RUMINAL FLORA STUDIES IN THE SHEEP

III. THE INFLUENCE OF DIFFERENT SOURCES OF NITROGEN UPON NITROGEN RETENTION AND UPON THE TOTAL NUMBER OF FREE MICROORGANISMS IN THE RUMEN

By V. J. Williams* and R. J. Moir*

[*Manuscript received April* 9, 1951]

Summary

The data are presented from a replicated feeding trial designed to determine the influence of different sources of nitrogen, fed at a constant level, upon the nitrogen balances and numbers and types of ruminal microorganisms in growing lambs. Six Merino lambs were fed a basal ration of oaten chaff and wheaten grain supplemented with six sources of nitrogen: linseed meal, subterranean clover seed, whole powdered egg, casein, urea, and urea plus methionine. In each diet the test nitrogen contributed 40 per cent. of the total nitrogen and the crude protein (N $\times$ 6.25) content of the whole diet was very close to 10 per cent. All diets were very similar in crude fibre and gross energy content. Additional data for certain of these diets were obtained with seven mature Merino wethers.

The mean biological values of the nitrogen of the different rations as fed to the lambs were: linseed 79.7 $\pm$ 2.59; subterranean clover seed 83.0 $\pm$ 3.43; egg 86.7 $\pm$ 4.28; casein 82.0 $\pm$ 6.05; urea 68.6 $\pm$ 1.52; urea plus methionine 75.2 $\pm$ 1.48. The biological value of the nitrogen of the whole powdered egg ration was significantly greater ($P < 0.01$) than that of linseed, of linseed significantly greater ($P < 0.01$) than that of urea plus methionine, and this significantly greater ($P < 0.01$) than that of urea. The values for the casein and subterranean clover seed rations were significantly greater than that of the urea ($P < 0.01$) and urea plus methionine ($P < 0.05$) rations but were not significantly different from each other nor from the other protein nitrogen sources.

The mean concentrations of ruminal bacteria on the different rations were found to be: linseed 23.9 $\pm$ 8.46; subterranean clover seed 25.2 $\pm$ 10.5; egg 41.2 $\pm$ 5.23; casein 42.8 $\pm$ 9.94; urea 17.7 $\pm$ 2.09; urea plus methionine 43.7 $\pm$ 9.12 million per cu. mm. The ruminal bacterial numbers were highly significantly greater ($P < 0.01$) for the egg, casein, and urea plus methionine diets than for the linseed, subterranean clover seed, and urea diets.

It is concluded that:

(i) Different sources of nitrogen can vary markedly in their biological value, i.e. capacity to promote nitrogen retention in growing lambs.

(ii) Different sources of nitrogen can vary markedly in their capacity to promote bacterial growth in the rumen of both growing lambs and mature sheep.

(iii) The value of methionine, as a supplement to urea, in improving nitrogen retention in growing lambs is due largely to its stimulating effect on bacterial growth in the rumen, thus increasing the amount of bacterial protein available to the host.

* Institute of Agriculture, University of Western Australia, Nedlands, W.A.

The numbers of ruminal protozoa were found to be highly variable and to bear no obvious relationship to the diets fed.

Some of the morphological characteristics of the ruminal bacteria on the various diets are presented and discussed.

I. Introduction

In an experiment designed to determine the effects of varying intakes of protein, from a restricted source, upon the numbers of "free" ruminal microorganisms in the sheep Moir and Williams 1950) found an extremely high correlation between the levels of protein intake and the total numbers of free microorganisms in the rumen. They concluded that, under the conditions of the experiment, the number of organisms was determined by the protein intake and that a relatively constant proportion of the dietary protein (about 50 per cent.) was converted to bacterial protein. It was realized that these results might apply to only one type of ration and to one source of protein (casein) supplementing a basal diet of oaten hay and starch. No data are available, so far as is known, on the effect of different sources of nitrogen, fed at a constant level, upon ruminal flora numbers, although there is considerable information from American sources on the utilization by the sheep of different nitrogen sources.

Johnson *et al.* (1942, 1944) presented evidence indicating that, up to a level of 10-12 per cent. crude protein (N × 6.25), a considerable proportion of the protein ultimately utilized by the ruminant is microbial protein regardless of the nature of the dietary nitrogen. They comment on the frequency with which biological values close to 60 have been obtained with ruminants fed a wide variety of rations in which the protein level is about 10-12 per cent. Exceptions to this generalization occurred, however, in the work of Sotola (1930) and Turk, Morrison, and Maynard (1934, 1935). Loosli and Harris (1945) obtained a marked improvement in the nitrogen balance of lambs when methionine was added to a urea ration. In a more extensive study Lofgreen, Loosli, and Maynard (1947) confirmed this result with respect to urea and urea plus methionine, and also found significant differences in the biological values of various nitrogen sources for the growth of lambs. A biological value of 80 was found for whole egg protein, 76 for linseed meal protein, 74 for urea plus methionine, and 71 for urea. It should be noted that all these values are appreciably higher than 60, the figure quoted by Johnson *et al.* (1942, 1944), although the nitrogen intake in each case was equivalent to 10 per cent. crude protein (N × 6.25).

