SODIUM BENTONITE IN UREA-CONTAINING RATIONS FOR RUMINANTS

By

LETHAL CONRAD MARTIN

Bachelor of Science

University of Kentucky

Lexington, Kentucky

1967

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
May, 1969

SODIUM BENTONITE IN UREA-CONTAINING RATIONS FOR RUMINANTS

Thesis Approved:

Allen D. Tillman
Thesis Adviser

Donald G. Wagner

D. D. Durham
Dean of the Graduate College

ACKNOWLEDGMENTS

The author wishes to express his appreciation to Dr. Allen D. Tillman, Professor of Animal Science, for his advice, encouragement and guidance during the course of this study and in the preparation of this thesis.

Appreciation is also extended to Mr. A. J. Clifford, Graduate Assistant, Mr. T. E. Nelson, Chief Technician, and Mrs. Jane Witte, Laboratory Technician, for their assistance during this study.

The author is indeed grateful to Mr. D. L. Williams, Mr. James Word, Jr. and other fellow Graduate Students for their never failing assistance and encouragement during the course of the study.

Sincere appreciation is extended to the author's parents for their encouragement throughout this program of graduate study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Sodium bentonite is an inert colloidal clay of volcanic origin and is composed chiefly of the mineral montmorillonite, which is a hydrated form of aluminum silicate. The inclusion of this material in pelleted feeds improves the physical consistency by either acting as a pellet binder or as an adsorbent for high moisture pellets. Bentonite is able to adsorb ten to fifteen times its own weight of water. Also it increases the life span of pelleting equipment by acting as a lubricating agent.

The clay also has an inherent affinity for adsorbing certain cations such as ammonium (NH_4^+), potassium (K^+) and calcium (Ca^{++}) and retaining these ions in an exchangeable state. This property suggests that bentonite could be a beneficial feed ingredient other than as a binder. Its adsorptive capacity could be extremely beneficial when used in urea-containing rations for ruminants, since these rations predispose high levels of ammonium ions in the rumen. If this product could adsorb the ammonium ions, when concentration is high, shortly after feeding, and then release these ions gradually when the concentration decreases, more efficient utilization of the urea might be obtained. The studies described herein were undertaken to investigate the possible beneficial effects of sodium bentonite in urea-containing diets for ruminants.

CHAPTER II

LITERATURE REVIEW

Properties of Sodium Bentonite

Sodium bentonite has adsorptive properties which are basically those of montmorillonite. Montmorillonite, which makes up approximately 90% of the total bentonite substance, has the approximate chemical formula:

$$(\mathrm{Al,Fe}_{1.67}\ \mathrm{Mg}_{.33})\ \mathrm{Si}_4\mathrm{O}_{10}(\mathrm{OH})_2\mathrm{Na,Ca}_{.33}$$

The remaining 10% of bentonite consist of small fragments of other minerals, the most abundant being feldspar. There are also small fractions of gypsum, calcium carbonate, and quartz and traces of partially altered volcanic glass, biotite mica, magnetite, limonite, hematite, leucoxene, apatite, zircon, pyrite, titanite and tremolite (Gustaveson, 1963). A typical chemical analysis of bentonite is shown in Table I.

The basic chemical and physical properties of bentonite, its adsorptive capacity and its ability to expand, are due mainly to its structure. All clays are generally composed of the same compounds; however, the structural arrangement of these compounds varies markedly from clay to clay. This creates materials with widely varying chemical and physical properties. The basic particles of all clays are hydrated

TABLE I

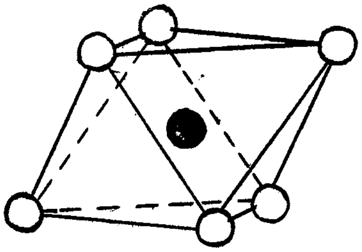
TYPICAL CHEMICAL ANALYSIS OF SODIUM BENTONITE[a, b]

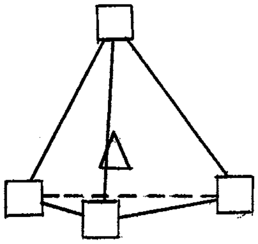
Compound	Percent
H_2O, Mechanically Held	0.00
Silica (SiO_2)	63.07
Alumina (Al_2O_3)	21.08
Ferric Oxide (Fe_2O_3)	3.25
Ferrous Oxide (FeO)	0.35
Titanium (TiO_2)	0.14
Lime (CaO)	0.65
Magnesia (MgO)	2.67
Soda (NaO_2)	2.20
Potash (K_2O)	0.37
Other Minor Constituents	0.58
H_2O, Chemically Held	5.64

[a] The exact percentages shown above may vary slightly from sample to sample.

[b] Chemical analysis courtesy of American Colloid Co., Skokie, Illinois.

aluminum and magnesium, and silicon oxides. The structure of these particles are:





Hydroxyls ○

Aluminum, Magnesium, etc. ●

Oxygen □

Silicon △

These basic particles are brought together to form distinct layers of two kinds: 1) Silicon oxygen sheets, and 2) Aluminum oxygen sheets. The sheets then combine to form unit cells and at this point there is a radical difference in the properties exhibited by the different clays. The unit cells of montmorillonite have two silicon sheets with an aluminum sheet sandwiched between. This arrangement is termed a "Si-Al-Si" structure. This "Si-Al-Si" sheet structure composes the basic flake unit of the mineral and produces a unit of definite thickness, but indefinite width and breadth. Laterally, the plates extend until they reach broken edges; vertically, they extend only one cell unit in thickness. This plate structure provides a cell unit which has an estimated ratio of thickness to depth and breadth of from 1:100 to 1:300, thus it has an almost astronomical surface area. It has been estimated that one cubic inch of bentonite, when suspended in water, yields 9,500 billion individual flakes with a total area of approximately one acre (Gustaveson, 1963). The "Si-Al-Si" structure also predisposes a flake with a uniform negative surface charge which causes it to disperse readily in water and further enhances its adsorptive capacity.

Under dry conditions the flakes of bentonite stack together, in a fashion similar to playing cards lying together on a surface, and in this way forms the structure of the clay particles. However, even when dry the flakes possess a molecular coat of chemically bound water. If more water is made available to the clay particles, it crowds between the individual flakes, forms layer upon layer in a semi-rigid position, pushes the flakes apart and causes the mass to swell. This will continue until a water coat of sufficient thickness to overcome the attractive forces of the clay flakes has been provided. This allows free water to move between the semi-rigid water sheaths and frees the clay along with its surrounding atmosphere of bound water and forms a dispersed system. Upon removal of the water, the mass contracts and the flakes resume approximately their original spacing.

The hydrated clay flakes with their bound coat of water are of prime importance in determining the adsorptive capacity of the material. The bound atmosphere around each flake provides a miniature environment into which compounds may migrate and be retained. Obviously as the surface area is increased, along with the charge of the material, there will be an increase in the total volume of bound water, thereby providing a greater area for adsorption. Therefore, bentonite with its tremendous surface area and charged surface is an extremely adsorptive compound.

The negative surface charges of the dry sodium bentonite flakes form ionic bonds mainly with sodium, and to a much lesser extent with potassium. As the flakes hydrate the sodium and potassium ions become exchangeable for other metallic bases. The order of exchange of these bases or the degree of affinity that the clay flake shows for each base is determined by electrical charge: monovalent cations are mildly attracted to bentonite with di-, and tri-valent cations being more strongly attracted. The relative attractive forces for mono-, di-, and tri-valent cations has sometimes been estimated as 1 to 30 to 1000 (Gustaveson, 1963). The base exchange sequence of bentonite is as follows: calcium, magnesium, potassium, hydrogen, sodium and lithium.

