FACTORS AFFECTING BIURET ADAPTATION BY RUMEN MICROORGANISMS IN SHEEP

By

ROGER DANIEL WYATT

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West Texas State University

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CHAPTER I

INTRODUCTION

Ruminant animals will occupy an even more unique position in terms of their future role as food sources for human consumption. The ruminant has the ability to convert plant material into human food without competing with the human for his plant food supply nor even competing for the land on which he grows his food. In addition to utilizing land which is unsuitable for crop production, the ruminant animal utilizes many by-products of plant food production. For example, field stubble and hulls from growing and processing of many grains for human consumption would have little or no value if they were not used as feed ingredients for ruminant diets.

Two unique qualities of the ruminant which are responsible for its prominent position are: the capacity to utilize cellulose and hemicellulose, and the ability of ruminal microorganisms to synthesize relatively high quality protein from lower quality protein or from non-protein-nitrogen.

In recent years considerable time and effort have been spent investigating the utilization of non-protein-nitrogen as a supplement to ruminant diets. Urea has long been and

will no doubt continue to be one of the most widely used non-protein-nitrogen supplements. The rapid hydrolysis of urea in the rumen and the resulting increase in ammonia concentration may result in a low efficiency of utilization as well as toxicity to the animal. These problems are particularly troublesome when high roughage diets are consumed. The desire to take advantage of non-protein-nitrogen supplementation and avoid the problems of wastage and toxicity from urea feeding has stimulated interest in biuret as a possible nitrogen source.

Biuret is a condensation product of urea. It is non-toxic, slowly hydrolyzed, and is particularly attractive as a supplement to low quality roughage type rations. One of the main obstacles to the wide acceptance of biuret as a nitrogen supplement is the lag period between the initiation of biuret feeding and the time when it is efficiently utilized.

The purpose of this study was to further characterize the effect of varying levels of biuret and the addition of low levels of soluble carbohydrate sources upon the rate and extent of adaptation to biuret in sheep fed high roughage rations.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Because of the economic advantages in substituting simple forms of nitrogen for protein in ruminant ration, a large number of experiments have been conducted in an attempt to more clearly define the extent to which this substitution can be practiced.

Since urea has emerged as the most commonly employed non-protein-nitrogen source, it has become the generally accepted reference source to which other non-protein-nitrogen sources are compared. The following review will include a discussion of nitrogen metabolism in the ruminant along with a comparison of biuret with urea as a non-protein-nitrogen source for ruminants. Particular attention will be directed toward the adaptation period required for efficient biuret utilization, and its importance when considering biuret as a possible protein substitute.

Nitrogen Metabolism in the Ruminant

The Nitrogen Cycle

McDonald (1948) reported that while the general circulatory system contained only traces of ammonia, the blood of the ruminal veins contained significant amounts (the values averaged 1.7 mg. ammonia N/100 ml. blood). Evacuation of rumen contents and replacement with warm water reduced ammonia concentration of blood in the ruminal vein to 0.93 mg./100 ml.; the addition of dilute ammonium acetate solution to the evacuated rumen resulted in a large increase in ruminal vein ammonia concentration. In addition to this evidence for ruminal absorption of ammonia, McDonald noted substantial urea content in the saliva of sheep. With these facts at hand McDonald proposed the nitrogen cycle for the ruminant animal. Since this proposal was made an extensive amount of literature concerning nitrogen metabolism of the ruminant has accumulated.

Nitrogen Transfer Between Blood and Rumen

Houpt (1959) showed that when urea was administered into the jugular vein of sheep, 48-53 percent was utilized when the animals were receiving a carbohydrate supplemented diet. Sheep fed low quality hay alone only utilized 22 percent of the injected urea. When the rumen-reticular cavity was isolated from adjacent digestive tract parts and the contents replaced with a saline (0.9 percent NaCl)

solution, an overall urea transfer rate from blood to the rumen of 5.2 mmole urea-N/hr. was observed in sheep. Since salivary urea secretion rates were only 0.3 mmoles urea-N/hr. it was emphasized that 16 times as much urea passed directly from the blood to rumen as moved by way of saliva. Nolan and Leng (1972) reported data which departed rather drastically from those of Houpt, in which they proposed that the quantity of urea appearing as ruminal ammonia could be completely accounted for by a total salivary flow of 8 l./d. assuming that the concentration of urea in mixed salivary secretions was 60 percent of that in plasma. Using an isotope dilution technique, Cocimano and Leng (1967) have shown that 73-92 percent of the urea entering the body pool is degraded in the alimentary tract, and that about 84 percent of this is converted into compounds more complex than ammonia.

Houpt and Houpt (1968), using surgically constructed rumen pouches have shown that there is a linear relationship between the net transfer of urea across the rumen wall and the concentration difference of urea between the blood and rumen contents. It was hypothesized that urea passes through the rumen wall by a diffusion process which is facilitated by urease penetration of the epithelial layers of rumen wall tissues. Urea is thus hydrolyzed to ammonia and carbon dioxide within the rumen wall which enhances the rate of nitrogen transfer into the rumen. Weston and Hogan (1967) showed that with sheep on a low protein diet a limit

to the net transfer of urea from blood to the rumen occurred at plasma urea concentrations of 16-18 mg. N/100 ml. This suggests a more complex mechanism than simple diffusion is involved. Vercoe (1968) confirmed that a similar situation exists in cattle, and reported that the limit to net transfer occurred at a plasma urea concentration of 12 mg. N/100 ml.

Urinary Excretion of Urea and Biuret

Much of the nitrogen which enters the plasma pool is excreted in the urine and is unavailable for utilization by rumen microorganisms or by body tissues. Energy availability is an important limiting factor involved in nitrogen utilization. Houpt (1959) reported that 78 percent of intravenously injected urea was excreted in sheep receiving diets not supplemented with carbohydrate. When the diet was supplemented with carbohydrate only 48 percent of the injected urea was excreted in the urine. Cocimano and Leng (1967) showed that urea excretion varied with protein intake. Sheep on a low-protein ration excreted 10-25 percent of urea formed in the body. This percentage increased as the protein intake increased. Hatfield et al. (1959) reported that 27 percent of the biuret intake of lambs fed a biuret supplemented ration, appeared as biuret reacting material in the urine. Gray and Clark (1964) showed that 20-30 percent of the biuret dosed via rumen cannulae to lambs was excreted as such in the urine. Hinman (1971) reported 70.4 and 80.3

percent recovery of intravenously-infused biuret in the urine of sheep within 48 hours when fed cottonseed meal and biuret plus cottonseed meal rations respectively. Farlin et al. (1968) demonstrated urinary recovery of 94 percent of the ^{14}C intravenously-injected as biuret-^{14}C within 48 hours post-injection.

Non-Protein Nitrogen Utilization

Hydrolysis of Urea and Biuret

Pearson and Smith (1943) found that ruminal urease activity is consistently high and the enzyme is not secreted by the ruminal mucosa. They reported that 100 g. of rumen contents could convert 100 mg. of urea to ammonia in one hour. Similar results obtained by Bloomfield et al. (1960) placed urea hydrolyzed at 80 mg. urea-N per hour per 100 ml. of rumen fluid. Hungate (1966) states that high concentrations of urease are not necessary, since this enzyme is extremely active. Bauriedel (1971) reported that urease activity was not localized in any fraction of the rumen contents, while biuretase activity was greater in the plant debris fraction of the rumen contents.

Biuretolytic activity in the ruminant when biuret is not a component of the diet is negligible (Gilchrist et al., 1968; Oltjen et al., 1968; Schroder and Gilchrist, 1969; Bauriedel, 1971; Johnson and Clemens, 1973), however, biuretolytic activity is developed subsequent to its incorporation into the diet. Using ^{15}N-biuret in an

in vitro fermentation system, Bauriedel (1971) presented evidence that the ^{15}N of biuret appears primarily as $^{15}NH_3$, but if nutrients are added to rumen fluid medium to promote microbial growth, incorporation of $^{15}NH_3$ into microbial protein is simultaneously promoted. Waite and Wilson (1968) reported that the replacement of oilcake nitrogen with biuret nitrogen resulted in increased non-protein-nitrogen concentrations in the rumen fluid and decreased ruminal ammonia concentrations. If the oilcake was replaced with urea non-protein-nitrogen concentrations were intermediate between those produced by oilcake and those produced by biuret, however, ruminal ammonia levels were the highest produced by any of the three diets. Bauriedel (1971) isolated cell-free extracts from rumen microorganisms that were shown to possess biuretase activity. The enzyme was characterized as distinct from urease, but urease may participate in the complete hydrolysis of biuret to CO_2 and NH_3.

Toxicity of Urea and Biuret

Ruminal hydrolysis of urea usually proceeds at a faster rate than ammonia assimilation. This results in a decreased efficiency of utilization and produces toxic effects in the animal if blood ammonia nitrogen levels become sufficiently high. Repp *et al.* (1955) reported that jugular blood ammonia nitrogen levels exceeding 1.158 mg./100 ml. resulted in acute toxicity which leads to death of the lamb.

Clinical symptoms of ammonia toxicity were observed when blood ammonia nitrogen levels exceeded 1.0 mg./100 ml. Administration of 40 g. of urea to lambs resulted in toxicity and death. Twenty-five g. of urea administered to lambs as a drench (Meiske et al., 1955) resulted in slow feed consumption and labored breathing. Drenching with 28.5 g. resulted in acute toxicity and death. Word et al. (1969) showed that drenching of pregnant cows with urea at 0.44 g./kg. body weight resulted in toxicity and death. Severe toxicity occurred in cows (Webb et al., 1972) when blood ammonia nitrogen concentrations rose above 0.7 to 0.8 mg./100 ml.

Berry et al. (1955) demonstrated that biuret fed to lambs at levels up to 68 g./day had no toxic effect. Steers fed biuret at 1.75 percent of their total diet showed no signs of toxic effects. The feeding of 375 g. of biuret to a 146 pound ewe (Hatfield et al., 1959) did not produce any visible toxicity symptoms or indications of unusual after effects. In another test, no toxic symptoms were exhibited by ewes receiving 30, 60 or 90 g. of biuret by drench. In a third test 275 g. of biuret administered as a drench caused some signs of distress within the first 36 hours after drenching and a large amount of biuret crystallized out of the urine. Clark et al. (1963) showed that biuret was non-toxic in sheep that were adapted to biuret.

Adaptation

It has often been observed that there is a lag phase involved in which there is little or no beneficial response to biuret feeding. McLaren *et al.* (1959) found that retention of absorbed nitrogen by lambs fed biuret, increased as a function of the length of the preliminary period. Maximal nitrogen retention was obtained after 30-40 days of receiving the diet. An increase in nitrogen balance when lambs started on a biuret containing diet were inoculated with rumen fluid from lambs previously adapted to biuret was demonstrated by Ewan *et al.* (1958). This indicated that the feeding of biuret enhanced biuretolytic activity and that the factors responsible for the increased activity could be transferred with ruminal ingesta from one animal to another. Mackenzie and Altona (1964) showed that addition of biuret to a basal ration of low quality veld hay reduced the weights lost by yearling ewes, but the live-weight responses were delayed by four weeks. The maximal response was not obtained until the eighth week of supplementation. Using an *in vitro* biuret disappearance technique, Gilchrist *et al.* (1968) reported that no biuretolytic activity was present in lambs that were not fed biuret. After biuret feeding began, measurable activity was observed within one to two weeks. Maximal activity was not reached until the third to fifth week of biuret feeding. Many workers, including Gilchrist *et al.* (1968); Schroder and Gilchrist (1969) and Clemens and Johnson (1973), have shown that addition of soluble

carbohydrate to the diet enhances the rate of adaptation to biuret by varying degrees. The amount of preformed protein present in the diet also plays a role in biuret adaptation. Schroder and Gilchrist (1969) encountered a decreased adaptation rate when lambs on a high protein basal diet were dosed with 15 g. of biuret as compared to lambs receiving lower levels of protein in their basal diet. Lambs receiving basal diets containing 10.3 percent crude protein reached peak activity after 13 days of biuret dosage. Clemens and Johnson (1973) reported that when biuret supplied less than 15 percent of the digestible nitrogen requirement little or no biuretolytic activity was observed.