The work reported in this paper constitutes a repetition of the work of Lofgreen, Loosli, and Maynard (1947), although a wider range of nitrogen sources is employed, but it goes much further, since the influence of the rations on the microflora and fauna of the rumen is also assessed.

II. Experimental

(*a*) *Design of Experiment*

The original feeding trial was designed on the basis of a 6×6 latin square in which six lambs, six trials, and six rations with different nitrogen sources were used. Unfortunately, this design could not be adhered to because of the poor consumption of certain of the diets by some of the lambs. The number of trials was therefore extended to obtain a sufficient number of satisfactory determinations. The significance of the results was tested by the use of Fisher's "*t*" test, no analysis of variance being possible.

In addition some further trials were carried out with seven mature wethers because of the great variability shown by the lambs on certain of the diets with respect to numbers of ruminal microorganisms. The mature wethers are distinguished from the lambs by the letter X following the number of the animal in the tables.

(*b*) *Experimental Animals*

Six Merino wether lambs were selected from a larger group of similar breeding for evenness as to age and appearance. They were all between 5 and 7 months of age and 60 and 70 lb. live weight. The seven mature Merino wethers were also selected from a larger group for evenness of size and appearance.

(*c*) *Rations*

The following six sources of supplementary nitrogen were used: linseed meal, subterranean clover seed, whole dried egg, casein (acid-precipitated), urea, and urea plus methionine. In each of the rations fed, approximately 40 per cent. of the total nitrogen of the ration came from one of these sources. The rest of each ration was made up of oaten chaff (40 per cent.), wheaten grain (33.3 per cent.), a mineral supplement, a small amount of molasses, and varying amounts of starch. The starch was varied in order that the diets should be of similar gross energy value, dry matter, nitrogen, and crude fibre contents. The crude protein ($N \times 6.25$) content of all the rations was very close to 10 per cent. The compositions of these rations are given in Table 1.

For all rations except the egg, the constituents other than chaff were thoroughly mixed and bound together in the form of "nuts" with a small amount of watered molasses, the resultant mix being dried for 48 hours at 55°C. The powdered egg was kept separate in air-tight tins in a refrigerator and the required weight added to the rations each day. The rest of the constituents of the egg ration were treated in the same way as the other rations.

The diets were weighed into paper bags as required, a known weight of the mixture being added to the required weight of chaff. In each case the ratio of chaff to mixture was 2 : 3. Samples were taken of each mix and of each bag of chaff for analysis.

The actual daily consumption by each animal, i.e. after making allowance for food residues, is given in Appendix I.

(*d*) *Treatment of Animals*

Each feeding period extended over 24 days, the last 10 days of which constituted the collection period. There were no rest periods between treatments. Previous experience (Moir and Williams 1950) has shown that a 14-day pre-collection period is normally sufficient to allow for adjustments of numbers of microorganisms to dietary changes where sheep have been fed for some months on dry feed. Certain types of organisms, however, persist for much longer periods in some cases, as will be described later.

TABLE 1
PERCENTAGE CONSTITUENTS IN DIET

Diet	*C* Egg	*E* Urea	*F* Urea and Methionine	*B* Sub. Clover Seed	*D* Casein	*A* Linseed
Chaff	40	40	40	40	40	40
Wheat	33.33	33.33	33.33	33.33	33.33	33.33
Egg	9					
Urea		1.44	1.43			
DL-Methionine			0.2			
Sub. clover				12.62		
Casein					5.13	
Linseed						11.75
Starch	8.42	16.0	15.78	4.8	12.28	5.67
Molasses	6.67	6.67	6.67	6.67	6.67	6.67
NaCl	0.58	0.58	0.58	0.58	0.58	0.58
Dicalcic phosphate	1.0	1.0	1.0	1.0	1.0	1.0
$CaCO_3$	1.0	1.0	1.0	1.0	1.0	1.0
Total	100.0	100.0	100.0	100.0	100.0	100.0
Crude protein (%)	9.95	9.92	10.03	9.67	9.67	9.62

The sheep were maintained in metabolism crates throughout and were fed the whole of their daily ration at 9 a.m. each day. During the 10-day collection period urine, faeces, and any feed residues were collected daily immediately prior to feeding. On the seventh and ninth days of this period, samples of rumen contents were withdrawn by stomach-tube (Moir and Williams 1950) at 3 p.m., i.e. six hours after feeding. The sheep were weighed on the first, thirteenth, and twenty-fourth days of each treatment. The average of the last two weights was used in the calculations of the biological values of the nitrogen of the diets. Adequate tap water was before the sheep at all times.

(*e*) *Treatment of Urine, Faeces, and Feed Residues*

The daily collections of urine, faeces, and feed residues were treated in the manner described by Moir and Williams (1950).

