Table 3. Performance of Lambs Weaned at 30 Days of Age and Lambs Weaned at 70 Days of Age.

	Weaned at 30 days of age	Weaned at 70 days of age
ADG birth to 70-days	0.55	0.63
70-day weight	48.4	56.9
ADG 70-days to mkt.	0.52	0.57
Avg. mkt. age	156	147
Avg. mkt. wt.	92.8	97.2

lowing fall, early weaning could prove useful in some situations. If a sheepman wished to convert a group of spring lambing ewes to fall lambing schedule without sacrificing a complete lambing, early weaning could aid in this conversion.

Adaptation of Sheep to Biuret as a Nitrogen Source When Fed Low Quality Roughages

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Story in Brief

Rumen fistulated lambs were utilized to study the adaptation of rumen microflora to biuret as a source of nitrogen. The lambs were fed a low quality bermudagrass hay plus supplements containing either (1) cottonseed meal, (2) biuret, (3) biuret + cornmeal or (4) urea + cottonseed meal. There was little apparent effect of the nitrogen supplements on digestibility of dry matter, organic matter or fiber. Nitrogen retention data was highly variable.

When rumen contents from the lambs were utilized as inoculum for *in vitro* cellulose digestion studies, there appeared to be no adaptation

to the biuret as a source of nitrogen since after feeding biuret for over 80 days, the inoculum still would not digest cellulose when biuret served as the only *in vitro* source of nitrogen. On the other hand, when biuret disappearance and ammonia appearance data were considered during the conduct of the trial, there was definite evidence for adaptation of the microflóra to biuret. Ammonia appearance *in vivo* from biuret was considerably more rapid on day 87 than on day 4 of the supplemental feeding period.

The *in vitro* biuretolytic activity of the inoculum from non-biuret fed animals was negligible throughout the entire study. Release of ammonia from biuret was obvious at day 42 when the inoculum from biuret fed animals was tested *in vitro* and was even greater on day 87. Disappearance of biuret *in vitro* was slightly greater for the inoculum from biuret fed animals at day 14 and was markedly improved in these inocula in later periods of the study. The significance of these observations are presently being studied in additional experiments.

Introduction

Previous work in Oklahoma and elsewhere has clearly demonstrated the need for supplemental protein for ruminant animals during the winter grazing periods. This is true whether grazing native grasses or introduced species such as bermudagrass. In fact, this protein supplementation constitutes, in many cases, the major portion of the feed cost for grazing animals. It is of interest, therefore, to continue to search for lower cost supplements which will be acceptable and high in nutritive value.

Non-protein nitrogen has been utilized very successfully in protein supplements for cattle and sheep being fed in the feedlot. The major source of non-protein nitrogen in these supplements has been urea. The relatively low cost of urea in recent years has made the substitution of natural protein by urea a very economical practice.

Numerous experiments have also been carried out in attempts to utilize urea in range supplements with somewhat less success. Urea is very quickly hydrolyzed in the rumen to the ammonia form of nitrogen. This form of nitrogen can be rapidly absorbed from the rumen and may be excreted or, under some circumstances, may cause an ammonia toxicity in the animal. This condition is very unlikely in animals on high concentrate rations since the ammonia is rapidly utilized by the microorganisms digesting the highly available forms of carbohydrates.

When dry roughages, such as those used in Oklahoma winter grazing, are consumed there is very little of the highly available form of carbohydrate. As a consequence, much greater chance for nitrogen loss and for possible toxicity exist. Therefore, searches have been underway

for other forms of nitrogen which are less toxic and still available for utilization by the rumen microorganisms. One form which has been studied recently is biuret. Biuret is a non-protein nitrogen source similar chemically to urea but generally hydrolyzed much slower in the rumen. Previous workers have shown that a considerable adaptation period is necessary prior to the development of the ability for ruminants to utilize biuret efficiently.

The project being reported here was initiated in early 1970 to study the adaptation of ruminants to biuret when different forms of roughages and concentrates were being fed. The results of the first years studies are reported here.

Materials and Methods

Twelve lambs were fitted with rumen cannulas in the winter of 1970. Bermudagrass hay harvested in February from an ungrazed bermudagrass plot was utilized as base feed throughout the entire study. Although intended to be a very low quality roughage, the crude protein content of this bermudagrass hay was approximately 9 percent on a dry matter basis. The twelve lambs were divided into four groups and fed the rations shown in Table 1. All lambs received 450 gm of chopped bermudagrass hay plus the supplements as indicated daily. Since the biuret supplement was very unpalatable, it was poured into the rumen through the cannula each morning prior to feeding. During the course of the experiment, one animal on supplement 2 died.

