Further Studies on Adaptation and Loss of Adaptation of Rumen Microorganisms to Biuret as a Nitrogen Source Fed with Low Quality Roughages

R. R. Johnson and E. T. Clemens

Story in Brief

The utilization of biuret as a nitrogen source for ruminants consuming low quality roughage is continuing to be studied. This years results suggest that the length of time required for adaptation to biuret by rumen microorganisms on roughage rations is directly related to the level of natural protein in the rations, that is, the more natural protein present, the longer the time required for adaptation. Furthermore, removal of the biuret from the ration caused an almost immediate loss of biuret hydrolyzing ability by rumen contents. In one growth trial with steers, biuret appeared to be utilized only partially as well as cottonseed meal.

Introduction

The search continues for lower cost nitrogen supplements that can be substituted for natural protein in protein supplements provided for grazing cattle and sheep. Recent studies in our department (Misc. Pub. No. 85, 1971) have shown that biuret will provide a less toxic, more slowly released form of nitrogen than urea, but considerable time is required for the rumen microorganisms to acquire the ability to break the biuret down to ammonia which is the useful form of nitrogen. For example, in the 1971 report, "biuretolytic" activity was not evident until after 42 days of supplementation with biuret. This years studies were designed to further investigate this time required for adaptation as well at the loss of adaptation after biuret is removed from the ration.

Materials and Methods

Trial 1. The rations shown in Table 1 were fed to rumen fistulated sheep for a period of 91 days. The objective was to compare the development of biuretolytic activity (ability to utilize biuret) when sheep received various combinations of biuret with natural protein, starch and urea.

Apparent digestibilities of ration components were determined during three different 7 day periods beginning at day 7, 42 and 84 of supplementation. The ability of the rumen microorganisms to hydrolyze biuret to ammonia was determined on days 6, 20, 42, 62 and 83 after beginning supplementation. This was accomplished by incubating a biuret solution with whole rumen contents in the laboratory and measuring disappearance of biuret. The data reported is percent disappearance of the original biuret in the flask.

Trial 2. Three fistulated dairy steers were fed prairie hay for 20 days during which time the level of biuretolytic activity in their rumen contents was determined. The biuret supplement shown in Table 2 was then fed to all three steers. Biuretolytic activity was determined on days 7, 16, 30, 44, 60 and 75 after the start of biuret feeding.

Two deadaptation trials were conducted with these steers. In the first, the adapted animals were returned to the cottonseed meal supplement and biuretolytic activity was determined on days 1, 2, 4, 7 and 10 after removal of biuret. They were then readapted to biuret. After readapting, the biuret supplement was again removed but no other supplement was given during the deadaptation study to determine the effect of

Table 1. Composition of Supplements and Feed Offerings—Trial 1

Ingredient	Supplement[1] composition, %				
	1 CSM	2 B+LS	3 CSM+HS	4 CSM+B	5 B+U
Cottonseed meal	89.3	–	42.4	37.7	–
Corn starch	–	75.4	52.2	37.7	75.8
Biuret, pure	–	15.4	–	15.4	12.3
Urea, 281	–	–	–	–	2.7
Limestone	3.6	3.1	1.8	3.1	3.1
Dical, phos.	3.6	3.1	1.8	3.1	3.1
T.M. salt	3.6	3.1	1.8	3.1	3.1
Vit. A & D	+	+	+	+	+
Daily ration, gm					
Chopped prairie hay	500	500	500	500	500
Supplement	170	170	340	170	170

[1] Abbreviations refer to CSM-cottonseed meal; B-biuret, LS-low starch, HS-high starch; U-urea.

Table 2. Composition of Supplements Fed to Fistulated Steers in Trial 2

Ingredient	Grams in mixture	
	Control supplement	Biuret supplement
Cottonseed meal	10,000	–
Corn starch	–	9800
Biuret	–	2000
Limestone	400	400
Dicalcium phosphate	400	400
T.M. salt	400	400
Vit A (30,000 I.U./gm)	2	2
	11,202	13002
Daily Ration		
Hay, prairie	7000 gm	7000 gm
Supplement	1700 gm	1700 gm
N obtained from supplement	95 gm	93 gm

the presence or absence of other nitrogen sources on the rate of loss of adaptation.

Trial 3. Twenty "black baldy" steers were alloted by weight to 4 lots and fed the rations shown in Table 3 free choice. These were designed as high roughage rations to support wintering type gains but feed consumption far exceeding expectations, as will be discussed later. Shrunk weights were taken before and after the 96 day feeding period.

