen in the synthesis of bacterial protein which in turn is rendered available to the host animal. Burroughs and Mitchell have shown that the maintenance requirement of indispensible amino-acids are indeed small, being correlated with the

species/.....

species of animal as well as to the functional activity of the individual. It can easily be appreciated, therefore, that despite its deficiency in cystine, lucerne nevertheless can satisfy the maintenance requirements of adult sheep. On the other hand, when growth is superimposed on maintenance, the cystine requirements are increased to such an extent that lucerne by itself can no longer satisfy these demands. Hence, the addition of cystine to lucerne increases its biological value by as much as 10 per cent when fed to growing sheep (Table 19). Consequently, from the experiments conducted both on rats and sheep, there is no conclusive evidence of specific cystine synthesis in the forestomachs of sheep. On the other hand, there are definite indications, that crude plant proteins, including other nitrogenous substances as shown in the work on urea, are transformed by these ruminal organisms into proteins which are more readily digested and assimilated by the host and which consequently are of a higher biological value.

4. SUMMARY AND CONCLUSIONS.

1. Metabolism experiments in which rumen ingesta collected from sheep kept on a diet of lucerne hay only, was subsequently fed to white rats, showed that the biological value of this material was definitely higher (by 15 per cent) than that of untreated lucerne similarly fed to white rats. Moreover, the addition of cystine to rumen ingesta caused further enhancement of its biological value to the extent of 13 per cent.

2. In the case of growing sheep, the supplementation of lucerne by cystine increased its biological value by 10 per cent, whereas in adult animals, Smuts and Marais, in their researches, failed to produce any such improvement.

From/.....

From the evidence at present available, it is reasonable to conclude therefore, that as far as protein metabolism is concerned, the role of the ruminal microflora appears to be primarily that of assimilation of proteins and other nitrogenous compounds which are built up into the protoplasm of these organisms. With the onward passage of the ingesta from the forestomachs, large numbers of these micro-organisms, amounting to as many as 1900 million per cubic centimetre on a diet of lucerne, are similarly swept to the more distal parts of the digestive tract where they are exposed to the action of potent proteolytic enzymes, thus rendering their breakdown products available for assimilation by the host.

III. B. (5) THE UTILISATION OF UREA BY RUMINAL MICRO-ORGANISMS.

1. Literature.
2. Conversion of urea into protein by bacteria.
   (i) Experimental.
   (ii) Results of the incubation of rumen ingesta with glucose and urea.
   (iii) Discussion and Conclusions.
3. The utilisation of urea by mature sheep.
   (i) Experimental.
   (ii) Results of the supplementation of nitrogen low rations with urea.
   (iii) Discussion and Conclusions.

1. LITERATURE.

In spite of experiments dating back to 1895, the value of non-protein-nitrogen in feeds has not yet been satisfactorily assessed. In Germany, where proteins have been scarce for many years, nutritionists were compelled to search for protein substitutes and consequently, much attention was paid to the possible substitution of animal or plant proteins by synthetic products like urea, ammonium acetate, ammonium bicarbonate, glycine, asparagine, succinamide and other similar substances. The synthetic production of urea by artificial fixation of atmospheric nitrogen has already been carried out in Germany and America on a commercial scale at a very low cost of production. Its use as a partial protein substitute was found both economical and physiological by the German Forschungs Dienst (1937), and it was, therefore, incorporated in ruminant feeds in the form of 'amide-oilcake' and 'amide-molasses' etc.

Owing to war conditions, proteins became limited

for/.....

III: B. (2)

for animal feeds in Great Britain, so that active steps had to be taken in order to ensure adequate supplies of proteins or of protein-substitutes (Benesch, 1941). Accordingly, extensive and well planned experiments were initiated both at the Hannah Dairy Research Institute, Ayr, by Owen, Smith and others, and in America by Harris and Mitchell, to determine the value of urea as a protein substitute. In a preliminary report published in 1941, Owen et al found urea to be of "unquestionable value as a partial substitute for protein in the feeding of dairy cattle".

The investigations carried out so far were all practical feeding experiments, designed primarily to ascertain the result of this 'amide' substitution on:

(a) Milk production in dairy cattle and goats.

(b) Meat production in heifers, bullocks, etc.

(c) Meat and wool production in sheep.

(d) Humans and poultry.

(e) THE EFFECTS OF 'AMIDE' FEEDING ON MILK PRODUCTION.

Krebs (1937) presented an exhaustive review of German literature, on the subject up to 1937. He quoted many recent experiments, e.g. Schmidt et al (1937), Carstens and Mehner (1937), Mehring (1937) all being from the German Forschungs Dienst, in which subminimal rationing of nitrogen was used and in which milk yield was stimulated by the feeding of urea. While admitting that the feeding of such compounds as urea and glycocoll had some effect in augmenting the protein value of the diet, Krebs was reluctant to accept any considerable protein synthesis, or that the bacterial protein produced was of any appreciable value to the animal.

Schmidt et al (1937) investigated the availability of urea for the production of milk. In planning their work

they/.....

they assumed a 50 per cent utilisation of urea by the cow. Only one normally fed, and five urea-fed animals were used. A 14 day control period, during which all the animals were fed alike, was followed by a 76 day experimental period. During this time the milk yield of the normally fed animal fell from 25.9 to 18.1, i.e. by 7.8 Kg., and that of the experimental animals from 26.7 to 17.6, i.e. by 9.1 Kg. The average yields in the course of the experimental periods were 22.5 and 22.1 Kg. per animal per day respectively. The experimental group lost 4 Kg. in body weight per animal in the experimental period, while the normally fed animal gained 5 Kg. At the end of this experiment a group of animals on a nitrogen deficient diet was also included in order to show that the control animal had not been overfed. The milk yields of these decreased from 18.4 to 14.4 Kg. in 10 days.

Similarly, experiments were planned by Ehrenberg et al (1938) on four groups of eight cows. These confirmed previous German work as to the protein-sparing effect of urea. Ehrenberg and his colleagues claimed that glycine also had a sparing effect.

Nehring (1939) in further experiments, came to the same conclusion as Ehrenberg et al, viz. that there was a limit to the extent to which the protein of the production ration could be replaced by urea. Nehring found a beneficial effect when urea was given in sufficient quantity to replace the protein of the production ration for 5 Kg. milk, but a deliterious effect when urea was given for 10 Kg. milk. In these experiments in which eighteen cows were used, the protein equivalent of the urea was calculated on the basis of a 50% utilisation.

Results of experiments of Richter and Herbst (1938)

support/.....

support the claim of Ehrenberg et al, that glycine has a protein-sparing effect. Richter and Herbst used four groups each of eight cows. One group was deprived of part of the production ration, a second was normally fed, a third had 50% of its production ration replaced by urea, and in the fourth group 50% of the production ration was replaced by glycine. The use of supplements resultedin a fall in milk yield which was less evident with glycine than with urea.

