NITROGEN BALANCE STUDIES WITH
MATURE RAMS

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INTRODUCTION

The biological importance of the bacteria which live in the paunch of the ruminant has been appreciated only during the last few years. Since they are plant cells, rather than animal, they have the ability to synthesize certain nutrients deficient in the normal diet of the ruminant. Apparently the bacteria can also utilize certain inorganic forms of nitrogen, from which they can build their own cellular proteins. As the bacteria are digested in their passage through the intestinal tract, these proteins become available to the host.

The shortage of natural forms of proteins for livestock feeds during the two World Wars stimulated much research on this phase of ruminant nutrition. Of the various nitrogenous compounds studied, urea -- a diamide of low molecular weight which could be commercially synthesized -- appeared to give the best results.

The extent to which the ruminant can utilize urea nitrogen has been measured principally by growth and maintenance studies, rumen fistula analyses, and nitrogen balance studies. The latter have been the most widely used criteria. A high positive nitrogen balance supposedly indicates a high retention of nitrogen for tissue synthesis, as well as a high biological value for the urea nitrogen, and an overall beneficial effect of the nitrogen.

A nitrogen balance might be affected by errors from many sources. Among these are: (1) errors in calculating and measuring the nitrogen intake; (2) errors in the collection, sampling, and analysis of the excreta; (3) differences in the energy content of otherwise comparable rations; (4) environmental effects, such as those of temperature, and (5) obscure factors in protein metabolism not yet understood. This study was undertaken with the above sources of error in mind as a critical evaluation of the nitrogen balance technique when urea supplied a part of the nitrogen in the rations of sheep.

REVIEW OF LITERATURE

Armsby (1911) was one of the first to summarize the early experimental work on the utilization of non-protein forms of nitrogen by ruminants. He cited work which indicated that if the level of natural proteins in the diet were low, the ruminant could utilize certain non-protein forms of nitrogen. Armsby was critical, however, of the actual value of urea as compared to natural proteins.

Mitchell and Hamilton (1929) summarized the literature available at that time on non-protein forms of nitrogen. They concluded that the practical value of protein synthesis in the rumen was still in doubt and that much further experimental work was needed on the problem. Later, Krebs (1937) published an extensive review of the literature dealing with the utilization of urea nitrogen and stressed the difficulty of drawing reliable conclusions from the mass of conflicting data. He admitted that the evidence indicated some protein-sparing effect by such nitrogenous compounds as urea. However, he felt that the quantity of bacterial protein synthesized was too small to be significant and of little value to the host.

Recently, Hastings (1944), McNaught and Smith (1947), and Elsden and Phillipson (1948) have published extensive reviews of the experimental work with urea. They have cited work which definitely indicates that urea can be utilized by the ruminant as a source of nitrogen.

The Mechanism of Urea Utilization

Many workers have attempted to show that urea can be utilized by the bacteria of the paunch. Both "in vivo" and "in vitro" techniques have been developed; the former through the use of the rumen fistula, and the latter by means of artificial conditions of fermentation. Regardless of method, an attempt has been made to estimate the synthesis of protein in the fermenting mass. However, the difficulties involved in obtaining uniform rumen samples, or in duplicating the exact conditions found in the paunch through artificial means, are so formidable that the results obtained from such studies are inconsistent.

Wegner and co-workers (1941), using the rumen fistula technique, observed that urea, when ingested at levels of from 1 to 5 per cent of the dry matter of the ration, disappeared from the rumen within 4 to 6 hours after feeding. In further experiments, they found that the ammonia released in the breakdown of urea was efficiently converted to bacterial protein only when the diet was low in natural forms of protein. At low levels of protein intake, the addition of urea caused an increase in the crude protein content of the ingesta of the paunch, proportional to the decrease in ammonia nitrogen. They found little evidence of protein synthesis at high levels of protein intake.

Mills, et. al. (1942) have been able to show from their rumen fistula experiments that only when urea is ingested in the presence of starch does the crude protein content of the rumen show a measurable increase, and the ammonia concentration decrease appreciably. With timothy hay as the sole ingredient of the basal ration, urea was utilized only to a limited extent. In the presence of starch, a suitable substrate was apparently established for the development of an active microflora and urea was much more efficiently utilized. They concluded that the insoluble polysaccharide, cellulose, was apparently attacked too slowly during the first few hours after ingestion to make it a readily available source of energy for the bacteria. They noted that when casein was added to a timothy hay-starch-urea ration, the utilization of urea was markedly reduced. The authors concluded that to obtain maximum efficiency from urea, the ration should contain a fermentable carbohydrate, such as starch, together with proteins less available to the bacteria, such as those found in the natural feeds of the ruminant.

Pearson and Smith (1943) have pointed out the difficulties of drawing reliable conclusions from the analysis of rumen samples. They found that successive samples, withdrawn through a fistula from different sections of the rumen, varied as much as 20 per cent in protein content. They have shown that the urease activity of the rumen liquor of the cow is very great at all times of the day. They have further demonstrated that ammonia is the first product formed when urea breaks down, and have suggested that the efficiency of protein synthesis varies inversely with the concentration of ammonia in the fermenting mass -- high concentrations of ammonia resulting in lowered protein synthesis. They were able to show that the two reactions -- urea breakdown to form ammonia, and protein synthesis from the ammonia liberated -- proceed at the same time in the rumen medium. It was noted, however, that protein synthesis was equal to the ammonia produced only when starch, maltose, or simple sugars were present. They calculated that the synthesis of protein was approximately 8 mgm. of nitrogen per 100 grams of rumen liquor during the first two hours after ingestion. This would be equivalent to 300 grams of protein synthesized during 24 hours of such activity. It was further shown that the availability of the proteins in the ration had an adverse affect on the utilization of urea nitrogen. Their work indicated that when rations were already high in readily available forms of proteins, such as casein, an inefficient utilization of urea-nitrogen resulted. Ammonia formation thus appeared to exceed the capacity of the bacteria to synthesize their own cellular proteins.

Smith and Baker (1944) have shown that the synthesis of protein "in vitro" in the presence of added carbohydrate, is accompanied by a corresponding increase in the bacterial content of the fermenting mass.

Johnson and co-workers (1944) used defaunated lambs to study urea utilization. Samples taken through a rumen fistula indicated that the bacterial population was highest shortly after the ingestion of the ration. Their results have shown that the bacterial population decreases in proportion to the increase in the protozoan content of the paunch, but they believed that the protozoa played no active part in the synthesis of protein. Defaunated lambs were able to utilize urea nitrogen with the same apprent efficiency as normal lambs fed the same ration, with an average biological value of 49. The protozoan and bacterial fractions of the rumen contents, when isolated and fed to rats, gave biological values of 68 for the protozoan protein, and 66 for the bacterial fraction.

McDonald (1948) showed that the utilization of urea in the paunch is a normal digestive function. He has shown that the parotid gland of the sheep secretes an appreciable quantity of urea (approximately 0.5 grams) during a 24 hour period. Using sheep on normal rations, he found that the ammonia content of the portal blood draining the rumen was much higher than that of the systemic circulation. He postulated that ammonia was not only produced, but absorbed in significant quantities from the rumen, in amounts quivalent to 4 to 5 grams of ammonia nitrogen during a 24 hour period.

Dinning and associates (1948) have shown that the oral administration of 40 grams of urea in water solution to a sheep under light anesthesia produced a rapid rise in the urea and ammonia content of the portal blood. The urea-nitrogen content of the portal blood rose from 7.77 mgm. per cent to 15.26 mgm.

per cent within 15 minutes after administration of the drench. The ammonia-nitrogen values rose from 1.75 to 4.06 mgm. per cent during the same period, indicating a very rapid absorption of both ammonia and urea from the rumen.

The fate of the absorbed urea or ammonia is not clear at the present time. Schoenheimer (1946) fed ammonium citrate, labeled with isotopic nitrogen to rats and was able to recover appreciable quantities of the isotope in amino acids isolated from the tissue proteins. Recent work by Leifer, Roth, and Hempelman (1949) has shown that when urea, with labeled C^{14}, was injected intraperitoneally into mice, approximately 21 per cent of the isotopic carbon appeared in the exhaled CO_2 within 3 hours. On tissue analysis, it was found that the remainder of the labeled CO_2 was uniformly distributed in the liver, heart, spleen, muscle, brain and blood.

McNaught and Smith (1947), in summarizing the literature on the mechanism of bacterial protein synthesis, commented that "the economy of utilization of nitrogen in the rumen will depend on (a) the rate of growth of the bacteria, which in turn depends on a liberal supply of starch, and (b) the balance between the nitrogen requirements of the bacteria and the supply of nitrogenous compounds." They further state that if excess quantities of ammonia are formed, either from the breakdown of urea or from such sources as casein, in amounts greater than the bacteria can utilize in protein synthesis, this excess will be wasted. A portion of the ammonia entering the blood stream may be utilized again by the ruminant through the urea in the saliva, but the remainder will probably be excreted in the urine. The authors felt that it was inevitable that a certain portion of the urea and ammonia fractions of the rumen mass should pass directly through the rumen wall and into the blood stream before the bacteria could utilize it. They felt that this possibly explained the fact that non-protein forms of nitrogen were of less value to the ruminant than true proteins.

The Effect of Urea on the Ration

Experimental work with urea has proven almost conclusively that when this substance is added to low-protein rations, a positive nitrogen balance is promoted and the digestibility of the other feed nutrients in the ration is enhanced.

Hart, Bohstedt, Deobold and Wegner (1939) were among the first to make a comprehensive study of the utilization of urea by growing dairy calves. They found very little difference in the daily growth rates of the calves on rations where 43 per cent of the nitrogen was supplied by either casein or urea.

Harris and Mitchell (1941) studied nitrogen equilibrium with 8 wethers, 15 to 18 months of age, and averaging 27 to 40 kilograms in body weight. They calculated the endogenous urinary nitrogen to be 0.0333 grams daily per kilogram of body weight, while the metabolic fecal nitrogen values obtained were 0.555 grams per 100 grams of dry matter intake. They found that the wethers used in their experiment could be maintained in nitrogen equilibrium with an intake of approximately 202 mgm. of urea nitrogen, or 161 mgm. of casein nitrogen, per kilogram of live weight per day. At nitrogen equilibrium, the biological value of urea was 62, as compared to 79 for casein. They concluded that urea nitrogen was only 80 per cent as valuable as casein nitrogen in replacing the loss of endogenous nitrogen from sheep. However, they were able to show that urea nitrogen could replace at least 90 per cent of the endogenous nitrogen loss of the wethers, with an efficiency of utilization of approximately 60 per cent. The authors state that since the utilization of urea is a function of the paunch bacteria, it may vary widely from time to time, and from animal to animal.

In further experiments, Harris and Mitchell (1941a) worked with growing lambs and concluded that at protein levels exceeding 11 per cent of the ration, bacterial synthesis of proteins from urea nitrogen was retarded. Biological values computed from rations containing urea tended to bear out this assumption. With the percentage of nitrogen in the ration supplied by urea remaining the same, they found biological values for the proteins of rations containing 8, 11, and 15 per cent crude protein to be 74, 60 and 44, respectively. Two tests with casein nitrogen and urea nitrogen at the 15 per cent protein level of the ration, indicated a wide superiority in favor of the casein. The authors noted that the addition of urea to low-protein rations increased the digestibility of the cellulose.

Johnson, Hamilton, Mitchell and Robinson (1942) working with growing lambs, found that the addition of urea to a basal ration of red top hay, corn, and molasses to produce the equivalent of 12 per cent crude protein in the ration, induced a retention of nitrogen which could not be improved by further additions of urea. However, when the true protein content of the ration was increased by the addition of casein, the retention of nitrogen did improve. They postulated that the conversion of urea to bacterial protein in the paunch did not proceed at a sufficiently rapid rate to meet the protein requirements of the growing lambs.