It should be noted that a bentonite water system, such as that provided when the compound enters the rumen or other parts of the gastrointestinal tract, may contain both positively and negatively charged electrolytes, and also electrolytes with high and low valences. Bentonite is an electrolyte but, if complicated by other electrolytes in a solution, the reactions are predictable only in a general way.

Bentonite in Feeds

The fact that acid clays of the bentonite type adsorb polyene pigments was established as early as 1923 by Tadahashi and Kawakami (Laughland and Phillips, 1954b). Bentonite will adsorb vitamin A and render it unavailable for use by animals (Laughland and Phillips, 1954a, 1956; Briggs and Fox, 1954, 1956; Blakely *et al*., 1965; Erwin *et al*., 1957), but the mechanism by which vitamin A is tied up is not clear. Erwin *et al*. (1957) suggested that vitamin A is physically bound to the bentonite and that no destruction occurs, while Laughland and Phillips (1956) concluded that, in addition to the physical adsorption, there is a chemical alteration. They suggested that anhydro-vitamin A is formed and that this product, when removed from the bentonite, forms a secondary compound not available to the animal.

The actual point of vitamin A destruction or adsorption also remains in question. Laughland and Phillips (1954a) indicated that the actual physical destruction or adsorption of vitamin A may be accounted for in the small intestine. Briggs and Fox (1956), on the other hand, found that there was substantial disappearance of vitamin A prior to the consumption of the feed.

Laughland and Phillips (1954a), Briggs and Fox (1954, 1956) and Blakely et al. (1965) have produced vitamin A deficiency symptoms in chicks, turkey poults, rats and mice by including bentonite in synthetic diets with a normally adequate supply of vitamin A. As would be expected, the animals receiving bentonite also achieved lesser weight gains and had an increased mortality rate. However, in later work Laughland and Phillips (1954a, 1956) and Briggs and Fox (1956) found that all signs of vitamin A deficiency, decreased weight gains, and increased mortality rates were overcome by adding a stabilized form of vitamin A. However, in all diets bentonite decreased liver storage of the vitamin (Laughland and Phillips, 1954a, 1956). In no case did any worker produce vitamin A deficiency symptoms or significant decreases in performance when bentonite was added to natural diets. Thus it appears that, in the presence of other natural pigments, bentonite does not adsorb sufficient amounts of vitamin A to produce any detrimental effects upon the health or performance of animals.

Ershoff and Bajwa (1965), who supplemented a low calcium, low protein, low fat, non-heat processed, wheat flour-containing diet, with from one to four percent clay, which contained primarily silica and aluminum oxides with some montmorillonite, found that the animals which received the clay supplements had highly significant increases in weight gains. They also noted that pathological changes which were observed in the long bones of the immature rats, hamsters and mice which had been fed the diet were prevented. It was also observed that, the beneficial effects of the supplements were proportional to the level of clay being fed.

Kurnich and Reid (1960), Quisenberry and Bradley (1964) and Quisenberry (1966) studied the effects of the addition of sodium bentonite to laying rations and found favorable results. Kurnich and Reid (1960) fed low, medium and high energy levels and found that the addition of bentonite produced highly significant increases in weight gains and slightly improved feed conversion with both the low and medium energy levels. They noted increased feed consumption in the low energy diets and found that bentonite decreased the rate of passage of this diet in the intestinal tract.

Quisenberry and Bradley (1964) and Quisenberry (1966) found that the addition of 2.5 percent bentonite to laying rations brought about increased weight gains and larger egg size without a significant decrease in hen-day production. Both reports also indicate a slight increase in feed efficiency and a highly significant decrease in percentage of water in the feces.

Almquist _et_ _al_. (1967) fed one and two percent of bentonite to 10-12 day old turkey poults and found bentonite consistently improved nutrient digestibility and feed utilization. The effects were small and the results indicate that the optimal level was two percent of the diet.

Bentonite can be fed to ruminants without detrimental effects (Jordan, 1953, 1954; Erwin _et_ _al_., 1957). Jordan used bentonite to control protein intake in two trials, and found in both cases that lambs were easier to keep on feed and consumed slightly more feed when bentonite was used. In one case, slight increases in weight gain and efficiency of gain were noted: Erwin _et_ _al_. (1957), fed a ration to steers which contained three percent bentonite and found that there was no significant influence on rate of gain, feed efficiency, digestible dry matter, digestible crude fiber, hepatic vitamin A storage, or carotene retention; however, there appeared to be a trend toward increased weight gain and improved feed efficiency when bentonite was fed.

Inconsistent results have been obtained when bentonite was added to urea-containing feedlot type rations for steers. Vetter _et_ _al_. (1967) and Perry _et_ _al_. (1968), found that the addition of 150 gm. of bentonite per day to a steer ration increased weight gain and feed consumption. However, subsequent studies (Vetter _et_ _al_., 1968; Perry _et_ _al_., 1968; Beeson _et_ _al_., 1968) found that the addition of bentonite was without effect.

As ruminant studies are limited and have yielded inconsistent results, further information is needed on the value of sodium bentonite as a feed additive for ruminants. With this in mind the present studies were undertaken.

CHAPTER III

EXPERIMENTAL

This study consists of six trials, two of which were *in vitro* trials and were carried out to observe the effect of bentonite on ammonia production, adsorption and release. The other four trials were *in vivo* studies which determined the effect of bentonite on growth; the retention of nitrogen, calcium, and phosphorus; digestibility of dry matter and organic matter; and intraruminal Ca:P ratios.

Trial 1. Five liters of rumen fluid were obtained per fistulum from a steer, which had been fed a 1:1 mixture of bermuda hay and a commercially prepared concentrate. The rumen fluid was strained through four layers of cheesecloth and 400 ml. of the strained fluid placed in each of eight incubation flasks. Forty gm. of ration 1 and 2 (Table II) were added to flasks 1, 2, 3, 4 and 5, 6, 7, 8, respectively. In addition 0.4, 0.8 and 1.2 gm. of sodium bentonite was added to flasks 2 and 6, 3 and 7, 4 and 8, respectively, providing supplemental bentonite at levels of 1, 2 and 3% of each ration. All flasks were thoroughly shaken and incubated at 39°C. Carbon dioxide was continuously circulated through all flasks, which were constantly shaken during the incubation. Twenty-five ml. aliquots were removed from each flask at 0, 0.5, 1.0, 1.5, 2.0, 6.0, 12.0, 18.0 and 24.0 hours after the initiation of incubation and a pH reading immediately taken. Ten ml. of each sample were placed in tubes containing 1.0 ml. of saturated mercuric

TABLE II

PERCENTAGE COMPOSITION OF CONTROL RATIONS USED IN ALL TRIALS

	Rations	
Items	1 High-Concentrate	2 High-Roughage
Milo	81.58	19.57
Molasses	5.00	5.00
Cottonseed hulls	10.00	70.00
Urea[a]	1.15	3.00
Dicalcium phosphate	0.50	1.66
Sodium chloride	0.75	0.75
Calcium carbonate	1.00	----
Vitamins A and D[b]	0.02	0.02
Sodium bentonite	----	----

[a] Crystalline urea. Courtesy Nipak Chemical Co., Pryor, Oklahoma.

