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Sodium Bentonite in Ruminant Rations

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SODIUM BENTONITE IN RUMINANT RATIONS

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INTRODUCTION

Sodium bentonite, an expanding clay is being used in many different ways in industry today. The wine industry makes use of its protein-binding properties in the clarification of wine. Bentonite is also used in the decolorization of oils, in preparation of oil-well drilling muds, and is used as a sealing agent in the bottom of earth dams and bottomless tanks. The livestock and feed industry use it in many different ways. It is used as a pellet binder, to maintain fat test in dairy animals, as a digestive aid in the poultry industry and as a "buffering agent" in feedlot rations.

Bentonite has the ability to swell to many times its original volume upon mixing with water. This is due to a unique accordian-like lattice structure and to its ion exchange capacity.

A two pronged investigation was undertaken to determine the effect sodium bentonite could have on the ruminant feeding industry. One part of the investigation sought to determine if bentonite could partially or entirely alleviate subclinical lactic acidosis with problem rations. The other part of the investigation involved the effect of bentonite on NPN and natural protein utilization.

Economic losses suffered by the feedlot industry due to acute and sub-acute lactic acidosis are incalculable. Sub-acute acidosis is characterized by lowered feedlot performance. Feedlot operators are

able to identify problem pens due to their lowered feed intake but cannot easily identify individual animals suffering sub-acute acidosis. Including buffers in the rations is one of many methods that has been proposed to combat this problem. Sodium bentonite exhibits alkaline properties in aqueous solution. If including it in feedlot rations would serve to buffer or neutralize rumen pH, it might be beneficial in reducing the economic losses from sub-acute lactic acidosis.

Burroughs and colleagues have proposed a new method for evaluating ruminant protein utilization. Their proposed system involves balancing rumen ammonia release with the energy availability of the ration. A part of their system is the concept that a certain quantity of the protein in the ration escapes microbial degradation and is passed to and absorbed from the lower tract. Different protein sources have bypass values characteristic of their solubility in the rumen. Burroughs postulates that more efficient utilization of NPN and natural protein will result from the use of the metabolizable protein concept. NPN can be used to fulfill the needs of the rumen microorganisms, while high quality protein can be bypassed to the lower tract without undergoing microbial degradation and resynthesis. The bypassed high quality protein can be absorbed as amino acids and used to meet the ruminants' amino acid requirement. Various methods have been used to slow ruminal degradation of high quality protein in order to obtain higher bypass values. Sodium bentonite: protein complexes have been shown to retard microbial degradation in vitro. If this would be true in vivo,

a high quality protein could be complexed and then bypassed to the lower tract. If amino acids from the protein:bentonite complex were available in the lower tract, a more efficient utilization of preformed protein could result.