Lardinois, et. al. (1944) have shown that the addition of urea to the ration increased the synthesis of riboflavin, nicotinic acid, biotin, and pantothenic acid in the bovine rumen, when corn molasses and starch were present as readily available sources of energy for the bacteria.

Kinney (1946) studied the effect of substituting urea nitrogen for 25 and 50 per cent of the nitrogen of cottonseed meal in the rations of fattening steers. He found that urea had no significant effect on the apparent digestibility of the crude protein, ether extract, crude fiber, nitrogen-free extract, or organic dry matter of the ration. He concluded that the biological value of a ration in which urea was fed to supply 50 per cent of the supplemental nitrogen of the ration was as efficient as that of a similar ration supplemented with cottonseed meal. There were no observed ill effects on any of the urea rations, and the excretion of urinary nitrogen did not change.

Loosli and Harris (1945) found that the addition of small amounts of methionine to rations containing urea, increased the rate of gain and nitrogen retention of lambs. Urea rations so supplemented were equivalent to linseed meal rations. Urea without supplementation failed to promote as good gains, or as high a positive nitrogen balance as linseed meal. Lofgreen and associates (1947) later confirmed this work. They were able to show that the addition of 0.2 per cent methionine to a ration containing 10 per cent protein (with urea furnishing 40 per cent of the total nitrogen) significantly increased the nitrogen retained by lambs. They obtained biological values of 42, 49, 58 when urea, urea fortified with methionine, and dried egg, respectively, were used as sources of nitrogen. The lambs used in this experiment utilized the nitrogen of the dried eggs better than the nitrogen supplied by either linseed meal or urea. This suggested to the authors that, for lambs at least, quality of protein might be important.

Briggs, et. al. (1947) found that pellets containing 25 per cent of their nitrogen as urea, were equal to cottonseed meal in promoting nitrogen storage for beef steers on wintering rations, while pellets contining 50 per cent of their nitrogen in the form of urea, were not as satisfactory. Owen and associates (1943) compared blood meal and urea as supplemental sources of nitrogen for lactating dairy cows and found that at the 25 per cent level of substitution, there was no significant difference between the two in sustaining milk production and weight gains. The authors noted, however, that with the urea supplemented rations about 25 per cent more nitrogen appeared in the urine of the animals.

Briggs and associates (1948) found that the addition of urea to a low-protein ration of prairie hay for lambs increased the apparent digestibility of the hay nutrients and changed a negative nitrogen balance to one slightly positive. However, a uniform decrease in the nitrogen stored by the lambs was noted as the percentage of nitrogen in the ration supplied by urea was increased from 50 to 75 per cent. It was concluded that the lambs were not as efficient in storing nitrogen from urea as from cottonseed meal.

Hamilton, Robinson and Johnson (1948) compared the utilization of urea nitrogen with other proteins by sheep. They concluded that urea is a satisfactory source of nitrogen for the growing lamb if (1) at least 25 per cent of the nitrogen in the ration is in the form of preformed protein, and (2) the total protein equivalent of the ration is above the minimum requirements of the ruminant and does not exceed a level of approximately 12 per cent. In their experiments, the authors found that nitrogen from linseed meal was more efficiently utilized than nitrogen from urea. However, urea nitrogen was as well utilized as the nitrogen of casein, or casein fortified with cystine. They were able to show that the nitrogen in a ration containing 16.2 per cent protein (with 63 per cent of the nitrogen supplied by urea) was less efficiently utilized than the nitrogen of a ration containing 11.4 per cent protein (46 per cent supplied by urea).

Nitrogen Balance as a Method of Evaluating Protein Utilization

Harris and Mitchell (1941) have pointed out a number of possible sources of error in the results obtained from nitrogen balance studies when urea makes up a large portion of the nitrogen content of the ration. One possible source of error would be the loss of nitrogen from the body other than by the normal pathways of excretion. However, they could detect no loss of ammonia in the expired air of ruminants on urea-supplemented rations, and have noted that the lung alveoli are relatively impermeable to ammonia. Neither could they detect an appreciable loss of nitrogen through the skin of sheep on rations high in urea.

The possibility that nitrogen is lost in the accumulation of urea or ammonia in the blood and tissues was largely disproven by Hart, et. al. (1939). Dinning (1948) could obtain no evidence of ammonia loss through the formation and elimination of gases from the paunch of two-year-old steers.

Mitchell and associates (1936) have attributed the variability of individual determinations of the biological values of a protein to two sources; first, technical errors in the methods used; and secondly, individual differences in protein utilization and metabolism. From the results of 30 determinations with 10 rats each, they were able to obtain biological values for beef and pecan proteins with an average standard deviation of 3.7. They concluded from their study that statistically significant differences in biological values between two proteins of less than 4 per cent might be clearly demonstrated by the nitrogen balance technique. They were also able to show that the relative nutritive equivalence of two protein mixtures for maintenance and growth of rats were practically the same when evaluated by the nitrogen balance method, or by the paired-feeding technique with supplementary carcass analysis.

Recent work by Anderson and Nasset (1948) has shown that an imbalance in the amino acid concentrations necessary for optimum protein utilisation by adult rats may cause a wide variation in the nitrogen balance data obtained.

Protein Requirements of Sheep

Sheep must have proteins for wool growth in addition to body requirements for the growth and maintenance of the tissues. The protein in wool is largely in the form of keratin, of which cystine comprises nearly 13 per cent. It is the belief of many workers that cystine can be synthesized by the microorganisms of the paunch. Frazer (1934) has pointed out that the normal cystine consumption in the ration of the average sheep is far below the amount required for wool production.

Armsby (1917) suggested that the protein required for normal wool growth is approximately 0.0135 pounds per 100 pounds of live weight per day. This would be equivalent to 0.978 grams of nitrogen per day, if the standard conversion factor of 6.25 is used to calculate protein quantity from nitrogen content.

Mitchell, Kammlade and Hamilton (1928) proposed that the daily wool growth of sheep requires approximately 0.0149 pounds of protein for each 100 pounds of live weight. This would be equivalent to 6.749 grams of protein per day, or 1.079 grams of nitrogen for each 100 pounds of live weight. They also postulated that with mature sheep, wool growth accounts for a greater percentage of all added nutrients than with the growing lamb — a comparison of 0.0149 pounds protein for mature sheep to 0.0086 pounds for the growing lamb (these requirements being based on 100 pounds of live weight). They have shown that wethers in negative nitrogen balance will continue to store nitrogen in wool growth at approximately normal rates. They found that the average wool from experimental sheep contained approximately 60 per cent protein, on a grease weight basis.

The National Research Council (1946), through their subcommittee on sheep nutrition, have suggested that a 150 pound ram should have a daily feed intake of 4.3 pounds, of which 5.3 per cent should be digestible protein. This is equivalent to 0.23 pounds of digestible protein per day, or 16.6 grams of available nitrogen (244 mgm. nitrogen per kilogram body weight).

Morrison (1948) does not give the recommended protein allowance for mature rams. He does give the digestible protein allowance for ewes weighing 150 pounds, prior to 4 to 6 weeks before lambing, as 0.21 to 0.25 pounds of digestible protein per day. This would be equivalent to 15.22 to 18.12 grams of nitrogen per day.

Mitchell, et al. (1932) have postulated a protein requirement far below the above recommendations. While they do not give values for a 150 pound mature ram, they have placed the minimum digestible protein requirement for a 140 pound ram at 0.081 pounds per day. This is equivalent to 5.87 grams of nitrogen (92,56 mgm. nitrogen per kilogram of body weight).

It appears that there is a great deal of disagreement as to the exact protein requirements of sheep during growth and maintenance. Blaxter and Mitchell (1948) have critically evaluated the Morrison feeding standards and concluded that in following the recommended protein allowances there is a definite tendency to underfeed the immature sheep and to overfeed the mature animal.

EXPERIMENTAL OBJECTIVES

Biological values for urea, as computed by the nitrogen balance technique with growing animals, have varied widely. These differences may have been partially due to errors in experimental technique, and thus nitrogen balance data may not be dependable if urea supplies a large share of the nitrogen in the ration.

This study was primarily designed to test the accuracy of the nitrogen balance data obtained in measuring the utilization of protein and non-protein forms of nitrogen by ruminants. It also offered an opportunity to obtain further information on the comparative value of urea as a partial substitute for protein in the rations of sheep. Since the amount of nitrogen needed by mature sheep is confined to that required for maintenance and wool growth, dietary nitrogen in excess of these requirements should be largely excreted in the urine.

It was planned to determine: (1) the minimum amount of crude protein required to maintain mature sheep in nitrogen equilibrium; (2) the effect on nitrogen balance of additional nitrogen supplied as urea and as cottonseed meal at this level of protein intake; (3) the effect of urea on nitrogen balance at relatively high levels of protein intake, and (4) the time required to reach nitrogen equilibrium following the addition and withdrawal of urea from the ration.

EXPERIMENTAL PROCEDURES AND METHODS

A total of 11 metabolism trials were completed during the course of the experiment. Two mature rams were used in each trial, with the exception of Trials VII, X, and XI, which were replications of previous trials. The rams were placed in metabolism stalls and daily collections of feces and urine were made for a period of time sufficient to rule out temporary variations in nitrogen balance.

Animals Used and Methods of Feeding

Three rams were used during the course of the experiment. In June, 1948, a grade Shropshire ram was purchased from the Oklahoma National Stockyards in Oklahoma City. This ram is referred to as Ram I. At the same time, a purebred Shropshire ram was obtained from the college flock, and is designated Ram II. Since Ram II was frequently off-feed while in the metabolism stall, an accurate record of feed intake could not be secured and it was decided to substitute another ram during Trial V and subsequent trials. A grade Shropshire ram was obtained from the Oklahoma National Stockyards in October and substituted for Ram II in Trials, V, VI, VIII, IX, and IXr. This ram will be referred to as Ram III in this study. Two other rams were tried without success and were not used in any of the trials reported.

Rams I and II were drenched with 25 grams of phenothiazine two weeks before the first trial was attempted. Ram III was not drenched until the preliminary period preceding Trial VIII, but had been confined to a box stall for about a month before the first metabolism trial was attempted. The rams were fed twice daily in all trials, one-half the daily allowance to a feed. The approximate times of feeding were 7:30 a.m. and 5.45 p.m. The feed containers were left before the rams for at least one hour, and somewhat longer if they did not show a strong inclination to eat. After the feed had been consumed, the containers were removed and the rams were watered in gallon cans. An additional watering period was scheduled at noon each day.

The rams were weighed between trials. During Trial IX, when it became apparent that Ram I had lost weight, daily weights were taken for a period of time until a constant weight was established. The weights of the rams fluctuated only slightly and, with the exception of the above mentioned period, were within expected variations.

Equipment Used

All collections were made in a new type of metabolism stall designed by Briggs and Gallup (1949). The stalls were patterned after those successfully used with steers at the Oklahoma station. The only major change in the general design as given by Briggs and Gallup was the replacement of the plywood feed containers with metal containers of the same dimensions, but lacking the covered top.

Rations and fecal collections were weighed to the nearest gram on a Toledo balance. Fecal aliquots were stored in quart jars under refrigeration. A 1000 ml. graduated cylinder was used to measure the daily amounts of urine excreted. Readings were taken to the nearest 5 ml. and the aliquots measured out in a 50 ml. graduated cylinder. Urine aliquots were collected in pint glass jars with tight fitting lids and stored under refrigeration. When urea was added to the ration, the daily allowance was weighed to the nearest milligram and stored in small glass vials until used. The salt mixture was made up in 5 kilogram lots and stored in a gallon jar.