When biuret is removed from the ration there is a rapid loss of biuretolytic activity. Schroder and Gilchrist (1969) described de-adaptation as a rapid process, occurring within 1-5 days after removal of biuret from the ration. Clemens and Johnson (1973) observed that biuretolytic activity was greatly reduced by day 2 and completely lost by day 4 after biuret removal from the ration. After de-adaptation, the re-establishment of biuretolytic activity is a progressive process similar to that for initial adaptation (Schroder and Gilchrist, 1969).

In view of the rather prolonged biuret adaptation period and its rapid de-adaptation response, a problem is posed regarding the most effective manner of administration of the supplement. General feeding practices under range conditions vary from daily feeding of supplements to once or

twice weekly feeding. Clemens and Johnson (1973) reported that lambs receiving biuret containing supplement at intervals of one or two days were able to develop and maintain a substantial amount of biuretolytic activity. Animals supplemented at four day intervals were unable to develop or maintain a substantial amount of biuretolytic activity. In another experiment they demonstrated that steers which had been previously adapted to biuret could maintain biuretolytic activity when supplemented at one and two day intervals, but not when supplemented at four day intervals.

Another possible method of supplementation is ad libitum feeding. Mackenzie and Altona (1964) found that voluntary intake of a lick containing three parts crude biuret, two parts bonemeal and one part salt was sufficient to meet the nitrogen requirements for maintenance of body weight of sheep and cattle on poor quality roughage.

When lambs on the range had access to a biuret containing lick three times weekly (Torrell et al., 1972), no beneficial response was observed. No improvement in lambing performance was observed when biuret and salt were fed in comparison with salt alone. Two possibilities exist here, first, the lambs may not have voluntarily consumed adequate biuret to maintain biuretolytic activity, and second, the lambs may not have been fed at close enough intervals to maintain activity.

Biuret in Ruminant Rations

Urea has generally performed well when used to supplement high energy or fattening type rations. Performance of urea, however, has been disappointing and hazardous in low energy, high roughage feeding programs for cattle and sheep. Biuret has created considerable interest as a potential nitrogen supplement under the latter conditions. Its properties of being non-toxic and palatable when fed with low quality roughage make biuret an attractive possibility for this type of feeding program.

Belasco (1954) found that *in vitro* cellulose digestion when biuret was added as a nitrogen source was only seven percent of that occurring when urea was added as a nitrogen source. He was unaware, however, of the adaptation requirement for biuretolytic activity. The rumen ingesta used for inoculum were from unadapted animals, thus his lack of response is not surprising.

The fact that biuret was non-toxic (Repp *et al.*, 1955) still held the interest of several investigators. Meiske *et al.* (1955) found that addition of biuret or crude biuret to a 7.13 percent crude protein diet, significantly increased average daily gain and feed efficiency of lambs. These increases were equal to those produced by the addition of urea, or replacing part of the corn with soybean oil meal.

Many workers (Meiske *et al.*, 1955; Anderson *et al.*, 1959; Clark *et al.*, 1963; Mackenzie and Altona, 1964;

Oltjen et al., 1969) have demonstrated that biuret is effective in maintaining positive nitrogen balance and growth under a variety of feeding regimes. Anderson et al. (1959) reported the digestibility of organic matter, protein, crude fiber and nitrogen free extract were not significantly changed when crude biuret supplied 50 percent of the supplemental nitrogen. However, when 100 percent of the supplemental nitrogen was supplied by crude biuret, the apparent digestibility of protein in the diet decreased. When pure biuret supplied 100 percent of the supplemental nitrogen instead of urea, nutrient digestibility and nitrogen utilization decreased. Clark et al., (1963) observed that no differences in nitrogen balance or apparent nitrogen absorption occurred between biuret and urea supplementation. Hay consumption was increased by both urea and biuret but the response due to biuret was more delayed. Mackenzie and Altona (1964) showed that biuret was a suitable substitute for urea in steers and lambs fed low quality (4.12 percent crude protein) veld hay. Oltjen et al. (1969) reported that biuret fed to steers was retained as efficiently as urea after adaptation had occurred. In a growth study where twice daily feeding was practiced biuret-fed steers consumed 15 percent more feed than did the urea-fed animals (non-protein-nitrogen sources comprised 50 percent of the total dietary nitrogen). Ammerman et al. (1972) presented evidence showing that biuret plus an energy source increased hay intake and

improved apparent digestibility of nitrogen in lambs. Biuret gave a response similar to that obtained with either soybean meal or cottonseed meal.

Several researchers (Raleigh and Turner, 1969; Mackenzie and Altona, 1964; Van Horn et al., 1969) have shown that biuret can be utilized as a nitrogen supplement to low quality roughage diets under range conditions. Raleigh and Turner (1969) reported that heifers on a low quality roughage diet supplemented with biuret gained significantly more (0.11 kg./hd./day) than groups receiving either urea or cottonseed meal as nitrogen supplements. Using a lick composed of crude biuret, bonemeal and salt, Mackenzie and Altona (1964) demonstrated that sheep allowed to consume the lick gained weight, while those not supplemented suffered rapid weight loss. Liveweight response was immediate (first week) contrary to expected performance of unadapted animals. In a second experiment with cattle, the liveweight response was slower, and only became evident four weeks after supplementation began. Van Horn et al. (1969) reported that biuret was as efficient as urea in maintaining weight gain, fleece weight, percent lambs weaned and pounds of lamb weaned per ewe, when fed as nitrogen supplement for range ewes. Gray et al. (1972) reported that biuret was as effective as natural protein for maintaining growth rate in steers fed finishing rations.

Some workers (Gilchrist et al., 1968; Schroder and Gilchrist, 1969) have shown an increased response to biuret

when the addition of a readily fermentable carbohydrate source is made. Gilchrist et al. (1968) reported that addition of maize meal to the diet resulted in a three fold increase in biuretolytic activity. Schroder and Gilchrist (1969) demonstrated that addition of maize meal resulted in the maintenance of higher levels of biuretolytic activity. However, Clemens and Johnson (1973) when investigating the rate of adaptation to biuret, reported that high levels of concentrate (40 and 60 percent) were favored through day 4. By day seven and for sampling days subsequent to day seven, the rate of adaptation and extent of biuret hydrolysis progressively favored the 20 percent corn starch diet. Mackenzie and Altona (1964) showed that a biuret lick was effectively utilized without the addition of readily fermentable carbohydrate.

CHAPTER III

MATERIALS AND METHODS

Introduction

Two experiments were conducted to study the effect of level of biuret supplementation and low level addition of soluble carbohydrate upon the rate of adaptation to biuret as a nitrogen source for ruminants. Wether lambs fitted with permanent rumen cannulae were used in each experiment.

Rumen contents were removed at intervals throughout the trial for use in the in vitro biuret disappearance studies. After a 35 day adaptation period, rumen contents from the experimental animals were used to study in vitro cellulose digestion when urea, biuret or no nitrogen source was added.

Experimental Animals, Equipment and Diets

Experiment 1

This experiment was designed to measure the effect of three levels of biuret supplementation upon the rate of adaptation to biuret. Nine wether lambs equipped with permanent rumen cannulae were alloted to three treatments of three lambs each and placed in metabolism stalls. Each animal was individually fed approximately 1,000 g./day of a

high roughage ration during the experiment. Water was available at all times. All rations contained about 80 percent cottonseed hulls and biuret provided 20, 50 and 80 percent of the total nitrogen in rations 1, 2 and 3 respectively as shown in Table I. The rations were iso-nitrogenous and each contained about the same concentrate level. Sodium sulfate was added to give a nitrogen:sulfur ratio of about 12:1. The lambs had not received biuret prior to the beginning of this trial and were without biuretolytic activity at the start of the experiment.

Experiment 2

This experiment was designed to measure the effect upon biuret adaptation, of low level additions of two soluble carbohydrate sources. Either dehydrated cane molasses, dehydrated alfalfa or a combination of the two were utilized as the soluble carbohydrate sources. Twelve wether lambs fitted with permanent rumen cannulae were alloted to six treatments (two lambs per treatment) in a 2 x 3 factorial arrangement. The treatments employed are shown in Table II. Treatment 1 was a control diet which contained no molasses or alfalfa. Treatments 2 and 3 contained no molasses and alfalfa was added as 2 and 8 percent of the rations, respectively. Treatments 4, 5 and 6 contained 5 percent molasses and 0, 2 and 8 percent alfalfa, respectively. The lambs were kept in metal pens on concrete slatted floors. They were individually fed approximately 1,000 g./day of the

TABLE I

COMPOSITION OF RATIONS [1,2] FED TO SHEEP IN EXPERIMENT 1

	% composition, air dry basis		
	1	2	3
Ground corn	6.66	11.09	13.06
Soybean meal	10.14	4.84	--
Cottonseed hulls	81.63	81.30	83.00
Biuret[3]	0.54	1.38	2.14
$Na_2 SO_4$	0.19	0.49	0.77
Dical. phos.	0.11	0.22	0.39
$Ca CO_3$	0.12	0.10	0.02
Trace mineral salt	0.61	0.61	0.61

[1]In addition 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.

[2]All rations were isonitrogenous.

[3]Biuret was "pure," containing greater than 95 percent of nitrogen as biuret.

TABLE II

TREATMENTS EMPLOYED IN EXPERIMENT 2

Treatment[1]	% of ration, air dry basis	
	Dehydrated Molasses	Dehydrated Alfalfa
1	0	0
2	0	2
3	0	8
4	5	0
5	5	2
6	5	8

[1]Rations fed to animals in experiment 2 are shown in Table III (two animals/treatment).

rations shown in Table III. All rations contained approximately 80 percent cottonseed hulls and were calculated to be isonitrogenous and isocaloric. Biuret supplied 50 percent of the nitrogen present in each ration. As in experiment 1, water was available at all times and sodium sulfate was added to give a nitrogen:sulfur ratio of about 12:1. Lambs employed in this experiment had not previously received biuret and were without biuretolytic activity at the beginning of the trial.

Rumen Sampling and *In Vitro* Procedures

In Vitro Biuret Disappearance

Biuretolytic activity of the rumen contents was determined on selected days within each experiment. Day zero samples were taken in the morning, prior to feeding on the first day of biuret supplementation. During experiment 1, subsequent sampling of rumen contents was performed on days 3, 5, 7, 10, 14, 21, 28, and 35 after initiation of biuret feeding. Rumen contents were collected using a rubber hose inserted through the cannula and attached to a 500 ml. collecting flask. All collections were performed prior to feeding on the day specified. Immediately after sampling, 375 ml. of whole rumen contents were mixed thoroughly with 125 ml. of 1 percent biuret solution in a 500 ml. wide mouth flask. A 100 ml. zero time sample was removed and the flasks were placed in a water bath and incubated at 39°C. with a constant bubbling of carbon dioxide through the

TABLE III

COMPOSITION OF RATIONS [1,2] FED TO SHEEP IN EXPERIMENT 2

	% 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[3]	2.41	2.41	2.41	2.41	2.41	2.36
Dical. phos.	0.28	0.17	0.08	0.14	0.07	0.05
Monosodium phos.	0.05	0.14	0.44	0.19	0.29	0.57
Sodium sulfate	0.92	0.89	0.80	0.76	0.74	0.63
Trace mineral salt	0.60	0.60	0.60	0.60	0.60	0.60

[1]In addition 2.75 g. of Vitamin A (30,000 I.U./gm.) and 1.05 g. of Vitamin D (12,000 I.U./gm.) were added per 100 kg. of ration.

[2]Rations were isonitrogenous and isocaloric.