Results and Discussion

Trial 1. Digestibility coefficients for the three periods of testing are shown in Table 4. Except for ration 3, there was a general increase in digestibility of dry matter and organic matter as the trial progressed. The reasons for this are not obvious but the most likely explanation would be

Table 3. Composition of Rations for Steers, Trial 3

Ingredient	Composition (%, as is basis)			
	1	2	3	4
Cottonseed hulls	70.2	70.2	70.1	70.1
Cottonseed meal	15.0	7.0	–	8.0
Ground corn	11.1	18.8	24.1	16.9
Molasses	2.0	2.0	2.0	2.0
Biuret	–	–	1.8	1.1
Limestone	0.5	0.3	0.3	0.4
Dicalcium phosphate	0.5	1.0	1.0	0.8
Sodium sulfate	0.1	0.1	0.1	0.1
T.M. salt	0.6	0.6	0.6	0.6
Vitamin A (30,000 I.U./gm)	5 gm/100 lb	5 gm/100 lb	5 gm/100 lb	5gm/100 lb

Table 4. Coefficients of Digestibility for Rations in Trial 1

Ration	Period[2]	Digestibility coefficients[1]			
		DMD	OMD	ADFD	N-dig
		%	%	%	%
1 CSM	1	53.8[a]	55.6[a]	46.1[a]	60.6[a]
	2	57.2[ab]	59.7[ab]	51.2[ab]	64.2[ab]
	3	68.7[b]	70.9[b]	63.7[b]	76.1[b]
2 B + LS	1	50.2[x]	51.7[xa]	45.5[a]	65.8[x]
	2	57.4[xy]	60.2[xyb]	49.1[a]	68.4[xy]
	3	62.3[y]	65.2[y]	51.8[a]	74.7[y]
3 CSM + HS	1	61.5[a]	63.2[a]	41.4[a]	60.6[a]
	2	62.0[a]	64.1[a]	53.9[b]	60.1[a]
	3	62.0[a]	61.4[a]	45.2[ab]	51.3[b]
4 CSM + B	1	48.7[a]	50.9[a]	34.8[a]	63.2[a]
	2	57.6[a]	60.4[a]	49.8[a]	76.3[a]
	3	66.2[b]	68.5[a]	57.6[a]	73.9[a]
5 B + U	1	48.0[a]	49.7[a]	51.4[a]	69.9[ab]
	2	61.4[b]	63.5[b]	52.6[a]	72.9[b]
	3	57.1[ab]	59.8[ab]	45.5[a]	62.5[a]

[1] DMD, OMD, ADFD refer to dry matter digestibility, organic matter digestibility, acid detergent fiber digestibility, RESP.

[2] Periods refer to 7 day collection periods starting with (1) day 7, (2) day 42 and (3) day 84 of supplemental feeding.

A, B
$P < .05$—Between Periods
X, Y
$P < .01$—Between Periods

that of a change in the quality of hay component in the ration. Although the bales of hay were taken from one lot, quality of prairie hay can vary considerably between bales. Statistically significant increases in nitrogen digestibility with time on feed were noted for the cottonseed meal, the biuret and a non-significant increase was noted for the CSM + biuret ration.

Nitrogen digestibility for the biuret plus urea ration increased in period 2 but decreased again in period 3. It is difficult to associate these changes directly with the ration supplement since the quality of hay may have been variable. However, the increases in nitrogen digestibility of the biuret rations agrees with previous observations and would obviously correlate with possible adaptation phenomena.

The biuretolytic activity of the rumen contents from the lambs fed these rations is illustrated in Table 5. There was no biuretolytic activity apparent at anytime in the animals fed rations 1 and 3 (no biuret in rations). On the other hand, biuretolytic activity did appear in all animals fed biuret but in somewhat of an erratic fashion. Rumen contents from animals on ration 2 showed low activity by 20 days (32 percent in 24

Table 5. Biuret Disappearance During Biuretolytic Tests—Trial 1

Period	Ration	Percent biuret loss from zero time									
		1 CSM		2 B+LS		3 CSM+HS		4 CSM+B		5 B+U	
	Time, hrs[1]	8	24	8	24	8	24	8	24	8	24
		%		%		%		%		%	
1	(6)[2]	1.1	7.5	1.2	8.9	0	—0.6	2.0	9.0	2.6	8.6
2	(20)	0.4	3.4	6.2	31.5	—2.6	—2.6	2.1	11.2	2.2	9.2
3	(42)	—1.8	—1.8	6.2	24.1	—0.9	—2.6	10.3	32.9	12.8	72.6
4	(62)	—1.0	0.5	1.6	22.6	—3.0	—3.0	3.6	34.8	0.0	13.7
5	(83)	—0.4	0	7.4	16.0	—7.3	—6.7	8.5	55.9	—2.3	12.7

[1] Hours after initiation of in vitro biuretolytic fermentation.
[2] Days after start of supplemental feeding.

hrs) but never increased beyond that level. The activity in contents from ration 4 fed animals increased steadily from 42 to 83 days on feed. With ration 5, a high activity was noted on day 42 but this disappeared by 62 days and did not reappear. As will be seen later, this may have been due to accidental removal of biuret from the ration for a few days which causes an immediate loss of activity.