Rations Used

The general experimental plan was first to establish the nitrogen equilibrium point of the rams. This was to be accomplished by stepwise reductions in the protein content of a ration consisting of low-quality prairie hay, cottonseed meal, starch, and finely ground corn. Reductions were made from a crude protein level of 8.5 per cent in Trial I, to 4.5 per cent in Trial V. Since the analysis of the excreta indicated that the rams were slightly below their nitrogen equilibrium point during Trial V, no further reductions in protein levels were made.

In Trial VI, and arbitrary level of 8 grams of urea was added to the ration of Ram I, while 6.66 grams of urea were added to the ration of Ram III. In Trial VII, Ram I was returned to a ration similar to the one used in Trial V in an attempt to further study his nitrogen equilibrium point. Due to the shortage of time, Trial VII was not attempted with Ram III.

During Trial VIII, a quantity of cottonseed meal equivalent in nitrogen content to 8 grams of urea for Ram I, or 6.66 grams of urea for Ram III, was added to the basal ration. In Trial IX, 8 grams of urea for Ram I, or 6.66 grams of urea Ram III, were added to the ration used in Trial VIII which was already adequate in protein. Quantitative recovery of the excess nitrogen in the urine was attempted during this trial. Trial IXr was a repetition of Trial IX with Ram III in an attempt to check a possible loss of fecal nitrogen during the drying of the samples. No further trials were attempted with Ram III.

Trial X was a repetition of Trial VIII, using the same ration. In Trial XI, a higher level of urea (12 grams) was added to the ration used in Trial X and quantitative recovery of excess nitrogen was studied. During Trials VIII, IX, and X with Ram I, daily urinary collections were analyzed for nitrogen in order to study changes in urinary nitrogen excretion following the addition and withdrawal of urea from the ration.

The composition of the various feeds used in the experiment is given in Table 1. It was impossible, due to the length of the experiment, to use the same lot of feed during all trials. Three different lots of prairie hay and two lots of corn were used. The same lot of cottonseed meal was used in all trials, and the same analysis was used for all components except the nitrogen content, which was determined four times during the experiment. In all cases, an attempt was made to analyze the feeds to be used before the trial had started.

Table 2 gives the daily feed allowance of the ration constituents for each ram with the percentage of crude protein in the ration. Tables 7 and 8 in the appendix give these same values, together with the per cent composition of the entire ration, and the percentage of the ration furnished by each ingredient.

Due to the low palatability of the high starch diets used in Trials V, VI, and VII, it was decided to substitute equal amounts of cane sugar for starch in the ration. In later trials, the corn and cottonseed meal levels of the ration were increased and it was not necessary to include sugar in the ration. In an attempt to keep the energy and total feed intake constant during the last six trials of the experiment, an equal amount of starch was removed whenever the levels of corn or cottonseed meal were increased. The nearly constant weight of the rams during the experiment was taken as an indication that the rations were adequate in energy to meet maintenance requirements.

Beginning with Trial III, an attempt was made to adjust the feed intake of the other rams used in the experiment to an equivalent amount (body weight basis) of that consumed by Ram I. The daily allowance of feed allotted to Ram III was based on a body weight of 125 pounds. Since the ram actually weighed less during the trials in which he was used, his nitrogen intake

Table 1. Composition of feeds (air-dry basis) and rations in which they were used.

Feed	Used in rations	Chemical composition								
		Moisture %	Ash %	Protein %	Ether extract %	Crude fiber %	N-free extract %	Nitrogen %	Calcium %	Phosphorus %
Prairie hay	1 to 3 inc.	6.09	6.21	7.15	1.89	30.52	48.15	1.15	0.40	0.05
	4 to 5	6.72	5.89	3.68	2.54	31.91	49.26	0.59	do.	do.
	6 to 10 inc.	7.24	5.86	3.62	2.60	30.18	50.50	0.59	do.	do.
Corn	1 to 3 inc.	12.15	1.46	9.65	2.28	1.75	72.72	1.55	0.01	0.30
	4 to 6 inc.*			9.62			72.75	1.54	do.	do.
	6b to 7	8.99	1.50	8.66	2.28	1.75	76.82	1.38	do.	do.
	8 to 10 inc.*			7.90			77.58	1.27	do.	do.
Cottonseed meal	1 to 3 inc.	8.56	7.67	37.13	6.14	12.05	28.45	5.94	0.20	1.00
	4 to 5*			35.86			29.72	5.74	do.	do.
	6 to 7 inc.*			36.44			29.14	5.83	do.	do.
	8 to 10 inc.*			35.91			29.67	5.75	do.	do.
Starch	1 to 10 inc.	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00
Sucrose	6 to 7 inc.	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00
NaCl	1 to 10 inc.	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
$NaH_2PO_4 \cdot H_2O$	5 to 6b inc.	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	22.50
Bonemeal	7 to 10 inc.	0.00	100.00	0.00	0.00	0.00	0.00	0.00	24.20	13.10
Urea	6, 6b, 9, 10	0.00	0.00	291.60	0.00	0.00	0.00	46.66	0.00	0.00

* Nitrogen was the only constituent determined, other constituents being calculated from previous analysis.

Table 2. Daily allowance of feeds, with per cent crude protein of each ration, used in nitrogen balance studies with mature rams.

Ram I

Feed		Ration number										
		1	2	3	4	5	6	6b	7	8	9	10
Prairie hay	gms.	680	680	622	622	550	550	550	550	550	550	550
C. S. meal	gms.	84	84	46	71	28	28	28	28	94	94	94
Corn	gms.	340	226	192	207	228	228	252	252	274	274	274
Starch	gms.	226	340	356	318	340	120	120	120	152	152	152
Sugar	gms.	00	00	00	00	00	120	120	120	00	00	00
NaCl	gms.	8	8	8	8	7	7	7	8	8	8	8
$NaH_2PO_4 \cdot H_2O$	gms.	0	0	0	0	8	8	8	0	0	0	0
Bonemeal	gms.	0	0	0	0	0	0	0	12	12	12	12
Urea	gms.	0	0	0	0	0	8	8	0	0	8	12
Total	gms.	1338	1338	1224	1226	1161	1069	1093	1090	1090	1098	1102
Crude protein	%	8.42	7.60	6.54	5.57	4.50	7.06	6.90	4.76	6.91	8.99	10.01

		Ram II				Ram III						
Prairie hay	gms.	680	680	680	680	450	450			450	450	
C. S. meal	gms.	84	84	50	77	32	32			86	86	
Corn	gms.	340	226	210	226	210	233			254	254	
Starch	gms.	226	340	390	348	340	170			265	265	
Sugar	gms.	00	00	00	00	00	170			00	00	
NaCl	gms.	8	8	8	8	7	8			8	8	
$NaH_2PO_4 \cdot H_2O$	gms.	0	0	0	0	8	0			0	0	
Bonemeal	gms.	0	0	0	0	0	12			12	12	
Urea	gms.	0	0	0	0	0	6.66			0	6.66	
Total	gms.	1338	1338	1338	1339	1047	1082			1075	1082	
Crude protein	%	8.42	7.59	6.54	5.56	4.61	6.26			6.25	8.12	

per kilogram of body weight is higher than that of Ram I. Thus the rations for the two rams are not strictly comparable.

Vitamins A and D were supplied during all trials by the addition of approximately 0.75 grams of cod liver oil per day to the ration. During Trials V and VI, 8 grams of $NaH_2PO_4 \cdot H_2O$ were added to the ration to raise the phosphorus level above the minimum requirements. Twelve grams of bonemeal were added to the daily ration during Trials VII to XI. These supplements were fed in a salt mixture which provided the rams approximately 8 grams of salt per day.

Refusals of feed were encountered with Ram III in all trials. The total feed refused during the entire trial, including the preliminary period, was weighed and analyzed for nitrogen at the end of the trial. An average daily refusal of feed was calculated and its nitrogen content subtracted from the daily nitrogen intake. Hence, it was possible to determine the average daily feed and nitrogen intake of the ram, even though some feed was refused.

Collections

Trials I and II were completed while the metabolism stall was located in a box stall in the basement of the Animal Husbandry building. Since the accuracy of collections was impaired by the conditions present, the stall was moved to the concrete floor in the southeast corner of the basement. Trials III and IV were completed at this location. At the end of Trial IV, the rams were shorn and, due to the low temperature of the basement area, it was decided to move the stall to an enclosed room in the southwest corner of the basement. All further trials were completed at this location.

A preliminary period of 6 to 8 days preceded each collection period, with the exception of Trials IX and X with Ram I where daily collections of urine were analyzed. During these trials, the first 6 days of the collection period were deducted as the preliminary period in computing the average nitrogen balance. Collections were made at approximately 11:30 a.m. during all trials.

Aliquots of feces and urine were taken for three days and then analyzed, with the exception of the daily urinary studies made with Ram I. At least three 3-day collections were completed in all trials. Five per cent aliquots of urine and feces were taken for analysis. The urine was tested for acidity with litmus before storage in the refrigerator. It was found that when a few milliliters of concentrated HCl were added to the collection jar beneath the stall, the urine was invariably acid to litmus. A few drops of toluene were added to the fecal collections before storage in the refrigerator. This procedure assisted in keeping bacterial action at a minimum.

Analysis of Collections

Total nitrogen in the excreta was determined by the Kjeldahl method, using a 5 ml. sample of urine and a 5 gram sample of fresh feces. The feces were forced through an 1/8-inch screen to break up the pellets. Feces samples for Kjeldahl analysis were weighed out on a torsion balance. In the case of soft feces encountered with Ram III, the entire 3-day collection was dried at approximately 100°F for 24 hours, ground in a Wiley mill, and mixed thoroughly. A dry feces sample equivalent to 5 grams of fresh feces was then taken for analysis. During Trial IXr, a fresh feces sample of 10 grams was taken for analysis, while the same quantity was dried in the oven for 24 hours and then analyzed for nitrogen. Thus it was possible to calculate a probable loss due to drying.

Analysis of Wool

Ram I was shorn three times during the experiment. The successive dates of shearing were: July 10, October 8, and April 9. The first period of wool growth, July 10 to October 8, included the first four trials of the experiment. The last period, October 8 to April 9, included the last 7 metabolism trials. Ram II was shorn twice, July 10 and October 8. This period included the first four trials of the experiment. Ram III was shorn on October 8 and again on April 9. This period included all trials in which Ram III was used. The fleece was weighed to the nearest gram and representative portions of the wool were taken for analysis.

Various methods of analyzing wool by the Kjeldahl technique were attempted. Large variations were noted when a one gram sample of grease wool was taken for analysis. On the other hand, large losses of wool were encountered when an attempt was made to wash the sample and determine the nitrogen content of the clean, dry wool. A third method of analysis was employed in which a larger sample of wool (10 grams) was dissolved in 200 ml. of concentrated sulfuric acid and a 10 ml. aliquot of the mixture measured into the Kjeldahl flask for analysis. Accurate checks between successive samples were difficult in all cases. It was decided that an average of the results of all methods used would give the best estimate of the true nitrogen content of the wool.

EXPERIMENTAL RESULTS

The average daily nitrogen balance values obtained for each trial with Ram I are summarized in Table 3. The individual collections from which these average values have been computed are shown in detail in Table 9 (appendix). With the exception of a single feed refusal during Trial V, Ram I consumed all feed placed before him. Collections were made for at least nine days in each trial, with the exception of the first trial which was regarded as preliminary in nature. Since it was possible to accurately measure the feed consumed by Ram I, and since the collection periods were of sufficient length to overcome unusual fluctuations in nitrogen output, a relatively high degree of consistency was achieved in calculating the average daily nitrogen retention.