[3]Biuret was "pure," containing greater than 95 percent of nitrogen as biuret.

contents to maintain anaerobic conditions. Additional 100 ml. samples were taken from each flask after 8 and 24 hours of incubation. The samples were mixed with 2 ml. of 10 percent sulfuric acid, strained through two layers of cheesecloth and stored until analysis was performed. Samples collected during experiment 1 were frozen at -20°C. until analysis. Samples collected during experiment 2 were not frozen, but were refrigerated until the biuret analyses were performed. Prior to analysis the samples were centrifuged at 14,000 x g. for 10 minutes. Biuret was determined by a modification of the Gilchrist et al. (1968) technique, as described by Johnson and Clemens (1973). The biuret determination procedure is described in detail in the Appendix. Biuret present in the sample was expressed as mg. of biuret/100 ml. of rumen contents. Calculation of the biuret content in samples was made using the following formula:

$$\text{mg. Biuret/100 ml.} = \frac{\text{optical density unknown}}{\text{optical density standard}} \times \text{standard concentration} \times \text{dilution factor} \times 10$$

In Vitro Cellulose Digestion

After the lambs in each experiment had received the biuret supplementation for 35 days, an in vitro cellulose digestion trial was performed. Two replicates were conducted for each experiment. In vitro cellulose digestibility was determined by methods described by Johnson et al. (1969)

using purified wood cellulose[1] as a substrate. Tubes containing 0.5 g. cellulose plus a mineral and buffer media (Table IV) and either no nitrogen source biuret or urea were inoculated with 10 ml. of strained rumen fluid. In nitrogen supplemented tubes, biuret or urea was added so that 12 mg. of nitrogen was supplied by each of the respective sources. Rumen liquor was collected in the morning prior to feeding in the same manner described for measurement of biuretolytic activity. The tubes were incubated at 39°C. with continuous carbon dioxide gassing for 30 hours. The pH was adjusted to 6.9 at the beginning of each trial and again at the 8 and 24 hour points during incubation. At the termination time, fermentation in all tubes was stopped with mercuric chloride (0.5 ml.) and the samples were refrigerated until analysis was conducted. Cellulose was determined on the tube contents by the Crampton and Maynard (1938) method.

Statistical Analysis

Analyses of variance were conducted according to the methods outlined by Snedecor and Cochran (1967). In experiment 1, levels of significance were determined for differences between level of biuret feeding and sampling days. In experiment 2, levels of significance were determined for differences between carbohydrate sources and sampling days. For cellulose digestion data in both

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[1]Solka Floc, Brown Paper Co., Berlin, N. H.

TABLE IV

MINERAL AND BUFFER MEDIA FOR CELLULOSE DIGESTION

Reagent		Amount per tube
Na_2CO_3 , 200 g./l.		0.25 ml.
Biotin , 10 mg./ml.		0.25 ml.
Valeric Acid , 5 mg./ml.		1.50 ml.
$FeCl_3 \cdot 6 H_2O$, 4.4 mg./ml.		0.25 ml.
$CaCl_2 \cdot 2 H_2O$, 5.29 mg./ml.		0.25 ml.
Mineral Mix		5.00 ml.
Na_2HPO_4	56.5 g./10 l.	
NaH_2PO_4	54.5 g./10 l.	
KCL	21.5 g./10 l.	
NaCl	21.5 g./10 l.	
$MgSO_4 \cdot 7 H_2O$	5.82 g./10 l.	
$NaSO_4$	7.5 g./10 l.	
	Total	7.50 ml.

experiments, levels of significance were determined for differences between nitrogen sources. In both experiments, the location of significant treatment differences was determined using Duncan's New Multiple-Range Test (Duncan, 1955).

CHAPTER IV

RESULTS AND DISCUSSION

Experiment 1

In Vitro Biuretolytic Activity

The biuret concentrations of *in vitro* media prepared from lambs in these experiments were quite variable due to the variation in the liquid composition of rumen liquor. This inherent variation makes biuret concentration data somewhat confusing. In the interest of acquainting the reader with biuret levels that were generally encountered, average biuret concentrations of *in vitro* media are shown in Table V. Biuret concentrations are expressed as the mg. of biuret present per 100 ml. of *in vitro* media. Initial biuret concentrations ranged from 217-345 mg. per 100 ml.

The lowest concentrations were observed after incubation of media prepared from adapted animals (collection days 28 and 35) and were in the range of 40-50 mg. per 100 ml. of *in vitro* media. Adapted animals receiving the higher levels of biuret supplementation (50 and 80 percent of total dietary nitrogen) were able to degrade about 183 mg. of biuret per 100 ml. of *in vitro* media in a 24 hour incubation period on collection day 35, while animals receiving the low level of

TABLE V

AVERAGE IN VITRO BIURET CONCENTRATION OF FLASKS PREPARED FROM ANIMALS IN EXPERIMENT 2 AFTER VARIOUS PERIODS OF FEEDING THREE LEVELS OF BIURET[1]

Collection day	Level of biuret[2] and hours of incubation					
	20		50		80	
	0	24	0	24	0	24
0	345	340	284	324	244	340
3	247	205	267	205	305	281
5	346	242	309	189	303	227
7	246	192	237	160	266	156
10	251	170	217	67	233	127
14	233	167	264	108	262	122
21	245	161	265	92	241	104
28	250	169	232	84	233	51
35	241	146	242	59	225	42

[1]Biuret concentrations expressed as mg. biuret/100 ml. of in vitro media.

[2]Biuret levels are expressed as the percent of dietary nitrogen furnished by biuret.

biuret (20 percent of total dietary nitrogen) only degraded about 95 mg. of biuret per 100 ml. of *in vitro* media.

To facilitate the comparison of biuretolytic activities on different treatment-time combinations, the percent disappearance of the original biuret concentration (zero time) during the incubation period was calculated and is reported in Table VI and Figures 1, 2 and 3. On collection day 0, an apparent net increase in biuret present in solution was observed with incubation of *in vitro* media prepared from animals receiving 50 and 80 percent of their dietary nitrogen as biuret (Table VI). *In vitro* media prepared from lambs receiving 20 percent of their dietary nitrogen as biuret did not show a similar increase on day 0. Four possible explanations for the increased biuret concentrations observed in media prepared from lambs on ration treatments 2 and 3 will be offered. However, none of these possibilities was tested by this author. First, biuret could be synthesized by the microbial population during incubation. This appears extremely unlikely however, in view of the fact that biuret is not a common constituent of rumen liquor in animals not fed biuret. If indeed, the rumen microorganisms did synthesize biuret, it is doubtful that a marked adaptation response would be observed since the animal would be inherently pre-conditioned to biuret. A second possibility which exists is a determination error, either caused by an analytical error or the presence of an interfering substance. This possibility seems remote, since duplicate determinations

TABLE VI

PERCENT OF INITIAL BIURET REMAINING IN THE *IN VITRO* MEDIA AFTER INCUBATION[1]

Collection day	Level of biuret[2] and hours of incubation					
	20		50		80	
	8	24	8	24	8	24
0	96.2	98.7	104.4	114.1	128.3	144.4
3	92.5	83.7	90.4	76.3	96.9	92.3
5	75.6	71.1	80.9	61.8	91.1	74.9
7	82.0	79.2	91.2	69.4	81.7	59.1
10	82.1	67.9	83.9	29.6	87.6	53.9
14	79.1	71.9	74.4	40.3	79.2	47.1
21	85.6	66.1	76.4	34.6	82.7	42.7
28	86.7	67.9	78.5	33.9	69.7	23.2
35	85.2	60.3	75.0	24.0	73.9	18.5

[1]Biuret expressed as the percent of initial biuret still present after 8 and 24 hours of incubation.

[2]Biuret levels are expressed as the percent of dietary nitrogen furnished by biuret.

were made on two separate days with similar results, and this problem was not encountered with any other samples collected during the two experiments. A third possibility is that of formation of a complex at the time of biuret addition to the media which is subsequently dissociated resulting in the release of biuret. Tiwari et al. (1972) reported the disappearance of 20 percent of the biuret added to rumen fluid within 15 minutes. They proposed the formation of a relatively stable protein-biuret complex when biuret was first mixed with fresh rumen fluid. Should this occur, this complex would be removed from the fluid in the protein precipitation step in the biuret analysis, yielding falsely low values for initial biuret. If this biuret was subsequently released after 8 or 24 hours of incubation, then there would be an apparent increase in biuret content at those times. The low zero time biuret levels for the 50 and 80 percent groups in Table VI support this theory. In vitro media prepared from lambs on ration 3 showed a 44 percent increase in biuret content after 24 hours incubation which is over twice that reported by Tiwari et al. (1972). A fourth feasible explanation would be the solubilization of insoluble biuret inadvertently added to the in vitro system during sample preparation. The one percent biuret solution used in preparing the in vitro flasks had been prepared during a previous trial and had been standing for about 3 weeks prior to the initiation of this trial. A small amount of biuret had precipitated out of solution and was present

as sediment in the bottom of the bottle. It appears possible that some of this biuret was added to the flask but was insoluble and removed during preparation for analysis of zero hour samples. Placing the *in vitro* flasks in a warm water bath (39°C.) under presumably, slightly acid conditions and a bubbling stream of carbon dioxide may have resulted in solubilization of the biuret and measurement in samples removed subsequent to the zero hour sample. On the remaining sampling days, biuret was prepared fresh, filtered and no increases in biuret concentration during incubation were observed. It is of further interest to note that the flasks in question were among the last prepared and some of the sedimented material had no doubt been mixed during removal of the bottle contents. For unexplained reasons, the phenomena did not occur in the lambs fed rations containing 20 percent of their dietary nitrogen as biuret nor did it appear to be a significant problem on subsequent test days.

The biuret adaptation response was evident in this experiment as indicated by the highly significant ($P<.01$) period effect. Table VII and Figures 1, 2 and 3 illustrate the rate of biuret adaptation of rumen contents of lambs in experiment 1 as measured *in vitro*. No biuretolytic activity was evident on day zero. This agrees with work reported previously by Schroder and Gilchrist (1969), Johnson and Clemens (1973) and Clemens and Johnson (1973). Lambs receiving ration 1 (20 percent total nitrogen as biuret) showed a significant ($P<.05$) increase in biuretolytic

TABLE VII

IN VITRO BIURET DISAPPEARANCE FROM ANIMALS IN EXPERIMENT 1 AFTER VARIOUS PERIODS OF BIURET SUPPLEMENTATION

Ration[1]	Period[2]								
	0	3	5	7	10	14	21	28	35
	Percent loss of initial biuret[3]								
1	1.3^{a}	16.3^{ab}	28.9^{bc}	20.8^{bc}	32.1^{bc}	28.1^{bc}	33.9^{bc}	32.1^{bc}	39.6^{c}
2	-14.1^{a}	23.7^{b}	38.2^{bc}	30.6^{bc}	70.4^{cd}	59.7^{cd}	65.4^{cd}	66.1^{cd}	76.0^{d}
3	-44.4^{a}	7.7^{b}	25.1^{bc}	40.9^{bc}	46.1^{cd}	52.9^{cd}	57.3^{cd}	76.8^{d}	81.5^{d}

[1]Rations 1, 2 and 3 contained 20, 50 and 80 percent of total nitrogen as biuret, respectively.

[2]When reading across the periods within a given ration, numbers without a common superscript are significantly ($P < .05$) different.

[3]Biuret expressed as the percent of initial biuret disappearing after 24 hours of incubation.

activity by day 5. At this time they were showing disappearance of about 29 percent of the initial biuret in 24 hours of incubation. Although the increase in biuret adaptation between days 5 and 35 was non-significant ($P>.05$), lambs fed ration 1 displayed maximum biuretolytic activity on day 35 with about 40 percent disappearance. The slow rate of adaptation and low level of biuret utilization observed here support speculation by Clemens and Johnson (1973) that low biuret utilization observed when lambs received an 80 percent ground corn diet was the result of insufficient biuret to maintain biuretolytic activity (their ration contained 0.40 percent biuret). Their observation was confounded by the fact that high concentrate levels also decrease biuret utilization. These data also agree with the findings of Schroder and Gilchrist (1968) showing decreased adaptation rates when crude protein content of the diet was increased while holding the biuret level in the diet at 15 g. per day. That level of biuret intake was considerably higher than employed in ration 1 of this experiment (5.4 g./day). Schroder and Gilchrist reported biuret utilization of about 150 mg. %/24 hours in lambs receiving good quality (10.3% crude protein) hay and 15 g. of biuret per day, when activity was measured on day 35 of supplementation. In this experiment, lambs fed ration 1 utilized only about 95 mg. %/24 hours (Table V; 241 - 146 = 95 mg./100 ml./24 hours) on collection day 35. This apparent difference in activity may be due to the different biuret levels fed in the two experiments.