Although biuretolytic activity was not as high in this study as noted in last years work, the results suggest that development of the activity is more rapid if there are no other sources of nitrogen such as cottonseed meal or urea. This agrees with results by other workers.

Trial 2. The biuretolytic activity of the rumen contents from the fistulated steers in this trial are shown in Figure 1. The points on this curve represent averages of the three animals. On days 7 and 16 there was practically no biuretolytic activity observed. By day 30, however, maximum biuretolytic activity was developed with no further increases up to 75 days. This is more rapid than observed with the lambs in last years tests. Again, however, this was with a prairie hay with 4-5 percent crude protein while last years study was conducted with a bermuda grass hay with 8-9 percent protein. The presence of natural protein is known to delay development of biuretolytic activity.

We were interested in how rapidly the biuretolytic activity could be lost if biuret was removed from the ration. Figure 2 illustrates these results when the biuret supplement was replaced with the cottonseed meal supplement. Biuretolytic activity decreased markedly by day 1 and completely by day 4 after the switchover. Since considerable natural protein was present (cottonseed meal) it was decided to repeat this test using no protein supplement. The steers were readapted to the biuret supplement and then this supplement was removed without any replacement. These results are shown in Figure 3. Again most of the biuretolytic activity was lost after 2 days and all was lost by 4 days after removal.

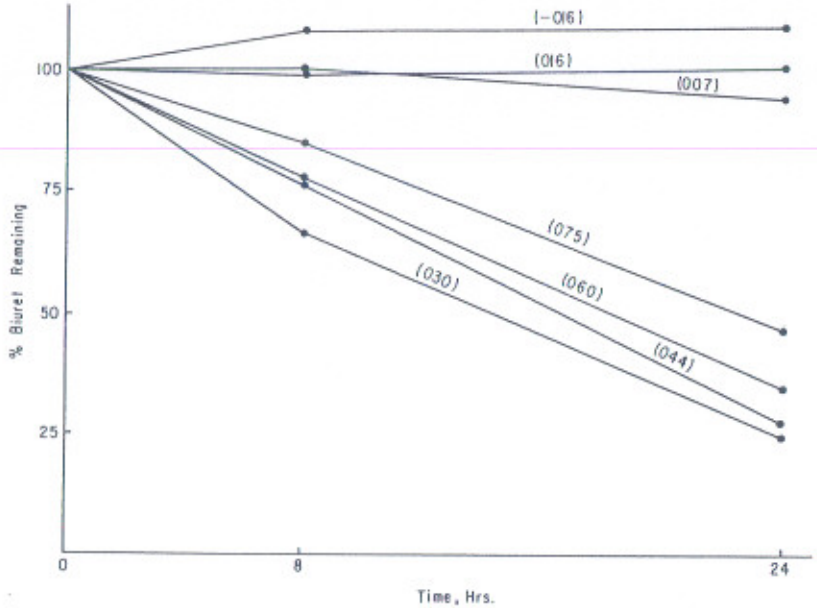

Figure 1. Biuretolytic activity of rumen contents from seers being fed a biuret supplement with prairie hay. Numbers refer to days before (–) or after (+) start of biuret supplement feeding.

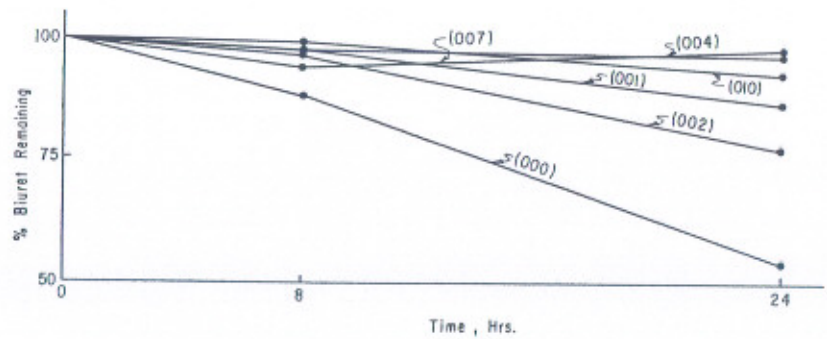

Figure 2. Biuretolytic activity of rumen contents from steers on various days following removal of biuret from the supplement (CSM supplement replaced biuret supplement).