(*f*) *Counting Techniques*

The counts for the total concentrations of free ruminal bacteria and of protozoa were made as described by Moir (1951) following the method of Gall,

Stark, and Loosli (1947). Carbol-fuchsin smears were used to study the morphology of the bacteria. A qualitative estimate of the proportion of different types of organism was made by six separate counts of the numbers of each type in a defined field over the smeared area. Gram stains were also made for one sample from each treatment, from which an estimate of the proportion of Gram-negative to Gram-positive organisms was made.

Some comment on the accuracy of the counts for total free bacteria and of the extent of diurnal and day-to-day variations in individual animals on a fixed dietary régime is appropriate at this point, since the validity of any dietary treatment differences is obviously influenced by these factors.

The satisfactory relationship that exists between stomach-tube samples and the free microorganisms within the rumen has been discussed by Moir and Williams (1950) and need not be mentioned again. The relationship between the numbers of organisms counted in a nigrosine smear and the numbers actually present in the sample, however, is not nearly so satisfactory. It is exceedingly difficult to distinguish with certainty between artifacts and bacteria when their size is less than about 0.5 μ. The counts presented in this study arbitrarily exclude all bacteria less than about 0.5 μ, even though the presence of more minute organisms can be demonstrated in stained preparations. As a result, the numbers of organisms counted are slightly underestimated. Nevertheless, repeated checks by different workers using this technique, both in this laboratory and elsewhere, have given very similar results on the same samples and it is not unreasonable to argue that they are valid for comparative purposes, i.e. for comparing the effects of different dietary treatments. The value of phase contrast microscopy in overcoming this difficulty is being investigated at the present time.

Day-to-day variations in counts made on samples taken from the same sheep at the same time in relation to feeding are, in the experience of this laboratory, usually small. Moreover, the counts presented in this paper are the average of two counts made on *each* of two samples, one taken on the seventh and one on the ninth days of the collection period. Diurnal variations are considerable and are the subject of a separate study to be reported later, but it can be stated that for most types of rations there is a diurnal pattern in which the concentration of ruminal bacteria is at a minimum in the early morning before feeding and is maintained at a significantly higher level for a period of about seven hours, i.e. from three to ten hours after feeding. The sampling time used in this investigation, namely six hours after feeding, represents the time at which the concentration of ruminal bacteria is, in our experience, most likely to be near its maximum.

III. Results

The complete nitrogen balance data for each lamb for each experimental period are presented in Appendix I. In Appendix II the individual ruminal bacteria and protozoa counts are presented for both the lambs and the mature wethers included in the later stages of the experiment. The mean values for each treatment, together with their standard errors, are given in Table 2.

The biological values of the nitrogen of the rations were calculated from the nitrogen balance data by using the figures for metabolic faecal nitrogen (5.55 mg. N per 100 g. dry matter intake) and for endogenous urinary nitrogen (0.035 g. N per kg. body weight) given for lambs by Harris and Mitchell (1941).

TABLE 2
AVERAGE RESULTS FOR EACH TREATMENT

Diets	*A* Linseed	*B* Subterranean Clover	*C* Egg	*D* Casein	*E* Urea	*F* Urea and Methionine
Calc. biological value (%)	79.7 ± 2.59	83.0 ± 3.43	86.7 ± 4.28	82.0 ± 6.05	68.6 ± 1.52	75.2 ± 1.48
"True" digestibility of nitrogen (%)	89.5 ± 4.71	86.6 ± 3.43	94.2 ± 5.25	90.8 ± 2.92	92.5 ± 2.93	92.2 ± 2.16
Digestibility of dry matter (%)	73.1 ± 2.21	68.5 ± 3.76	73.9 ±4.65	74.5 ± 3.08	74.6 ± 2.19	73.8 ± 2.21
Rumen bacteria (millions per cu. mm.)	23.9 ± 8.46	25.2 ± 10.5	41.2 ± 5.23	42.8 ± 9.94	17.7 ± 3.09	43.7 ± 9.12
Methionine (g.) (estimated) per 650 g. dry matter ration	1.6	1.0	2.1	2.0	0.9	2.3

Inspection of the results of Table 2 suggests that there are real differences in the biological values of the various nitrogen sources. Statistical analysis of these data reveals the fact that certain of these differences are highly significant. Thus the mean biological value of the egg protein is significantly ($P < 0.01$) higher than that of the linseed meal protein, the linseed meal protein significantly ($P < 0.01$) higher than the urea plus methionine, and the urea plus methionine significantly ($P < 0.001$) higher than the urea. The biological values of the casein and the subterranean clover seed diets were not significantly different from each other or from the egg and linseed meal diets but they were both significantly higher than the urea plus methionine ($P < 0.05$) and the urea ($P < 0.001$).