Eventually experiments were conducted on a large scale by the German Forschungs Dienst, according to a common experimental plan, in which the protein demand for about two gallons of milk was covered by 'amides'. In general, the 'amides' were found to possess half the efficiency of protein. Therefore, a complementary value (Wertigkeit) of 50 was allotted to urea nitrogen. These results led finally to the commercial manufacture of the amide-oil-cake by the German I.G.

The most recent work in this respect is being carried out at the Hannah Dairy Research Institute, Ayr, by Owen, Smith and Wright (1941) who in a preliminary report published the following findings:

(1) Urea which was first rapidly converted into ammonium salts in the rumen, proved to be of unquestionable value as a partial substitute for protein in the feeding of dairy cattle, provided that it was fed in proper amounts and in a suitable mixture. It was readily eaten by the animals and was witnout ill-effects either on the general health or in the yield and quality of their milk.

(2) In the course of in-vitro incubation of rumen contents inwhich the general conditions of the medium and the

microbiological/.....

microbiological picture (so far as could be judged) were similar to those in the intact rumen, the conversion of non-protein-nitrogen to protein was clearly demonstrable. This conversion, though small, was sufficient to account for the effective utilisation of much more urea than would normally be included in the diet of the lactating cow.

(b) Cattle: The effect of 'amide' feeding on meat production.

At the end of his exhaustive review, Krebs summed up by stating that evidence had not been produced of any increase of flesh as a result of urea feeding, and that, until such an increase had been demonstrated, it could not be said that there was any substitution of protein by urea. However, experiments published since the review of Krebs have supplied convincing evidence that urea and ammonium salts may, to a considerable extent, assume the role of protein in the ruminant. Hart et al (1939) have subjected urea and ammonium bicarbonate to a very stringent test by comparing them with casein. In a preliminary experiment four male calves were used. The basal ration contained only 6% protein. This was supplemented in the case of the experimental calves with urea, ammonium bicarbonate or casein in sufficient quantities to raise the crude protein content to 18%. The control animal showed an increase in weight of 120 lb. in 28 weeks, during which period the ammonium bicarbonate, urea and casein-fed animals showed increases of 170, 220 and 310 lb. respectively. On analysis the moisture, fat and protein contents of the tissues of the different groups were very similar. The experiment was repeated with six heifer calves on somewhat similar rations.

Comparable/.....

Comparable rates of growth were observed in all the animals except the one receiving a basal ration. After 12 weeks its weight remained constant at 290 lb. while the appetite was poor. A supplement of 1.4 lb. urea per 100 lb. of feed was then added to its ration. The appetite improved and growth continued for 9 months at the rate of 1 lb. per day, which was but slightly less than that for the casein-fed calf (1.2 lb. per day). The animals showed regularly recurring oestrus. Samples of rumen content were collected at post mortem for pH determination. This showed no significant difference. Bacterial cultures from the same samples also revealed no essential qualitative or quantitative differences.

Fingerling (1937), Bartlett and Cotton (1938), Murray and Romyn (1939) and Work and Henke (1939) have all reported favourably on amides as promoters of growth in cattle.

(c) Sheep: The effect of 'amide' feeding on meat and wool production.

Nehring and Schramm (1937) using growing lambs replaced one-third of the total nitrogen by urea, glycine and ammonium acetate respectively. Whilst the nitrogen-balances are negative in the basal and low protein periods, they became positive in the 'amide' periods reaching a maximum with ammonium acetate. Up to 200 grams of urea was fed daily to the experimental sheep, without any ill effects.

Very similar results were obtained by Mangold and Stotz (1937) who experimented with linseed cake plus urea, and with potato flakes plus urea. The amount of urea present in both preparations was about 15 per cent.

Liliencron (1938) feeding glycine to lambs for 150

days/.....

days, obtained very satisfactory results.

Kirsch and Sauer (1938) studying the effect of different 'amides' on growing sheep, with 20-30 per cent of the nitrogen was replaced, found no differences between the normal and urea groups with regard to live-weight and wool production.

Harris and Mitchell (1941) planned a series of experiments

(1) to determine whether the rate of conversion of urea was sufficient to cover the maintenance requirements of sheep,

(2) to measure the efficiency of conversion by comparing its biological value with that of casein,

(3) to determine the extent to which urea nitrogen could be used in satisfying the growth requirements of sheep.

They concluded that

(1) Sheep may be maintained in body and nitrogen equilibrium for well over 100 days on rations containing urea and minimal amounts of protein, where the latter provides only one-tenth of the amount of nitrogen needed for equilibrium.

(2) Nitrogen equilibrium may be maintained on 202 mgm. of urea nitrogen and 161 mgm. of casein nitrogen per Kg. of body weight daily.

(3) At nitrogen equilibrium the biological value of urea nitrogen is 62, and of casein nitrogen 79.

(4) The addition of urea to a low-nitrogen ration which in itself is unable to support appreciable growth in lambs or even to maintain consistant nitrogen equilibrium, converts it into a ration capable of promoting a normal or nearly normal rate of growth. Such a ration need

contain/.....

contain no more than 11% of conventional protein (N x 6.25), in which urea provides 50% of nitrogen.

(5) Rations used in the experiment and containing up to 3.16% urea on the dry basis do not exert any notable toxic effects on lambs over a feeding period of 110 days. Some renal hypertrophy resulted in lambs on the higher urea levels.

(d) The effect of 'amide' feeding to rats and chickens.

Utitilisation of urea in rats (Kriss and Marcy, 1930) and chickens (Bice and Dean, 1942) could not be demonstrated.

THE INCLUFENCE OF MICRO-ORGANISMS ON THE UTILISATION OF 'AMIDES'.

As far back as 1843 Delafond exoressed the view that infusoria might have a special nutritional significance. Later, Pasteur and A Mayer (1907) succeeded in producing micro-organic protein from ammonia, using yeast moulds. These results were confirmed by M. Mueller (1909) with asparagine and ammonium hydrogen tartrate, by showing that rumen bacteria prefer these substances to protein for anabolic processes, thereby producing considerable quantities of protein. Zuntz (1913, 1917) assumed that 'amides' could be used by the ruminant as a result of assimilation of the 'amides' by ruminal bacteria which were in turn digested in the truly enzymatic digestive tract. (Zuntz's bacteria hypothesis).

In 1920, Voeltz, on the basis of extensive experiments, concluded that urea could be utilised as protein by the ruminant. He also assumed that urea was first built up into bacterial protein in the digestive tract and that 80-90 percent of this was afterwards absorbed from the intestines.

Klein et al (1936, 1937, 1938) concluded on the basis of the results of their metabolism experiments, that

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in certain protein deficient rations, protein could be synthesised in the rumen from the nitrogenous fractions of 'molasses-amides'.