Increased rates of biuret adaptation were observed when the level of biuret in the diet was increased. When biuret supplied 50 and 80 percent of the total dietary nitrogen (rations two and three respectively), significant ($P<.05$) increases in biuretolytic activity were registered by day three of the trial. In order to minimize the problems associated with interpretation of changes in biuretolytic activity associated with collection day 0, day 3 of the experiment will be used as a reference point. Lambs fed ration two showed significant ($P<.05$) increases in biuretolytic activity between days 3 and 10 and between days 7 and 35. Lambs fed ration 2 showed maximum biuretolytic activity on collection day 35 when about 76 percent of the initial biuret was hydrolyzed during the 24 hour incubation period. Lambs fed ration 3 showed significant ($P<.05$) increases in biuretolytic activity between days 3 and 10 and between days 7 and 28. Maximum biuretolytic activity was recorded on day 35 when about 82 percent of the initial biuret was hydrolyzed in the 24 hour incubation period.

Generally speaking, the adaptation response evident in this experiment can be described as a two phased process. An early phase was evident during the first five to ten days of biuret supplementation. This phase was characterized by rapidly increasing biuretolytic activity. This early phase was followed by a second period in which activity increased at a somewhat slower rate.

Figures 1, 2 and 3 illustrate the biuretolytic activity of the rumen contents from lambs receiving the three biuret levels, as measured on the various collection days during experiment 1. No biuretolytic activity was evident on day 0 (Figure 1). Biuretolytic activity was evident on days three, five (Figure 1) and seven (Figure 2); however, treatment differences were non-significant ($P>.05$) on these days. By day ten, substantial biuretolytic activity was observed in lambs receiving ration 2. Lambs receiving ration 3 showed intermediate activity, and those fed ration 1 registered the least amount of activity. On day 14, lambs receiving rations 2 and 3 showed significantly ($P<.05$) greater biuretolytic activity than lambs receiving ration 1. The trend favoring greater biuret adaptation in animals fed the higher levels of biuret was evident throughout the remainder of the experiment. Variability in biuretolytic activity showed a marked increase on day 21 (Figure 3) and apparent differences in activity were non-significant ($P>.05$). Biuretolytic activity was significantly greater in animals fed ration 3 as compared to ration 1 on day 28. Activity in lambs fed ration 2 was not significantly ($P>.05$) different from either of the other treatments on day 28; however, activity in these lambs was substantial and indicative of a high degree of biuret utilization. On day 35, lambs fed 50 and 80 percent of their total dietary nitrogen as biuret again registered significantly ($P<.05$) greater biuretolytic activity than lambs fed biuret as 20 percent of their total nitrogen intake.

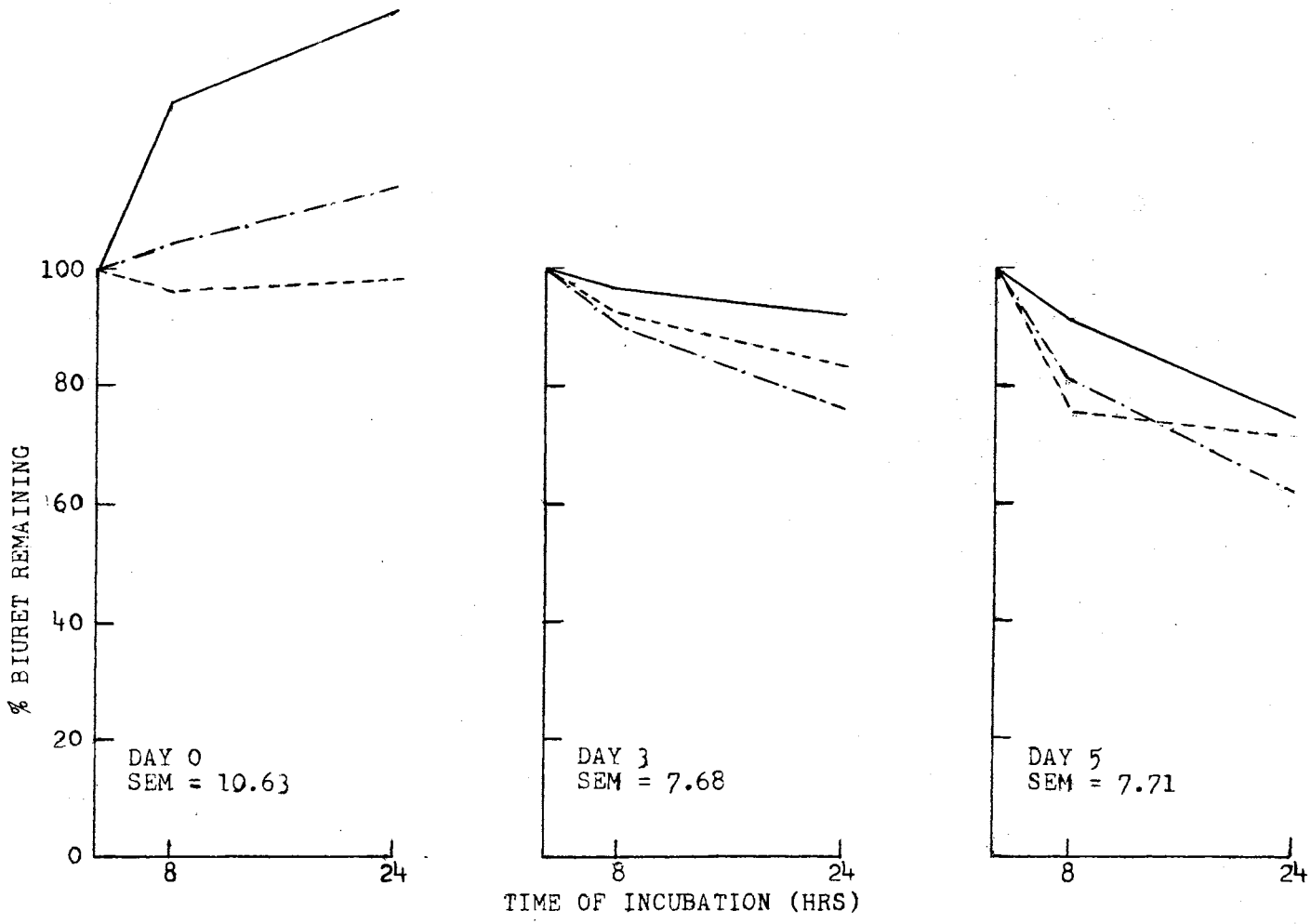

Figure 1. In Vitro Biuret Disappearance for Sheep Fed Three Levels of Biuret (Experiment 1). The designation of lines refers to the diets fed to source animals: 20 (----), 50 (-·-·), and 80 (——) precent of the dietary nitrogen as biuret. Each line represents the average value of 3 sheep.

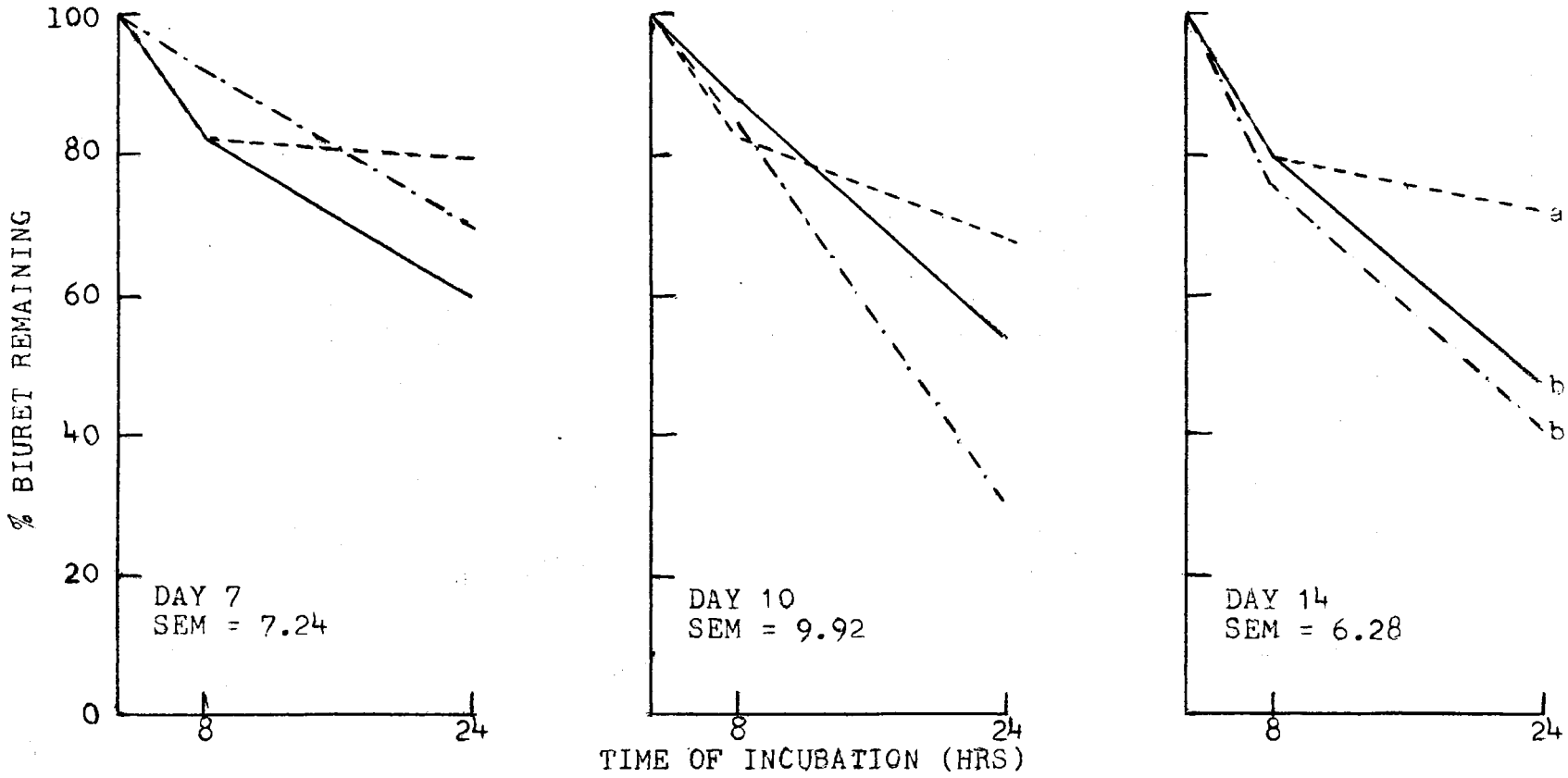

Figure 2. In vitro biuret disappearance for sheep fed three levels of biuret (Experiment 1). The designation of lines refers to the diets fed to source animals: 20 (----), 50 (-·-·), and 80 (——) percent of the dietary nitrogen as biuret. Each line represents the average value of three sheep. Values (24 hour) with unlike superscripts (a, b) are significantly ($P < .05$) different.