[b] Containing 20,000 I.U. and 2,500 USP units/gm. of vitamins A and D, respectively. Courtesy NOPCO Chemical Co., Harrison, New Jersey.

chloride to stop all enzyme activity and this aliquot was used for ammonia-nitrogen determination (Conway, 1957). The remaining 15 ml. were immediately chilled to below 0°C and assayed for urea-nitrogen (Conway, 1957). Chemical analyses were conducted immediately after all samples were collected. This entire trial was replicated twice and the data were analyzed statistically by analysis of variance and treatment means compared using Duncan's New Multiple Range Test as outlined by Steele and Torrie (1960).

Trial 2. Sixteen adult wether lambs were allotted at random into four treatment groups for a nitrogen retention study. Diets 1 and 2 (Table II) were fed to groups 1, 2 and 3, 4 respectively. The diets fed groups 2 and 4 were supplemented with 2% sodium bentonite. All animals were treated with a antihelminth preparation prior to the initiation of the experiment and fed their respective rations at a level of 1.0 Kg. per head per day during the trial. A 14-day preliminary period preceded the 10-day collection period, during which feces and urine were collected. Standard procedures were used in collecting and preparing feed, feces and urine for chemical analysis. Total nitrogen was determined by standard Kjeldahl procedures (A.O.A.C., 1960) and data were statistically analyzed as in Trial 1.

Trial 3. Twenty lambs, average weight 30 Kg., were treated with a antihelminth preparation and allotted at random into four groups for a 2 x 2 factorial arrangement of treatments during a 32-day growth trial. Diets 1 and 2 (Table II) were fed to groups 1, 2, and 3, 4, respectively. Diets fed to groups 2 and 4 were supplemented with 2% sodium bentonite. All lambs were individually-penned and had free access to feed and water throughout the trial. A 16-hour fast from feed and water preceded initial and final weighings. Gain and feed consumption were the response criteria.

Trial 4. Twenty crossbred wether lambs were divided randomly into four groups of five each, for the determination of calcium, phosphorus, and nitrogen retention and the digestibility of dietary dry matter, organic matter, and nitrogen. All animals were fed diet 2 (Table II) at a level of 1.2 times daily maintenance requirement, which was calculated by the use of the formula TDN = $0.036W^{.75}$ (Garrett *et al.*, 1959). Rations fed to groups 1 through 4 were supplemented with either 0, 2, 4 or 8% added bentonite, respectively. All lambs were treated with an antihelminth prior to being placed on trial. A 14-day period was used to allow the sheep to adjust to the metabolism crates and to establish a constant feed intake. The adjustment period was followed by successive 7-day preliminary and collection periods. All animals were provided deionized water during the preliminary and collection periods. The rations and collected samples were analyzed for calcium, phosphorus, nitrogen dry matter, and organic matter. Calcium was determined by atomic absorption spectrophotometry and phosphorus by the method of Fiske and Subbarow (1925). Dry matter and organic matter was determined by standard procedures (A.O.A.C., 1960). Other details are as described in Trials 1 and 2.

Trial 5. An ammonium chloride solution which contained approximately 100 mg. of nitrogen per 100 ml. of solution, was used to determine if bentonite would adsorb and retain ammonia from solution and later release this ammonia when the ammonia concentration of the solution was reduced. In the first part of the trial, two hundred ml. of the ammonium chloride was added to flasks containing either 0, 4, 8 or

16 gm. of bentonite. The flasks were shaken for six hours, then refrigerated for 12 hours to allow the bentonite to settle. The concentration of nitrogen in the supernatant was then determined by the Kjeldahl procedure (A.O.A.C., 1960).

In the second portion of the trial, either 0 or 2 gm. of bentonite were added to 100 ml. of the ammonium chloride solution. The flasks were then shaken and refrigerated as described previously and 75 ml. of the supernatant was removed by pipette for determination of nitrogen concentration. The remaining 25 ml. which contained the bentonite, was then diluted to 100 ml. The flasks were again shaken for 6 hours and refrigerated for 12 hours and the concentration of nitrogen in the supernatant determined.

Trial 6. Four mature rumen-fistulated steers were fed diet 2 (Table II) for 10 days. The animals were then removed from feed for 12 hours prior to being fed 3.6 Kg. of feed via fistula. Two of the steers received in addition, 8% bentonite, mixed with the feed. Rumen samples were then taken via the fistula at 0, 1, 2, 3, 4, 5, and 6 hours after feeding. The samples were then separated into solid and liquid portions and analyzed for calcium and phosphorus as previously described.

CHAPTER IV

RESULTS

Addition of sodium bentonite in Trial 1 had no significant affect ($P > .05$) on the pH, ammonia- or urea-nitrogen levels in rumen fluid incubated with either high-concentrate or high-roughage type diets (Figure 1, 2 and 3). The high-roughage diets promoted greater ($P < .05$) levels of pH, ammonia- and urea-nitrogen than the high-concentrate diets. These results should be expected since the high-roughage diets contained a greater level of urea than did the high-concentrate diets. High-roughage rations, regardless of nitrogen source, also produce higher pH values than high-concentrate rations (Annison and Lewis, 1959). Urea in both rations was almost completely hydrolyzed in 1.0 hour; nevertheless, the ammonia-nitrogen levels continued to increase and reached maximum values after approximately 10 hours in the high-roughage diets and 6 hours in the high-concentrate diets. An explanation of this is not readily apparent.

In Trial 2, bentonite did not affect ($P > .05$) (Table III) the digestibility or retention of dietary nitrogen, but there appeared to be a trend toward improved nitrogen retention when bentonite was added to the high-roughage diet. The high-concentrate ration decreased urinary nitrogen excretion ($P < .05$) and increased nitrogen retention ($P < .05$) when compared to the high-roughage ration (Table III).