Comparison of the results of Table 2 with those of Lofgreen, Loosli, and Maynard (1947) is of interest in view of the fact that these workers used rations comparable to our own in many respects, and especially in that the various nitrogen sources being compared comprised 40 per cent. of the total nitrogen of the rations. Their figures for urea, urea plus methionine, linseed meal, and whole dried egg were 71 ± 1.2, 74 ± 1.8, 76 ± 1.7, and 80 ± 2.1 respectively. It is apparent that the first three of these are very similar to those of Table 2 but our figure for egg protein (86.7 ± 4.28) is appreciably higher and more variable.

No data are available with which our figure for the biological value of subterranean clover seed protein can be compared but there are a number for casein. Thus Harris and Mitchell (1941) obtained for casein a figure of 59 and Johnson *et al.* (1942, 1944) a figure of 60 for the growth of lambs. In both these cases the casein comprised 50 per cent. of the total nitrogen of the ration and the percentage total protein in the diets was higher than ours. This would tend to depress the biological values (Mitchell 1924) but even taking these factors into consideration, our figure of 82 ± 6.05 appears extremely high. It is possible that this is related, at least in part, to the nature of the casein preparation, since Reed, Moir, and Underwood (1949), using the same source of casein, obtained a biological value of 79 for the growth of rats. This is much higher than the figures of 62, 65, and 73 obtained by Olson and Palmer (1940), Hughes and Hauge (1945), and Beadles *et al.* (1933) respectively.

(*b*) *Counts for Total Free Ruminal Bacteria*

The outstanding characteristic of the figures for the total concentrations of ruminal bacteria, presented in Appendix II, is the marked individual variability between sheep on the same diet. The very high standard errors of the means given in Table 2, particularly for the linseed, casein, subterranean clover seed, and urea plus methionine rations, show this very clearly. This variability is difficult to explain in the present state of our knowledge, although a better understanding of the nature and cause of diurnal fluctuations, especially in relation to time and rate of food consumption, should throw considerable light on this problem. It should be pointed out, however, that marked individual fluctuation was shown by the mature wethers, as well as the lambs, although only those wethers were included in the results of Table 2 that had completely consumed their diets within 24 hours on all days previous to sampling and on sampling days had completely consumed their diets within six hours of feeding, i.e. before sampling.

Statistical examination of the mean results of Table 2 shows that the bacterial counts fall into two distinct groups. The first of these groups contains the counts from the urea plus methionine, casein, and egg rations. These do not differ significantly from each other but are all highly significantly greater ($P < 0.01$) than the counts of the second group. This group contains the urea, linseed meal, and subterranean clover seed rations, which again do not differ significantly from each other. The effect of the addition of methionine to the urea diet is very great—the mean count is raised from 17.7 ± 3.09 to 43.7 ± 9.12 million bacteria per cu. mm.

(*c*) *Morphological Characteristics of the Bacteria*

As accurate differential counts of the various organisms present were not made it is difficult to present the results of the morphological observations in a concise form. In Appendix III, however, an attempt is made to present the main features. In examining this it should be appreciated that morphological characteristics serve only as a guide to the predominating forms of microorganisms present.

On the urea, linseed, egg, and subterranean clover seed diets, most of the sheep showed a similar morphological picture for each diet but the sheep on the casein and urea plus methionine diets were less consistent. It appears that in these two groups the sheep were still showing the residual effects of previous dietary treatments. There is evidence that 24 days, which was the length of the treatments with our diets with the lambs, is not always sufficient to allow a proper expression of morphological changes induced by dietary means. Thus the results for wether 1X (shown in Appendix III) indicate that the effect of green grazing, as evidenced by a high concentration of yeast-like forms (see Quin 1943; Van der Westhuizen, Oxford, and Quin 1950) can persist through two treatments. No other mature sheep or lamb had yeast-like forms present after 24 days on either the linseed or casein diets. In another experiment, yeast-like forms have been found in the rumen of one sheep after four months on a wheat gluten diet, whereas these organisms had entirely disappeared from its 19 companions under the same dietary conditions within 3-8 weeks.

A number of the mature sheep were continued on the various diets for a total of 40 days. During this extended period, on certain of the diets, a number of interesting changes in the balance of the morphological types of bacteria took place. These are indicated in Appendix III. It is important to note, however, that in no cases did the extra period of time on any of the diets result in any significant changes in the total concentrations of ruminal bacteria, compared with the 24-day period used for most of the sheep.

(*d*) *Protozoal Counts*

The individual protozoal counts are given in Appendix II both for the lambs and the mature sheep, but the mean figures for each treatment are not given in Table 2 because the variability shown, both between sheep on the same diet and between samples taken from the same sheep on different days, is so large that averages would, in most cases, be meaningless. No explanation of this tremendous variability can be given, although it can hardly be due to the counting technique or to the method of sampling, since protozoa are free movers in the rumen liquor. However, there appears to be no relationship between the diets fed and protozoal numbers.