Table 1. Composition of Rations and Daily Feed Allowance for Biuret Trial 1.

	Daily Feed Allowances (Grams)			
	Ration 1	Ration 2	Ration 3	Ration 4
Low Quality Bermuda grass hay	450	450	450	450
Supplement 1	168	---	---	---
Supplement 2	---	45	---	---
Supplement 3	---	---	195	---
Supplement 4	---	---	---	179
Supplement Composition	1 %	2 %	3 %	4 %
Cottonseed meal	89.3	---	---	41.9
Ground corn	---	---	77.0	41.9
Biuret	---	60.0	13.8	---
Urea, 281	---	---	---	6.1
Dicalcium phosphate	3.6	13.3	3.1	3.3
Limestone	3.6	13.3	3.1	3.3
Trace mineralized salt	3.6	13.3	3.1	3.3

Prior to the initiation of supplemental feeding all animals were placed on bermudagrass hay plus cottonseed meal supplement for 14 days. During this period of time, one digestion trial was conducted consisting of a 7-day collection period in which total feces and urine collections were made in addition to accurate observations of feed consumption. After the initiation of supplementation, digestion trials were conducted at three different periods during the course of the 87-day feeding period.

On days 4 and 87 of the supplemental feeding period, rumen samples were removed from each animal at 0, 1, 2, 4 and 8 hours after feeding for ammonia and biuret analyses. On days 4, 14, 21, 42, 66 and 84 of the supplemental feeding period, the ability of the rumen microorganisms to release ammonia from biuret was measured *in vitro* in the laboratory. This was accomplished by taking a sample of the rumen contents from each animal and mixing it with a known quantity of biuret and incubating the mixture at 39°C for 24 hours. Samples were removed from flasks at 0, 8 and 24 hours to determine the disappearance of the biuret and the appearance of ammonia.

The ability of the rumen microorganisms to utilize biuret as a source of nitrogen for the digestion of cellulose was measured using an *in vitro* system in which the rumen microorganisms taken from each animal were used to innoculate flasks containing purified cellulose and all of the nutrients required for their growth except for nitrogen. Cellulose digestion was then measured in flasks which contained either (1) no nitrogen source, (2) urea, which served as a positive control, or (3) biuret.

Results and Discussion

Apparent Digestibilities. The coefficients of digestibility of dry matter, organic matter, acid detergent fiber and kjeldahl nitrogen as well as nitrogen balance are shown in Table 2. Statistical analyses of these data have not been conducted as yet. It would appear that the digestibilities of ration 2, the biuret supplemented ration, are definitely lower than the digestibilities for the other three rations. The most likely explanation for this result, however, is not due to the nitrogen supplement itself but to the fact that the three rations with the highest digestibility contained either cornmeal, cottonseed meal or both. Cornmeal and cottonseed meal both have high digestibilities, whereas ration 2 did not contain either of these high energy supplements.

The nitrogen digestibility of rations 2, 3 and 4 appear to be higher than that for ration 1. Again this explanation is very likely due to the fact that the latter three rations contain non-protein nitrogen which

Table 2. Apparent Digestibilities and N-balance For Biuret Supplemented Rations (Trial 1).

Measurement	Period[1]	Ration 1	Ration 2	Ration 3	Ration 4
Dry Matter, %	1	47.2	58.5	47.8	52.2
	2	54.2	46.5	51.8	55.6
	3	52.8	45.8	54.3	52.8
	4	50.5	46.7	55.1	51.8
	$\bar{x}$[2]	52.5	46.3	53.7	53.4
Organic Matter, %	1	49.4	60.4	50.2	53.9
	2	57.1	49.3	54.9	58.0
	3	54.1	47.5	56.0	54.8
	4	52.3	48.0	56.9	53.9
	$\bar{x}$[2]	54.5	48.3	55.9	55.6
Acid Detergent fiber, %	1	40.2	46.5	39.7	40.4
	2	48.1	38.8	36.7	43.2
	3	44.0	36.7	35.5	37.9
	4	44.7	38.0	39.5	40.7
	$\bar{x}$[2]	45.6	37.8	37.2	40.6
Nitrogen, %	1	66.3	70.1	62.3	68.4
	2	66.5	73.8	68.6	73.4
	3	69.6	76.7	76.2	72.1
	4	68.4	78.0	78.1	74.5
	$\bar{x}$[2]	68.2	76.2	74.3	73.3
N-balance, gm/day	1	3.66	2.52	3.34	3.02
	2	4.26	2.06	3.38	2.70
	3	3.07	1.61	3.81	2.13
	4	2.89	7.94	1.28	3.35
	$\bar{x}$[2]	3.41	3.87	2.82	2.73