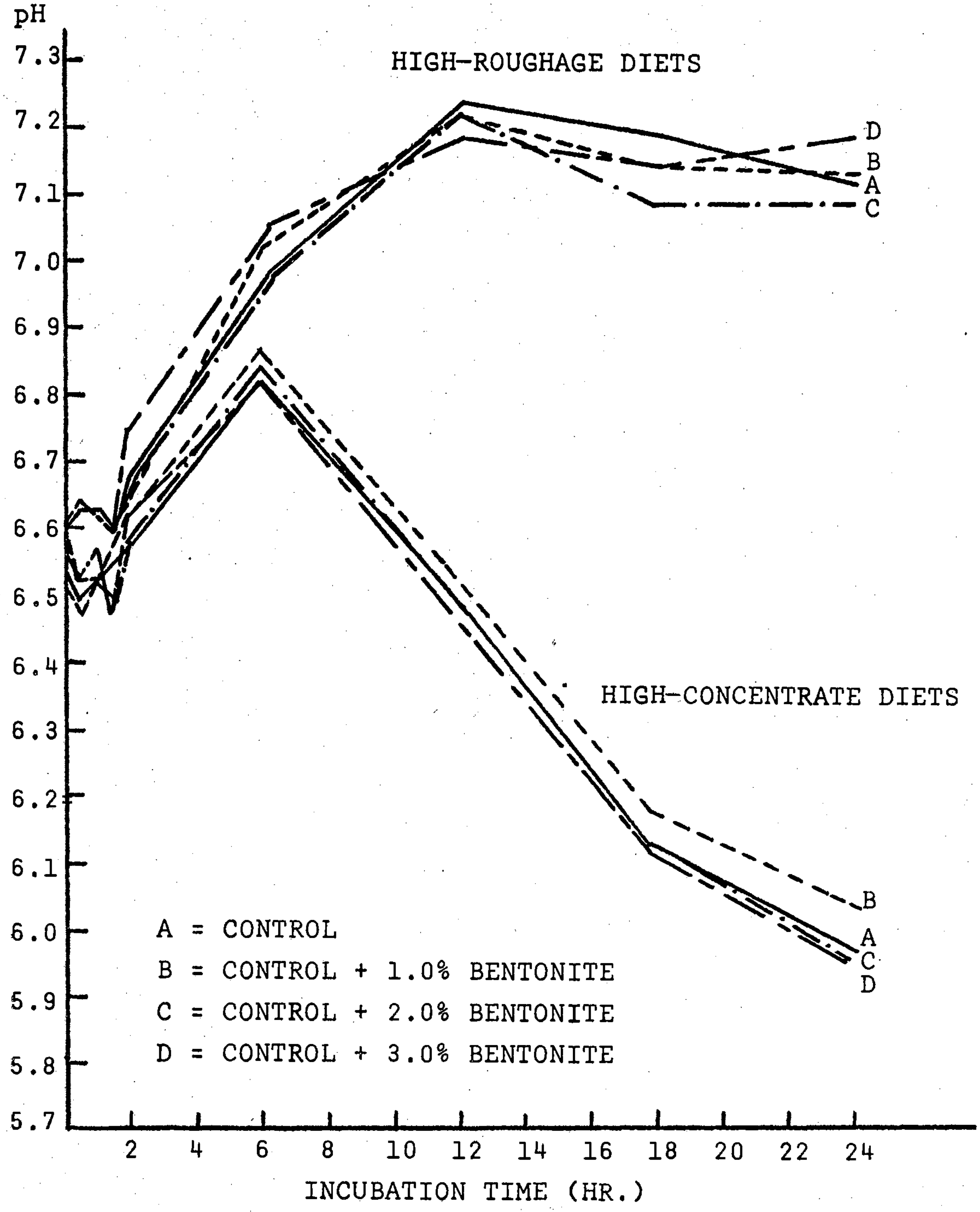


Figure 1. Effect of Sodium Bentonite on pH of In Vitro Mixtures of Rumen Fluid With High-Roughage and High-Concentrate Type Diets

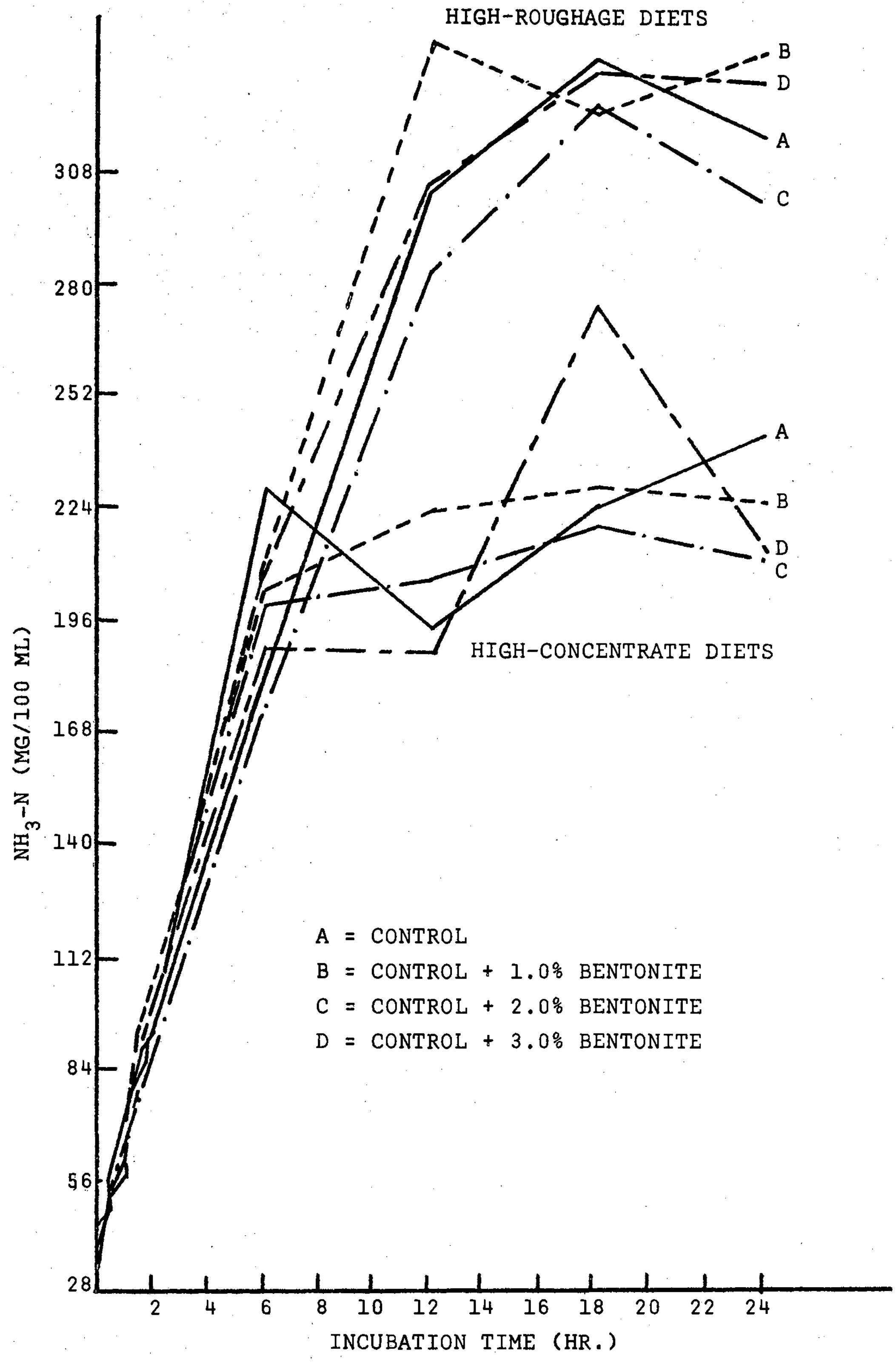


Figure 2. Effect of Sodium Bentonite on Ammonia Levels From *In Vitro* Mixtures of Rumen Fluid With High-Roughage and High-Concentrate Diets