IV. Discussion

It is clear from the results given in the preceding section that different sources of nitrogen can vary significantly in their value for the promotion of nitrogen retention in growing lambs. To this extent our results are merely a confirmation and extension of the findings of Lofgreen, Loosli, and Maynard (1947) and in regard to the effect of methionine supplements in increasing the value of urea, also those of Loosli and Harris (1945). They are, however, in contrast to those of Johnson *et al.* (1942, 1944) and do not support the claim of these workers that with sheep "the biological value of the nitrogen of rations containing 10-12 per cent. crude protein ($N \times 6.25$) generally varies only within a few per cent. of 60." Nor do they support the implication of this claim that

the proportion of dietary nitrogen converted to bacterial protein is always high and relatively constant for all nitrogen sources. In fact the significant variation in the capacity of certain of our nitrogen sources to promote bacterial growth shows that there can be marked differences in this regard. If the concentration of ruminal bacteria is an indication of the extent of conversion of dietary nitrogen in the rumen to bacterial protein then it is obvious that this conversion was, with the linseed and subterranean clover seed diets, only about one-half that of the casein and egg diets. The difference was even greater with the urea plus methionine diet compared with the urea diet, in spite of the fact the only difference between the two rations was that the former contained a supplement of pure DL-methionine at a level of 0.2 per cent.

Although these results are quite definite, they are by no means easy to explain. However, it is of some interest to compare certain of the biological values obtained by us for lambs with those given in the literature for rats, although it must be recognized that in our work the test nitrogen made up only 40 per cent. of the total nitrogen. It can be assumed, for the purposes of preliminary argument, that these values would be much the same, if it were not for the intervention of the ruminal bacteria. The average biological value of the protein of flax seed (equivalent to linseed meal) is given by Block and Mitchell (1946) as 78 for growing rats. This is very close to our figure of 79.7 for growing lambs. It would appear that in this case the net effect of ruminal microflora on the nitrogen retention of the host has been negligible. This is not unexpected in view of the relatively low bacterial numbers on this diet. For the proteins of whole egg, Block and Mitchell (1946) give a biological value of 96 for the rat. This is 10 per cent. higher than our figure for this protein for lambs and 16 per cent. higher than that of Lofgreen, Loosli, and Maynard (1947). On this ration the numbers of bacteria in the rumen were high and their net effect appears to have been detrimental to the nitrogen balance of the host. This could have been due to such factors as preferential deamination of certain of the essential amino acids or loss by absorption of ammonia, owing to deamination of the egg protein by the bacteria more rapidly than it can be built up again into bacterial protein, and to the lower value of the bacterial protein.

The position with respect to the urea and urea plus methionine diets is slightly less complex because in these the supplementary nitrogen source is not directly available to the host. In our experiments the effect of the addition of methionine was to raise the mean concentration of ruminal bacteria from 17.7 ± 3.09 to 43.7 ± 9.12 million per cu. mm. and to increase the biological value of the nitrogen from 68.6 ± 1.52 to 75.2 ± 1.48. It seems certain therefore that the improved nitrogen retention that occurs when methionine supplements urea as a source of nitrogen to the sheep can be largely explained by the methionine acting as a growth factor for the ruminal bacteria, resulting in greatly increased numbers of organisms and therefore greatly increased synthesis, from the urea, of bacterial protein. The possibility of some direct absorption of the methionine by the host, either from the rumen itself or from

lower parts of the digestive tract, cannot be excluded, however. This absorbed methionine would supplement the bacterial protein because this protein is somewhat deficient in methionine, as Reed, Moir, and Underwood (1949) have shown. It is suggested that any such supplementary effect must be small, compared with the effect of the methionine on bacterial growth and bacterial protein synthesis.

These results led us to examine the rest of the diets to see if the low bacterial numbers obtained with some of them could be explained by a low methionine intake. The approximate amounts of methionine contained in the six diets, calculated from the figures of Block and Bolling (1945) and Johanson and Lugg (1946), are included in Table 2. A comparison of these amounts with the mean concentrations of ruminal bacteria on the various diets suggests that lack of methionine has acted as a limiting factor on bacterial growth in the subterranean clover and possibly the linseed diets, as well as the urea diet. Examination of the individual data given in Appendix II, however, casts considerable doubt on this explanation because certain of the sheep in the linseed and subterranean clover groups showed concentrations of ruminal bacteria similar to those shown by most of the sheep in the groups with a high methionine intake. It is obvious that the relationship of methionine intake to bacterial growth in the rumen warrants further study. This problem, together with the possible significance of other essential amino acids, is under investigation in this laboratory at present.

V. Acknowledgments

Grateful acknowledgment is made to the Wool Research Trust Funds for financial assistance; to Professor E. J. Underwood, Director of the Institute of Agriculture, for assistance and advice in the planning of this work and in the preparation of the manuscript; and to Mr. M. C. Nottle for carrying out some of the nitrogen estimations.

VI. References

Beadles, J. R., Quisenberry, J. H., Nakamura, F. I., and Mitchell, H. H. (1933).—*J. Agric. Res.* **47**: 947.