Table 3 shows the nitrogen supplied Ram I by the natural feeds of the ration, as well as the nitrogen supplied by urea. The nitrogen excretion values represent the average daily nitrogen output over the entire collection period. The nitrogen balance values were obtained by subtracting the daily nitrogen excreted from the average daily intake. It was felt that the last nine days of each trial might be a more satisfactory criteria from which to evaluate nitrogen retention. These values are also shown in Table 3. However, it will be noted that in nearly every case the values obtained for the last nine days closely approach the average values for the entire period. The milligrams of nitrogen consumed per kilogram of body weight were calculated for purposes of comparison with similar values reported by other workers.

During the first four trials of the experiment, an attempt was made to establish the nitrogen equilibrium point of the rams. The estimated minimum quantity of nitrogen required for equilibrium, on a body weight basis, was computed from the values suggested by Harris and Mitchell (1941). Reductions

Table 3. Average daily feed intake, nitrogen intake, and nitrogen excreted by Ram I during successive nitrogen balance trials.

Trial No.	Ration No.	Collection period[1]	Body weight	Feed intake	Nitrogen intake			Mgm. N per kg. body weight	Nitrogen excreted[2]			Nitrogen retained	
					Total	As feed	As urea		Feces	Urine	Total	Ave. for the total period	Ave. for the last 9 days of period
		Days	Kg.	Gm.	Gm.	Gm.	Gm.		Gm.	Gm.	Gm.	Gm.	Gm.
I	1.	6	67.5	1338	18.00	18.00	0.00	267	11.17	5.98	17.15	+0.85	
II	2.	9	67.5	1338	16.25	16.25	0.00	241	8.63	4.80	13.43	+2.82	+2.82
III	3.	9	67.5	1224	12.80	12.80	0.00	190	6.54	3.84	10.38	+2.42	+2.42
IV	4.	9	70.0	1226	10.93	10.93	0.00	156	5.92	3.30	9.22	+1.71	+1.71
V	5.	15	67.0	1132[3]	8.22	8.22	0.00	123	5.73	2.96	8.69	-0.47	-0.12
VI	6.	18	67.1	1069	12.04	8.31	3.73	179	6.16	4.29	10.45	+1.59	+1.57
VII	7.	18	67.5	1090	8.30	8.30	0.00	123	5.78	2.60	8.38	-0.08	-0.15
VIII	8.	12	68.0	1090	12.06	12.06	0.00	177	6.46	3.86	10.32	+1.74	+1.60
IX	9.	27[4]	66.4	1098	15.79	12.06	3.73	238	7.38	7.62	15.00	+0.79	+1.73
X	8.	12	66.2	1090	12.06	12.06	0.00	182	6.78	3.99	10.77	+1.29	+1.26
XI	10.	12	66.6	1102	17.66	12.06	5.60	265	6.98	8.94	15.92	+1.74	+1.61

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[1] Each collection period was preceded by a preliminary period of 8 days or longer, during which the same rations were fed in the same amounts as in the collection period.

[2] Analyses were made on composite 3-day samples, except during Trials VIII, IX, and X when analyses were made on daily samples of urine.

[3] Feed intake corrected for a feed refusal.

[4] Ram lost weight during the trial.

in nitrogen intake were made from a level of 18 grams per day (Trial I) to 10.93 grams per day (Trial IV). The positive nitrogen balance values obtained in each of the first four trials indicated that the rations supplied more than the minimum nitrogen requirements of Ram I.

During Trial V, with a daily nitrogen intake of 8.22 grams, Ram I showed an average daily nitrogen balance of -0.47 grams for the entire trial, with a daily average of -0.12 grams during the last nine days of the trial. Thus, it was apparent that a ration supplying 8.22 grams of nitrogen per day, or the equivalent of 123 milligrams of nitrogen per kilogram of body weight, was below the bodily nitrogen requirements of Ram I.

During Trial VI, 3.73 grams of urea nitrogen were added to the ration used in Trial V and a positive nitrogen balance resulted which was equivalent to ≠1.59 grams per day for the entire period, or ≠1.57 grams per day for the last nine days. Thus the addition of this quantity of urea nitrogen changed the nitrogen balance from one slightly negative to one definitely positive. The favorable effect due to the presence of urea in the ration was in agreement with previous work at this station (Briggs, et. al. 1948). Upon the withdrawal of the urea nitrogen from the ration (Trial VII, ration 7) Ram I showed an average daily nitrogen balance of -0.08 grams for the 18-day collection period, with a daily average of -0.15 grams for the last nine days of the period.

During Trial VIII, a quantity of cottonseed meal equivalent in nitrogen content to 8 grams of urea was added to ration 7, and the results showed an average daily nitrogen balance of ≠1.74 grams for the entire period, with a daily average of ≠1.60 grams for the last nine days of the trial. These values are very similar to those obtained in Trial VI where 8 grams of urea were added to the basal ration. Detailed results presented in Table 9 (appendix) show that variations between individual 3-day collections were much greater than these small differences between trials.

In Trial IX, 8 grams of urea were added to ration 8 which had already been demonstrated to be adequate in nitrogen. The average daily nitrogen balance was +0.79 grams for the entire collection period, or +1.73 grams for the last nine days. Since the weather during the first part of the trial was unusually cold and the ram lost weight, these factors may have been primarily responsible for the small quantity of nitrogen retained during the first 18 days of the collection period. During Trial X, the 8 grams of urea added to ration 8 were removed from the ration. The result was an average daily nitrogen balance of +1.29 grams over the entire period, with a daily average of +1.26 grams during the last nine days.

The possibility that the addition of urea to the ration might have had a depressing effect on the utilization of the natural feed proteins was further investigated in Trial XI. In this trial, a larger quantity of urea (12 grams) was added to ration 8. The average daily storage of nitrogen was +1.74 grams for the entire period, with an average of +1.61 grams per day for the last nine days. Since these values are similar to those obtained in Trials VI, VIII and X, it would appear that urea was not responsible for the low nitrogen retention during the first part of Trial IX.

Table 4 shows the average daily nitrogen balance data obtained with Rams II and III during nine metabolism trials. This table also gives the average daily amount and nitrogen content of the feed refused during each trial. The data presented in Table 4 are summarized from the complete results of each trial as shown in Tables 10 and 11 (appendix).

During the first four metabolism trials with Ram II, the positive nitrogen balances which resulted were similar to those obtained with Ram I. These results indicate that rations containing as little as 10.34 grams of nitrogen (equivalent to 4.5 per cent of crude protein) were sufficient to meet the

Table 4. Average daily feed intake, nitrogen intake (corrected for feed refused) and nitrogen excreted by Rams II and III during successive nitrogen balance trials

Trial No.	Ration No.	Collection period[1]	Body weight	Feed refused		Feed intake	Nitrogen intake			Mgm. N per kg. body weight	Nitrogen excreted[2]			Nitrogen retained	
				Total	Nitrogen		Total	As feed	As urea		Feces	Urine	Total	Ave. for total period	Ave. for last 9 days of period
		Days	Kg.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.		Gm.	Gm.	Gm.	Gm.	Gm.
							RAM II								
I	1.	6	75.2	00	0.00	1338	18.00	18.00	0.00	240	8.70	7.19	15.89	+2.11	
II	2.	9	74.3	00	0.00	1338	16.25	16.25	0.00	218	7.71	5.71	13.42	+2.83	+2.83
III	3.	9	74.8	00	0.00	1338	13.99	13.99	0.00	187	7.87	4.22	12.09	+1.90	+1.90
IV	4.	9	76.1	197	1.16	1142	10.76	10.76	0.00	141	5.93	3.49	9.42	+1.34	+1.34
							RAM III								
V	5.	9	54.4	65	0.36	983	7.74	7.74	0.00	142	5.68	2.08	7.76	-0.02	-0.02
VI	6.	18	54.8	40	0.24	1042	10.56	7.40	3.10	193	6.26	2.51	8.77	+1.79	+1.30
VIII	8.	12	52.5	27	0.17	1048	10.59	10.59	0.00	202	6.68	2.49	9.18	+1.41	+1.32
IX	9.	9	53.5	33	0.22	1049	13.65	10.55	3.10	255	6.53	4.16	10.69	+2.96	+2.96
IXr	9.	12	56.2	46	0.34	1036	13.53	10.43	3.10	235	6.69	5.62	12.31	+1.22	+1.01

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[1] Each collection period was preceded by a preliminary period of 8 days or longer, during which the same rations were fed in the same amounts as in the collection period.

[2] Analyses were made on composite 3-day samples of feces and urine.

bodily requirements of Ram II. During Trial V, Ram II went completely off-feed and was removed from the experiment. Ram III was substituted for Ram II at this point and was placed on ration 5, which was calculated to be very close to his minimum nitrogen requirements.

Although feed refusals were encountered in all trials in which Ram III was used, the nitrogen balance data, after the nitrogen content of the refused feed was deducted, were very similar to those obtained with Ram I. During Trial V, with the ration supplying 7.74 grams of nitrogen per day, an average daily nitrogen balance of -0.02 grams resulted from the nine-day collection period. This compares favorably with the nitrogen balance values obtained with Ram I, which were -0.47 grams per day during Trial V, and -0.08 grams per day during Trial VII. However, the nitrogen intake of Ram III was greater than that of Ram I, when expressed in milligrams of nitrogen per kilogram of body weight. Thus a difference appeared to exist in the nitrogen requirements for equilibrium between the two rams.

The nitrogen balance values obtained with Ram III in Trials VI and VIII were positive, but somewhat lower than those obtained with Ram I. With Ram III, supplementing the ration used in Trial V with 3.10 grams of nitrogen in the form of urea promoted a greater storage of nitrogen than supplementing the same ration with an equivalent quantity of nitrogen in the form of cottonseed meal.

In Trial IX, 6.66 grams of urea were added to ration 8 which had been demonstrated to be adequate in nitrogen. The results showed an average daily nitrogen balance of +2.96 grams for the nine-day collection period. Since this average was much greater than values obtained during Trials VI and VIII, as well as the nitrogen balance values obtained with Ram I, it was decided to repeat the trial. In this repetition of Trial IX, which was designated Trial

IXr, the average daily nitrogen balance was +1.22 grams per day over the entire period and +1.01 grams per day for the last nine days. These latter results were in agreement with nitrogen balance values obtained with Ram I. They show almost complete recovery of urea nitrogen when added to rations which contained adequate amounts of protein.

Table 5 gives the daily nitrogen balance data for Ram I during periods when urea was added to, or removed from, the ration. The urine was collected and analyzed daily. Average daily fecal nitrogen values were computed from the analysis of 3-day collections. It will be noted that in Trial IX, when 8 grams of urea were added to the ration, urinary nitrogen values increased markedly on the first day of the trial, and by the end of the third day (Jan. 14) had reached a level of 7.92 grams which was slightly above the mean of the urinary nitrogen values for Trial IX (7.62 grams per day). Fecal nitrogen values increased slowly during the entire trial.

Following the withdrawal of the urea from the ration, in Trial X, urinary nitrogen values dropped very markedly on the first day, and by the end of the third day (Feb. 16) had decreased to 3.86 grams per day. This value was slightly below the mean of the urinary nitrogen values for Trial X (3.99 grams per day).

Table 5 also shows the average daily temperatures during the period of daily urinary collections and analyses. It will be noted that the extreme negative nitrogen balances on January 30 and 31 are associated with the extremely low temperatures at that time.

Table 5. Average daily nitrogen balance for Ram I and average daily temperature during Trials VIII, IX and X.