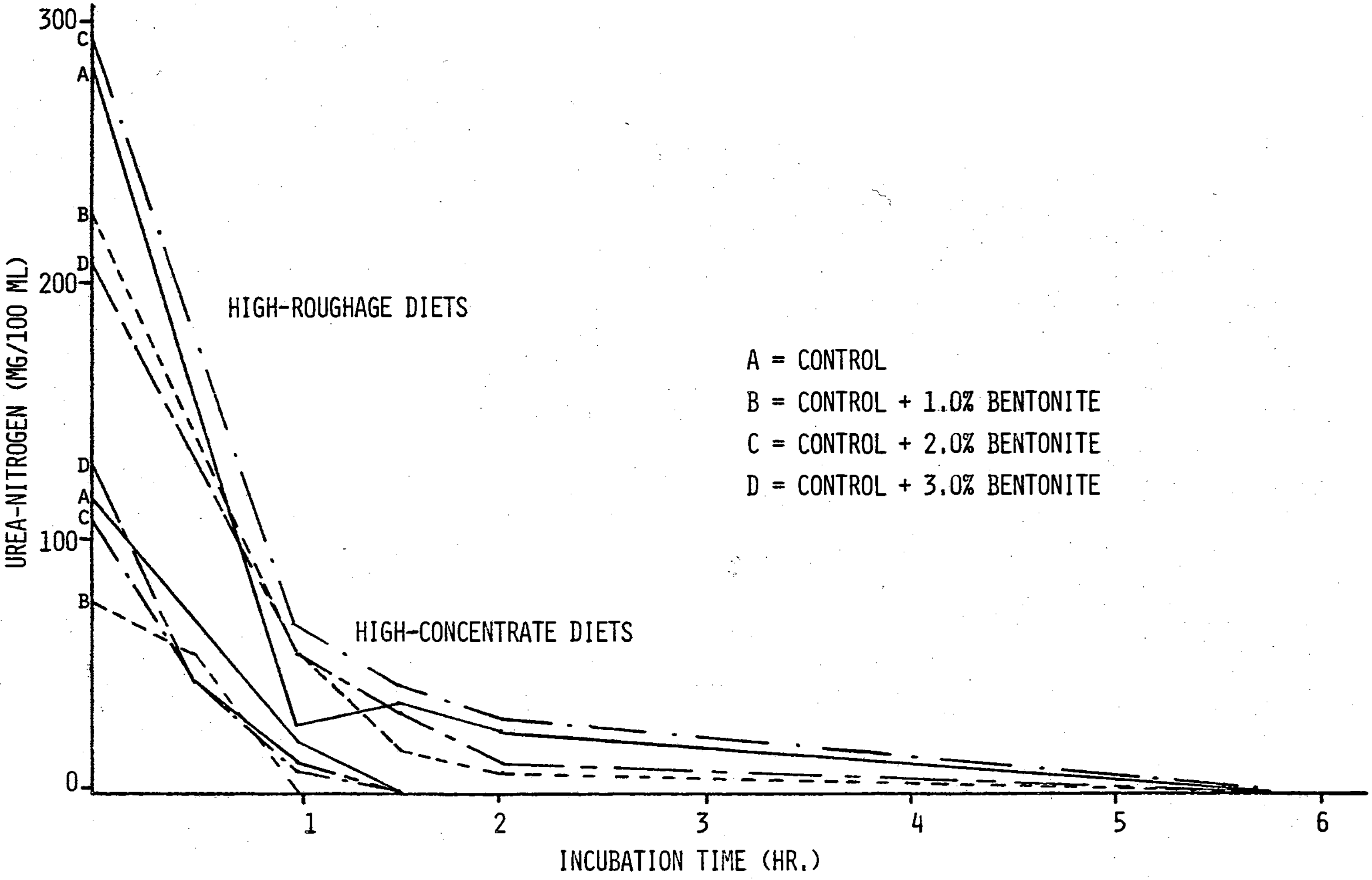


Figure 3. Effect of Sodium Bentonite on Urea Hydrolysis From *In Vitro* Mixtures of Rumen Fluid With High-Roughage and High-Concentrate Diets

TABLE III

EFFECT OF SODIUM BENTONITE ON NITROGEN DIGESTIBILITY AND RETENTION IN LAMBS FED HIGH-CONCENTRATE AND HIGH-ROUGHAGE RATIONS (TRIAL 2)

Ration	High-Concentrate			High-Roughage			
Level of Bentonite	0	2	Mean	0	2	Mean	SE[a]
N intake, gm.	22.2	22.6	22.4	25.7	26.4	26.1	
Feces nitrogen, %[b]	33.7	28.6	31.2	35.3	33.4	34.4	1.7
Urine nitrogen, %[b]	27.1	36.2	31.7	41.9	38.1	40.0*	2.8
Nitrogen retention, %[b]	39.2	35.2	37.2*	22.8	28.5	25.7	2.9

[a] Standard error

[b] Expressed as percent of intake

*Greater ($P < .05$) than corresponding mean

Bentonite did not affect ($P > .05$) gains or feed consumption in Trial 3; however, there appeared to be a trend (Table IV) toward improvement in weight gains when the product was included in the high-roughage diet. The high-concentrate diet promoted faster gains ($P < .05$) and more efficient feed utilization ($P < .05$) than did the high-roughage one. Increasing levels of bentonite in Trial 4 (Table V) decreased the digestibility of dry matter in a manner which did not differ from linearity ($P < .05$); these results agree with those of Erwin et al. (1957). Organic matter digestibility was not affected ($P > .05$) by level of bentonite; however, there appeared to be a trend toward decreasing digestibility with increasing levels of bentonite. As the level of dry matter in all rations was increased in accord with level of added bentonite, the observed trend toward a decreased organic matter digestibility as bentonite level increased might be explained on the basis of an expected increase in fecal endogenous nutrient losses in the bentonite-containing diets. Digestibility of nitrogen was not affected ($P > .05$) by level of bentonite; however, the same trend noted in the case of organic matter was also found in nitrogen and the same explanation is offered. Nitrogen retention was not affected ($P > .05$) by level of bentonite; however, the 2% level of bentonite appeared to promote greater nitrogen retention than the control diet or the other two levels of bentonite.

TABLE IV

EFFECTS OF SODIUM BENTONITE ON FEED INTAKE AND GAINS OF LAMBS FED HIGH-CONCENTRATES AND HIGH-ROUGHAGE RATIONS (TRIAL 3)

Ration	High-Concentrate			High-Roughage			
Level of Bentonite	0	2	Mean	0	2	Mean	SE[a]
Feed intake, gm./day	1356	1566	1461*	1217	1214	1216	77
Daily gain, gm.	230	195	213*	91	98	95	54
Feed/gain, gm.	6.4	8.1	7.3	13.4	12.4	12.9*	3.1

[a] Standard error

*Greater ($P < .05$) than corresponding mean

TABLE V

EFFECTS OF LEVELS OF BENTONITE UPON THE DIGESTIBILITY OF DRY MATTER, ORGANIC MATTER AND NITROGEN AND THE RETENTION OF CALCIUM, NITROGEN AND PHOSPHORUS BY SHEEP FED A HIGH-ROUGHAGE, UREA-CONTAINING DIET (TRIAL 4)

Item	Level of Bentonite, %				
	0	2	4	8	SE[a]
Dry matter intake, gm./day[b]	854	890	887	966	
Digested, %[c]	56.5	52.7	50.9	50.2	2.0
Organic matter intake, gm./day[b]	812	834	818	865	
Digested, %	57.1	54.3	53.7	53.6	1.8
Nitrogen intake, gm./day[b]	22.5	22.6	21.4	22.7	
Digested, %[c]	61.9	61.1	58.8	57.2	2.8
Urine, %	47.5	43.8	45.4	44.2	1.9
Retained, % intake	14.4	17.3	13.4	13.0	2.7
Calcium intake, gm./day[b]	5.51	5.03	5.40	6.33	
Digested, %[c]	28.8	18.2	21.6	10.6	4.6
Urine, %[c]	2.7	1.8	1.4	2.4	0.5
Retained, %[c]	26.1	16.4	20.2	8.2	4.7
Phosphorus intake, gm./day[b]	3.61	3.84	4.17	4.30	
Digested, %[c]	11.5	16.4	18.3	34.5	7.3
Urine, %[c]	0.9	0.6	0.7	0.7	0.3
Retained, %[c]	10.6	15.8	17.6	33.8	7.3