Block, R. J., and Bolling, D. (1945).—"The Amino Acid Composition of Proteins and Foods." 1st Ed. (Ryerson Press: Toronto.)

Block, R. J., and Mitchell, H. H. (1946).—*Nutr. Abstr. Rev.* **16**: 249.

Gall, L. S., Stark, C. N., and Loosli, J. K. (1947).—*J. Dairy Sci.* **30**: 891.

Harris, L. E., and Mitchell, H. H. (1941).—*J. Nutrit.* **22**: 183.

Hughes, C. W., and Hauge, S. M. (1945).—*J. Nutrit.* **30**: 245.

Johanson, R., and Lugg, J. W. H. (1946).—*Aust. J. Exp. Biol. Med. Sci.* **24**: 321.

Johnson, B. C., Hamilton, T. S., Mitchell, H. H., and Robinson, W. B. (1942).—*J. Anim. Sci.* **1**: 236.

Johnson, B. C., Hamilton, T. S., Robinson, W. B., and Garey, J. C. (1944).—*J. Anim. Sci.* **3**: 287.

Lofgreen, G. P., Loosli, J. K., and Maynard L. A. (1947).—*J. Anim. Sci.* **6**: 343.

Loosli, J. K., and Harris, L. E. (1945).—*J. Anim. Sci.* **4**: 435.

MITCHELL, H. H. (1924).—*J. Biol. Chem.* **58**: 905.
MOIR, R. J. (1951).—*Aust. J. Agric. Res.* **2** (3): 322.
MOIR, R. J., and WILLIAMS, V. J. (1950).—*Aust. J. Sci. Res.* B **3**: 381.
OLSON, F. C., and PALMER, L. S. (1940).—*J. Agric. Res.* **60**: 331.
QUIN, J. I. (1943).—*Onderstepoort J. Vet. Sci.* **18**: 91.
REED, F. M., MOIR, R. J., and UNDERWOOD, E. J. (1949).—*Aust. J. Sci. Res.* B **2**: 304.
SOTOLA, J. (1930).—*J. Agric. Res.* **40**: 79.
TURK, K. L., MORRISON, F. B., and MAYNARD, L. A. (1934).—*J. Agric. Res.* **48**: 555.
TURK, K. L., MORRISON, F. B., and MAYNARD, L. A. (1935).—*J. Agric. Res.* **51**: 401.
VAN DER WESTHUIZEN, G. C. A., OXFORD, A. E. T., and QUIN, J. I. (1950).—*Onderstepoort J. Vet. Sci.* **24**: 119.

APPENDIX I

NITROGEN BALANCE RESULTS PER 10-DAY PERIOD

Sheep	Initial Weight (lb.)	Final Weight (lb.)	Average Weight (lb.)	Dry Matter Intake (g.)	Nitrogen Intake (g.)	Nitrogen Faeces (g.)	Metabolic Nitrogen (g.)	Nitrogen Urine (g.)	Endogenous Nitrogen (g.)	Apparent Digestible Nitrogen (%)	True Digestible Nitrogen (%)	Nitrogen Balance (g.)	Biological Value (%)	Digestible Dry Matter (%)
Diet *A* (Linseed)														
714	70	71	70.5	5392	83.0	34.2	21.6	23.9	10.7	58.5	84.7	+31.1	81.9	74.9
321	66	68	67	5841	89.9	26.0	23.4	29.9	10.2	71.1	97.1	+34.0	77.4	74.3
724	83	84	83.5	5841	89.9	32.6	23.4	26.8	12.7	63.7	89.7	+30.5	82.5	72.8
719	72	74	73	5151	77.8	33.9	20.6	23.1	11.5	56.5	83.2	+20.8	82.0	74.2
304	72	74	73	6500	107.7	38.3	26.0	32.3	11.1	64.4	88.6	+37.1	78.0	69.9
316	76	78	77	6500	107.7	35.9	26.0	37.8	11.7	66.7	90.8	+34.0	75.6	70.4
301	79	81	80	6500	107.7	33.8	26.0	32.9	12.2	68.6	92.7	+41.0	79.2	75.5
Diet *B* (Subterranean Clover Seed)														
304	58	61	59.5	5533	85.5	34.0	22.1	21.3	9.0	60.2	86.1	+30.2	83.3	65.9
725	69	70	69.5	5533	85.5	38.2	22.3	20.2	10.6	55.4	81.5	+27.2	86.2	69.6
723	59	64	61.5	5221	78.8	33.3	20.9	18.6	9.4	57.8	84.3	+25.9	86.0	69.5
721	76	79	78	5995	92.6	34.4	24.0	24.0	11.9	62.9	88.8	+34.3	85.2	70.0
301	80	81	80.5	6550	122.7	35.3	26.0	42.6	12.2	71.2	92.4	+44.8	79.0	70.3
312	65	68	66.5	6550	122.7	43.5	26.0	32.15	10.1	64.5	85.7	+47.1	79.0	68.5
316	72.5	76	74	6285	122.7	44.2	25.1	33.1	11.3	64.0	84.5	+45.5	79.0	60.8
714	69	72	70.5	5344	84.7	30.5	21.4	21.2	10.7	64.0	89.3	+33.1	86.2	73.5
Diet *C* (Powdered Whole Egg)														
719	67	71	69	4669	75.4	21.1	18.7	18.0	10.5	72.0	90.6	+36.3	89.7	70.7
724	73	77	75	5562	86.6	24.4	22.7	22.6	11.4	71.8	98.1	+39.7	86.8	79.8
722	68	69	68.5	4563	72.2	19.8	18.2	14.9	10.3	72.6	97.9	+37.5	93.7	77.4
312	59	61	60	5683	76.3	24.9	22.7	19.6	9.1	67.4	97.2	+31.9	85.9	76.0
306	54	56	55	5416	83.7	36.6	21.7	18.1	8.4	56.3	82.2	+29.0	87.2	65.7
321	77	78	77.5	6312	115.9	28.9	25.2	32.2	11.7	75.1	96.8	+54.9	81.0	73.2
318	77	79	78	6460	117.8	29.7	25.8	31.9	11.9	74.8	96.7	+56.1	82.4	74.3