Collection dates	Body weight	Nitrogen intake	Nitrogen excreted[1]			Nitrogen retained	Ave. daily temperature[2]
			Feces	Urine	Total		
	Kg.	Gm.	Gm.	Gm.	Gm.	Gm.	°F
			Trial VIII, ration 8, 6.91% protein; no urea.				
Jan. 6	68.0	12.06	7.29	3.75	11.04	+1.02	31
Jan. 7		do.	7.29	3.77	11.06	+1.00	44
Jan. 8		do.	7.29	3.97	11.26	+0.80	49
Jan. 9		do.	6.28	3.72	10.00	+2.06	49
Jan. 10		do.	6.28	3.38	9.66	+2.40	24
Jan. 11		do.	6.28	3.16	9.44	+2.62	26
			Trial IX, ration 9 (ration 8 plus 3.73 gm. of urea nitrogen) 8.99% protein				
Jan. 12	66.4	15.79	6.52	4.38	10.90	+4.89	25
Jan. 13		do.	6.52	6.56	13.08	+2.71	27
Jan. 14		do.	6.52	7.92	14.45	+1.34	38
Jan. 15		do.	6.85	7.96	14.81	+0.98	49
Jan. 16		do.	6.85	8.88	15.73	+0.06	46
Jan. 17		do.	6.85	8.94	15.79	+0.00	21
Jan. 18		do.	7.21	7.96	15.17	+0.62	19
Jan. 19		do.	7.21	7.65	14.86	+0.93	17
Jan. 20		do.	7.21	7.71	14.92	+0.87	15
Jan. 21		do.	7.47	8.14	15.61	+0.18	28
Jan. 22		do.	7.47	8.06	15.53	+0.26	28
Jan. 23		do.	7.47	7.42	14.89	+0.90	40
Jan. 24	66.6	do.	6.89	7.74	14.63	+1.16	36
Jan. 25		do.	6.89	8.58	15.47	+0.32	15
Jan. 26		do.	6.89	7.95	14.84	+0.95	16
Jan. 27		do.	6.61	8.54	15.15	+0.64	24
Jan. 28		do.	6.61	9.32	15.93	-0.14	25
Jan. 29		do.	6.61	8.94	15.55	+0.24	11
Jan. 30		do.	7.49	9.46	16.95	-1.16	3
Jan. 31		do.	7.49	9.36	16.85	-1.06	8
Feb. 1		do.	7.49	8.35	15.84	-0.05	19
Feb. 2	62.5	do.	7.86	8.64	16.50	-0.71	23
Feb. 3		do.	7.86	6.98	14.84	+0.95	26
Feb. 4		do.	7.86	6.92	14.78	+1.01	37
Feb. 5-6[3]	64.3	do.	7.33	6.52	13.85	+1.94	35
Feb. 7	64.8	do.	7.33	7.12	14.45	+1.34	32

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[1] Average daily fecal nitrogen computed from 3-day collection.

[2] Average daily temperatures supplied by Meteorology Department, Oklahoma Agricultural and Mechanical College.

[3] Average of 2-day urinary collection.

Table 5 (cont'd)

Collection dates	Body weight	Nitrogen intake	Nitrogen excreted			Nitrogen retained	Ave. daily temperature
			Feces	Urine	Total		
	Kg.	Gm.	Gm.	Gm.	Gm.	Gm.	°F
			Trial IX cont'd.				
Feb. 8	65.2	15.79	7.51	5.10	12.61	+3.18	40
Feb. 9	65.7	do.	7.51	6.40	13.91	+1.88	35
Feb. 10		do.	7.51	6.34	13.85	+1.94	36
Feb. 11		do.	8.01	6.19	14.20	+1.59	41
Feb. 12		do.	8.01	7.19	15.20	+0.59	55
Feb. 13		do.	8.01	6.58	14.59	+1.20	43
			Trial X, ration 8, 6.91% protein, no urea.				
Feb. 14	65.7	12.06	7.22	5.61	12.83	-0.77	22
Feb. 15		do.	7.22	4.01	11.23	+0.83	22
Feb. 16		do.	7.22	3.86	11.08	+0.98	37
Feb. 17	66.1	do.	7.55	3.80	11.35	+0.71	40
Feb. 18		do.	7.55	4.04	11.59	+0.47	48
Feb. 19		do.	7.55	3.34	10.89	+1.17	55
Feb. 20		do.	7.00	3.83	10.83	+1.23	51
Feb. 21		do.	7.00	3.99	10.99	+1.07	31
Feb. 22		do.	7.00	3.24	10.24	+1.82	36

The daily wool growth of the rams used in the experiment is shown in Table 6, together with the nitrogen content of the wool and the calculated daily storage of nitrogen in wool growth. Ram I stored an average of 0.846 grams of nitrogen per day in wool growth, while Ram III stored an average of 1.10 grams of nitrogen per day. Ram II stored 1.260 grams of nitrogen per day in the form of wool. It will be noted that with Rams I and III, the amount of nitrogen stored daily in wool growth during the last period, which included Trials V to XI inclusive, was less than that stored during the initial period, which included the first four trials of the experiment. Since the last 6 trials of the experiment included two trials in which the rams were in negative nitrogen balance, this deficiency in nitrogen intake may have had some bearing on the apparent decreased nitrogen stored in wool.

Table 6. Daily wool growth of rams used in the experiment, with the nitrogen content of the wool and the calculated daily storage of nitrogen in wool growth.[1]

Ram number	Dates of shearing	Trials completed during period of wool growth	Total period of wool growth	Fleece weight	Average daily wool growth	Nitrogen content of wool	Nitrogen stored in wool growth	
							Total	Daily ave.
			Days	Gm.	Gm.	per cent	Gm.	Gm.
I	July 10 — Oct. 8	I to IV inc.	90	1000	11.11	8.23	82.30	0.914
	Oct. 8 — April 9	V to XI inc.	183	1890	10.33	7.53	142.32	0.778
II	July 10 — Oct. 8	I to IV inc.	90	920	10.22	12.33	113.44	1.260
III	April 1 — Oct. 8[2]	none	191	2164	11.33	11.39	246.48	1.290
	Oct. 8 — April 9	V to IXr inc.	183	1635	8.93	10.22	167.10	0.913

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[1] All values in Table 6 are based on grease wool.

[2] April 1 was taken as the probable date of shearing.

DISCUSSION

The positive nitrogen balances obtained during the first four trials of the experiment indicate that the rations used were adequate in nitrogen to meet the body requirements of the rams. When a further reduction in the nitrogen content of the rations was made during Trial V, the negative nitrogen balance values obtained indicate that rations supplying only 8.22 grams of nitrogen per day for Ram I, or 7.74 grams of nitrogen per day for Ram III, were below the minimum nitrogen requirements of the rams. During Trial V, Ram I had a daily nitrogen intake of 123 mgm. per kgm. body weight, while Ram III received a daily nitrogen allowance of 142 mgm. per kgm. body weight. The average daily nitrogen balances resulting from these intakes, based on the last nine days of the collection period, were -0.15 grams for Ram I and -0.02 grams for Ram III. The difference in nitrogen intake during this period, as contrasted to the similarity of the nitrogen balances obtained, would lead to the conclusion that a difference existed between the two rams as to the minimum quantity of nitrogen required for equilibrium. It will also be noted that a slightly different nitrogen balance was obtained with Ram I in Trial VII, while on the same nitrogen intake as in Trial V. Likewise, it is apparent from Table 4 that large differences in nitrogen storage existed between Rams II and III while on nearly equivalent nitrogen intakes.

These individual variations between mature ruminants as to the minimum quantity of nitrogen required for equilibrium as not out of line with those noted by Harris and Mitchell (1941). They observed large differences in the minimum nitrogen requirements of mature wethers. By using a statistical method, they calculated the nitrogen intake required for equilibrium and obtained values ranging from 122 mgm. to 248 mgm. nitrogen per kgm. body weight. These differences between mature ruminants are probably related to differences in microfloral activity in the paunch, as well as to the inherent ability of the animal to digest and utilize the proteins of both feed and bacterial origin. Considering the many factors that may alter the ability of the ruminant to utilize the proteins of its ration, it is not surprising that variations should exist between animals as to the minimum nitrogen intake required for maintenance.

It was not anticipated, under the conditions of the experiment in which natural feed proteins were the sources of nitrogen, that the nitrogen requirements for maintenance would be less than the minimum found by Harris and Mitchell (1941). These workers concluded that 160 mgm. of casein nitrogen, or 202 mgm. of urea nitrogen, per kgm. of body weight were the minimum quantities of nitrogen which would promote equilibrium in wethers 15 to 18 months of age and weighing approximately 35 kilograms. It would have seemed logical to expect the nitrogen requirements of the rams used in this experiment to have approached an average of the two values reported by Harris and Mitchell. However, from the data presented in Tables 3 and 4, it is apparent that the minimum nitrogen intake necessary for equilibrium for the rams used in this experiment was below the level of 160 mgm. of casein nitrogen suggested by Harris and Mitchell. During Trial IV, Rams I and II showed positive nitrogen balances of +1.71 and +1.34 grams per day on nitrogen intakes of 156 and 141 mgm. per kgm. body weight, respectively. In Trial V, Rams I and III showed negative nitrogen balances of only -0.47 and -0.02 grams per day on nitrogen intakes of 123 and 142 mgm. per kgm. body weight, respectively. A possible explanation of the difference in the minimum values obtained in this study, as compared with those obtained by Harris and Mitchell, may be due to the age and greater weight of the sheep used in this experiment.

The results discussed above indicate that the nitrogen requirements of mature sheep for maintenance are below the values suggested by Morrison (1948) and the National Research Council (1946). The National Research Council has recommended that a 150 pound ram should have a daily allowance of 244 mgm. digestible nitrogen per kgm. body weight. Morrison's values for sheep of equivalent weight are very similar. On the other hand, Mitchell and associates (1932) have stated that a 140 pound ram requires a digestible nitrogen intake of 94.56 mgm. per kgm. body weight per day. However, since the above values are based on digestible protein, with certain assumptions as to the digestibility and biological values of the protein, while the results of this study are based on total nitrogen intake, no direct comparison can be made. Moreover, the values given by Morrison and the National Research Council are based on production requirements which may be considerably above the nitrogen required for maintenance. It is probable that when allowances as low as those suggested by Mitchell and his associates (1932) are accepted, no consideration is given to the actual requirements of the bacteria within the paunch in order that they may make the most efficient use of the carbohydrates of the ration. The results obtained from this study tend to support the values given by Harris and Mitchell (1941) as to the minimum nitrogen requirements of mature sheep.

The nitrogen which goes into wool growth greatly effects the nitrogen retention values obtained with sheep. Nitrogen stored in the form of wool must be considered as lost to the body tissues and this quantity of nitrogen should be added to that quantity excreted from the body if a more accurate measure of nitrogen retention is desired. The values obtained for the average daily quantity of nitrogen stored in wool growth, as shown in Table 6, are considerably below the values suggested by Armsby (1917) and Mitchell et. al. (1928). Armsby has suggested that the daily wool growth of sheep weighing

150 pounds requires 1.47 grams of nitrogen, while Mitchell and his associates have postulated a daily requirement of 1.62 grams of nitrogen. If Mitchell's value of 1.62 grams of nitrogen per day required for wool growth were added to the nitrogen excreted by Ram I, the average daily nitrogen balance for Trial V would be changed from -0.47 grams to -2.09 grams. The average quantity of nitrogen which went into the daily wool growth of Ram I, as shown in Table 6, was equivalent to 0.846 grams per day, or 12.45 mgm. of nitrogen per kgm. body weight. If this quantity were added to the nitrogen excreted during Trial V, the nitrogen balance would become -1.32 grams per day. Thus it is apparent that the minimum intake requirements necessary to produce nitrogen equilibrium, as determined by the nitrogen balance technique, do not take into consideration the quantity of nitrogen lost to the tissues in the form of wool growth.