[1] Period 1 (Day −19 to −12), Period 2 (Day 6 to 11), Period 3 (Day 24 to 30), Period 4 (Day 74 to 81) with Day 1 being the start of supplemental feeding.
[2] Averages of last 3 periods.

almost invariably increases the apparent digestibility of nitrogen. These forms of nitrogen are absorbed and even though they may be excreted for the large part they appear as digested nitrogen. The nitrogen balance data is quite inconsistent and does not show any trends which are useful. When one compares periods 2, 3 and 4 within these data, there would appear to be a slight decrease in dry matter and organic matter digestibilities during the conduct of the trial, at least for rations 1 and 4. No particular explanation can be offered for this at the moment.

In contrast, nitrogen digestibility appeared to increase during the conduct of the trial for rations 2 and 3, the biuret supplemented ration, whereas there was no significant change for rations 1 and 4. This result is not likely due to increased absorption of the nitrogen since the biuret form of nitrogen would presumably be absorbable at any period of the trial.

In vitro cellulose digestibility data. In vitro cellulose digestibility by the rumen microorganisms from two of the three animals on each treatment was determined three days prior to the commencement of supplementation and on days 12, 19, 39 and 80 after the commencement of supplementation. The data from these determinations are shown in Table 3. In all cases, the tubes containing urea as the source of nitrogen had cellulose digestibilities which were considerably higher than the control tube, the one without nitrogen. On the other hand, those tubes containing biuret rarely had cellulose digestibilities which were any higher than the control tubes suggesting that the biuret nitrogen could not be utilized for cellulose digestion by these microorganisms. This was generally true for all of the animals whether they had been adapted to biuret or not.

On days 39 and 80 there appeared to be slightly higher cellulose digestibility in the tubes containing biuret nitrogen as compared to the tubes containing no source of nitrogen. These slight apparent increases in no way come close to the digestibilities supported by urea as a source

Table 3. Utilization of Biuret and Urea Nitrogen to Support In Vitro Cellulose digestion[1] by Inoculum from Biuret Adapted and Non-Adapted Lambs.

Ration	N-Source[2]	Trial Day (Percent)				
		−3	12	19	39	80[3]
1	O	19.4	21.0	23.8	18.7	19.7
	U	44.7	46.4	46.1	42.0	---
	B	23.2	21.1	23.2	18.9	20.0
2	O	23.0	12.9	26.1	20.2	22.6
	U	41.3	29.2	46.2	42.6	---
	B	27.7	13.9	25.9	22.6	27.4
3	O	18.5	14.4	15.1	16.5	19.0
	U	37.4	37.8	47.8	38.3	---
	B	18.6	14.1	14.7	24.3	25.3
4	O	23.0	22.0	22.4	13.3	21.9
	U	43.9	55.4	53.1	40.4	
	B	20.9	21.7	21.6	12.4	21.8

[1] Data reported at % digestion of a change of 0.5 gm cellulose in a 30 ml volume *in vitro* tube. Values are averages of two lambs.
[2] O=No N-Source, U=urea, B=Biuret.
[3] Urea was omitted inadvertantly. Biuret-control comparison is valid, however.

of nitrogen. Thus, biuret was considerably less than optimal as a nitrogen source. In this case, then, there appeared to be no marked evidence of adaptation of the cellulose digesting microorganisms to biuret as a nitrogen source.

In vivo rumen ammonia release from biuret. Figure 1 shows the results of the ammonia analyses on rumen samples taken from the supplemented animal on days 4 and day 87 after the initiation of supplemental feeding. The curves for rations 1 and 4 represent rather typical curves in which natural protein (ration 1) or urea (ration 4) are utilized as nitrogen sources. With the natural protein, there is a rather slow release of the ammonia and usually a falling off of the rumen ammonia level due to utilization by the microorganisms.

With the urea supplemented rations, there is always a very rapid rise in rumen ammonia due to the hydrolysis of the urea and a subsequent gradual decrease due to both absorption and excretion as well as utilization by the rumen microorganisms. There is no particular difference between the curves for days 4 and 87 for the cottonseed meal supplement. The release of the ammonia from urea and cottonseed meal combinations in ration 4 was very rapid on both days 4 and 87, but appeared to fall off more rapidly on day 4 than 87.