Lenkeit and Becker (1938) studied the fate of urea in 'amide-flakes' in the rumen. They carried out experiments in vivo and in vitro. In the former the decomposition of urea into ammonia was instantaneous, but in the latter, working with ruminal juice, it could be followed analytically. There was a progressive decrease of urea nitrogen and increase of ammonia nitrogen, leading to the complete decomposition of urea after twenty four hours; 10 to 20 per cent of the original urea, however, did not appear in the form of ammonia. The fixation of ammonia nitrogen in the micro-organisms themselves would probably explain this.

Hart et al (1938) showed experimentally that urea and ammonium carbonate nitrogen could be utilised for at least a part of the supply of protein nitrogen required by growing calves. They concluded that the protein were produced by bacteria while multiplying in the rumen and that subsequent digestion of the bacteria in the fourth stomach and intestine made the proteins available to the host.

Harris and Mitchell (1941) found the average true digestibility of casein nitrogen to be 86.9% and that of urea nitrogen 88.8%. Urea is a readily soluble compound and its nitrogen should have been digested very nearly 100 per cent Harris and Mitchell concluded that the portion of urea nitrogen that could not be accounted for was probably converted into bacterial protein in accordance with the hypothesis that urea nitrogen is changed into bacterial nitrogen in the paunch.

From the literature consulted there are strong indications that the ruminal bacteria are responsible for the

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synthesis of protein from non-protein-nitrogen. However, the crucial point as to whether there is an actual increase in the ruminal bacteria and hence in bacterial protein during 'amide' feeding, has not been directly investigated. Similarly, the biochemical reactions involved in the rumen during this conversion of non-protein-nitrogen into true protein still remains to be explained.

Experiments were accordingly planned to throw light on:

(1) the mechanism of the conversion of urea into protein in the rumen, and

(2) the utilisation of urea by mature sheep.

2. THE MECHANISM OF THE CONVERSION OF UREA INTO BACTERIAL PROTEIN.

Bunge (1937) proved by means of filtrates of rumen ingesta that an enzyme "urease" was present in the ruminal juice. The mode of action of this enzyme was, however, obscure until Lenkeit and Becker (1938) showed that it acted by hydrolysing urea to CO_2 and NH_3.

$$CO(NH_2)_2 + 2H_2O \xrightarrow{\text{urease}} CO_2 + H_2O + 2\,NH_3$$

In an attempt to elucidate the conditions under which these reactions can take place and the mechanism where urea is utilised in the production of micro-organic nitrogen, the following experiments, both *in vitro* and *in vivo* were carried out in order to obtain quantitative data.

(i) Experimental.

Experiments may be conducted *in vitro* with ruminal ingesta without departing significantly from the normal course of fermentation in the rumen provided the period during which observations are made does not exceed three to four hours. After this period of time the absence of saliva,

which is continually being swallowed and added to the ruminal contents by the animal, alters the normal course of fermentation due to a change in the hydrogen-ion concentration. Furthermore, even if the hydrogen-ion concentration is controlled by chemical substances, e.g. chalk, putrefaction sets in as a result of the accumulation of metabolic products and dead micro-organisms which are usually diluted in the rumen by saliva and also continually passed out of the rumen, together with food particles and ruminal juice to the lower compartments of the digestive tract.

For the purposes of this experiment rumen ingesta was incubated at 39°C in a thermostatically controlled water bath. The waterbath again was kept in continuous gentle to-and-fro movement throughout the period of incubation by means of a mechanical shaker. The rumen ingesta was withdrawn from the rumen of sheep with permanent artificial fistulae immediately before the commencement of the experiment in the morning. These sheep were fed 600 grams veld hay, plus bone ash, salt and yeast, daily.

In each of four 250 cc conical flasks 100 cc. of fresh ruminal ingesta was placed. Flask 1 was kept as control. To flask 2 .166 grams of urea was added. A similar quantity of urea plus one gram of glucose was added to flask 3. The fourth received one gram of glucose only. Small samples were withdrawn from the control material for hydrogen-ion concentration determination and bacterial counts. All four flasks were then incubated in the waterbath at 39°C. Further sampling was done at half-hourly intervals for pH determination, and after one and three hours for bacterial counts.

H-ion concentration values were obtained with Beckman's Glass Electrode pH meter.

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The experiment was repeated on different sheep with varying amounts of urea, but with essentially the same results.

The results of typical reactions encountered in experiments where the minimal and maximal quantities of urea were used are presented in table No. 20 and will be discussed below.

(ii) RESULTS OF THE INCUBATION OF RUMEN INGESTA WITH GLUCOSE AND UREA.

(1) Effects on hydrogen-ion concentration.

The effects of incubation on the pH of the rumen ingesta are revealed as follows:-

(a) Control sample : The pH remained fairly constant, with a definite tendency to acidity towards the third hour of incubation.

(b) Rumen ingesta + urea: The pH increased on the average from an initial value of 6.94 to 7.83, i.e. by .89, and shifted therefore from the acid to the alkaline side of neutrality. The ingesta acquired a musty odour and turned dark brown.

(c) Rumen ingesta + urea + glucose: The pH compares very well with that of the control. The fluctuations are between narrow limits, e.g. pH 7.02 to 6.55 at the end of the period of incubation. It therefore also has a definite tendency towards acidity.

(d) Rumen ingesta + glucose: The pH falls well below neutrality, viz. from 7.02 to 5.7. In one trial in which .33 gram urea was used, the pH of the mixture rose from 6.7 to 8.45, i.e. by 1.75. A slight ammoniacal odour was perceived. In the flask containing glucose as well as urea, the usual slightly sour odour as well as active fermentation was present.

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TABLE 20: EFFECT OF UREA AND GLUCOSE ON BACTERIAL POPULATION AND pH OF RUMEN INGESTA IN VITRO.