The second objective of the experiment was to determine the nitrogen storage resulting from the addition of equal quantities of urea and cottonseed meal nitrogen to rations which supplied less than the minimum nitrogen requirements of the rams. In Trial VI, the addition of 3.73 grams of urea nitrogen to the ration of Ram I, or 3.10 grams of urea nitrogen to the ration of Ram III, promoted an average nitrogen retention of +1.57 grams per day for Ram I and +1.30 grams per day for Ram III, during the last nine days of the trial. In Trial VIII, the addition of an equivalent quantity of nitrogen in the form of cottonseed meal resulted in a positive daily nitrogen balance of +1.60 grams for Ram I and +1.32 grams for Ram III during the last nine days of the trial. It was apparent that at this level of protein intake, the addition of equal quantities of urea or cottonseed meal nitrogen to rations which supplied less than the minimum amount of nitrogen necessary for equilibrium, gave relatively the same positive balance regardless of the source of nitrogen.

The third objective of the experiment was to study the effect of urea when added to rations already adequate in protein. The addition of 8 grams of urea to the ration of Ram I during Trial IX resulted in a lower average nitrogen balance of +0.79 grams per day over the entire period. During the last nine days of the period, however, the average positive nitrogen balance of +1.73 grams per day compares favorably with the +1.57 grams and +1.60 grams per day which resulted during the last nine days of Trials VI and VIII, respectively. It has been previously mentioned that the average daily temperatures during the first part of Trial IX were quite low and the ram lost weight. Since the energy content of the ration remained unchanged, it would seem logical to attribute the decreased nitrogen balance during the early part of the period to an insufficient supply of energy to meet the body requirements, with a resultant loss of weight and lowered nitrogen retention.

The addition of 6.66 grams of urea to the ration of Ram III during Trial IX increased nitrogen retention from an average of +1.41 grams (Trial VIII) to +2.96 grams per day. No satisfactory explanation can be given to account for this increased retention. When the trial was repeated, Trial IXr, the average daily quantity of nitrogen retained over the entire trial was +1.22 grams, or +1.01 grams for the last nine days of the period. These values are in much closer agreement with the results of Trials VI and VIII. No appreciable loss of nitrogen could be detected in drying the feces samples during Trial IXr. It is apparent from the values given in Table 11 (appendix) that an increased excretion of urinary nitrogen occured in Trial IXr, as compared to Trial IX, and that this was primarily responsible for the decreased positive nitrogen balance.

The possibility of urea depressing the utilization of the natural feed proteins of the ration was investigated in Trial XI with Ram I. During this trial, 12 grams of urea were added to ration 8. The average daily nitrogen balance over the entire period was +1.74 grams, or +1.61 grams for the last nine days of the trial. The average daily nitrogen balance for the last nine days of the trial is very similar to the averages obtained during similar periods in Trials VI, VIII, and IX; these values being +1.57 grams, +1.60 grams, and +1.73 grams per day, respectively. This result is taken as further evidence that the low nitrogen balance values obtained during the early part of Trial IX with Ram I were not due to the effect of urea in the ration. From the above results, it would seem logical to conclude that the addition of urea to a ration already adequate in protein does not alter the nitrogen balance values obtained to any appreciable extent.

During Trials IX and XI, it was possible to estimate the percentage of excess urea nitrogen added to the ration which could be recovered by the nitrogen balance technique. In computing these values, it was assumed that the quantity of nitrogen in the excreta which resulted from the basal ration was the same when urea was added to the ration as in the previous trial without urea. If complete recovery of the urea nitrogen was achieved, the difference between the total nitrogen excreted and that portion assumed to be due to the basal ration would have been equal to the urea nitrogen added.

The per cent recovery of urea nitrogen, using this method of calculation, gave the following results:

Ram number	Trial number	Per cent recovery of urea nitrogen for	
		Entire period	Last 9 days
I	IX	125.0	96.2
	XI	91.8	93.0
III	IX	49.0	49.0
	IXr	101.3	96.8

The average per cent recovery of the urea nitrogen added to the ration, based on the last nine days of each trial, was 95.3 per cent. Since it appeared that the per cent recovery during Trial IX, Ram III, was abnormally low in view of the other results, this period was not included in calculating the average. It would seem logical to conclude from the above results that excess urea added to rations already adequate in protein could be recovered quantitatively to the extent of approximately 95 per cent.

The final objective of the experiment was to study the daily urinary nitrogen excretion following the addition and withdrawal of urea from the ration. From the values given in Table 5, it is apparent that when 3.10 grams of urea nitrogen were added to the ration, the daily urinary nitrogen excretion of Ram I increased markedly; from an average of 3.63 grams per day during the preceding trial to 4.38 grams on the first day, 6.56 grams on the second day, and 7.92 grams on the third day. After the third day, urinary nitrogen values tended to become relatively constant. Considering the average daily urinary nitrogen excreted during the last six days of Trial VIII as the base (3.63 grams) the per cent increase in urinary nitrogen above the base level, following the addition of urea to the ration, was 21 per cent on the first day, 81 per cent on the second day, and 118 per cent on the third day.

Following the withdrawal of 3.10 grams of urea nitrogen from the ration, Trial X, the urinary nitrogen excretion decreased from an average of 6.30 grams per day during the last six days of Trial IX, to 5.61 grams on the first day, 4.01 grams on the second day, and 3.86 grams on the third day. These differences in urinary nitrogen excretion, when expressed as a percentage of the base value (6.30 grams), become 11 per cent for the first day, 36 per cent for the second day, and 39 per cent for the third day. Thus the general trend was a rapid decrease in urinary nitrogen output for the first three days after the removal of urea from the ration, followed by relatively constant urinary nitrogen excretions values during the remainder of the period.

Fecal nitrogen responded much more slowly than urinary nitrogen to the addition, or deletion, of urea from the ration. However, definite increases in the fecal nitrogen levels were apparent after the addition of urea. For example, the mean of the fecal nitrogen values during Trial VIII was 6.44 grams per day, as compared to 7.38 grams per day during Trial IX. Harris and Mitchell (1941) have noted a corresponding rise in the fecal nitrogen excretion following the addition of urea to the ration. They have suggested that it is an indication of bacterial utilization of the urea, with incomplete digestion of the proteins formed. The slow, but definite, increase in fecal nitrogen is apparent in each trial were urea was added to the ration.

It is generally assumed that when mature ruminants are receiving rations which supply more than their minimum nitrogen requirements, no storage of nitrogen takes place and that regardless of the level of protein intake above maintenance, nitrogen equilibrium will be maintained. It has already been noted that, with sheep, wool growth accounts for a significant part of the nitrogen intake required for maintenance. Thus an apparent positive nitrogen balance should be maintained at all levels of protein intake above the minimum. However, with the rams used in this experiment, the results obtained, as shown in Table 6, indicate that wool growth accounted for an average of only 50 to 75 per cent of the positive nitrogen balance values obtained. If the values for the daily storage of nitrogen in wool growth, as suggested by Armsby (1917) Mitchell et. al. (1928) are used, much closer agreement with the actual nitrogen balance values given in tables 3 and 4 are obtained. The nitrogen stored in wool growth proposed by Mitchell and his associates (1928), 1.62 grams of nitrogen per day for a 150 pound sheep, would account for nearly all the average daily nitrogen balance values obtained with Ram I during Trials IV, VI, VIII, IX, and XI, using the last nine days of each trial as the best measurement of actual nitrogen retention.

Although the values given in Table 6 are less than those postulated by Armsby and Mitchell, it was felt that this difference could not be explained solely as an error in analyzing the wool for nitrogen. No doubt wide variations occur in the quantity of nitrogen stored in wool by mature sheep of different sizes, breeds, and inherited ability to grow wool. Thus it was felt that the average daily nitrogen values for wool growth over the entire experiment, which are 0.846 grams per day for Ram I and 1.10 grams per day for Ram III, were close approximations of their daily nitrogen storage in wool. It was further believed that the difference between the positive nitrogen balances obtained and the amount which could be accounted for in wool growth, was not entirely an error in measuring the nitrogen intake and output of the rams. The relatively constant positive nitrogen balance values obtained over a wide range of nitrogen intakes above maintenance, support this assumption. More probably there was a loss of nitrogen through other channels, such as in the formation of sperm and genital fluids.

SUMMARY

A total of 11 nitrogen metabolism trials were completed with mature rams. Two rams were used in each trial, with the exception of three trials in which only one ram was used. The rams were placed in metabolism stalls of new design and separate daily collections of feces and urine were taken for a period of time sufficient to rule out unusual variations in nitrogen output. The basal ingredients of the rations fed were low-quality prairie hay, cottonseed meal, corn, and starch. Stepwise reductions were made in the protein content of the ration until an intake slightly below that required for maintenance was established. Equivalent quantities of nitrogen in the form of urea and cottonseed meal were then added to the ration. Urea was also added to rations already adequate in protein and quantitative recovery of the urea nitrogen was attempted.

The nitrogen equilibrium point of the rams used in the experiment was below the minimum nitrogen requirements suggested by other workers. Slightly negative nitrogen balances were obtained with rations supplying 123 and 142 mgm. nitrogen per kgm. body weight. The addition of either urea or cottonseed meal nitrogen in equivalent amounts to rations which supplied less than the minimum nitrogen requirements, promoted a positive nitrogen balance of the same relative proportions, regardless of the source of nitrogen.

The addition of urea to rations already adequate in protein caused no consistent change in nitrogen retention. The urea added to such a ration could be quantitatively recovered to the extent of approximately 95 per cent. Following the addition of urea to the ration, urinary nitrogen excretion increased markedly until the third day, at which level it tended to become relatively constant. The same general trend was noted for the decrease in urinary nitrogen excretion upon the removal of urea from the ration. Fecal nitrogen excretion likewise increased or decreased as urea was added to, or taken from, the ration. However, the response was much slower than with the urinary nitrogen excretion.

The nitrogen content of the daily wool growth of the rams during the experiment did not completely account for the positive nitrogen balance values obtained.

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APPENDIX

Table 7. Amounts and composition of daily rations fed to Ram I (air-dry basis) with the per cent of the daily feed intake supplied by each constituent.