[a] Standard error of means

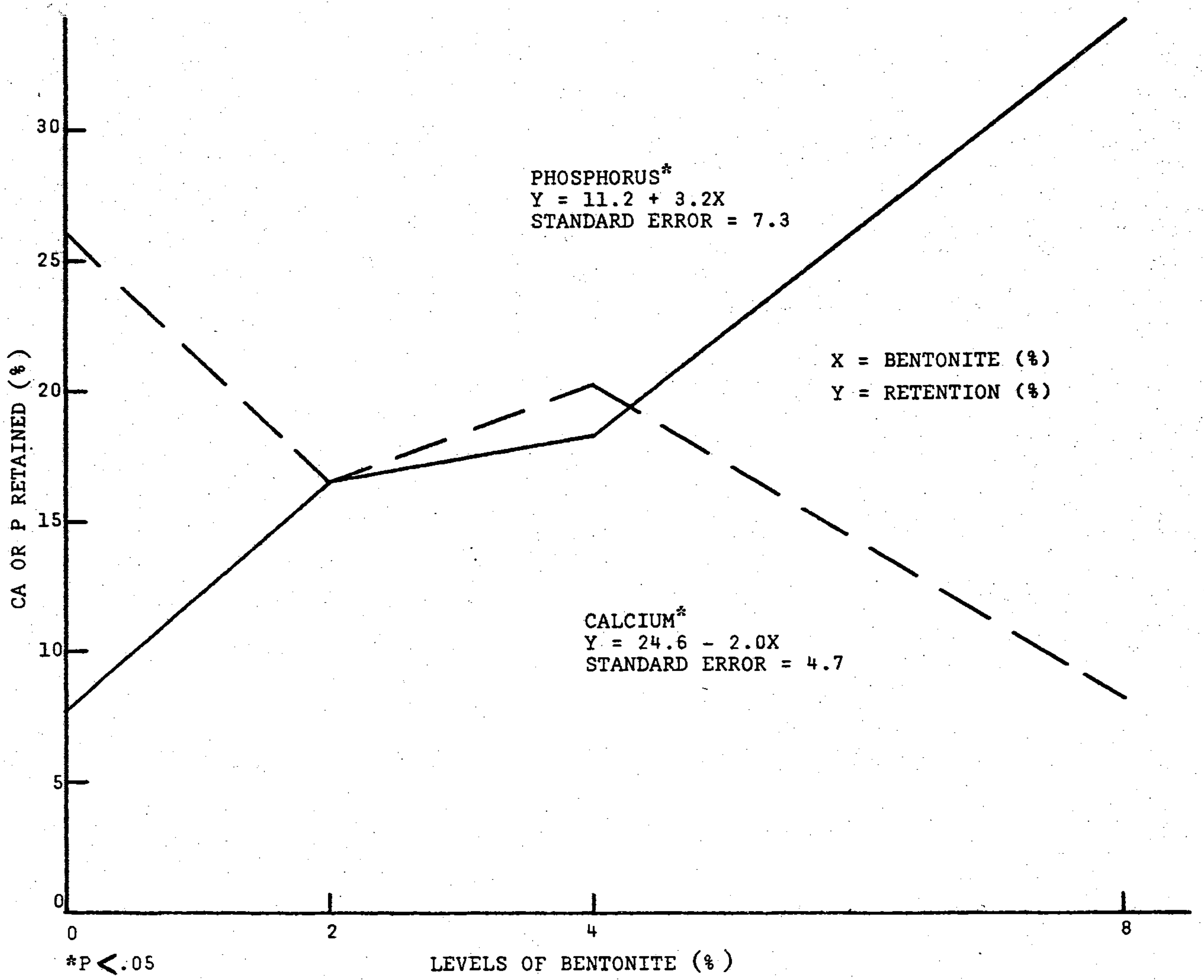
[b] Differences in intake were due to method of feed allotment (see text).

[c] Bentonite effect did not differ ($P < .05$) from linearity.

Bentonite reduced the retention of dietary calcium; the effect of bentonite level did not differ ($P < .05$) from linearity. Urinary excretion of calcium was not affected by level of bentonite, thus it only effected the fecal excretion of this element. A correlation coefficient (Figure 5) of 0.46 ($P < .05$) existed between levels of bentonite and percentage calcium retained and the regression equation was $Y = 24.6 - 2.0X$, in which X = level of bentonite and Y = percentage calcium retained. Levels of bentonite increased phosphorus retention in a manner not differing from linearity ($P < .05$). A correlation coefficient of 0.80 ($P < .01$) existed between levels of bentonite (Figure 4) and phosphorus retention and the regression equation was $Y = 11.2 + 3.2 X$, where X = level of bentonite and Y = percentage calcium retained.

The results of Trial 5 are shown in Figure 5 and Table VI. As the level of bentonite increased, the concentration of nitrogen in the supernatant decreased and the effect did not differ from linearity ($P < .05$). The results of the second part of Trial 5, in which only 2% bentonite was used, are shown in Table VI. Bentonite was able to adsorb ammonia when the ammonia concentration was high and then release it when the concentration of ammonia was decreased.

The results of Trial 6 are shown in Table VII. The inclusion of 8% bentonite in the diet of steers increased the Ca:P ratio ($P < .01$) in the solid portion of the rumen contents but had no affect ($P > .05$) upon Ca:P ratio in the liquid portion. The alteration of the Ca:P ratio in the solid material would be expected since bentonite should adsorb and retain calcium (Gustaveson, 1963) and thus prevent it from moving into the liquid portion in a soluble form. For the same reason a change in the Ca:P ratio in the liquid would be expected; however, was not found. An explanation concerns a possible variation in rumen water contents from animal to animal. Such a variation was evident when the samples were taken and also confirmed by the wide variation of actual calcium and phosphorus levels determined. The water in the rumen served as an inconsistent dilution factor and was removed from the solid portion by drying.



*P < .05

Figure 4. Effect of Levels of Bentonite on Retention of Ca and P in Sheep Fed a High-Roughage Ration