Trial	Time	Bact. count and pH.	Control	Rumen Ingesta + Urea.	Rumen ingesta + Urea + Glucose.	Rumen ingesta + Glucose.	Diet	REMARKS.
1 Sheep 8	0 hours.	Bact.	1000 x 10^6	1000	1000	1000	Veld hay	.166 gm.urea 100 cc.R.ingesta
		pH	7.02	7.02	7.02	7.02		
	½ hour	Bact	7.18	7.50	7.08	6.4		
		pH						
	1 hour	Bact.	1044 x 10^6	1033	1340	1167		
		pH	7.18	7.78	7.10	6.18		
	1½ hours	Bact.						
		pH	6.82	7.90	6.88	6.15		
	2 hours	Bact.						
		pH	6.92	7.90	6.80	6.02		
	3 hours	Bact.	1069 x 10^6	1189	1433	1188		
		pH	6.82	7.80	6.55	5.70		
2 Sheep 8	0 hours	Bact.	1133	1133	1133	1133	Veld hay	.166 gm.urea to 100 c.c. R. ingesta.
		pH	6.80	6.80	6.80	6.80		
	½ hour	Bact.						
		pH	6.82	7.90	6.90	6.10		
	1 hour	Bact.	1233	1433	1667	1378		
		pH						
	1½ hours	Bact.						
		pH	6.80	7.88	6.90	5.98		
	2 hours	Bact.						
		pH	6.68	7.85	6.50	5.55		
	3 hours.	Bact.	1248	1490	1778	1422		
		pH	6.22	7.60	6.22	5.20		
3 Sheep 8	0 hours	Bact.	1.67	1167	1167	1167	Veld hay	.166 gm. urea to 100 cc. r. ingesta.
		pH	7.02	7.02	7.02	7.02		
	½ hour	Bact.						
		pH	6.80	7.70	6.75	6.20		
	1 hour	Bact.	1211	1389	1678	1411		
		pH	6.80	7.70	6.68	5.98		
	1½ hours.	Bact.						
		pH	6.62	7.45	5.70	5.70		
	2mhours	Bact.						
		pH	6.42	7.42	6.32	5.52		
	3 hours	Bact.	1288	1442	1778	1409		
		pH	6.32	7.45	6.35	5.40		
4 Sheep 8	o Hours	Bact.	1189	1189	1189	1189	Veld hay	.33 gm. urea to 100 cc. rumen ingesta.
		pH	6.7	6.7	6.7	6.7		
	½ hour	Bact.						
		pH	6.7	7.08	6.82	6.25		
	1 hour	Bact.	1233	1456	1756	1478		
		pH	6.52	7.46	7.20	5.92		
	1½ hours	Bact.						
		pH	6.52	8.05	7.18	5.78		
	2 hours	Bact.						
		pH	6.38	8.30	6.95	5.52		
	3 hours	Bact.	1300	1511	1900	1544		
		pH	6.22	8.45	6.70	5.22		

(2) The effects on bacterial counts.

(a) Control: Bacterial populations increased on the average by 10 per cent throughout the period of incubation. No active fermentation was seen.

(b) Rumen ingesta + urea: The increase in bacterial population was 24 per cent. No fermentation was noticeable.

(c) Rumen ingesta + urea + glucose: The bacteria increased by 50 per cent. Very active fermentation took place. Large amounts of gas were liberated.

(d) Rumen ingesta + glucose: A bacterial increase of 23 per cent was produced. Fermentation was very active with evolution of much gas. The ingesta had a sour odour after fermentation.

In the trial in which .33 gram urea was used, the bacterial population of the rumen ingesta-urea mixture increased by 27 per cent as against 24 per cent on the lesser amount of urea. The material containing glucose as well, fermented actively; its bacteria increased by 60 per cent, as against 50 per cent on .166 grams of urea and 27 per cent when glucose was not added.

(iii) DISCUSSION AND CONCLUSIONS.

From the experimental results it appears that:

(1) The pH of the rumen ingesta containing urea definitely became alkaline, but not to a degree which is considered detrimental to ruminal bacteria. A pH 7.9 is frequently encountered in normal ruminal ingesta fermenting actively. (Unpublished data, S.J. Myburgh, Onderstepoort Laboratory).

(2) There was no perceptible fermentation in the ingesta-urea mixture throughout the period of incubation, and

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the ingesta developed an unusual dark brown colour as well as a musty odour. The indication is that some factor other than pH had inhibited the normal progress of fermentation and also partially that of bacterial multiplication. The bacteria increased by 24 per cent only compared to 50 per cent in other instances.

(3) In the case of rumen ingesta + urea + glucose the pH of the medium seems to have been controlled and kept within a range of hydrogen-ion concentration which was favourable for active fermentation and bacterial proliferation. Within 3 hours the bacterial flora was increased by 50 per cent compared to 24 per cent in the case of rumen ingesta + urea only. It seems, therefore, as if no inhibitory substances were formed, or, if they were formed, they were either removed or neutralised.

(4) The ingesta containing glucose only, fermented actively, but here also the bacteria merely increased by 23 per cent. After 2 hours the pH at 6.5 could not have been injurious to bacterial multiplication. A possible inhibitory factor which could be considered of significant influence is a deficiency of nitrogen. Similarly, in the case where urea only was added to the rumen ingesta, which could not have contained much nitrogen nor soluble carbohydrate at the stage at which it was withdrawn from the rumen, a deficiency of readily soluble carbohydrate is indicated. The medium to which both urea and glucose was added, fermented very well and also produced a 50 per cent bacterial multiplication. It seems, therefore, that if both nitrogen and glucose are present in ruminal juice, favourable conditions are created for fermentation and bacterial division,

whereas/.....

whereas if one or the other is absent or becomes deficient, the fermentative and proliferative processes are hampered at a stage when either the nitrogen or energy supply becomes depleted. For the maximal utilisation of urea nitrogen by ruminal bacteria it is necessary therefore, that a sufficient amount of readily soluble carbohydrate be present in the ruminal fluid. Urea, on hydrolysis in the rumen by the urease present there, gives rise to CO_2 and NH_3. The CO_2 thus formed is insufficient to bind the NH_3 to form ammonium carbonate. Calculated from their molecular weights, approximately .01 gram CO_2 must be derived from the rumen ingesta to bind the NH_3 set free. If it is considered that a large amount of CO_2 formed in the rumen is lost either by eructation or absorption from the rumen or by dissolving into the ruminal fluid, and that NH_3 may similarly be absorbed or dissolved into the fluid, it becomes so much more necessary to have an excess of CO_2 so as to bind as much of the NH_3 as possible and prevent its absorption into the circulatory system as a harmful agent.

It is possible to obtain .25 gram CO_2 from 1 gram of glucose so that the addition of 1 gram glucose to rumen ingesta to which .166 gram of urea had been added would ensure an excess of CO_2. Under these conditions ammonium bicarbonate (NH_4HCO_3) which acts as a buffer is conceivably formed. This assists the saliva in the control of the hydrogen-ion concentration of the rumen ingesta and favourably influences the proliferation of the flora as well as the fermentative processes occurring in the rumen.

In conclusion it may therefore be said that urea admixed with ruminal ingesta acts in a twofold

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way:

Firstly, by supplying the micro-organisms with a form of nitrogen which can be transformed by them into bacterial protein. This protein is utilisable by the ruminant to the extent of 90 per cent according to Voeltz (1920).

Secondly, urea, when hydrolysed into NH_3 and CO_2, probably contributes to the production of ammonium carbonate and ammonium bicarbonate in the presence of a sufficient amount of readily soluble carbohydrate in the rumen. These substances acting as buffers in a variable degree, in turn stabilise the hydrogen-ion concentration in the rumen and promote the vital processes of fermentation and bacterial proliferation.