Ration description		Ration number --										
		1	2	3	4	5	6	6b	7	8	9	10
Constituents												
Prairie hay	gms.	680	680	622	622	550	550	550	550	550	550	550
C. S. meal	gms.	84	84	46	71	28	28	28	28	94	94	94
Corn	gms.	340	226	192	207	228	228	252	252	274	274	274
Starch	gms.	226	340	356	318	340	120	120	120	152	152	152
Sugar	gms.	00	00	00	00	00	120	120	120	00	00	00
NaCl	gms.	8	8	8	8	7	7	7	8	8	8	8
$NaH_2PO_4 \cdot H_2O$	gms.	0	0	0	0	8	8	8	0	0	0	0
Bonemeal	gms.	0	0	0	0	0	0	0	12	12	12	12
Urea	gms.	0	0	0	0	0	8	8	0	0	8	12
Total	gms.	1338	1338	1224	1226	1161	1069	1093	1090	1090	1098	1102
Constituents												
Prairie hay	%	50.82	50.82	50.82	50.73	47.37	51.45	50.32	50.46	50.46	50.09	49.91
C. S. meal	%	6.28	6.28	3.76	5.79	2.41	2.62	2.56	2.57	8.62	8.56	8.53
Corn	%	25.41	16.89	15.68	16.88	19.64	21.33	23.05	23.12	25.14	24.95	24.86
Starch	%	16.89	25.41	29.08	25.94	29.28	11.22	10.98	11.01	13.94	13.84	13.79
Sugar	%	0.00	0.00	0.00	0.00	0.00	11.22	10.98	11.01	0.00	0.00	0.00
NaCl	%	0.59	0.59	0.65	0.65	0.60	0.65	0.64	0.73	0.73	0.73	0.73
$NaH_2PO_4 \cdot H_2O$	%	0.00	0.00	0.00	0.00	0.68	0.75	0.73	0.00	0.00	0.00	0.00
Bonemeal	%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.10	1.10	1.09	1.09
Urea	%	0.00	0.00	0.00	0.00	0.00	0.75	0.73	0.00	0.00	0.73	1.09
Composition												
Moisture	%	6.72	5.68	5.32	5.95	5.77	6.54	6.66	5.95	6.65	6.60	6.57
Ash	%	4.61	4.47	4.32	4.33	4.54	4.94	4.86	5.33	5.82	5.79	5.76
Protein	%	8.42	7.60	6.54	5.57	4.50	7.06	6.90	4.76	6.91	8.99	10.01
Ether extract	%	1.93	1.73	1.55	2.03	1.80	1.98	1.89	1.98	2.41	2.40	2.39
Crude fiber	%	16.71	16.56	16.24	17.18	15.75	16.22	15.90	15.94	16.71	15.59	16.53
N-free extract	%	61.62	63.95	66.02	64.93	67.62	64.70	65.83	66.21	61.48	61.48	60.81
Calcium	%	0.24	0.22	0.21	0.22	0.22	0.22	0.22	0.48	0.49	0.49	0.49
Phosphorus	%	0.16	0.14	0.11	0.13	0.13	0.13	0.13	0.32	0.39	0.39	0.39
Nitrogen	%	1.35	1.22	1.05	0.89	0.72	1.13	1.10	0.77	1.11	1.44	1.60

Table 8. Amounts and composition of daily rations fed to Rams II and III (Air-dry basis) with the per cent of the daily food intake supplied by each constituent

Ration description		Ration number —							
		1	2	3	4	5	6	8	9
		Ram II				Ram III			
Constituents									
Prairie hay	gms.	680	680	680	680	450	450	450	450
C. S. meal	gms.	84	84	50	77	32	32	36	86
Corn	gms.	340	226	210	226	210	233	254	254
Starch	gms.	226	340	390	348	340	170	265	265
Sugar	gms.	00	00	00	00	00	170	00	00
NaCl	gms.	8	8	8	8	7	8	8	8
$NaH_2PO_4 \cdot H_2O$	gms.	0	0	0	0	8	0	0	0
Bonemeal	gms.	0	0	0	0	0	12	12	12
Urea	gms.	0	0	0	0	0	6.66	0	6.66
Total	gms.	1338	1338	1338	1339	1047	1082	1075	1082
Constituents									
Prairie hay	%	50.82	50.82	50.82	50.78	42.98	41.59	41.86	41.59
C. S. meal	%	6.28	6.28	3.74	5.75	3.06	2.96	3.35	7.95
Corn	%	25.41	16.89	15.69	16.88	20.06	21.53	23.63	23.47
Starch	%	16.89	25.41	29.15	25.99	32.47	15.71	24.65	24.65
Sugar	%	0.00	0.00	0.00	0.00	0.00	15.71	0.00	0.00
NaCl	%	0.60	0.60	0.60	0.60	0.67	0.74	0.74	0.74
$NaH_2PO_4 \cdot H_2O$	%	0.00	0.00	0.00	0.00	0.76	0.00	0.00	0.00
Bonemeal	%	0.00	0.00	0.00	0.00	0.00	1.11	1.10	1.11
Urea	%	0.00	0.00	0.00	0.00	0.00	0.62	0.00	0.62
Composition									
Moisture	%	6.72	5.68	6.32	5.95	5.57	5.20	5.84	5.30
Ash	%	4.61	4.48	4.27	4.27	4.49	4.84	5.26	5.24
Protein	%	8.42	7.59	6.54	5.56	4.61	6.25	6.25	8.12
Ether extract	%	1.93	1.73	1.59	2.09	1.74	1.75	2.21	2.10
Crude fiber	%	16.71	16.56	16.24	17.19	14.43	13.28	14.01	13.92
N-free extract	%	61.62	63.96	66.09	64.99	69.49	69.24	66.50	66.22
Calcium	%	0.24	0.24	0.21	0.22	0.18	0.42	0.45	0.45
Phosphorus	%	0.16	0.14	0.11	0.13	0.28	0.32	0.37	0.37
Nitrogen	%	1.35	1.22	1.05	0.89	0.74	1.00	1.00	1.29

Table 9. Nitrogen intake, nitrogen output in feces and urine, and nitrogen retained by Man I during three-day collection periods.

Collection dates	Nitrogen intake	Feces			Urine			Total nitrogen excreted	Nitrogen retained	
		Weight	Nitrogen	Nitrogen	Volume	Nitrogen	Nitrogen		Total	Daily average
	Gm.	Gm.	per cent	Gm.	Ml.	Gm./L	Gm.	Gm.	Gm.	Gm.
	Trial I, Ration 1, feed intake 4014 grams, body weight 149 lbs.									
July 8,9,10	54.00	4351	0.870	37.85	1394	12.40	17.29	55.14	-1.14	-0.38
July 14,15,16	do.	3412	0.855	29.17	1323	14.06	18.60	47.77	+6.23	+2.08
	Trial II, Ration 2, feed intake 4014 grams, body weight 149 lbs.									
July 19,20,21	48.75	3356	0.800	26.85	1523	10.06	15.32	42.17	+6.58	+2.19
July 23,24,25	do.	3081	0.795	24.49	1444	9.66	13.95	38.44	+10.31	+3.43
July 26,27,28	do.	3562	0.740	26.36	1230	11.30	13.90	40.26	+8.49	+2.83
	Trial III, Ration 3, feed intake 3672 grams, body weight 149 lbs.									
Aug. 8,9,10	38.40	2381	0.712	16.95	2640	4.04	10.67	27.62	+10.78	+3.59
Aug. 11,12,13	do.	2713	0.772	20.94	2015	6.08	12.25	33.19	+5.21	+1.73
Aug. 14,15,16	do.	2770	0.756	20.94	1872	6.22	11.64	32.58	+5.82	+1.94
	Trial IV, Ration 4, feed intake 3678 grams, body weight 154 lbs.									
Sept. 27,28,29	32.79	2760	0.654	18.05	2208	4.04	8.92	26.97	+5.82	+1.94
Sept. 30, Oct. 1,2	do.	3157	0.568	17.93	3055	3.26	9.96	27.89	+4.90	+1.63
Oct. 3,4,5	do.	3198	0.542	17.33	2830	3.84	10.87	28.20	+4.59	+1.53
	Trial V, Ration 5, feed intake 3995 grams, body weight 148 lbs.									
Oct. 20,21,22	24.66	3299	0.522	17.19	1345	8.00	10.76	27.95	-3.29	-1.09
Oct. 23,24,25	do.	2777	0.624	17.33	1323	8.16	10.79	28.12	-3.46	-1.15
Oct. 26,27,28	do.	3223	0.544	17.53	1465	5.96	8.73	26.26	-1.60	-0.53
Oct. 29,30,31	do.	3098	0.546	16.92	1732	4.54	7.86	24.78	-0.12	-0.04
Nov. 4,5,6	do.	2810	0.602	16.92	1500	4.16	6.24	23.16	+1.50	+0.50

Table 9, Continued.

Collection dates	Nitrogen intake	Feces			Urine			Total nitrogen excreted	Nitrogen retained	
		Weight	Nitrogen per cent	Nitrogen	Volume	Nitrogen	Nitrogen		Total	Daily average
	Gm.	Gm.	per cent	Gm.	Ml.	Gm./L	Gm.	Gm.	Gm.	Gm.
Trial VI, ration 6, feed intake 3207 grams, body weight 148 lbs.										
Nov. 7,8	24.08	2345	0.594	13.93	885	5.84	5.17	19.10	+4.98	+2.49
Nov. 10,11,12[a]	36.12	2998	0.628	18.83	1500	7.16	11.46	30.29	+5.83	+1.94
Nov. 13,14,15	do.	2467	0.740	18.26	1535	8.48	13.02	31.28	+4.84	+1.61
Nov. 16,17,18	do.	2304	0.764	17.60	1335	9.39	12.54	30.14	+5.98	+1.99
Nov. 19,20	24.08	1549	0.789	12.22	1090	9.16	9.98	22.20	+1.88	+0.94
Nov. 21,22,23	36.12	2338	0.768	17.96	1370	10.05	13.77	31.73	+4.39	+1.46
Nov. 24,25,26	do.	2740	0.731	20.03	1550	7.78	12.06	32.09	+4.03	+1.34
Nov. 27,28,29	do.	2499	0.745	18.62	1675	6.85	11.47	30.09	+6.03	+2.01
Nov. 30	12.04	759	0.838	6.36	480	9.15	4.39	10.75	+1.29	+1.29
Trial VII, Ration 7, feed intake 3270 grams, body weight 149 lbs.										
Dec. 3,4,5	24.90	2518	0.684	17.22	1175	5.92	6.96	24.18	+0.72	+0.24
Dec. 6,7,8	do.	2764	0.612	16.92	1360	5.74	7.81	24.73	+0.17	+0.05
Dec. 9,10,11	do.	3199	0.580	18.55	1320	5.50	7.26	25.81	-0.91	-0.30
Dec. 12,13,14	do.	2865	0.583	16.70	1112	6.42	7.14	23.84	+1.06	+0.35
Dec. 15,16,17	do.	2722	0.598	16.28	1073	7.58	8.13	24.41	+0.49	+0.16
Dec. 18,19,20	do.	3012	0.608	18.31	1810	5.24	9.48	27.79	-2.89	-0.96
Trial VIII, Ration 8, feed intake 3270 grams, body weight 150 lbs.										
Dec. 31, Jan. 1,2	36.18	1998	0.899	17.96	1366	8.48	11.59	29.54	+6.64	+2.21
Jan. 3,4,5	do.	2002	0.945	18.92	1379	9.30	12.82	31.74	+4.44	+1.48
Jan. 6[b]				7.29	410	9.14	3.75	11.04		
Jan. 7				7.29	470	8.03	3.77	11.06		
Jan. 8				7.29	400	9.92	3.97	11.26		
Jan. 6,7,8	do.	2244	0.974	21.86	1280		11.49	33.35	+2.93	+0.97

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[a] Ration 6b substituted for ration 6, feed intake 3279 grams.

[b] Daily urine collections analyzed; daily fecal nitrogen an average of the three-day period.

Table 9, Continued.