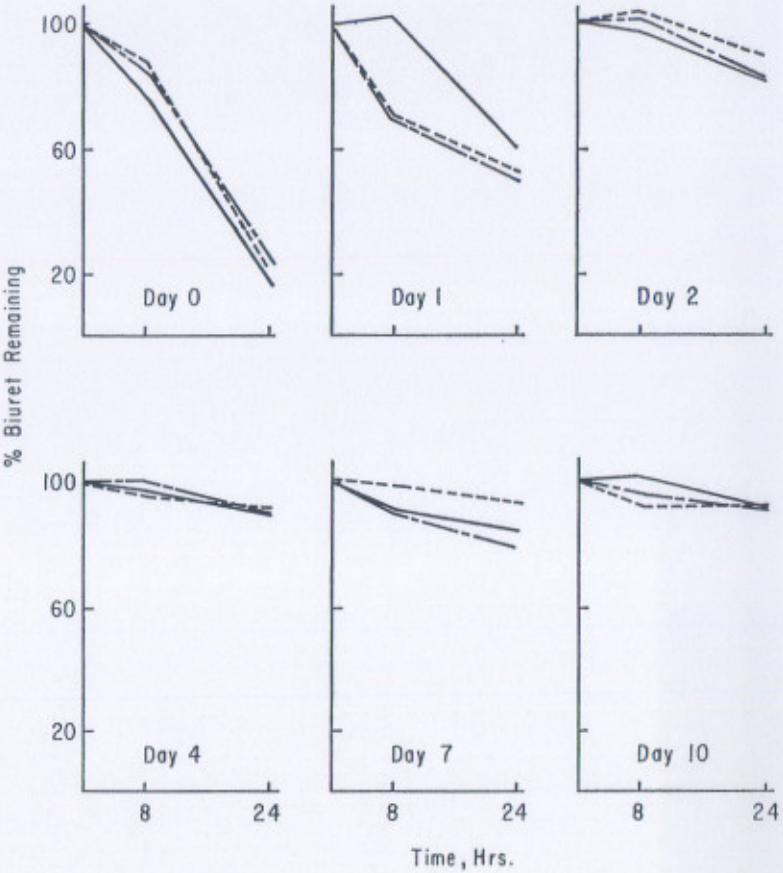

Figure 3. Biuretolytic activity of rumen contents of steers following second removal of biuret from the supplement (no other supplement provided).

These deadaptation results are significant from the standpoint that many beef cows are supplemented on alternate days or even only once or twice a week. This suggests that biuret adaptation might be difficult to maintain under these circumstances. This problem is being studied further at this time.

Trial 3. The steer growth trial was designed to stimulate a wintering situation in terms of ration and predicted gains. As such, the lot receiving one half the normal level of protein (ration 2 in Table 3) was a negative control utilized to estimate the extent of utilization of the test nitrogen sources. Table 6 presents the performance data for these steers. It is

Table 6. Performance of Steers on High Roughage Rations in Wintering Trial 3

Item[1]	1 CSM	2 ½ CSM	3 B	4 B+CSM
No. Animals	5	5	5	5
Days on feed	96	96	96	96
Total gain, lb.	332	256	290	275
Ave. daily gain, lb.	3.45	2.67	3.02	2.87
Ave. daily feed, lb., as is,	31.65	26.62	32.01	31.60
Feed per lb. gain, lb.	9.16	9.98	10.45	11.02

[1] CSM=cottonseed meal, B=biuret.

immediately obvious that the performance far exceeded predictions in that the average gain for all lots was over 2 lbs. per head per day. This is presumably due to the very high feed consumption exhibited by these steers, actually exceeding 4 percent of their body weight. Daily gains were highest for the CSM lot followed by the biuret lot and the CSM + biuret lot. The negative control gained at the lowest rate, but they also ate much less feed, typical in a protein deficiency situation. Their feed efficiency was second highest.

The steers on the biuret ration, lot 3, gained faster than the negative control but certainly less than the ones on CSM (lot 1). Furthermore, their feed efficiency was not as good since they ate as much feed as the CSM animals. Thus it is difficult to determine whether the biuret was well utilized or not. Looking at lot 4 where the feed consumption was still about the same, the average daily gain was slightly less than for lot 3 and the feed efficiency was the poorest of the four rations. Ration 4 contained the same quantity of CSM as ration 2, the negative control, plus half the amount of biuret fed to lot 3. Based on the feed consumption data plus the gains, it appears biuret did have an effect on rumen activity and was partially utilized but not as well as cottonseed meal.

Conclusions

Biuret adaptation is a slow process, the time for which can be influenced by the level of natural protein in the ration. The ability to hydrolyze and utilize biuret is quickly lost when biuret is removed from the ration. This may have important management implications. Biuret has been shown to be partially utilized in a growth study with steers but further tests are needed.