

Biuretolytic Activity of Rumen Microorganisms as Influenced by Dietary Molasses and Dehydrated Alfalfa

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Story in Brief

Two experiments were conducted to determine the effects of dietary additions of molasses and dehydrated alfalfa upon the rate of adaptation to and extent of hydrolysis of biuret by rumen microorganisms.

In experiment 1 high roughage (80 percent cottonseed hull) rations containing 50 percent of the total dietary nitrogen as biuret, were supplemented with 0, 2 and 8 percent dehydrated alfalfa both with and without 5 percent molasses. Biuret adaptation was rapid on all diets, generally being complete by day 3 of the trial. No significant treatment effects were observed.

In the second experiment, lambs receiving low quality prairie hay were fed biuret and alfalfa containing supplements so that biuret furnished 65 percent of the digestible protein requirement and alfalfa supplied 0, 3 and 10 percent of the total diet. Adaptation to biuret was rapid, being complete by day 4. The incorporation of dehydrated alfalfa into the supplement did not appear to have a substantial effect upon the rate of biuret adaptation.

Introduction

The adaptation period required for efficient biuret utilization is one of the major problems to be dealt with when using this NPN source. The length of this adaptation period is quite variable and may range from a few days to eight weeks.

Previous work has shown that addition of readily fermentable carbohydrate sources may decrease the length of the adaptation period and enhance the extent of biuret hydrolysis. Those studies were undertaken to characterize the effects of low levels of molasses and dehydrated alfalfa on the rate of adaptation to biuret and the extent of biuret hydrolysis by rumen microorganisms.

Experiment 1.

Twelve wether lambs fitted with permanent rumen cannulas were allotted into 6 groups and fed one of six experimental rations (Table 1). Ration 1 was a control diet which contained no molasses or alfalfa. In rations 2 and 3 alfalfa was added as 2 and 8 percent of the rations, respectively. Rations 4, 5 and 6 contained 5 percent molasses and 0, 2 and 8 percent alfalfa, respectively. All rations were calculated to be isonitrogenous and isocaloric and each contained about 80 percent cottonseed hulls. Biuret supplied 50 percent of the nitrogen content in each ration.

Rumen contents were withdrawn from each animal on days 0, 3, 5, 10, 14, 21, 28 and 35. The ability of the rumen microorganisms to degrade biuret to ammonia (biuretolytic activity) was determined by incubating the rumen contents with a biuret solution at 39°C and measuring the disappearance of biuret over a 24 hour period.

Experiment 2.

Twelve rumen cannulated sheep approximately 1 year of age were allotted to 3 experimental diets. All animals were fed 750 grams per day of poor quality prairie hay. Following an adjustment period, animals were fed 160 gram per day of the supplements shown in Table 2. The supplements were formulated so that dehydrated alfalfa provided 0, 3,

Table 1. Composition of Rations¹ Fed to Sheep in Experiment 1.

	% Composition, air dry basis					
	1	2	3	4	5	6
Ground corn	13.42	11.95	10.69	8.81	7.28	6.57
Soybean meal	2.32	1.84	0.02	2.09	1.62	---
Cottonseed hulls	80.00	80.00	76.95	80.00	80.00	76.22
Dehydrated molasses	---	---	---	5.00	5.00	5.00
Dehydrated alfalfa	---	2.00	8.00	---	2.00	8.00
Biuret ²	2.41	2.41	2.41	2.41	1.41	2.36
Dicalcium phosphate	0.28	0.17	0.08	0.14	0.07	0.05
Monosodium phosphate	0.05	0.14	0.44	0.19	0.29	0.63
Sodium sulfate	0.92	0.89	0.80	0.76	0.74	0.63
Trace mineral salt ³	0.60	0.60	0.60	0.60	0.60	0.60
Vitamins A and D ⁴	+	+	+	+	+	+

¹ Rations were isonitrogenous and isocaloric.

² Biuret was "pure", containing greater than 95 percent of nitrogen as biuret.

³ Trace mineral salt composition in %: NaCl 97.0, Mn 0.24, Fe 0.24, Mg 0.05, Ca 0.032, Co 0.011, I 0.007, Zn 0.005.

⁴ 2.75 g of vitamin A (30,000 I.U./gm) and 1.04 g of vitamin D (12,000 I.U./gm) were added per 100 kg of ration.

Table 2. Composition of Supplements Fed to Sheep in Experiment 2.

Ingredient	Supplement number		
	1	2	3
Dehydrated alfalfa ¹	0.0	16.2	54.0
Biuret ²	9.7	9.7	9.7
Ground corn	70.8	60.5	29.2
Soybean meal	16.8	10.8	4.3
Trace mineral salts ³	2.7	2.7	2.7

¹ Dehydrated alfalfa supplied 3 and 10 percent of the total feed intake of each animal of each diet, respectively.

² Biuret was "pure", containing greater than 95 percent of nitrogen as biuret, and supplied 65% of the digestible protein in each diet.

³ See footnote 3, Table 1.

and 10 percent of the total feed intake in sheep receiving supplements 1, 2 and 3 respectively. Biuret supplied 65 percent of the digestible protein in each diet.

Biureolytic activity in the rumen contents was determined on days 0, 2, 4, 7, 10, 14, 21 and 28 after initiation of the trial.

Results and Discussion

Experiment 1.