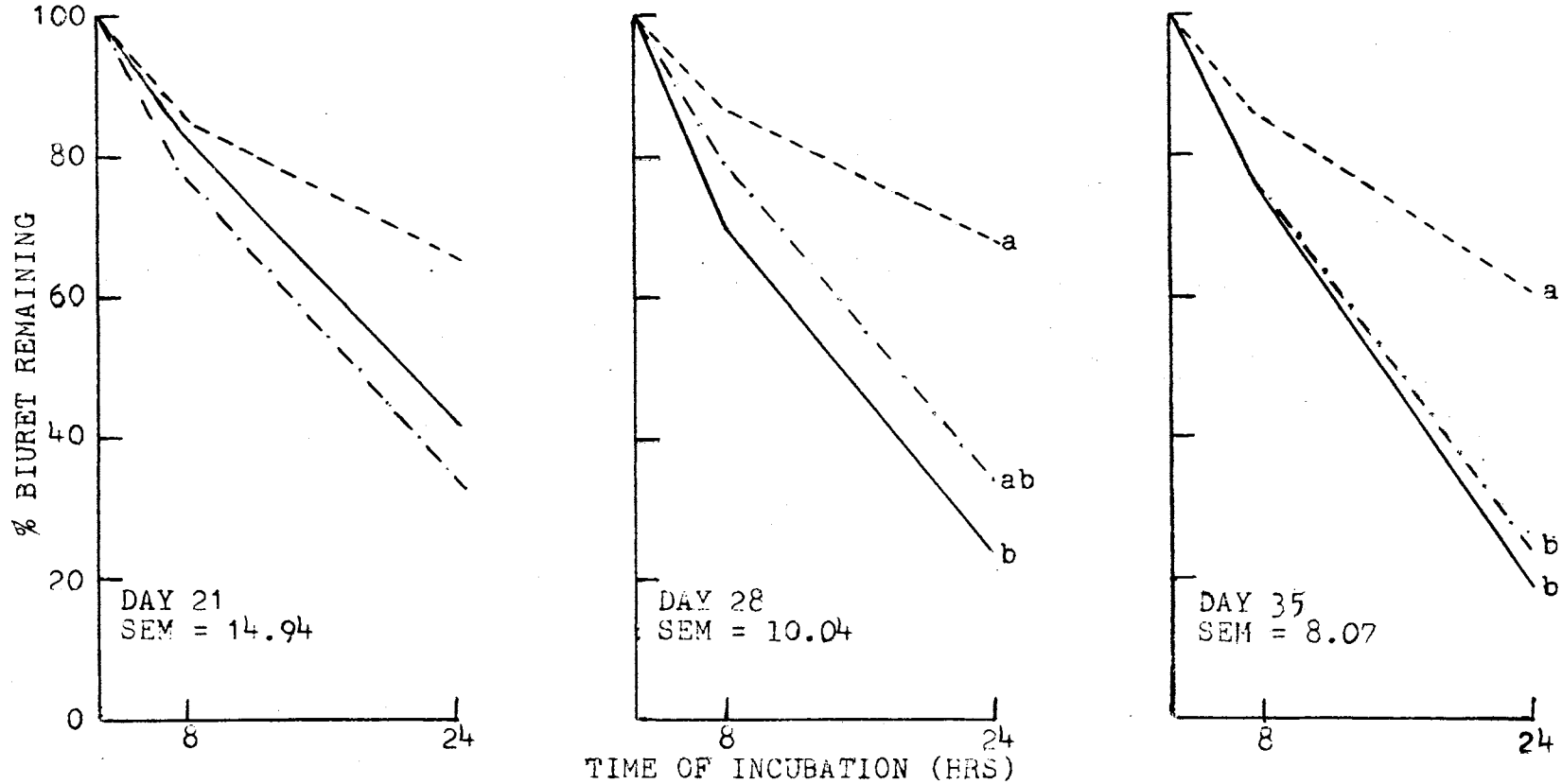

Figure 3. In vitro biuret disappearance for sheep fed three levels of biuret (Experiment 1). The designation of lines refers to the diets fed to source animals: 20 (----), 50 (-.-.), and 80 (——) percent of the dietary nitrogen as biuret. Each line represents the average value of three sheep. Values (24 hour) with unlike superscripts (a, b) are significantly ($P \leq .05$) different.

Both the rate of development of biuretolytic activity and the extent of this activity were favored by the higher levels of biuret supplementation. These data support the findings of Schroder and Gilchrist (1969), who observed that the adaptation rate of rumen microflora to biuret was inversely proportional to the digestible protein level in the basal diet. These results also provide additional support for the proposal by Clemens and Johnson (1973) that for efficient biuret utilization, biuret must supply more than 15 percent of the dietary nitrogen. This value may now be extended since from the data presented here, it would appear that the minimum amount of dietary nitrogen replacement required to stimulate enough biuretolytic activity to justify the use of biuret as a nitrogen source lies between 20 and 50 percent of the dietary nitrogen. These data indicate that biuret may be used to replace up to 80 percent of the dietary nitrogen requirement with no apparent ill effects. Whether or not this level of biuret supplementation will maintain reproductive performance and suitable lactation response has not yet been investigated.

The response observed here suggests that biuret may be a useful non-protein nitrogen source for roughage type rations if a high level of nitrogen replacement is practiced. An adaptation period of 10 to 14 days should probably be anticipated, and other limiting factors such as frequency of feeding (Clemens and Johnson, 1973) should be considered.

In Vitro Cellulose Digestion

The results of *in vitro* cellulose digestion trials conducted during this experiment were inconclusive. When lambs receiving ration 1 were used as inocula sources, a significant ($P<.05$) replicate effect was observed. There is no apparent explanation for this response, so no attempt was made to draw conclusions from these data. Average *in vitro* cellulose digestion observed during the two replicates of this trial is shown in Table VIII. Although these data allow little insight into the relative value of urea or biuret as nitrogen sources for the maintenance of cellulose digestion, certain characteristics of the data are noteworthy. The high level of cellulose digestion evident in the negative controls (no added nitrogen source) indicates that substantial amounts of nitrogen were present in inocula prior to supplemental nitrogen addition. The inocula apparently contained almost enough nitrogen to satisfy the requirement without the addition of a supplemental nitrogen source. These data also reflect a large amount of variation (Table VIII) which is characteristic of *in vitro* cellulose digestion measurements.

TABLE VIII

COMPARISON OF UREA AND BIURET AS NITROGEN SOURCES FOR *IN VITRO* CELLULOSE DIGESTION

Ration	*In Vitro* N-Source None	Urea	Biuret
(Replicate 1)		% cellulose digested[1]	
1	56.11	67.86	63.72
2	59.14	54.86	59.26
3	56.58	60.25	59.96
(Replicate 2)			
1	43.87	69.03	52.31
2	55.45	63.91	50.74
3	53.89	63.26	60.15
(Average[2])			
1	49.99	68.44	58.01
2	57.29	59.39	55.00
3	55.23	61.73	60.05

[1]Values within replicates represent the average cellulose digestion in media prepared from three lambs receiving the ration indicated.

[2]Coefficients of variation for cellulose digestibility in media prepared for lambs receiving rations 1, 2 and 3 were 11.19, 17.72 and 6.63 percent, respectively.

Experiment 2

In Vitro Biuretolytic Activity

Table IX shows the amount of initial biuret still remaining after the 24 hour *in vitro* incubation of biuret with inocula from lambs receiving the six rations employed in this experiment. Biuret adaptation was apparent in lambs receiving all rations as indicated by a highly significant ($P<.01$) period effect. Lambs used in this experiment showed a remarkable facility to adapt to biuret. Lambs receiving rations 2-5 appeared to be completely adapted by collection day 3. The rapid adaptation to biuret observed here was surprising in view of earlier reports from this laboratory (Johnson and Clemens, 1973) and by others (McLaren *et al.*, 1959; Oltjen *et al.*, 1969) investigating the biuret adaptation response. A few reports of rapid biuret adaptation are present in the literature. Clemens and Johnson (1973) reported that adaptation was complete by collection day 2 in lambs that had previously been fed biuret. Mackenzie and Altona (1964) observed an immediate liveweight response (first week) when biuret was fed as a lick. However, Schroder and Gilchrist (1969) reported that biuret re-adaptation was a slow process similar to the initial adaptation response. Animals employed in this experiment had not received biuret prior to initiation of this trial. Therefore, carry-over effects from previous biuret supplementation could not have been a factor.

TABLE IX

IN VITRO BIURETOLYTIC ACTIVITY OF RUMEN CONTENTS FROM LAMBS IN EXPERIMENT 2

Collection day	Ration[1] 1	2	3	4	5	6
	Percent of initial biuret remaining[2]					
0	100.2	97.0	101.6	96.0	99.2	103.1
3	62.1	15.8	26.1	28.2	0.0	31.1
5	50.6	19.0	21.5	29.5	10.3	67.0
10	23.5	16.3	49.3	61.3	34.4	72.6
14	4.6	21.7	36.8	34.8	44.7	60.9
21	36.7	27.2	25.8	31.8	24.4	51.1
28	24.0	12.8	12.7	17.6	11.0	44.2
35	19.5	8.0	20.1	8.0	32.1	41.0

[1]Rations 1, 2 and 3 contained no molasses and 0, 2 and 8 percent alfalfa respectively. Rations 4, 5 and 6 contained 5 percent molasses and 0, 2 and 8 percent alfalfa respectively.

[2]Each value represents the average of two lambs and designates the percent of initial biuret remaining after a 24 hour in vitro incubation.

No significant ($P>.05$) treatment effects were observed in this experiment. The biuretolytic activity at 8 and 24 hours of rumen contents from lambs used in this experiment are shown graphically for the various collection days in Figures 4-11. Rumen contents from all lambs showed little or no capacity for biuret hydrolysis on collection day 0 (Figure 4). By collection day 3, all lambs except those receiving the control diet (no molasses or alfalfa) registered a high degree of biuretolytic activity (Figure 5). On collection day 5 (Figure 6) and all subsequent collection days (Figures 7-11), lambs receiving ration 6 (5 percent molasses and 8 percent alfalfa), showed the lowest amount of biuretolytic activity. The results of this experiment are in agreement with earlier findings by Clemens and Johnson (1973), that higher levels of readily available carbohydrate enhance development of biuretolytic activity initially. As the supplementation period progressed, biuret utilization progressively favored the rations containing lower levels of readily fermentable carbohydrate. This initial response favoring the addition of readily fermentable carbohydrate is puzzling. The concurrent adaptation by rumen microorganisms to biuret and readily fermentable carbohydrate may be confounding. The possibility exists that alteration of existing microbial populations in the rumen caused by the change to a diet containing a readily fermentable carbohydrate creates a transient environment which allows biuretolytic microorganisms to flourish. Once this aberrant

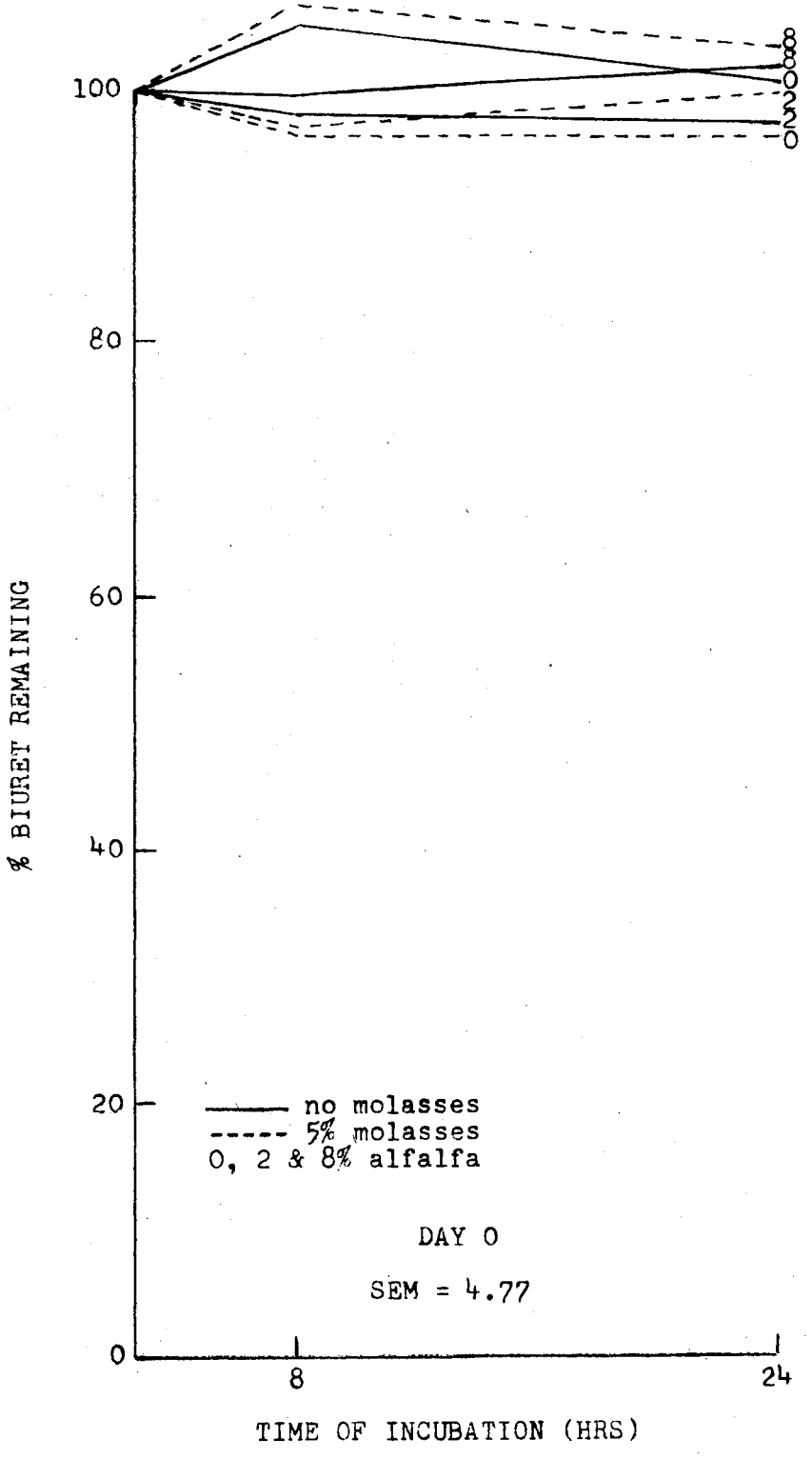

Figure 4. In vitro biuret disappearance for sheep fed various sources and levels of readily fermentable carbohydrate.