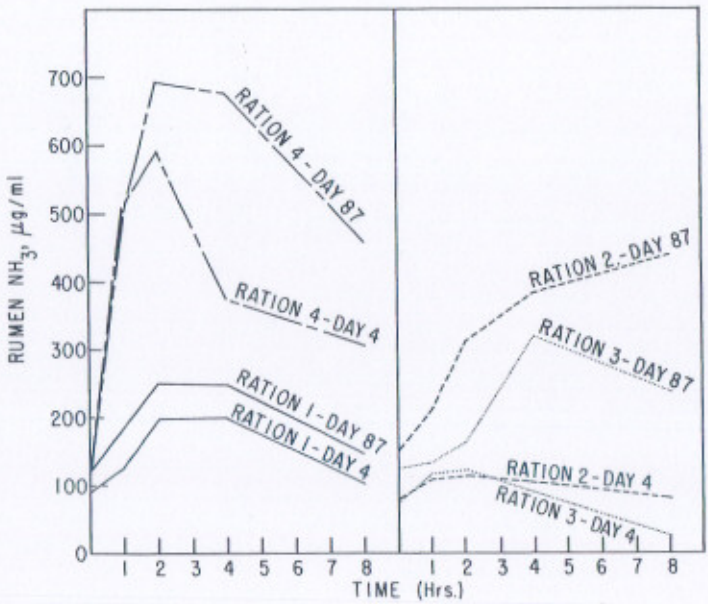

Figure 1. In Vivo NH_3 Release from N-Supplements.

The data for rations 2 and 3 are much more striking and revealing. In both cases, there is very little ammonia release on day 4 suggesting the biuret was not being hydrolyzed or at least was not being hydrolyzed fast enough for ammonia to accumulate. On day 87, however, with both biuret supplemented rations, there was an obvious increase in ammonia following feeding. Thus, these data show clearly that there had been an adaptation to biuret during this lengthy period and that on day 87 there was obviously some hydrolysis of the biuret to ammonia, which then suggests that utilization could occur.

Biuretolytic activity in vitro. The disappearance of biuret and the appearance of ammonia during the *in vitro* ammonia release tests would

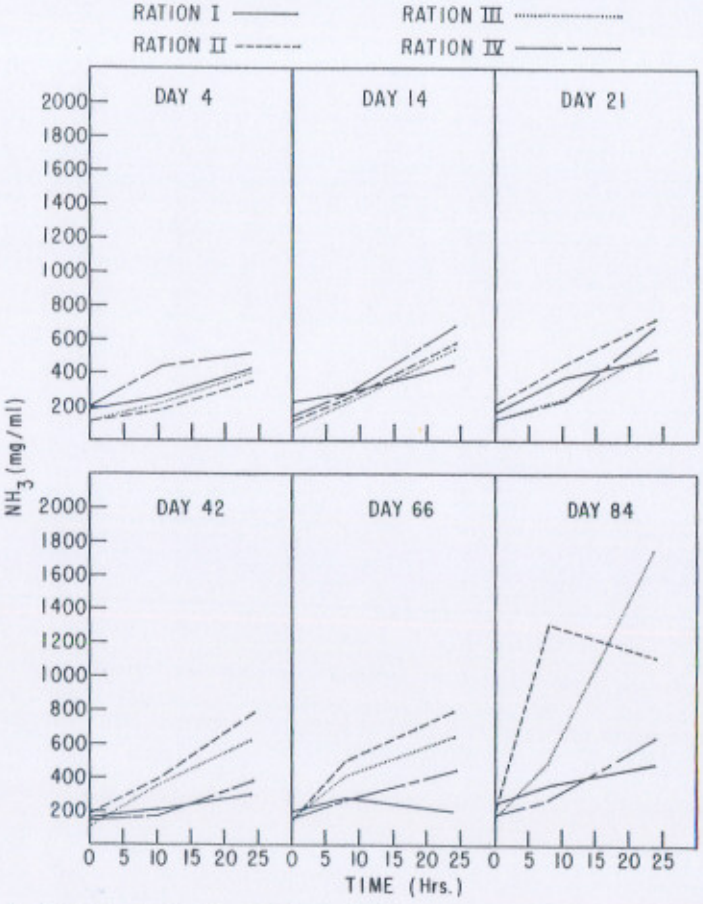

Figure 2. NH_3 Release From Biuret in Vitro.