APPENDIX I (*Continued*)

Sheep	Initial Weight (lb.)	Final Weight (lb.)	Average Weight (lb.)	Dry Matter Intake (g.)	Nitrogen Intake (g.)	Nitrogen Faeces (g.)	Metabolic Nitrogen (g.)	Nitrogen Urine (g.)	Endogenous Nitrogen (g.)	Apparent Digestible Nitrogen (%)	True Digestible Nitrogen (%)	Nitrogen Balance (g.)	Biological Value (%)	Digestible Dry Matter (%)
							Diet *D* (Casein)							
724	77	79	78	5935	89.8	31.8	23.7	20.4	11.9	64.6	89.4	+37.6	89.4	76.1
725	63	65	64	5386	83.3	26.8	21.5	18.5	9.7	67.8	93.6	+37.9	88.7	76.7
301	64	66	65	5395	83.5	32.7	21.6	21.2	9.9	60.8	86.6	+29.5	85.7	71.8
312	58	60	59	5395	83.5	30.2	21.6	20.8	9.1	63.9	89.7	+32.5	85.4	75.6
321	72	76	74	6500	109.9	36.3	26.0	31.6	11.3	67.0	90.6	+42.0	79.5	76.9
316	70	73	71.5	6410	105.0	35.2	25.6	31.5	10.9	66.5	90.9	+38.3	77.0	68.9
314	71	73	72	6500	109.9	37.7	26.0	34.8	10.9	65.7	89.4	+37.4	76.0	72.3
304	79	80	79.5	6484	103.9	29.8	25.9	38.2	12.2	72.2	96.2	+35.9	74.0	77.4
							Diet *E* (Urea)							
724	69	70	69.5	5436	84.9	24.9	21.7	36.4	10.6	70.8	96.4	+23.6	69.6	78.5
318	61	63	62	5911	92.3	33.0	23.6	36.0	9.4	64.2	89.9	+23.3	67.9	72.4
301	66	67	66.5	5911	92.3	30.9	23.6	37.3	10.2	66.5	92.1	+24.1	68.1	75.1
306	55	57	56	5911	92.3	33.9	23.6	36.0	8.5	63.3	88.9	+22.4	66.5	73.9
304	63	65	64	5911	92.3	30.6	23.6	36.4	9.4	66.8	92.4	+25.3	68.4	72.8
312	72	72	72	6500	114.5	31.3	26.0	42.6	10.9	72.6	95.3	+40.5	70.9	74.8
							Diet *F* (Urea plus Methionine)							
725	72	73	72.5	5907	93.2	31.6	23.6	30.1	11.0	66.1	91.5	+31.4	77.6	74.6
316	65	68	66.5	5453	86.0	27.6	21.8	30.3	10.2	68.0	93.3	+28.2	75.0	73.2
322	62	64	63	5042	78.3	29.0	23.4	26.6	9.6	63.0	92.8	+22.7	75.5	70.1
301	74	76	75	6500	117.6	40.0	26.0	37.5	11.4	66.0	88.1	+40.1	74.8	73.9
318	73	75	74	6500	117.6	33.8	26.0	40.9	11.2	71.6	93.7	+43.3	73.0	74.1
321	78	80	79	6500	104.5	25.7	26.0	27.0	12.0	75.5	93.7	+51.8	75.0	76.9