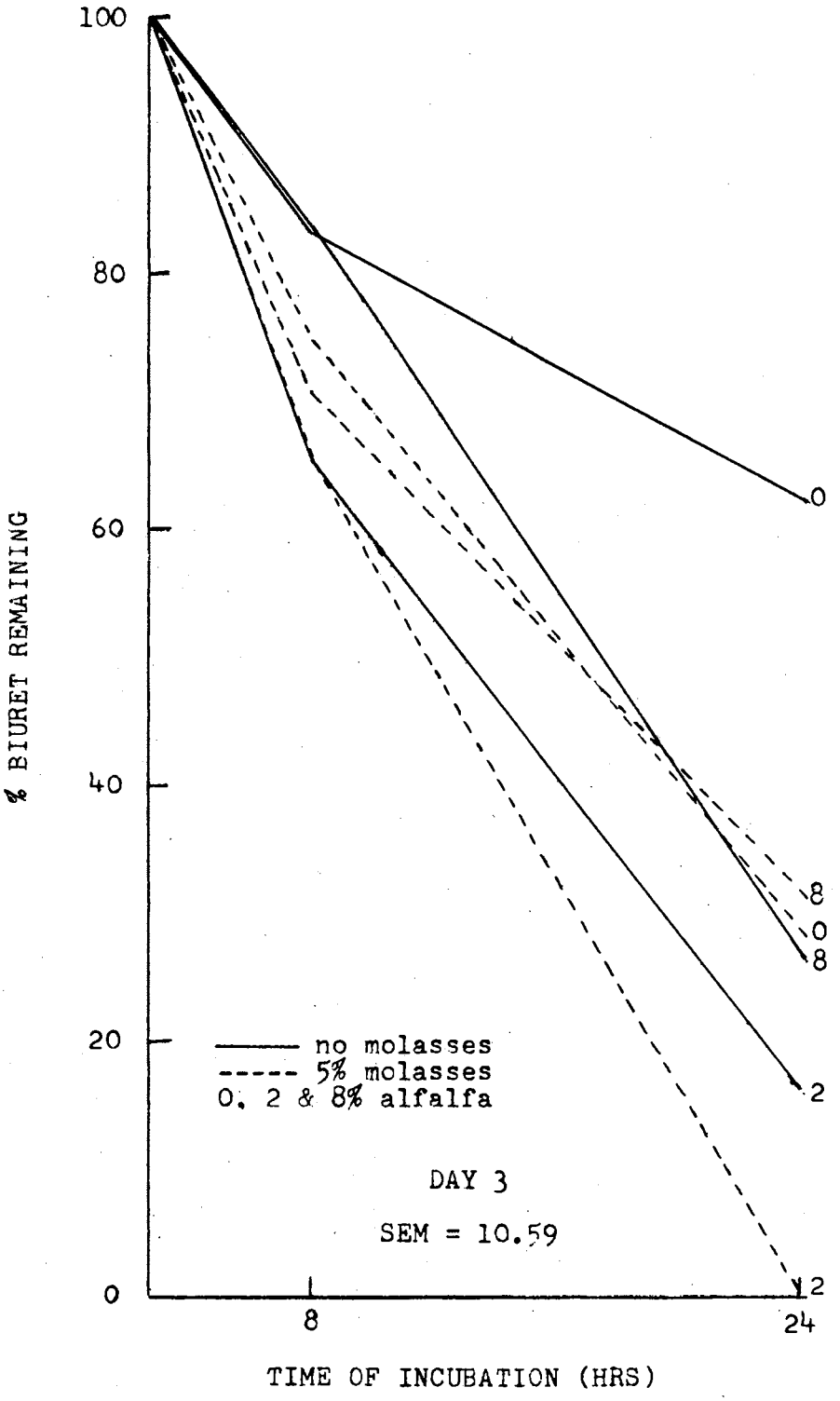

Figure 5. In vitro biuret disappearance for sheep fed various sources and levels of readily fermentable carbohydrate.

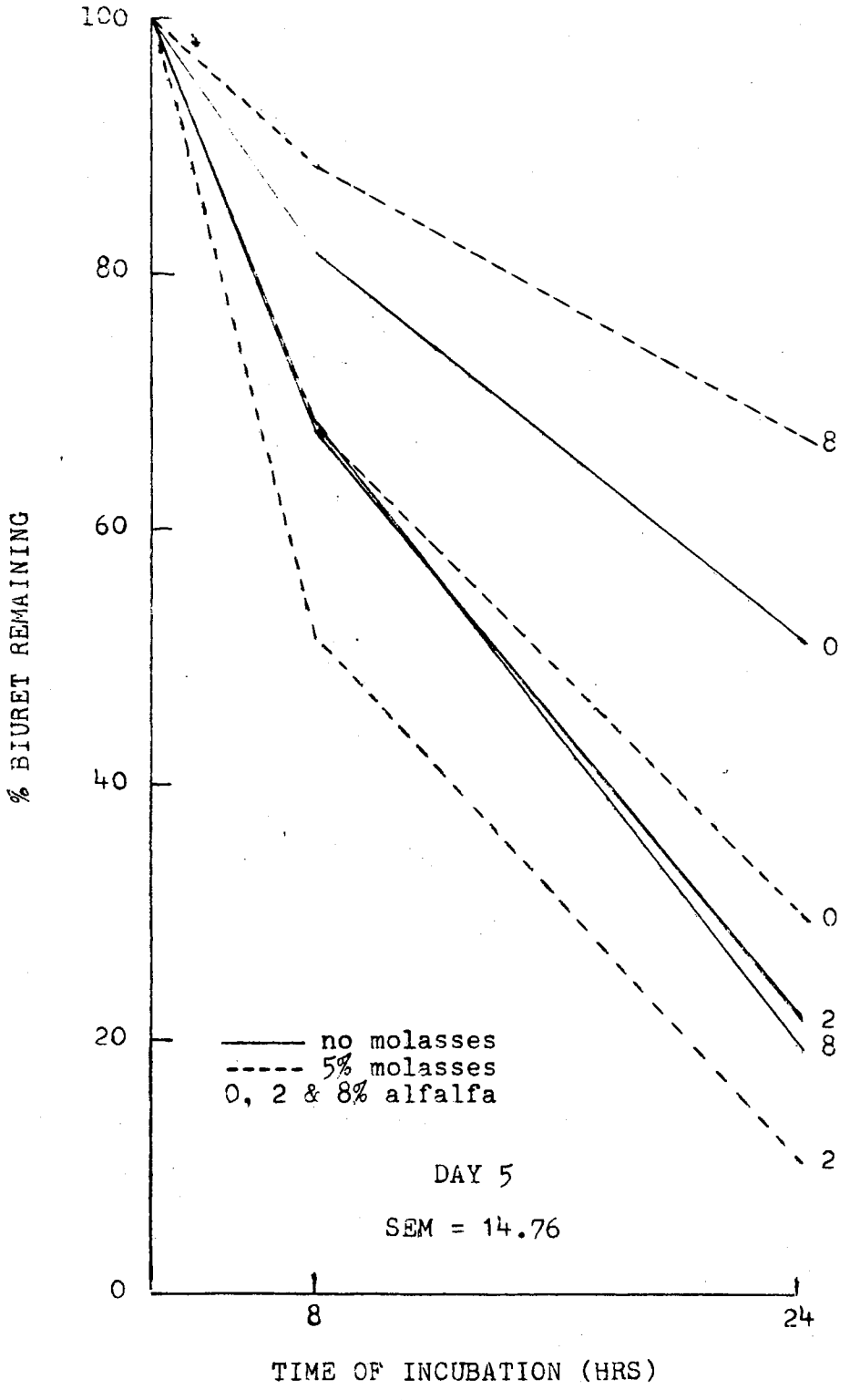

Figure 6. In vitro biuret disappearance for sheep fed various sources and levels of readily fermentable carbohydrate.

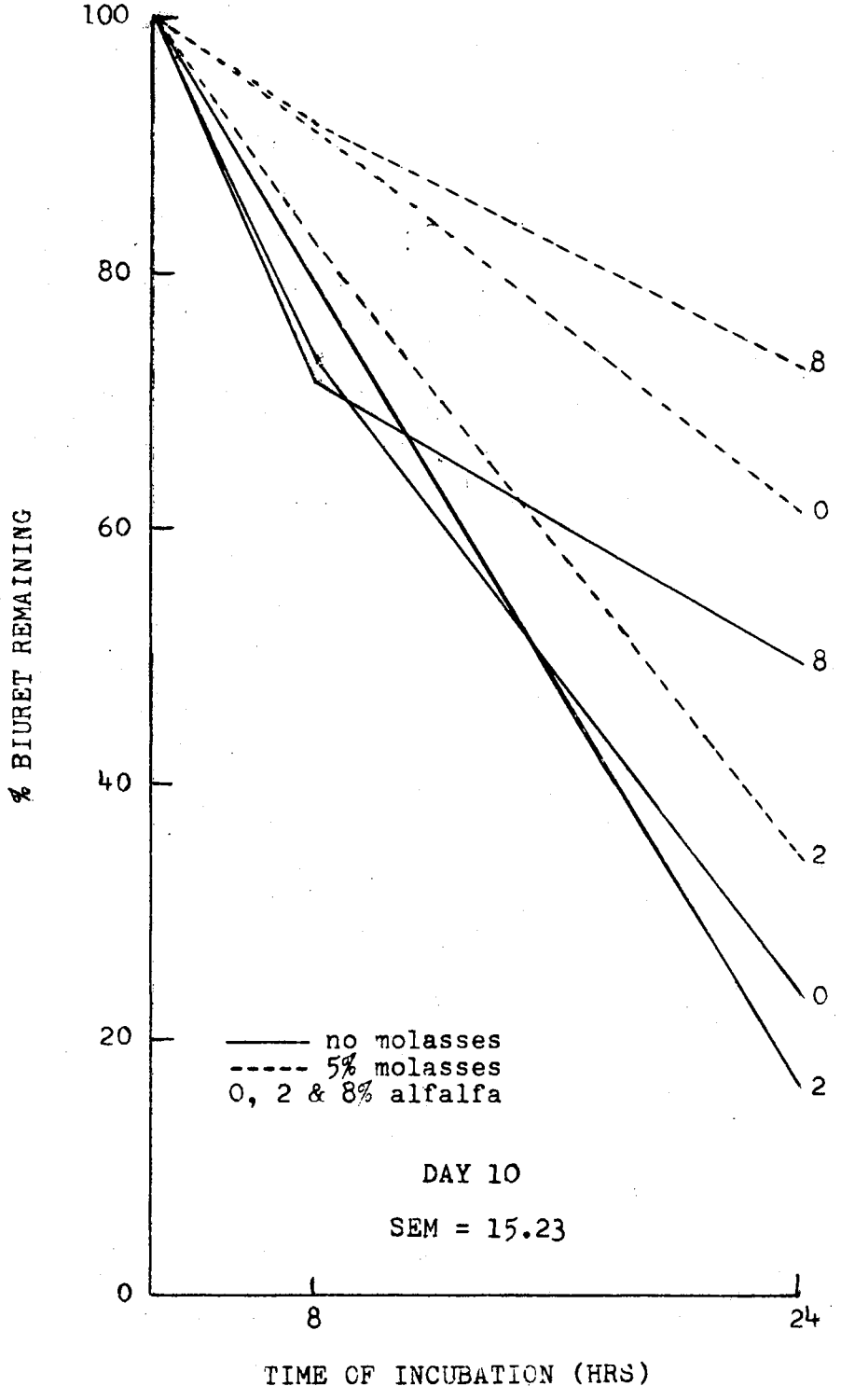

Figure 7. In vitro biuret disappearance for sheep fed various sources and levels of readily fermentable carbohydrate.