Further experiments are being undertaken to determine the most economical ratio between the amounts of urea and carbohydrate required for maximal utilisation of the urea.

3. The Utilisation of urea by mature sheep.

As a result of the information obtained in Experiment 1, a second experiment was planned to determine the effect of urea on the ruminal bacteria in vivo and to prove or disprove the hypothesis (Harris and Mitchell 1941) that the utilisation of urea by adult ruminants occurs through the formation of bacterial protein from urea nitrogen.

(i) Experimental:

For this purpose five fistula sheep were fed on a standard ration of 500 grams dry lucerne hay plus 300 grams of yellow crushed maize daily. During this period which lasted 14 days, bacterial counts were made to ascertain the density of the bacterial population under optimal

conditions/.....

TABLE 21: NITROGEN METABOLISM STUDIES ON FIVE ADULT FISTULA SHEEP. RESULTS EXPRESSED ON DAILY BASIS.

Sheep No.	Ration	Length of period.	Food consumed.	Nitrogen Intake. In basal ration.	Nitrogen Intake. In supplement.	Nitrogen Output. In faeces	Nitrogen Output. In Urine	Nitrogen Balance.
		Days.	gm.	gm.	gm.	gm.	gm.	gm.
2	Low Nitrogen	8	600	1.92	-	1.50	1.21	- .79
	Urea	10	480	1.36	2.33	1.60	1.68	+ .41
3	Low Nitrogen	8	600	1.92	-	1.26	2.07	- 1.41
	Urea	10	470	1.32	2.33	1.38	2.01	+ .26
4	Low Nitrogen	8	600	2.34	-	1.70	1.77	- 1.06
	Urea	10	760	2.66	2.33	1.64	1.52	+ 1.83
5	Low Nitrogen	8	620	2.77	-	1.85	2.87	- 1.95
	Urea	10	540	2.47	2.33	1.23	2.13	+ 1.39
6	Low Nitrogen	8	600	2.84	-	1.86	1.70	- .72
	Urea	10	720	2.47	2.33	1.18	2.01	+ 1.61

TABLE 22: AVERAGE BACTERIAL COUNTS PER cc. OF RUMEN INGESTA IN NITROGEN METABOLISM STUDIES.
BACTERIAL COUNTS EXPRESSED IN MILLION PER cc.

Period	Sheep No. 2	Sheep No. 3	Sheep No. 4	Sheep No. 5	Sheep No. 6	Average for 5 sheep.
1	1891	1838	1902	1933	1808	1874
2	613	585	613	629	620	612
3	1256	1088	833	1100	1063	1068
4	1711	1281	1118	1533	1244	1377
5	2091	1880	1702	1811	1891	1875

Period 1 - Standard ration of lucerne hay and crushed yellow maize.
" 2 - Nitrogen low ration.
" 3 - Nitrogen low plus 5 gm. urea.
" 4 - Nitrogen low plus 2.5 gm. urea plus 14.1 gm. white fish meal.
" 5 - Nitrogen low plus 28.2 gm. white fish meal.

conditions. On completion of the observations the animals were transferred to metabolism crates and put on to a nitrogen low diet (Ration No. 10). Collections of faeces and urine commenced after a pre-period of 12 days at which stage the animals were considered not to be in nitrogen equilibrium. During this N-low period, which was continued for eight days, bacterial counts were made daily. From the ninth day on, 5 grams urea was administered into the rumen of each sheep through the fistula. Feeding, counts and collections were continued as before, for a further period of ten days.'

With a view to gaining more information on the ability of urea to stimulate bacterial multiplication, the urea period was followed by two further periods. In the first, the nitrogen of half the urea was replaced by an equivalent amount of white fish meal calculated in terms of nitrogen. In the following period there was complete substitution of urea nitrogen by white fish meal nitrogen. During these periods bacteria were counted but excreta not collected.

(ii) Results of the supplementation of nitrogen low rations with urea.

The results obtained from the various supplementations are summarised in Tables 21 and 22.

In the nitrogen metabolism experiment it will be noted that none of the five sheep were in nitrogen equilibrium on the nitrogen low ration. By supplementing the ration with 5 grams urea the daily nitrogen intake was increased by 2.33 grams, with the result that a complete positive nitrogen balance was created. From these results it appears that the sheep utilised urea nitrogen to replenish the daily unavoidable nitrogenous losses associated with maintenance.

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The ruminal bacteria, as is shown clearly in Table 22, increased in number from 612 million per cc. to 1068 million per cc., representing an increase of 65 per cent.

On the substitution of 2.5 grams of urea with 14.1 grams of white fish meal, a further increase in ruminal bacteria occurred, viz. from 1068 million to 1377, which is an increase of 28 per cent. Complete substitution of urea by white fish meal resulted in a marked bacterial proliferation. From 1377 million the count rose to 1875 per cc. The bacterial population therefore increased from 612 million per cc. on a nitrogen low diet to 1875 on supplementing this diet with an amount of white fish meal having the same nitrogen value as 5 gram of urea. This is equivalent to the bacterial population on the standard ration of lucerne and yellow maize, and represents a bacterial increase of 206 per cent. The increase produced by urea was 55 per cent. This means that bacterial nitrogen, apart from any other micro-organic nitrogen that may have been formed at the same time, was increased by at least 65 per cent on supplementing with 5 grams of urea.

(iii) Discussion and Conclusions:

Schwarz (1925) found that the nitrogen of bacteria accounted for 11.7 per cent of the total nitrogen of the rumen in sheep on a poor hay diet (3-4 per cent protein). He calculated the weight of ruminal bacteria per 100 Kg. rumen content as 2.79 Kg., containing 41 gm. of N. This corresponds to 256 gram of protein (N x 6.25). For every 1 Kg. of rumen ingesta a sheep on a poor hay diet therefore has 2.55 gram of bacterial protein in its rumen. If the bacterial population is increased by 55 per cent when supplementing the ration with 5 grams of urea, the bacterial protein in the rumen would correspondingly be augmented by

65 per cent/.....

65 per cent. Thus, the rumen would then have 4.19 grams of bacterial protein per Kg. of ingesta. Assuming that the rumen contained 4 Kg. ingesta, its bacterial protein would be increased on urea feeding from 10.24 gram to 16.76 gram. In addition to this protein, the animal receives from its hay from 11.9 to 17.5 grams of protein (vide Table 21) daily, bringing the total protein in the rumen to between 28.6 and 33.2 grams.

According to Voeltz (loc. cit.), 90 per cent of the bacterial protein can be utilised, thus of the 16.76 grams the sheep would utilise 15 grams. Of the poor hay 50 per cent is utilised (Smuts, 1940), therefore actually 6-8.7 grams of protein. The total protein utilised by a 45 Kg. sheep would accordingly amount to 21.8-23.8 grams.