Appendix II

QUANTITATIVE RESULTS — RUMEN MICROORGANISMS

		725	724	723	722	721	719	714	322	321	318	316	312	306	304	301	314	4X	6X	1X	5X	3X	2X	7X
										Diet *A* (Linseed)														
Conc. rumen bacteria	Sample 1		34				20	18		20		14			41	35		21		20		21		
(millions per cu. mm.)	Sample 2		28				20	20		19		14			40	34								
	Average		31.0				20.0	19.0		19.5		14.0			40.5	34.5		21.0		20.0		21.0		
Protozoa (No. per cu. mm.)	Sample 1		165				1248	153		75		78			247	6								
	Sample 2		441				1336	153		135		18			434	12								
										Diet *B* (Subterranean Clover Seed)														
Conc. rumen bacteria	Sample 1	29		45		19		17				15	18		20	24			21		44			29
(millions per cu. mm.)	Sample 2	32		44		16		16				17	18		20	21								
	Average	30.5		44.5		17.5		16.5				16.0	18.0		20.0	22.5			21.0		44.0			29.0
Protozoa (No. per cu. mm.)	Sample 1	234		948		171		303				459	246		411	360								
	Sample 2	243		630		192		315				573	258		435	1425								
										Diet *C* (Powdered Egg)														
Conc. rumen bacteria	Sample 1		44		41		33			35	38		45	46										
(millions per cu. mm.)	Sample 2		50		46		39			32	39		44	45										
	Average		47.0		43.5		36.0			33.5	38.5		44.5	45.5										
Protozoa (No. per cu. mm.)	Sample 1		1167		909		762			420	30		0	6										
	Sample 2		1236		291		276			501	21		3	12										
										Diet *D* (Casein)														
Conc. rumen bacteria	Sample 1	32	57							44		36	51		40	31	32			41		55	60	
(millions per cu. mm.)	Sample 2	29	43							42		35	48		45	30	35							
	Average	30.5	50.0							43.0		35.5	49.5		42.5	30.5	33.5			41.0		55.0	60.0	
Protozoa (No. per cu. mm.)	Sample 1	2448	1175							795		93	45		1605	1620	162							
	Sample 2	963	1011							522		159	105		3438	1497	1221							
										Diet *E* (Urea)														
Conc. rumen bacteria	Sample 1		18								17		19	12	12	19			20		20			
(millions per cu. mm.)	Sample 2		18								19		19	13	13	19								
	Average		18.0								18.0		19.0	12.5	12.5	19.0			20.0		20.0			
Protozoa (No. per cu. mm.)	Sample 1		1251								2355		1605	1500	630	180								
	Sample 2		1221								1758		891	1671	765	1376								
										Diet *F* (Urea plus Methionine)														
Conc. rumen bacteria	Sample 1	61							44	34	44	40				35								
(millions per cu. mm.)	Sample 2	50								33	63	42				36								
	Average	55.5							44.0	33.5	53.5	41.0				35.5								
Protozoa (No. per cu. mm.)	Sample 1	977							1233	1002	1926	282				21								
	Sample 2	450								693	963	561				480								

APPENDIX III

QUALITATIVE MORPHOLOGICAL DESCRIPTIONS OF RUMEN BACTERIA (INCLUDING YEAST-LIKE FORMS)

Diet *A*

Linseed

24 days

Proportion of cocci to ovals and rods varies with individuals.
Large oval 1.6 μ × 0.8 μ always present.
Large curved rod 3.5 μ × 0.5 μ always present.
Exception 1X — contained yeast-like forms with otherwise normal flora.
Predominantly Gram —ve.

40 days

4X, Staining and floral balance unchanged with time

Diet *B*

Subterranean Clover Seed

24 days

Majority predominantly rod flora.
Rods mostly short 1.4 μ × 0.3 μ.
Exceptions 5X, massed cocci, few rods.
714, Oval, coccal flora, few rods.
Predominantly Gram —ve.

40 days

5X, Fewer cocci and more rods.
Trend towards same as majority.
Staining unchanged.
7X, Unchanged, in types and staining

Diet *C*

Egg

24 days

A balanced cocci, oval, rod flora.
Exception 724 — predominantly rods; straight, crescentic, and S-shaped.
Predominantly Gram —ve.

Diet *D*

Casein

24 days

Rod flora — 725, 2X, 3X.
Rod, oval, coccal — 316, 314, 312.
Cocci, oval — 301, 321, 304—small numbers rods.
Coccal flora — 724, 1X — small numbers rods.
1X, Large numbers of yeast-like forms superimposed over coccal flora.
Majority have conspicuous numbers of cocci (3 exceptions — 725, 2X, 3X).
Predominantly Gram —ve.

40 days

1X, Yeast-like forms persisted in high numbers.
Rods even smaller proportion of flora.
Cocci taken over.
Similar staining

Diet *E*

Urea

24 days

Majority dominated by curved rods.
Basal flora cocci.
306, Cocci — oval, few rods.
5X, Massed cocci — large curved rod.
6X, Massed cocci — few large curved rods.
Predominantly Gram —ve.

40 days

6X, Curved rod nearly entirely gone.
Massed cocci taken over

Diet *F*

Urea plus Methionine

24 days

Ovals always high proportion of flora.
Cocci as high as ovals in 4 cases.
Rods as high as ovals in 2 cases.
Predominantly Gram —ve.