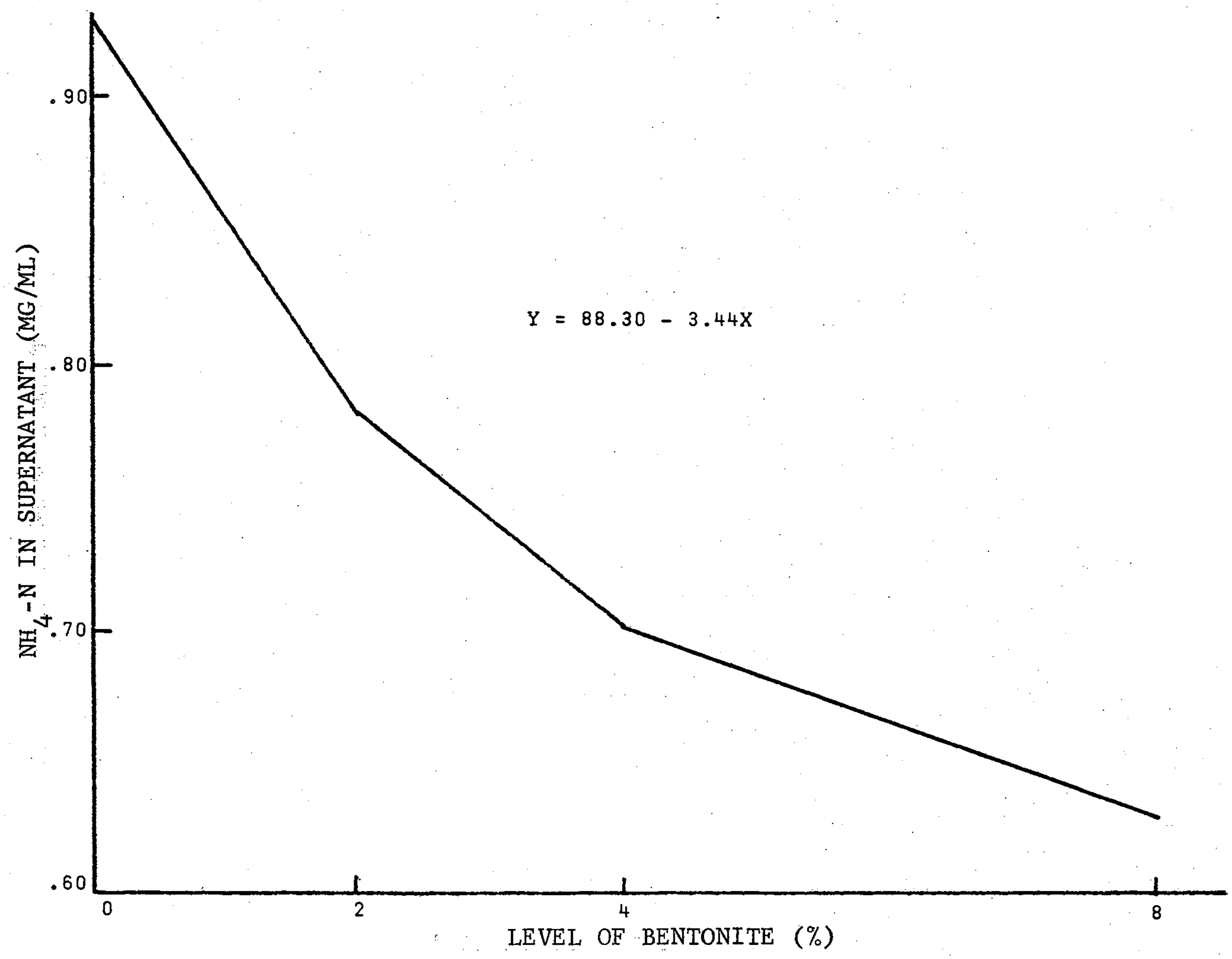


Figure 5. Effect of Level of Sodium Bentonite Upon Ammonia Level of Supernatant

TABLE VI

EFFECT OF SODIUM BENTONITE ON NH_4-N ADSORPTION AND RELEASE (TRIAL 5)

Item	Treatments	
	Control	2% Bentonite
Adsorption		
NH_4-N level		
Initial, mg/100 ml.	96.5	96.5
In 75 ml. of filtrate, Mg.[a]	72.4	61.4
In remaining 25 ml., Mg.[b]	24.1	35.1
In residue only, mg.	----	19.3[b]
Release[c]		
In filtrate, mg/100 ml.	24.0	24.0
In 5.7 ml. of residue, Mg.[d]	----	11.1
In original residue, Mg.	----	19.3
Released from residue	----	8.2

[a] Removed 75 ml. of filtrate from both by decanting.

[b] The remaining 25 ml. in the treated flasks contained 19.3 ml. of filtrate containing 15.8 mg. NH_4-N and 5.7 ml. of hydrated residue containing 19.3 mg. of NH_4-N.

[c] Distilled water (75 ml.) was added to the remaining 25 ml. of each flask to obtain the original volume of 100 ml.

[d] Volume of hydrated residue did not change.

TABLE VII

EFFECTS OF SODIUM BENTONITE ON INTRARUMINAL Ca:P RATIOS (TRIAL 6)

	Calcium:Phosphrous Ratios	
Portion of Rumen Content	0% Bentonite	8% Bentonite
Liquid	0.16:1	0.15:1
Solid	1.28:1[a]	1.44:1[b]

[a, b] Values with different superscripts differ significantly ($P < .05$).

CHAPTER V

DISCUSSION

Sodium bentonite finds its greatest use as a binder for the pelleting or cubing of animal feeds and the usual level is around 2 to 3% of the feed mixture. It is significant that this level of bentonite did not reduce the utilization of either the high-concentrate or high-roughage ration. In fact, when the high-roughage diet, containing urea was fed, 2% added bentonite apparently, though not significantly, improved nitrogen retention in two trials and growth in another. The results of Trial 5 which indicate that bentonite can adsorb ammonia when the compound is present in a high concentration and in turn release some of this ammonia when the concentration is lowered offers a possible explanation for the results found in Trial 2, 3 and 4. If this phenomenon holds true in the rumen, then peak ammonia concentrations would be less sharp resulting in less ammonia adsorption through the rumen wall (Hogan, 1961) and in an extension of protein synthesis. These effects would be of benefit to the animal as they increase the amount of microbial protein presented to the abomasum and small intestines. A second benefit would be realized because of less work by the liver in synthesis of urea from blood ammonia and by the kidney in the filtering of urea from the blood for excretion, thereby resulting in greater efficiency of feed utilization.

In Trial 1 (Figure 2) no depression in ammonia levels were noted when bentonite was added and these results appear to be at variance with those of Trials 2, 3, 4 and 5. An explanation concerns the high ammonia levels in this *in vitro* trial. These were approximately three times those found under similar *in vivo* conditions (Shidhu *et al*., 1968; Chalupa *et al*., 1964). As the total exchangeable base capacity of bentonite is only approximately 90 meq. per 100 gm. (Gustaveson, 1963), the 3% level of bentonite used, would produce a maximum ammonia level depression of only 2.7 mg. per 100 ml., or less than a 1% reduction in ammonia level. Thus detection of this small reduction would be improbable. This is also complicated by the presence of various other ions and pigments which would compete with ammonium ion for adsorption by the bentonite.

It was interesting to observe that bentonite was able to adsorb the ammonium ion (Trial 5) when its concentration was high. It is even more interesting to discover (Table IV), that the ammonium ion so adsorbed, was released in part when its concentration in the supernatant dropped. It would be logical to assume that ammonium ions released in the rumen would become available for microbial protein synthesis.

As shown previously (Gustaveson, 1963), and in Trials 4 and 6, sodium bentonite has a great affinity for divalent cations, especially calcium. The results of Trial 6 indicate that bentonite will adsorb a sufficient amount of calcium and/or release a sufficient amount of phosphorous to significantly alter the Ca:P ratio in the solid portion of the rumen content. This also indicates that there could be an increased number of phosphate ions free to combine with cations present.

If the ammonium ions were present, the formation of ammonium phosphate could take place, and thereby hold the ammonium ion in the rumen fluid for a longer period with the possibility of increasing microbial synthesis of protein in the rumen. Bentonite also could have a beneficial effect upon animals by removing ammonia from the lower portion of the intestinal tract. If there should be significant production of ammonia in the lower part of the tract and if bentonite came devoid or low in ammonia, it could decrease the passage of ammonia into the body by adsorbing it and thus reduce the energy required by the liver and kidney (Sidhu _et al_., 1968; Dang and Visek, 1960, 1964; Visek, 1962).

The vast adsorptive capacity of bentonite could predispose many other effects (Erwin _et al_., 1957; Briggs and Fox, 1954, 1956; and Laughland and Phillips, 1954a, b, 1956). When 2% bentonite was included in the high-roughage, urea-containing diet, nitrogen retention was apparently improved in two trials and growth in another. As the probability of three independent observations yielding positive results in one direction, when in fact, there were no treatment effects is small, it is suggested that the 2% level of bentonite improved animal performance when this ration was fed.

CHAPTER VI

SUMMARY

Six trials were conducted to determine the effect of sodium bentonite on *in vitro* pH, ammonia-, and urea-nitrogen levels; ammonia adsorption and release; and *in vivo* growth; dry matter and organic matter digestibility; calcium, nitrogen and phosphorus retention and intraruminal Ca:P ratios. High-concentrate and high-roughage urea-containing diets were used.