The minimum protein requirements of a mature sheep being 410 mgm. per Kg. of body weight (Klein, Schmidt, Studt and Müller, 1939) a sheep of 45 Kg. would require 18.45 grams of protein daily. The bacterial and food protein amounting to 21.8-23.8 grams daily would, therefore, keep a 45 Kg. sheep in positive nitrogen balance. This is borne out by the results recorded in table 21, and has also been confirmed by Harris and Mitchell (1941). Without supplementation by urea, the utilisable bacterial and food protein in the rumen amounts to 15.2-17.9 grams daily, which is insufficient for maintenance and produces a negative nitrogen balance.

Under the conditions investigated there is therefore no doubt that the feeding of urea results in increased bacterial protein in sufficient amounts to maintain mature sheep in nitrogen equilibrium, provided sufficient energy is available in the ration in the form of starch or sugar.

IV. SUMMARY/ .....

IV. SUMMARY.

Ruminal Infusoria:

1. A technique is described for the preservation and counting of ruminal infusoria.
2. Reactions of specific infusoria as well as total infusorial populations to changes in the diet of stable fed sheep are described.
3. Seasonal fluctuations in ruminal infusoria of sheep on pasture are recorded. The nutritive value of the pasture was shown to have a significant influence on the density of the infusorial population. The number of infusoria decreases almost proportionately to the decline in protein content and digestibility of dry matter.
4. Data are presented comparing the density and types of infusoria in sheep on pasture and different species of antelopes under natural conditions. It was found that browsing antelopes (Kudu, Duiker, Impala), harboured more than five times the number of infusoria counted in grazing antelopes (Wildebeest, Sassaby, Sable etc.). The number of infusoria present in the rumen of sheep on pasture was comparable to that in the antelopes with grazing habits. The average numbers per cubic millimetre being respectively 278 and 313. The genus Entodinium was commonly seen both in sheep and in antelopes, and was invariably the dominant organism in animals on diets rich in protein and carbohydrates. The genus Diplodinium, on the other hand, occurred with greater regularity in sheep, whereas the genus Epidinium occurred more frequently in antelopes, although it was also present in sheep.
5. The digestion of maize starch within an infusorium is described. The brown glycogen-like granules formed within/.....

within the foodsack and plasma of the infusorium have been shown to be glycogen-synthesising bacteria and not actual glycogen-granules synthesised by the infusorium as hitherto accepted. This important function previously assigned to the infusoria, is in reality performed by bacteria which normally may be ingested by the infusoria. Consequently, the infusoria can no longer be regarded as playing an important rôle in the synthesis of glycogen in the rumen.

6. The rate of digestion of starch within the rumen was shown to be the same whether infusoria were present or not. It is concluded therefore, that infusoria do not accelerate the rate of digestion of starch in the rumen.

7. It could not be proved that infusoria assist in the digestion of cellulose within the rumen. It is surmised that the digestion of cellulose within the infusorium occurs in lines similar to those described for starch, namely, by the agency of cellulose digesting bacteria which may normally be ingested by the infusoria or which are adherent to the cellulose particles when these are ingested.

Ruminal bacteria.

8. A technique for the counting of ruminal bacteria is described.

9. A diet deficient in nitrogen inhibits proliferation of the ruminal flora to a marked degree.

10. An excess of nitrogen in the diet, in the form of meat meal, proved harmful not only to the ruminal flora but also to the host animal.

11. Further a deficiency or an excess of starch has an inhibitory influence on bacterial proliferation.

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12. Optimal conditions for bacterial growth in the rumen are created when the animal receives a balanced ration.

13. The low cystine content of lucerne hay limits bacterial multiplication in the rumen of sheep subsisting on lucerne hay only.

14. The effect of bone meal feeding (10 grams daily) on the rumen flora, is to provide an increased amount of easily digestible bacterial protein to the extent of 2.55 grams per day. Whether this is of significance to the animal in such small amounts is doubtful.

Bacterial digestion and synthesis of starch.

15. The only diastases present in the rumen seem to be those secreted by ruminal bacteria, which would then be responsible for the entire starch disintegration seen there.

16. In the rumen of sheep receiving a regular supply of starch in the diet, the bacterial disintegration of starch granules commenced after 5 hours and was completed within 18–20 hours. In the case of sheep not receiving starch in the diet, disintegration of the starch administered through the fistula commenced after 7 hours and took 8–10 hours longer to complete.

17. Starch granules of the various cereals differ in size and shape. The diameter of starch granules influences their rate of disintegration within the rumen.

18. Some of the products of starch degradation are resynthesised into glycogen and starch-like polysaccharides within certain ruminal bacteria and pseudo-yeasts.

19. The iodophilic micro-organisms encountered in the rumen and associated with the disintegration and digestion of starch, are described.

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20. An iodophilic streptococcus closely associated with the disintegration of starch was isolated in pure culture and some of its characteristics described.

Cellulose digestion in the rumen.

21. The digestibility of cellulose in mature veld hay containing 3.0 per cent protein was not improved by nitrogen and starch supplementations, even though there was a significant increase in the number of ruminal bacteria.

22. The coefficient of digestibility of cellulose decreased with increasing amounts of starch in the diet.

23. The amount of starch or sugar present in the diet influences the utilisation of its nitrogen by the animal. On a diet low in starch the nitrogen was poorly utilised. However, this improved proportionately to the increase of starch (energy) in the ration.

24. Under optimal conditions for cellulose digestion, only 62.1 per cent of the cellulose present in the diet was digested. This incomplete digestion is dependant upon the degree of lignification of the plant. The mature veld hay used in these experiments showed that lignification was well advanced.

The possibility of cystine synthesis in the rumen and its relationship to nitrogen metabolism.

25. Metabolism experiments in which rumen ingesta collected from sheep kept on a diet of lucerne hay only, was subsequently fed to white rats, showed that the biological value of this material was definitely higher (by 15 per cent) than that of untreated lucerne similarly fed to white rats. Moreover, the addition of cystine to rumen ingesta caused further enhancement of its biological value to the extent of 13 per cent.

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26. In the case of growing sheep, the supplementation of lucerne by cystine increased its biological value by 10 per cent, whereas in adult animals, Smuts and Marais in their researches, failed to produce any such improvement.

27. The experiments conducted both on rats and sheep afford no evidence of a cystine synthesis by the microflora of the rumen.

28. As far as protein metabolism is concerned, the rôle of the ruminal microflora appears to be primarily that of assimilation of proteins and other nitrogenous compounds, which are built up into the protoplasm of these organisms. Such bacterial protein being rendered available for digestion and assimilation by the host.