The biureolytic activities of rumen contents for those animals in the first trial are shown in Table 3. Lambs used in this experiment showed a remarkable facility to adapt to biuret. Rumen contents from all lambs showed little or no capacity for biuret hydrolysis on collection day 0. Lambs fed rations 2-5 appeared to be completely adapted by collection day 3. Lambs receiving the control diet showed about 50 percent of the activity found in animals receiving diets 2-5 on collection day 3. However, activity was quite variable and the apparent differences were not statistically significant. No significant treatment effects were observed on any of the collection days. It is of interest to note, that on collection day 5 and all subsequent collection days, lambs receiving ration 6 (5 percent molasses and 8 percent alfalfa) showed the lowest level of activity. The reasons for this trend are not readily apparent.

Experiment 2.

Previous experiments conducted at this laboratory have shown that biuret adaptation was generally slower in lambs fed poor quality hay than when fed cottonseed hulls as a roughage source. This experiment was conducted to determine the effect of alfalfa feeding upon the rate of

Table 3. IN VITRO Biuret Disappearance in Rumen Contents from Lambs Fed Various Levels of Alfalfa and Molasses (Experiment 1).

Ration	Days on feed							
	0	3	5	10	14	21	28	35
	Percent loss of initial biuret ^a							
1	-0.2	37.9	49.4	76.5	95.4	63.3	76.0	80.5
2	3.0	84.1	81.0	83.7	78.3	72.8	87.2	79.9
3	-1.6	73.9	78.4	50.6	63.2	74.2	87.3	79.9
4	4.0	71.7	70.5	38.7	65.2	68.2	82.4	92.0
5	0.7	100.0	89.7	65.6	55.3	75.6	89.0	67.9
6	-3.1	68.8	33.0	27.4	39.1	48.9	55.8	59.0
SEM ^b	4.8	10.6	14.8	15.2	16.3	21.6	13.3	6.4

¹ Rations 1, 2 and 3 contained 0, 2 and 8 percent alfalfa, respectively. Rations 4, 5 and 6 contained 5 percent molasses each and 0, 2 and 8 percent alfalfa, respectively.

² Each value represents the average biuret disappearance in 24 hours from *in vitro* flasks prepared from two lambs receiving each treatment.

³ Pooled standard error of the means.

biuret adaptation when poor quality prairie hay served as the roughage source. The biuretolytic activities of rumen contents for sheep in this trial are shown in Table 4.

As in previous experiments, no biuretolytic activity was observed on collection day 0. All animals appeared to be fully adapted by day 4 of the trial. On collection day 2, sheep fed dehydrated alfalfa as 10 percent of their diets showed substantially more biuret hydrolysis (49.4 percent) than sheep receiving 3 percent alfalfa (16.5 percent) or the control ration (28.4 percent), however, variation was high and differences were not statistically significant.

Table 4. In vitro Biuret Disappearance in Rumen Contents from Sheep Fed Prairie Hay, Biuret and Various Levels of Dehydrated Alfalfa (Experiment 2).

Supplement	Days on feed							
	0	2	4	7	10	14	21	28
	Percent loss of initial biuret ^a							
1	-1.8	28.4	79.4	87.0	77.2	73.2 ⁴	77.8	78.2
2	4.0	16.5	82.6	93.0	78.7	84.8 ⁴	88.3	85.8
3	2.9	49.4	84.7	85.1	75.9	83.1 ⁴	86.3	59.3
SEM ^b	5.6	14.6	4.1	7.6	5.8	2.6	4.5	11.5

¹ Supplements 1, 2 and 3 provided dehydrated alfalfa as 0, 3 and 10 percent of the total diet.

² Each value represents the average biuret disappearance in 24 hours from *in vitro* flasks prepared from four lambs receiving each supplement.

³ Pooled standard error of the means.

⁴ Means with unlike superscripts are significantly ($P < .05$) different.

On collection day 14, sheep receiving alfalfa as 3 or 10 percent of their diets displayed significantly higher biureolytic activity than sheep not fed alfalfa. Data from collection days 4, 7, 10, 21 and 28 do not suggest a beneficial response from the feeding of dehydrated alfalfa. Thus, although alfalfa had a significant effect on day 14, this trend was not sufficiently consistent to conclude that dehydrated alfalfa has any substantial effect upon the rate of biuret adaptation.

The Influence of Treatment of Whole Fat Soybeans With Formaldehyde to Protect the Polyunsaturated Fatty Acids From Hydrogenation in the Ruminant.

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Story in Brief

The rumen microorganisms have the ability to biohydrogenate the polyunsaturated fatty acids (PUFA) in the diet. As a result, the highly saturated fatty acids produced are absorbed and deposited in the tissue fat. The results reported here are part of a study to determine if natural protein-fat complexes such as whole soybeans can be treated to protect the PUFA from biohydrogenation.

In trial 1, ground soybeans were treated with either 5.1 or 10.2 ml formaldehyde (HCHO) per 100 gm. of material for 30 min., 2 hr. or 6 hr. Incubation of these products in laboratory rumen fermentations showed that the treatment with HCHO markedly protected the PUFA against biohydrogenation compared to untreated soybeans in which all the PUFA were biohydrogenated.

In trial 2, larger quantities of ground soybeans were treated under the 5.1 ml/30 min. and 10.2 ml/6 hr. conditions. Incubation of these samples in the laboratory showed no protection against biohydrogenation. Growing lambs were fed rations containing: (1) soybean meal, (2)