Bentonite, at a level of 1, 2, or 3%, had no affect on *in vitro* pH, ammonia-, or urea-nitrogen levels. However, increasing levels of bentonite (0, 2, 4, or 8%), when added to a solution of ammonium chloride containing approximately 100 mg. of nitrogen per 100 ml., significantly decreased the nitrogen level in the supernatant; level of bentonite exerted a linear effect. Using this system, it was found that bentonite will adsorb ammonia, when ammonia concentration is high and then release a significant portion of it when ammonia concentration is decreased.

When added to either high-concentrate or high-roughage rations at a level of 2%, bentonite had no significant affect on nitrogen digestibility or retention, growth and feed consumption; however, there appeared to be a trend toward improved nitrogen retention and growth when the high-roughage ration was used. Increasing levels of bentonite (0, 2, 4, or 8%), added to the high-roughage ration decreased the digestibility of dry matter in a manner not differing from linearity,

but organic matter digestibility was not affected. Increasing levels of bentonite in the high-roughage ration had no affect on nitrogen retention; however, the 2% level appeared to increase nitrogen retention.

Increasing levels of bentonite, when added to the high-roughage ration, reduced the retention of dietary calcium and increased the retention of dietary phosphorus; these effects did not differ from linearity. The 8% level of bentonite when added to the high-roughage ration increased the ratio of Ca:P in the solid portion of the rumen content, but had no affect on the Ca:P ratio on the liquid portion.

LITERATURE CITED

A.O.A.C. 1960. Official Methods of Analysis (9th ed.). Association of Official Agricultural Chemists. Washington, D. C.

Almquist, H. J., H. L. Christensen and S. Maurer. 1967. The effect of bentonite on nutrient retention by turkeys. Feedstuffs 39:54.

Annison, E. F. and D. Lewis. 1959. Metabolism in the Rumen. John Wiley and Sons, Inc., New York.

Beeson, W. M., T. W. Perry, C. G. VanSlyke and M. T. Mohler. 1968. The value of dehydrated alfalfa products and distillers dried grains with solubles on the utilization of high-urea supplements for finishing beef cattle. Incomplete Data, Annal Indiana Cattle Feeders Day. April, 1968.

Blakely, R. M., J. R. Jowsey and H. I. MacGregor. 1965. The effect of sodium bentonite in the diet of turkey poults. Poul. Sci. 34:1181.

Briggs, G. M. and M. R. Spivey Fox. 1954. Vitamin A deficiency in chicks produced by feeding bentonite in diets. Poul. Sci. 33:1044.

Briggs, G. M. and M. R. Spivey Fox. 1956. Vitamin A deficiency in chicks produced by adding high levels of bentonite to synthetic diets. Poul. Sci. 35:507.

Chalupa, W., J. L. Evans and M. C. Stillions. 1964. Metabolic aspects of urea utilization by ruminant animals. J. Nutr. 84:77.

Conway, E. J. 1957. Microdiffusion Analysis and Volumetric Error (4th ed.). Crosby Lockwood and Son, London.

Dang, H. C.and W. J. Visek. 1960. Effect of urease injection on body weights of growing rats and chicks. Proc. Soc. Exp. Biol. Med. 105:164.

Dang, H. C. and W. J. Visek. 1964. Some effects of urease administration on laboratory animals. Am. J. Physiol. 206:731.

Ershoff, B. H. and G. S. Bajwa. 1965. Physiologic effects of dietary clay supplements. Final report on contract number NAS 9-3905. Submitted to: NASA Manned Spacecraft Center. Houston, Texas.

Erwin, E. S., C. J. Elam and I. A. Dyer. 1957. The influence of sodium bentonite *in vitro* and in the ration of steers. J. Animal Sci. 16:858.

Fiske, C. A. and I. Subbarow. 1925. The colormetric determination of phosphorus. J. Biol. Chem. 66:375.

Garrett, W. N., J. H. Meyer and G. P. Lofgreen. 1959. The comparative energy requirements of sheep and cattle for maintenance and gain. J. Animal Sci. 18:528.

Gustaveson, A. 1963. Volclay, Data Sheet 100. American Colloid Co., Skokie, Illinois.

Hogan, J. P. 1961. The absorption of ammonia through the rumen of the sheep. Australian J. Biol. Sci. 14:448.

Jordan, R. M. 1953. Tests indicate bentonite in lamb ration beneficial. Feedstuffs 25:53.

Jordan, R. M. 1954. Experiments show bentonite has no adverse effects on livestock. Feedstuffs 26:75.

Kurnick, A. A. and B. L. Reid. 1960. Poultry nutrition studies with bentonite. Feedstuffs 32:24.

Laughland, D. H. and W. E. J. Phillips. 1954a. The effect of sodium bentonite administration on vitamin A metabolism in the rat. Can. J. Biochem. and Physiol. 32:593.

Laughland, D. H. and W. E. J. Phillips. 1954b. The absorption of vitamin A by sodium bentonite. Can. J. Biochem. and Physiol. 32:610.

Laughland, D. H. and W. E. J. Phillips. 1956. The effect of dietary sodium bentonite on the rate of growth of chicks. Poul. Sci. 35:1050.

Perry, T. W., R. C. Peretson, M. T. Mohler and W. M. Beeson. 1968. Bentonite as an additive to high urea supplement for high energy beef cattle rations. Incomplete Data, Annual Indiana Cattle Feeders Day. April, 1968.

Quisenberry, J. H. and J. W. Bradley. 1964. Sodium bentonite feeding experiments. Feedstuffs 36:33.

Quisenberry, J. H. 1966. Research reports on factors that influence laying hen performance. Feedstuffs 38:28.

Sidhu, K. S., E. W. Jones and A. D. Tillman. 1968. Effect of urease immunity on growth, digestion and nitrogen metabolism in ruminant animals. J. Animal Sci. 27:1703.

Steele, R. G. D. and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., Inc., New York, New York.

Vetter, R. L., Nelson Gay and Floyd Ransom. 1967. Evaluation of bentonite in a high-concentrate finishing ration for steers. Iowa State Univ. A.S. R98.

Vetter, R. L., Nelson Gay, F. H. McGuire and Floyd Ransom. 1968. Further studies on the use of bentonite in ground ear corn-high urea rations for steers. Iowa State Univ. A.S. R110.

Visek, W. J. 1962. Studies on urea hydrolysis in birds and mammals. Am. J. Vet. Res. 23:569.

VITA

Lethal Conrad Martin

Candidate for the Degree of

Master of Science

Thesis: SODIUM BENTONITE IN UREA-CONTAINING RATIONS FOR RUMINANTS

Major Field: Animal Science

Biographical:

Personal Data: Born in Cadiz, Kentucky, March 25, 1945, the son of Mr. and Mrs. Lethal I. Martin, Jr.

Education: Graduated from Trigg County High School, Cadiz, Kentucky, in May, 1963; received the Bachelor of Science degree from the University of Kentucky, with a major in Animal Science, August, 1967; completed requirements for Master of Science degree at Oklahoma State University in May, 1969.

Professional Experience: Raised on a general farm in Kentucky; Undergraduate Assistant in Animal Nutrition at the University of Kentucky, 1963-1967; Graduate Research Assistant at Oklahoma State University, 1967-1969.

Professional Organizations: Member of the American Society of Animal Science and Gamma Sigma Delta.