THE UTILISATION OF UREA BY RUMINAL MICRO-ORGANISMS.

29. The effect of urea, admixed with ruminal ingesta is apparently twofold:

Firstly, it supplies the micro-organisms with a form of nitrogen which can be transformed by them into bacterial protein. This protein is then utilisable by the ruminant.

Secondly, urea, when hydrolysed into NH_3 and CO_2, probably contributes to the production of ammonium carbonate and ammonium bicarbonate in the presence of an adequate amount of sugar or starch in the rumen. The CO_2 derived from the urea has, on calculation, been found to be insufficient for this purpose.

30. The feeding of urea to sheep, results in increased bacterial protein in sufficient amounts to maintain mature sheep in nitrogen equilibrium, provided sufficient energy is available in the ration in the form of starch and sugar. Apart from the provision of CO_2 by these

carbohydrates/.....

carbohydrates, they also supply the micro-organisms with the energy required for their metabolism and proliferation.

V. DISCUSSION AND CONCLUSIONS:

THE SIGNIFICANCE OF THE RUMINAL FAUNA AND FLORA TO THE NUTRITION AND HEALTH OF THE ANIMAL.

In evaluating the significance of the ruminal micro-organisms in ruminant digestion, special attention was given throughout this study to the ability of the ruminal fauna and flora either to disintegrate or synthesise food substances within the forestomachs. The information gained from this investigation as well as the results obtained by other workers in this field will be considered in the following discussion in an attempt to elucidate and co-ordinate some of the functions of the ruminal micro-organisms.

(1) RUMINAL FAUNA.

The ruminal fauna is composed chiefly of infusoria and amoeba. As the latter organisms do not occur in significant numbers, only the presence of the infusoria will be considered as an index of the rôle played by the microfauna in the rumen.

With regard to the digestion of cellulose and fats, Mangold (1927) and Hennenberg (1926) have shown that these substances are digested by bacteria within the foodsack of the infusorium. These bacteria are normally ingested by infusoria. Moreover, the author could not demonstrate appreciable digestion of cellulose by infusoria in an experiment in which the degree of cellulose digestion in sheep harbouring a normal infusorial population was compared

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with the degree of digestion in the same sheep free of infusoria. It is reasonable, therefore, to conclude that infusoria do not significantly influence the digestion of cellulose within the rumen. Cleveland (1924) has shown that the protozoon Reticulotermes flavipes, which is a normal inhabitant of the intestinal tract of the termite, does possess the ability to digest cellulose. This has to be considered, however, as a rare exception of an individual species of protozoon, endowed with this specialised function.

Trier, Westphal and Mangold (loc. cit.) advanced the view that infusoria were able to synthesise glycogen from starch ingested by them. It was, however, shown in this study that bacteria, normally ingested by the infusoria, were responsible for the digestion of starch and synthesis of glycogen within the infusoria, as is the case with cellulose and fats. These organisms could, therefore, be regarded only as hosts in which starch, cellulose and fats are digested by bacteria.

Ferber (1925, 1929) held that these organisms served to convert plant proteins into more easily digestible animal proteins in the form of infusorial protoplasm. The amount of protein the ruminant could derive in this way is however insignificant and could not favourably influence the nitrogen balance of the animal.

Due to their amazing activity, ruminal infusoria dissipate a large supply of energy, and consequently require a larger calorific intake, thereby tending to elevate the temperature of the ruminal mass. The physical effect of the rapid mechanical movements of these organisms as well as the possible synthesis of vitamins and amino-acids, still needs investigation. These functions, however, do not appear to be of vital importance to the ruminant, as sheep

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kept free of infusoria for several months suffered no ill effects.

From the results of this study it may therefore reasonably be concluded that the ruminal infusoria are of no vital significance to digestion in the ruminant. This view confirms the conclusiosn drawn by Scheunert and Schieblich, Becker, Schulz and Emmerson, and Westphal.

(2) RUMINAL FLORA.

Physical disintegration, either by grinding or chewing, is an act which usually precedes chemical digestion. In the ruminant food is only partly broken down before swallowing, but is subsequently regurgitated to be thoroughly masticated during rumination. This process reduces the coarse material to a certain degree of fineness, leaving bruised and frayed edges in the plant material, which results in an increased surface exposed to bacterial action. This augments and accelerates the rate of bacterial disintegration (Woodman and Stewart, 1928, Meyer, 1927). Once the cellulose digesting bacteria have entered the bruised surface or edge of a cellulose particle, disintegration can be followed microscopically, step by step, until the cellulose particle loses its structure (Meyer, loc. cit., Baker and Martin, 1938). Although the nature of the breakdown products derived from cellulose has not as yet been fully established, the fact nevertheless remains that the digestion of cellulose as well as that of all other fibrous materials is determined solely by the presence of specific cellulolastic micro-organisms within the fore-stomachs of ruminants, seeing that the essential cellulase is not secreted in any part of the digestive tract of the host animal (Dukes, 1937). Moreover, the results of the present investigation show that (1) the maintenance of the

ruminal/.....

ruminal flora at a high level is essential to promote maximal utilisation of cellulose by the ruminant. This required density of the flora can be maintained by feeding the animal on a balanced ration. (2) The bacterial population in the rumen increased by as much as 74 per cent following the supplementation of extra protein to a basic diet consisting mainly of cellulose and starch, but otherwise low in available nitrogen. This significant increase in the bacterial population was in turn associated with an improvement in cellulose digestion to the extent of 21 per cent. On the other hand again, it was found that a diet which was rich in sugar or starch brought about a decrease in cellulose digestion, due probably to the fact that the micro-organisms diverted their attention from the cellulose to the more easily digestible carbohydrates. Hence, care should be exercised with the amount of soluble carbohydrates incorporated in a diet if maximal utilisation of cellulose is required. Furthermore, it was found that with a deficiency of starch (energy) in the diet, the available nitrogen is not fully utilised by the ruminant. Accordingly, the diet of animals on energy deficiency should be supplemented with adequate amounts of starch, or other easily digestible carbohydrates, e.g. molasses. Sheep on pasture during our winter months suffer from an energy as well as a nitrogen deficiency, consequently such animals should receive supplements of starch and protein, the feed indicated being yellow maize.

Lignification is another factor which has been shown to impede the digestion of cellulose contained in mature veld hay, even to the extent of 38 per cent. Hay should, therefore, be cut before lignification has become advanced. Louw (1938) has shown that if veld hay is cut during the flowering stage its nutritional value is high,

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the degree of lignification at this stage being small.