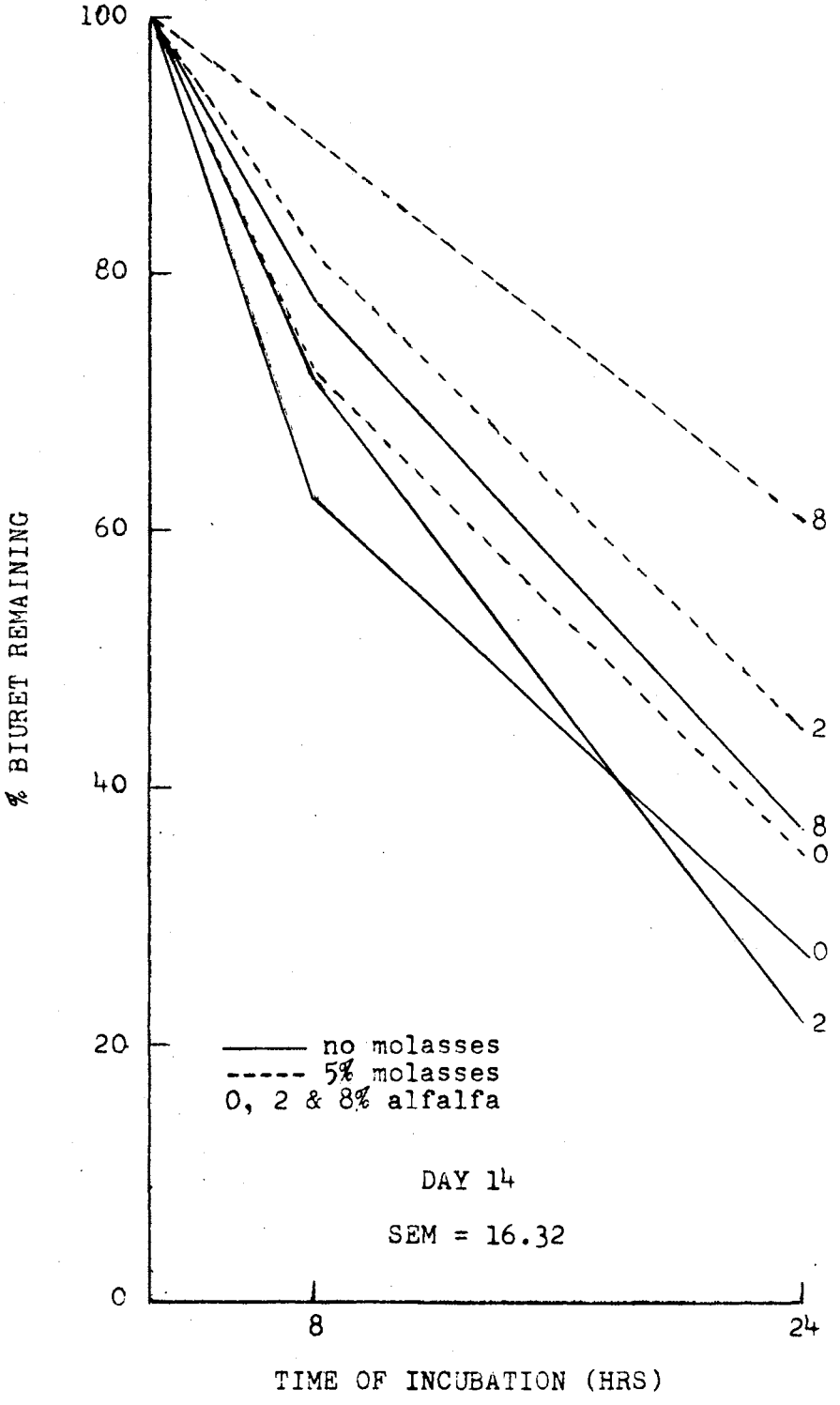

Figure 8. In vitro biuret disappearance for sheep fed various sources and levels of readily fermentable carbohydrate.

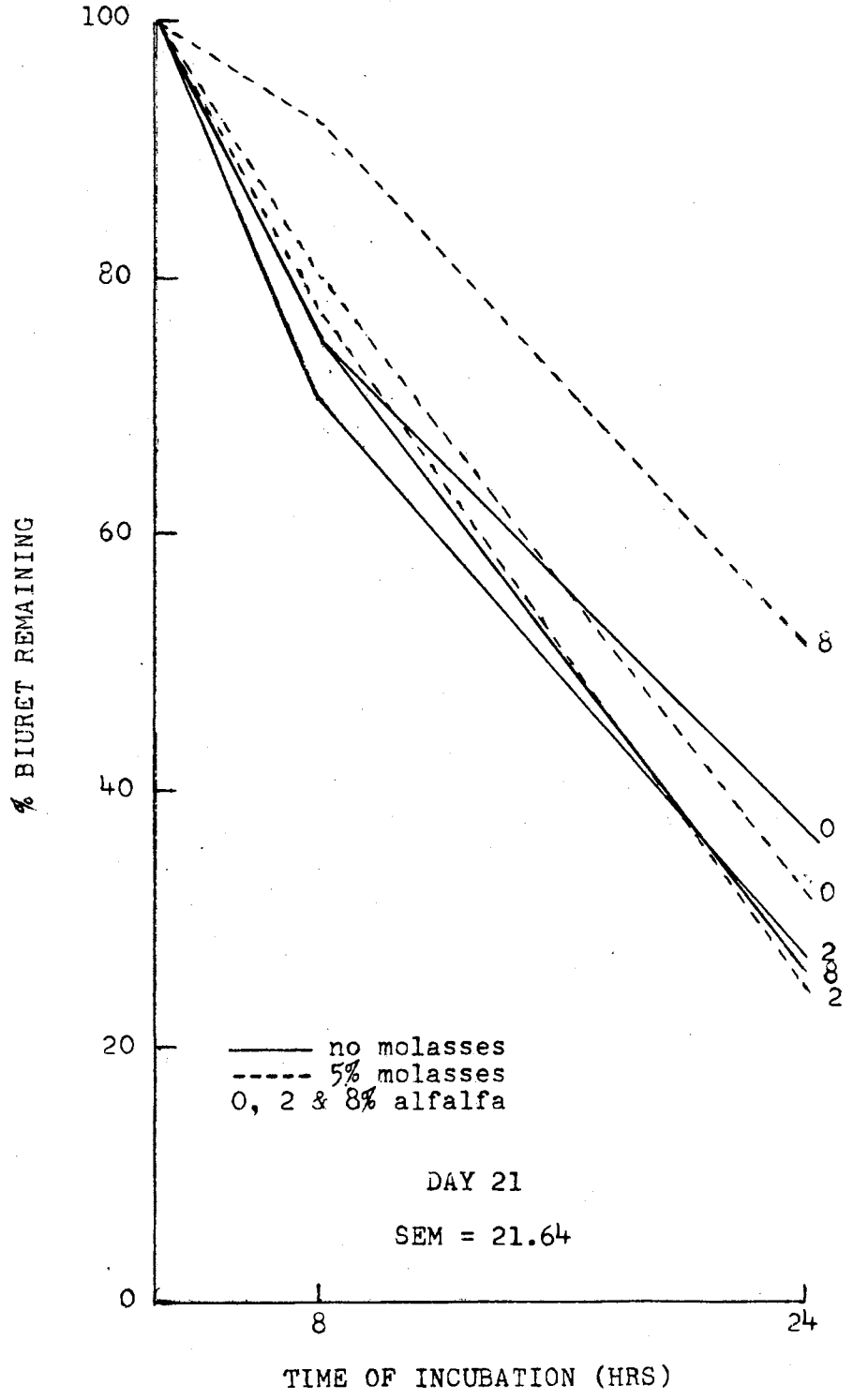

Figure 9. In vitro biuret disappearance for sheep fed various sources and levels of readily fermentable carbohydrate.

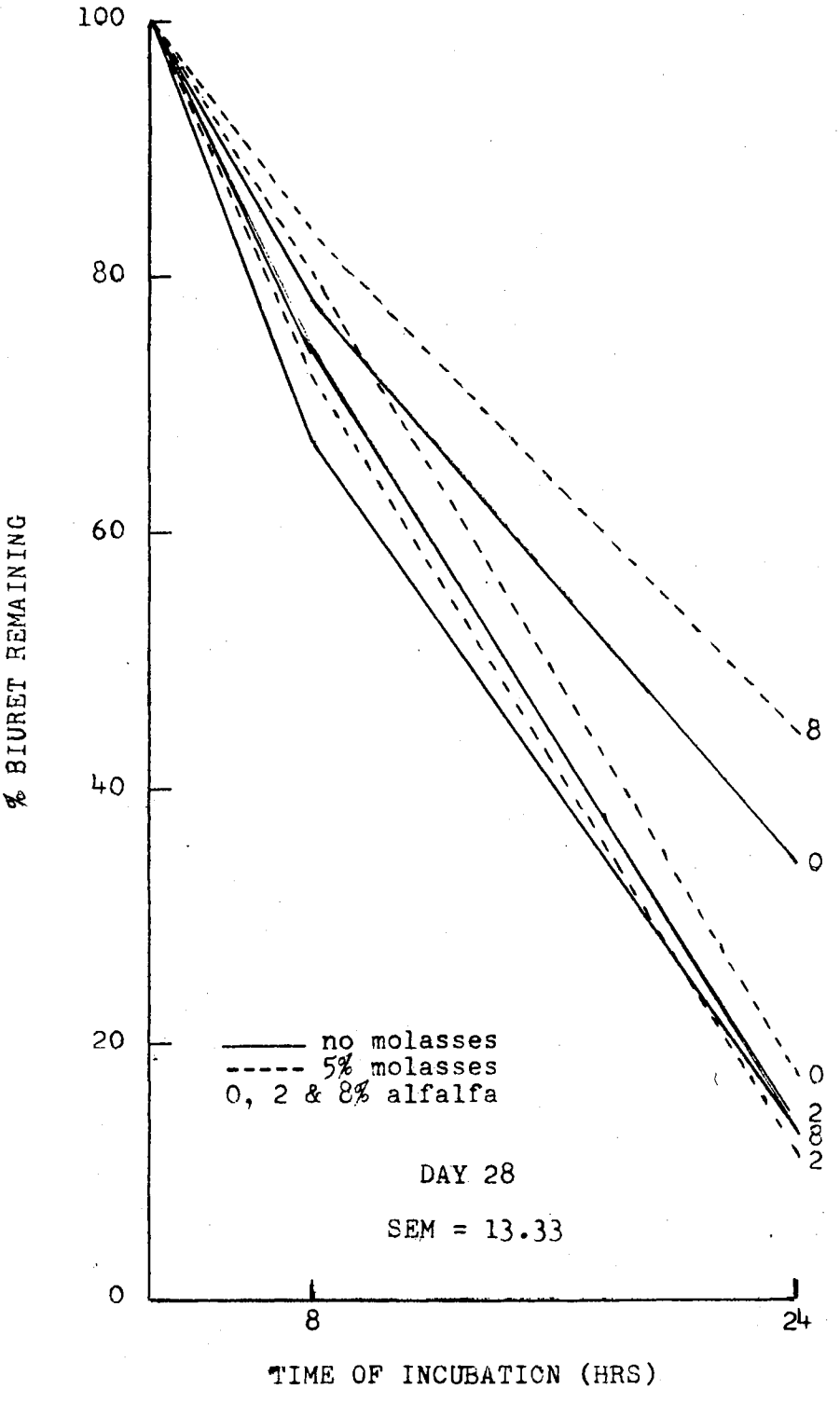

Figure 10. In vitro biuret disappearance for sheep fed various sources and levels of readily fermentable carbohydrate.