be an indication of biuretolytic activity by the rumen microorganisms. Figures 2 and 3 show the appearance of ammonia and the disappearance of biuret, respectively, as the feeding period progressed. In Figure 2, it can be seen that the ammonia appearance curves do not differ markedly until the measurement taken on day 42. At that time, it would appear that the inocula taken from biuret fed animals were beginning to release more ammonia from biuret than that taken from animals that had not been fed biuret. On day 66, the results are rather similar to day 42. On day 84, however, a considerable divergence in lines between those

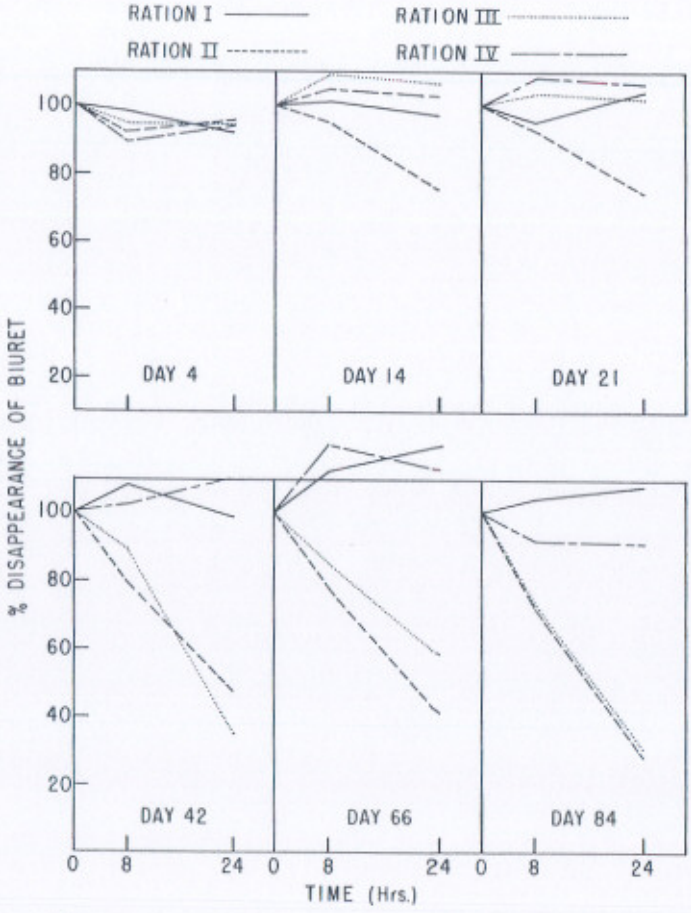

Figure 3. Biuret Disappearance in Vitro.

fed or not fed biuret appeared. There is a very obvious increase in ammonia appearance from the added biuret throughout the 24 hours with the inoculum from animals fed ration 3 and very rapid release up to 8 hours with the inoculum from ration 2, with an apparent leveling off at that point. Thus, these data also demonstrate adaptation of the rumen microorganisms to biuret.

Figure 3 shows the biuret disappearance at these same periods. These data suggest that biuretolytic activity started to a small degree by day 14 with ration 2 but was not markedly obvious until day 42 at which point inoculum from both rations 2 and 3 hydrolyzed significant amounts of biuret. In fact, on the basis of this evidence little additional biuretolytic activity could be found on day 84. Thus, although adaptation to biuret was indicated by both critera, biuret disappearance was evident before increased ammonia appearance could be observed.

Conclusions

It is evident from these results that there was definitely an adaptation of the rumen microflora to biuret as a source of non-protein nitrogen. The adaptation was not obvious in the digestion trial data, however. On the other hand, in those studies designed to measure the ability of the microflora to degrade the biuret to ammonia, there was very positive evidence of this adaptation. Although the biuret disappearance measurements suggested that adaptation might begin as early as 14 days, the ammonia appearance data would suggest that major adaptation took 42 days or longer.

This poses two interesting questions. First, is the possible lengthy adaptation of 42 days or longer impractical as far as useful feeding systems in the field. Secondly, even after adaptation, is the rate of release of ammonia rapid enough to support proper rumen digestion. The answers to these questions await further studies which are presently underway. This type of an experiment is presently being conducted again using a lower quality native grass hay and further studies are planned to evaluate the usefulness of this source of non-protein nitrogen.