Apart from the chemical digestion of cellulose by specific bacteria, the process of physical disintegration associated with this digestion is of paramount importance, in that it renders accessible and assimilable such vitally important nutriments as protein, sugars and minerals, which otherwise are locked up by cellulosic walls. During the process of disintegration the fragments of food particles become hydrated and softened in the rumen after which they are allowed to pass through to the abomasum and intestines. Due to the delicate nature of the abomasal and intestinal mucosa, in contrast to the horny lining of the forestomachs, this preliminary softening and maceration of the foodmass before leaving the rumen and reticulum aids in the protection of the digestive tract against excessive mechanical irritation. Consequently the food mass leaves the fore-stomachs as a fine watery colloidal suspension composed of the smallest food particles together with large numbers of micro-organisms. With the repeated onward passage of ingesta from the rumen, accommodation is made available for further amounts of food freshly consumed by the animal.

In addition to the disintegration and digestion of cellulose, the degradation of starch by micro-organisms has been shown above to occur in the rumen. As the endosperm of some cereals contains a maltase (Daish, 1916), it is possible that this enzyme assists in the later stages of starch hydrolysis within the rumen. The ruminant saliva does not influence starch digestion as it contains no starch splitting enzymes, nor are such enzymes secreted from tissue cells in the forestomachs. This deficiency is, however, compensated for by ruminal flora which also attacks and splits starch granules, the process being completed within 6½ to 18 hours. This is an advantage gained, especially

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by stall fed animals receiving a diet rich in unprepared raw starch, the digestion of which would be definitely delayed due to the absence of any salivary amylase. Unless partial disintegration of starch took place in the fore-stomachs, the digestion of this material would be dependent entirely upon the action of amylase present in the small intestine, in which case the whole process becomes unduly delayed. Whereas carbohydrates after digestion are normally absorbed as mono-saccharides, chiefly in the form of glucose, the intefvention of bacterial action in the forestomachs results in the elaboration of various organic acids such as lactic, acetic and propionic acids, which themselves are available for absorption by the animal. Part of the carbo-hydrate on the other hand, may be transformed into other intracellular polysaccharides such as glycogen, which, after the downward passage of the organisms also becomes available for assimilation.

An important group of chemical substances synthesised by the ruminal flora is the vitamin B complex. Thus, McElroy and Goss (loc. cit.) and Wegner et al (1940) have shown that these organisms can synthesise this vitamin complex even on a diet deficient in it. The significance of this function is not as yet fully realised. Ruminants are frequently dependant upon vitamin B deficient diets during our winter months when the vitamin B content of the pasture declines (Hunt, Record and Bethke, 1936). As a result of this function of the ruminal flora, avitaminosis B is a relatively unknown disease in ruminants as compared to such diseases as Polyneuritis in birds (Dobberstein and Haupt), (1927), "Black tongue", in dogs or dog pellagra (Wheeler and Sebrell, 1933), neurosis and ataxia in horses (Carlström et al, 1939).

Apart/.....

Apart from the synthesis of vitamins, it has been shown in the present investigation that ruminal micro-organisms are capable of utilising a non-protein-nitrogen e.g. urea, at least to some extent, for the purpose of building their own cellular proteins which in turn are at the disposal of the host. Consequently it enables the ruminant to utilise such a waste product of body metabolism as urea, which in the case of other animals is without any nutritive value. By virtue of its ruminal flora, and the protein-sparing effect which it displays, the ruminant animal can therefore be fed more economically than any other farm animals. This is of special importance in view of the expense and relative scarcity of proteins available in animal nutrition.

The true significance of the ruminal flora in animal health is a matter to be investigated still further, especially on account of the wide fluctuations in the nutritional value of the diet to which the ruminant animal may periodically be subjected. Thus it was found that while the ruminal flora was maintained at a high level of 1944 million per cc. in sheep on good summer pasture, the bacterial count consistantly declined to 1067 million when the nutritive value of the pasture (consumed ad lib,) increased owing to frosts. Should the consumption decline, however, as is the case during malnutrition, the number of ruminal micro-organisms decreases still further to a very low level. Ruminal paresis and ultimately complete cessation of rumination are conditions often associated with debility, which is probably aggravated by the accompanying low bacterial flora.

Moreover, it has frequently been observed in the course of this study that sudden unfavourable changes in

diet/.....

diet or the administration of such poisonous substances as copper sulphate, rapidly alters the nature of the ingesta, imparting to it a darker colour and a musty odour. At the same time the bacterial count drops from ca. 1900 to ca. 500 million per cc. and all signs of fermentation and gas production disappear. Thus the sudden intoxication of the ruminal micro-organisms, or reduction in the nutritive value of the ingesta, normally forming the substrate for the ruminal flora, is responsible for the death of vast numbers of organisms.

This is also attended by a complete change in the composition of the flora associated to some extent with progressively increased anaerobic conditions within the forestomachs. The above conditions may favour toxin production in the digestive tract with the result that liver function, which is already influenced by deficient glycogen supplies, is liable to varying degrees of derangement. There are indications that it is under these conditions that such ill defined metabolic disturbances as Domsiekte and Enzootic Icterus make their appearance. In the absence of critical experiments in this connection, it can only be assumed that the aetiology and pathogenesis of these conditions may be associated with some such disturbances in the forestomachs. Further investigations are essential, especially in regard to the behaviour of the ruminal flora under abnormal conditions.

By comparing the function of the ruminal fauna and flora, an attempt has been made to elucidate their relative importance in ruminant digestion, and it has been shown that:

(a) The infusoria which hitherto had been credited with the digestion of starch and synthesis of glycogen, in reality play a very minor rôle in this respect.

Mangold's/.....

Mangold's assumption that infusoria themselves did not directly digest cellulose but merely ingested cellulose splitting bacteria, is also confirmed by means of chemical determinations. The amount of protein the ruminant could derive daily from infusorial protoplasm has been shown to be insignificant.

(b) The part played by bacteria on the other hand seems very much more important in that they are capable of such widely different functions, as for instance, the transformation of non-protein-nitrogen, e.g. urea, into biologically more valuable proteins; the disintegration of starch and cellulose and the synthesis of glycogen and other polysaccharides.

From the results of the present study and of other workers in this field, the fact seems fairly well established that an intimate form of symbiosis exists between the ruminant and its ruminal flora. There is very little doubt that this symbiosis, is in fact, the most important feature which determines the ability of the ruminant to feed on and utilise a very much greater proportion of the lower, more abundant and therefore cheaper types of food, namely, cellulose compounds. Moreover, it enables the ruminant to build up assimilable proteins from non-protein-nitrogenous substances such as urea, and also to synthesise for itself the indispensible vitamin-B complex.

This fundamental physiological adaptation of the digestive tract has unfortunately, not received full consideration in the economic feeding of our stock. It is, therefore hoped, that this work will at least serve to focus the attention of nutritionists to the importance of ruminal biology and physiology in ruminant nutrition, and that the practical application of the present knowledge, although still incomplete, will go a long way in solving some of our nutritional problems.

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