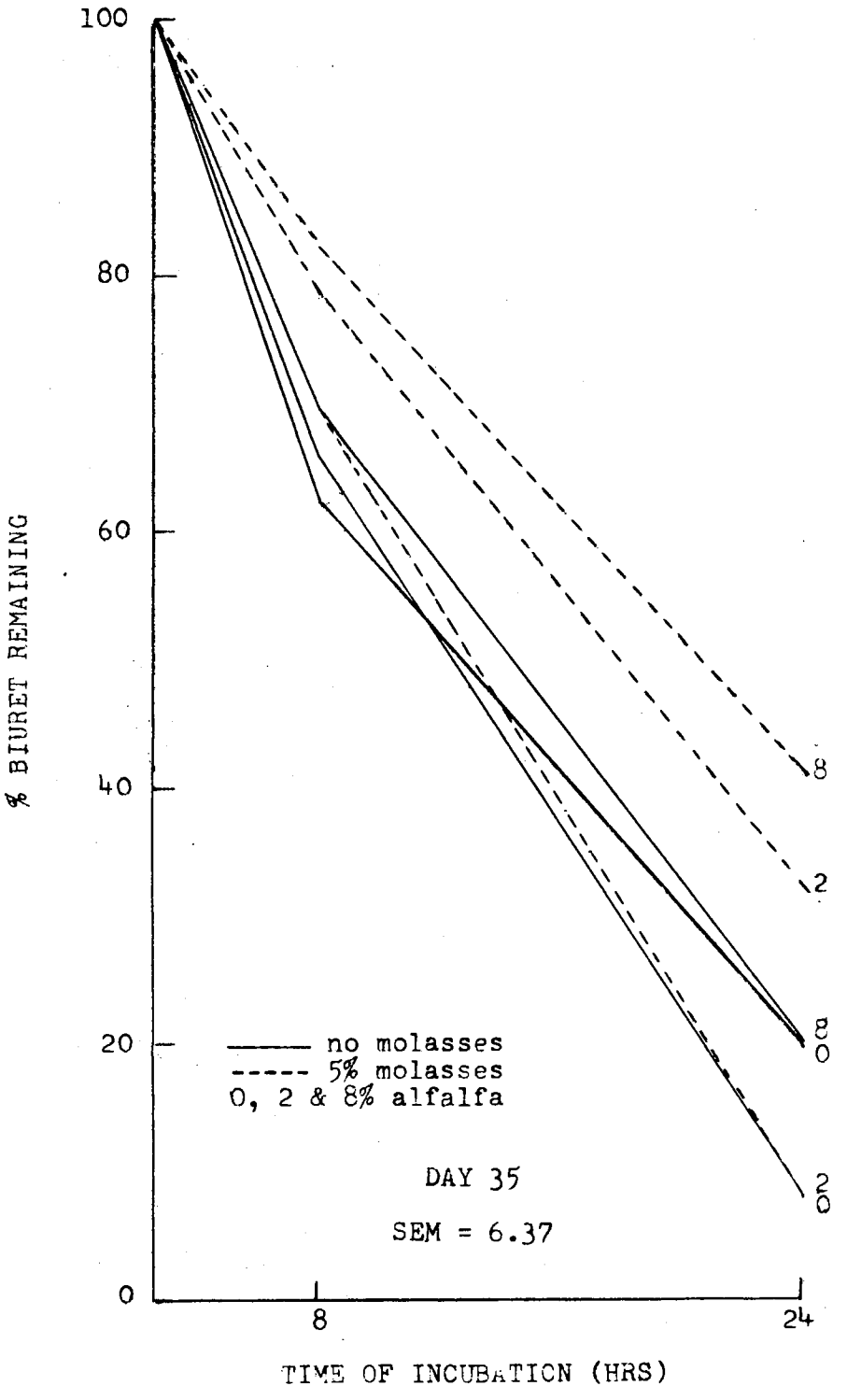

Figure 11. In vitro biuret disappearance for sheep fed various sources and levels of readily fermentable carbohydrate.

condition subsides, so does biuretolytic activity. In retrospect, a better measure of the effect of readily available carbohydrate upon biuret adaptation would be obtained if the animals were allowed to adapt to the carbohydrate source prior to biuret supplementation. This could be accomplished by substituting an alternate nitrogen source for biuret in the diet on an isonitrogenous basis until adaptation to the carbohydrate source was complete.

Schroder and Gilchrist (1969) reported that addition of 200 g. of maize meal or 1,000 g. of corn silage to a basal diet of poor quality teff hay increased the extent of biuretolytic activity. The duration of this experiment was much shorter than that conducted by Schroder and Gilchrist, but the increased extent of biuret utilization stimulated by readily fermentable carbohydrate was not evident in this experiment.

The results of this experiment suggest that a slight beneficial response may be obtained from feeding a readily fermentable carbohydrate source during the initial phase of biuret adaptation. This initial favorable response is lost or possibly even reversed as the supplementation period progresses.

In Vitro Cellulose Digestion

The average in vitro cellulose digestion values and coefficients of variation obtained in this experiment are shown in Table X. As in experiment 1, the cellulose digestion was

TABLE X

COMPARISON OF UREA AND BIURET AS A NITROGEN SOURCE FOR IN VITRO CELLULOSE DIGESTION BY RUMEN MICROORGANISMS FROM LAMBS ADAPTED TO BIURET

Ration	In Vitro N-Source: None	Urea	Biuret
(Replicate 1)	% cellulose digested[1]		
1	62.22	44.49	55.81
2	59.95	54.36	57.24
3	62.77	45.92	63.60
4	55.15	42.33	59.47
5	58.96	42.48	58.26
6	52.67	39.18	58.25
(Replicate 2)			
1	62.58	59.63	56.55
2	56.76	50.30	49.43
3	55.87	68.29	50.54
4	51.90	53.31	53.60
5	54.48	67.07	57.48
6	51.73	55.76	57.04
(Average[2])			
1	62.40	52.06	56.18
2	58.35	52.33	53.33
3	59.32	57.10	57.07
4	53.52	47.82	56.53
5	56.72	54.77	57.87
6	52.20	47.47	57.64

[1]Each value represents the average cellulose digestion by rumen microorganisms from two lambs receiving the same diet.

[2]Coefficients of variation for cellulose digestibility in media prepared for lambs receiving ration 1, 2, 3, 4, 5 and 6 were 5.83, 14.70, 12.53, 14.54, 14.44 and 7.11 percent, respectively.

quite variable and values for the negative controls were high. The high levels of cellulose digestion in the negative controls render this type of experiment difficult to interpret, and the large amounts of variation present result in a requirement of large differences to be significant.

As in experiment 1, *in vitro* cellulose digestion data were not meaningful. A significant ($P<.05$) replicate x non-protein nitrogen source interaction was observed when inocula were obtained from lambs fed rations 1, 3 and 6. No logical explanation for this interaction is apparent and no attempt to explain these results was made.

CHAPTER V

SUMMARY AND CONCLUSIONS

Two experiments were conducted to characterize the effects of varying levels of biuret and the addition of low levels of readily fermentable carbohydrate sources upon the rate and extent of biuret adaptation in lambs fed high roughage rations. Biuretolytic activity of rumen contents was determined *in vitro* by measuring biuret disappearance.

In experiment 1, biuret was fed as 20, 50 and 80 percent of the total dietary nitrogen. The rate and extent of biuretolytic activity development was highest at the higher levels of biuret supplementation. Biuret fed as 20 percent of the total dietary nitrogen resulted in low biuretolytic activity with no significant ($P>.05$) improvement registered after day 5 of supplementation. Lambs receiving 50 and 80 percent of their total dietary nitrogen as biuret showed significantly ($P<.05$) higher biuretolytic activity than lambs fed the 20 percent level by collection day 14 and this response held throughout the 35 day adaptation trial.

In experiment 2, six rations were employed with 50 percent of the total dietary nitrogen supplied as biuret. Rations 1, 2 and 3 contained no molasses and 0, 2 and 8 percent alfalfa; rations 4, 5 and 6 contained 5 percent

molasses and 0, 2 and 8 percent alfalfa, respectively. Rations were isonitrogenous and isocaloric. Biuret adaptation was rapid in animals receiving all diets, generally being complete by day 3 of the trial. No significant ($P>.05$) treatment effects were observed.

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PROCEDURE

BIURET DETERMINATION

Reagents:

1. 10N H_2SO_4
2. 10% NaOH. Adjusted so 4 ml. neutralizes 1 ml. 10N H_2SO_4
3. 7% $ZnSO_4$
4. 5% $Ba(OH)_2$. Adjusted so 10 ml. exactly neutralizes 7 ml. $ZnSO_4$ solution. Use CO_2 free water. Store under Mariot bulb of drierite.
5. Alkaline NaK Tartrate solution: Dissolve 110 gm. of NaOH pellets in 500 ml. distilled water. Add 100 g. of $NaKC_4H_4O_6.4\ H_2O$ and dissolve. Dilute to 1 liter.
6. $CuSO_4$ solution: Dissolve 35 g. of $CuSO_4.5\ H_2O$ in distilled water. Dilute to 1 liter.
7. Color reagent: Mix equal quantities of Tartrate and $CuSO_4$ solutions. Let stand for 48 hours before using.
8. Biuret standard: 1% biuret in water.
9. Activated charcoal: Powdered willow charcoal (pyramid brand, Ehrmann-Strauss Co., New York)

Procedure:

1. Centrifuge strained rumen fluid at 14,000 x g. for 10 minutes.
2. Pipet 10 ml. supernatant fluid into 50 ml. centrifuge tube.
3. Add 5 ml. distilled H_2O, 0.8 ml. 10% NaOH, 7.0 ml. $ZnSO_4$ and 10.0 ml. $Ba(OH)_2$.
4. Stopper and shake. Let stand for 10 minutes.
5. Centrifuge at 14,000 x g. for 15 minutes.
6. Decant supernatant fluid into beaker or flask containing 0.3 gm. of activated charcoal. Swirl and let stand for 10 minutes.
7. Filter through Whatman #40 filter paper.
8. Add 2 pellets NaOH swirl to dissolve. Let stand for 20 minutes.
9. Filter through Whatman #42 filter paper.
10. Pipet 15 ml. of filtrate and 5 ml. of the color reagent into a test tube. Allow to stand for 15 minutes.
11. Read optical density at 555 nm.
12. Blank prepared by adding water at step 2 and in step 3 add 3 ml. of H_2O instead of 5 ml., add 2 ml. of 1% biuret solution and continue procedure.

VITA

Roger Daniel Wyatt

Candidate for the Degree of

Master of Science

Thesis: FACTORS AFFECTING BIURET ADAPTATION BY RUMEN MICROORGANISMS IN SHEEP

Major Field: Animal Science

Biographical:

Personal Data: Born in Texarkana, Arkansas, July 21, 1944, the son of Leslie and Lucille Wyatt.

Education: Graduated from Broken Bow Senior High School, Broken Bow, Oklahoma, 1962. Received the Bachelor of Science degree from West Texas State University, Canyon, Texas, with a major in Animal Science, in May, 1971; completed the requirement for the Master of Science degree in 1973.

Professional Experience: Raised and worked on a ranch in southeastern Oklahoma. Employee of AT & SF Railway Company while attending West Texas State University, 1966-71; Research and Teaching Assistant in the Department of Animal Science, Oklahoma State University, Stillwater, Oklahoma, 1971-72.

Organizations: Member of American Society of Animal Science and Alpha Chi National Honor Society.