Collection dates	Nitrogen intake	Feces Weight	Feces Nitrogen per cent	Feces Nitrogen	Urine Volume	Urine Nitrogen	Urine Nitrogen	Total nitrogen excreted	Nitrogen retained Total	Nitrogen retained Daily average
	Gm.	Gm.	per cent	Gm.	Ml.	Gm./L	Gm.	Gm.	Gm.	Gm.
				Trial VIII, Continued.						
Jan. 9				6.28	540	6.88	3.72	10.00		
Jan. 10				6.28	405	8.34	3.38	9.66		
Jan. 11				6.28	460	6.88	3.16	9.44		
Jan. 9,10,11	36.18	2033	0.926	18.83	1405		10.26	29.09	+7.09	+2.36
				Trial IX, Ration 9, feed intake 3294 grams, body weight 146.5 lbs.						
Jan. 12				6.52	520	8.36	4.38	10.90		
Jan. 13				6.52	440	14.90	6.56	13.08		
Jan. 14				6.52	520	15.24	7.92	14.45		
Jan. 12,13,14	47.37	2047	0.956	19.57	1480		18.86	38.43	+8.94	+2.98
Jan. 15				6.85	525	15.16	7.96	14.81		
Jan. 16				6.85	520	17.08	8.88	15.73		
Jan. 17				6.85	740	12.08	8.94	15.79		
Jan. 15,16,17	47.37	2235	0.920	20.56	1785		25.78	46.34	+1.03	+0.34
Jan. 18				7.21	515	15.46	7.96	15.17		
Jan. 19				7.21	498	15.36	7.65	14.86		
Jan. 20				7.21	465	16.58	7.71	14.92		
Jan. 18,19,20	47.37	2390	0.905	21.63	1478		23.32	44.95	+2.42	+0.81
Jan. 21				7.47	455	17.90	8.14	15.61		
Jan. 22				7.47	580	13.90	8.06	15.53		
Jan. 23				7.47	525	14.14	7.42	14.89		
Jan. 21,22,23	47.37	2531	0.886	22.42	1560		23.62	46.04	+1.33	+0.44
Jan. 24				6.89	470	16.46	7.74	14.63		
Jan. 25				6.89	500	17.16	8.58	15.47		
Jan. 26				6.89	440	18.07	7.95	14.84		
Jan. 24,25,26	47.37	2291	0.902	20.66	1410		24.27	44.93	+2.44	+0.81
Jan. 27				6.61	400	21.35	8.54	15.15		
Jan. 28				6.61	410	22.73	9.32	15.93		
Jan. 29				6.61	580	15.41	8.94	15.55		
Jan. 27,28,29	47.37	2053	0.966	19.83	1390		26.80	46.63	+0.74	+0.25

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Table 9, Continued.

Collection dates	Nitrogen intake	Feces			Urine			Total nitrogen excreted	Nitrogen retained	
		Weight	Nitrogen per cent	Nitrogen	Volume	Nitrogen	Nitrogen		Total	Daily average
	Gm.	Gm.		Gm.	Ml.	Gm./L	Gm.	Gm.	Gm.	Gm.
Trial IX, Continued.										
Jan. 30				7.49	540	17.51	9.46	16.95		
Jan. 31				7.49	500	18.72	9.36	16.85		
Feb. 1				7.59	420	19.88	8.35	15.84		
Jan. 30,31, Feb. 1	47.37	2218	1.013	22.47	1460		27.17	49.64	-2.27	-0.76
Feb. 2				7.86	450	19.19	8.64	16.50		
Feb. 3				7.86	388	18.00	6.98	14.84		
Feb. 4				7.86	430	16.10	6.92	14.78		
Feb. 2,3,4	47.37	2360	0.999	23.58	1269		22.54	46.12	+1.25	+0.42
Feb. 5,6				14.66	815	16.00	13.04	27.70		
Feb. 7				7.33	415	17.15	7.12	14.45		
Feb. 5,6,7	47.37	2343	0.939	22.00	1230		20.16	42.16	+5.21	+1.74
Feb. 8				7.51	360	14.16	5.10	12.61		
Feb. 9				7.51	431	14.85	6.40	13.91		
Feb. 10				7.51	410	15.46	6.34	13.85		
Feb. 8,9,10	47.37	2358	0.955	22.52	1201		17.84	40.36	+7.01	+2.34
Feb. 11				8.01	435	14.22	6.19	14.20		
Feb. 12				8.01	635	11.32	7.19	15.20		
Feb. 13				8.01	495	13.30	6.58	14.59		
Feb. 11,12,13	47.37	2722	0883	24.04	1565		19.96	44.00	+3.37	+1.12
Trial X, Ration 8, feed intake 3270, body weight 146 lbs.										
Feb. 14				7.22	440	12.75	5.61	12.83		
Feb. 15				7.22	490	8.19	4.01	11.23		
Feb. 16				7.22	455	8.48	3.86	11.08		
Feb. 14,15,16	36.18	2372	0.913	21.66	1385		13.48	35.14	+1.04	+0.35
Feb. 17				7.55	490	7.76	3.80	11.35		
Feb. 18				7.55	535	7.56	4.04	11.59		
Feb. 19				7.55	530	6.30	3.34	10.89		
Feb. 17,18,19	36.18	2562	0.884	22.65	1555		11.18	33.83	+2.35	+0.78

Table 9, Continued.

Collection dates	Nitrogen intake	Feces			Urine			Total nitrogen excreted	Nitrogen retained	
		Weight	Nitrogen	Nitrogen	Volume	Nitrogen	Nitrogen		Total	Daily average
	Gm.	Gm.	per cent	Gm.	Ml.	Gm./L	Gm.	Gm.	Gm.	Gm.
Trial X, Continued.										
Feb. 20				7.00	665	5.76	3.83	10.83		
Feb. 21				7.00	535	7.46	3.99	10.99		
Feb. 22				7.00	410	7.90	3.24	10.24		
Feb. 20,21,22	36.18	2358	0.890	21.00	1610		11.06	32.05	+4.13	+1.38
Feb. 23,24,25	36.18	2506	0.822	20.60	1522	7.68	11.69	32.29	+3.89	+1.29
Feb. 26,27,28	36.18	2381	0.887	21.12	1750	6.89	12.06	33.18	+3.00	+1.00
Mar. 2,3,4	36.18	3077	0.900	18.69	1340	9.76	13.08	31.77	+4.41	+1.47
Trial XI, Ration 10, feed intake 3306, body weight 147 lbs.										
Mar. 19,20,21	52.98	2181	0.938	20.46	1785	14.70	26.24	46.70	+6.28	+2.09
Mar. 22,23,24	52.98	2322	0.926	21.50	1540	16.78	25.84	47.34	+5.64	+1.88
Mar. 25,26,27	52.98	2692	0.834	22.45	1890	14.65	27.69	50.14	+2.84	+0.94
Mar. 31, Apr. 1,2	52.98	2115	0.916	19.37	1564	17.62	27.56	46.93	+6.05	+2.02

Table 10. Nitrogen intake, nitrogen output in feces and urine, and nitrogen retained by Man II during three-day collection periods.

Collection dates	Nitrogen intake	Feces			Urine			Total nitrogen excreted	Nitrogen retained	
		Weight	Nitrogen per cent	Nitrogen	Volume	Nitrogen	Nitrogen		Total	Daily average
	Gm.	Gm.		Gm.	Ml.	Gm./L	Gm.	Gm.	Gm.	Gm.
Trial I, Ration 1, feed intake 4014 grams body weight 166 lbs.										
July 8,9,10	54.00	3134	0.902	28.27	2019	11.40	23.02	51.29	+2.71	+0.90
July 14,15,16	do.	3012	0.795	23.95	3170	6.36	20.16	44.11	+9.89	+3.29
Trial II, Ration 2, feed intake 4014 grams, body weight 164 lbs.										
July 19,20,21	48.75	3002	0.705	21.16	1672	10.30	17.22	38.38	+10.37	+3.46
July 23,24,25	do.	3713	0.670	24.88	2540	7.08	17.98	42.86	+5.89	+1.96
July 26,27,28	do.	3310	0.706	23.37	2460	6.58	16.19	39.56	+9.19	+3.06
Trial III, Ration 3, feed intake 4014 grams, body weight 165 lbs.										
Aug. 8,9,10	41.97	3582	0.618	22.14	4345	2.96	12.86	35.00	+6.97	+2.32
Aug. 11,12,13	do.	4658	0.536	24.97	3500	3.72	13.02	37.99	+3.98	+1.32
Aug. 14,15,16	do.	5879	0.404	23.75	2358	5.14	12.12	35.87	+6.10	+2.03
Trial IV Ration 4, feed intake 3426 grams, body weight 168 lbs.										
Sept. 27,28,29	32.28	3813	0.444	16.93	3317	3.18	10.55	27.48	+4.80	+1.60
Sept. 30, Oct. 1,2	do.	3848	0.478	18.39	3796	2.74	10.40	28.79	+3.49	+1.16
Oct. 3,4,5	do.	3879	0.466	18.08	3915	2.68	10.49	28.57	+4.71	+1.57

Table 11. Nitrogen intake, nitrogen output in feces and urine, and nitrogen retained by Ram III during three-day collection periods.

Collection Dates	Nitrogen intake	Feces			Urine			Total nitrogen excreted	Nitrogen retained	
		Weight	Nitrogen	Nitrogen	Volume	Nitrogen	Nitrogen		Total	Daily average
	Gm.	Gm.	per cent	Gm.	Ml.	Gm./L	Gm.	Gm.	Gm.	Gm.
Trial V, Ration 5, feed intake 2949 grams, body weight 120 lbs.										
Nov. 18,19,20	23.22	2412	0.732	17.66	2595	2.78	7.21	24.87	-1.65	-0.55
Nov. 21,22,23	do.	2670	0.532	14.20	1795	3.32	5.96	20.16	+3.06	+1.02
Nov. 24,25,26	do.	3210	0.602	19.32	2395	2.32	5.56	24.88	-1.66	-0.55
Trial VI, Ration 6, feed intake 3126 grams, body weight 121 lbs.										
Dec. 3,4,5	31.68	3555	0.484	17.21	3055	2.30	7.03	24.24	+7.44	+2.48
Dec. 6,7,8	do.	3476	0.472	16.41	3153	2.50	7.88	24.29	+7.39	+2.46
Dec. 9,10,11	do.	3786	0.524	19.84	1615	3.74	6.04	25.88	+5.80	+1.93
Dec. 12,13,14	do.	2565	0.680	17.44	3205	2.88	9.23	26.67	+5.10	+1.67
Dec. 15,16,17	do.	2800	0.702	19.66	4115	1.82	7.49	27.15	+4.53	+1.51
Dec. 18,19,20	do.	3338	0.660	22.03	1848	4.04	7.47	29.50	+2.18	+0.73
Trial VIII, Ration 8, feed intake 3144 grams, body weight 116 lbs.										
Dec. 31, Jan. 1,2	31.77	4232	0.455	19.26	2627	2.82	7.41	26.67	+5.10	+1.70
Jan. 3,4,5	do.	3420	0.553	18.91	2510	3.33	8.36	27.27	+4.50	+1.50
Jan. 6,7,8	do.	3965	0.538	21.33	2053	3.34	6.86	28.19	+3.58	+1.19
Jan. 9,10,11	do.	3946	0.525	20.72	2934	2.48	7.28	28.00	+3.77	+1.26
Trial IX, Ration 9, feed intake 3147 grams, body weight 118 lbs.										
Jan. 12,13,14	40.95	4960	0.477	23.66	2445	3.76	9.19	32.85	+8.10	+2.70
Jan. 15,16,17	do.	4302	0.451	19.40	3175	3.68	11.68	31.08	+9.87	+3.29
Jan. 18,19,20	do.	4121	0.490	20.19	2515	4.52	11.37	31.56	+9.39	+3.13
Jan. 21,22,23	do.	3351	0.559	18.73	2640	4.60	12.14	30.87	+10.08	+3.36
Jan. 24,25,26	do.	3554	0.559	19.87	2640	5.26	13.89	33.76	+7.19	+2.39
Trial IXr, Ration 9, feed intake 3108 grams, body weight 124 lbs.										
Feb. 25,26,27	40.59	3535	0.509	17.99	4180	4.82	20.15	38.14	+2.45	+0.81
Mar. 2,3,4	do.	3160	0.615	19.43	3260	4.96	16.17	35.60	+4.99	+1.66
Mar. 5,6,7	do.	3845	0.567	21.80	3475	4.92	17.10	38.90	+1.69	+0.56
Mar. 8,9,10	do.	3805	0.555	21.12	4150	3.38	14.03	35.15	+5.44	+1.81

Typed by: Mrs. Shirley L. Thomas