REVIEW OF LITERATURE

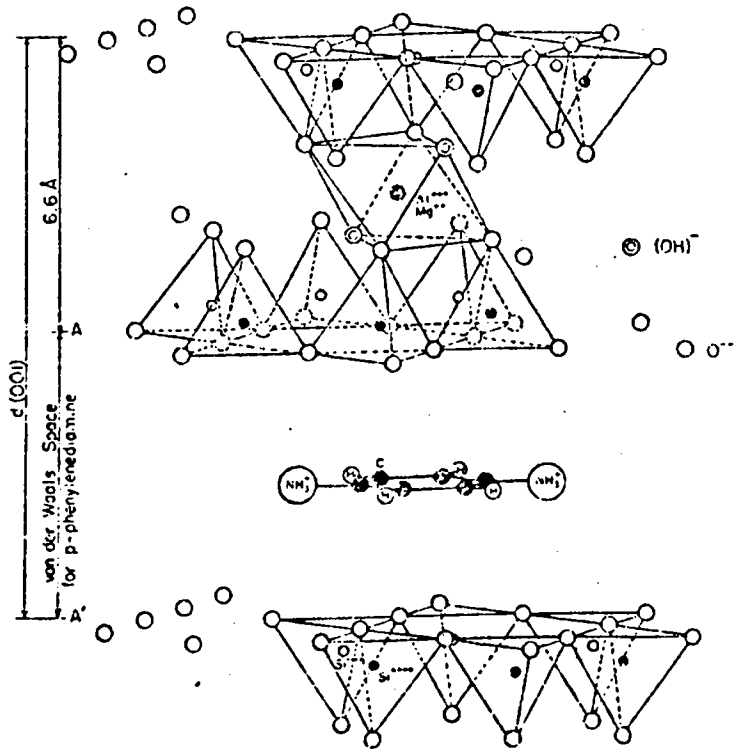
Description of Sodium Bentonite

Clays, by definition, are the products of silicate rocks which have been subjected to weathering processes for thousands of years (Buchman and Brady, 1969). They contain substantial amounts of softened hydrated aluminum silicate which has a plastic characteristic when water is added. Montmorillonite, the active mineral of bentonite, is a hydrous aluminum silicate with a tri-layered sheet structure. (Figure 1) The platelet structure is made up of two silicon-oxygen tetrahedral layers with a central layer of aluminum atoms and hydroxyl groups sandwiched between the silica layers. The approximate chemical formula is $\left[(Al, Fe)_{1.67}\ Mg_{0.33}\right]\ Si_4O_{10}\ (OH)_2\ (Na,\ Ca_{0.33})$. A pure aluminum silicate lattice structure would be in electrical balance, but weathering has substituted other metallic ions for aluminum in the central sheet of the structure. About one-sixth of the tri-valent aluminum positions in the central sheet are replaced by divalent magnesium ions. The remaining positive charge needed to balance the structure is provided by sodium or calcium ions which are loosely held in the outer silica layers of the lattice structure. In water, the exchange ions dissociate at varying rates creating a negative charge on the platelet surface. The ion exchange potential of bentonite is based on the replacement of the surface ions.

Sodium bentonite is a highly plastic colloidal clay found mainly in northern Wyoming as a naturally occurring alteration of volcanic ash. The percentage of montmorillonite in bentonites varies

Figure 1

Schematic Drawing of a Cationic Molecule Between the Hydrous Magnesium Aluminum Silicate Layers of Montmorillonite. From Hendricks, 1941.


d (001)
6.6 Å
A
A'
van der Waals Space for p-phenylenediamine
(OH)⁻
O⁻⁻
NH₂
NH₂
C

considerably depending on source. The calcium bentonites are much more broadly distributed but do not have the same properties as sodium bentonite relative to swelling or water holding capacity. In the United States, calcium bentonites are commonly found in southern states.

Structure and Properties

The pH values of most commercial bentonites are alkaline (Volclay technical data sheet 47-R). A 5% aqueous suspension of sodium bentonite has a pH between 8.5 and 10.5. The calcium bentonites will have a pH between 7.0 and 8.0.

Bentonites in dry form consist of varying sized groups of platelets adhering in clusters. When mixed in water most of the individual platelets separate but tend to remain in groups of 3 to 10 (Hendricks, 1941). The separation is believed to be triggered by the hydration and dissociation of the sodium ion itself. The negative charge of the platelets causes further separation. These factors cause expansion to proceed in an accordian-like manner as water is added to bentonite.

Sodium bentonite can hold at least 5 times its weight of water and expand 12 to 15 times its original dry volume (Buchman and Brady, 1969). In calcium bentonite, hydration of calcium and magnesium ions occurs but it is limited by the cross bonding of these divalent cations between platelets. This results in large bundles of platelets being tied together. Because of this, calcium bentonite cannot hold more than 1.5 times its weight of water.

The size of the individual platelets is about 9.5 A^{o} thick, whereas the width and length may vary from 10 to 100 times the thickness. As water is added, layers of water molecules build up around the lattice structure. The layers of water may develop to ten times the platelet thickness. Sodium bentonite has large internal and external surface area which is derived from the fact that it has a microscopic flat card-like structure. The surface area of sodium bentonite as determined by the glycerol retention method is 757 m^2 /g (Malik et al., 1972).

Protein Adsorption, X-ray Diffraction

Protein can be adsorbed to bentonite by taking advantage of the expansion of the lattice structure when water is added. Proteins, acting as cations, satisfy the electrical imbalance caused by the dissociation of sodium. Ensminger and Gieseking (1939) used this principle to see how much protein they could adsorb onto bentonite. They theorized that proteins, while satisfying the electrical imbalance, would be trapped in the expanded lattice structure. After adsorbing gelatin and albumin, they dried the resulting complex and determined by x-ray analysis that proteins were adsorbed on the bentonite.

The hydrogen-ion concentration of the suspensions during preparation is important in determining the degree of adsorption of proteins by montmorillonite (Ensminger and Gieseking, 1939). The adsorption is more nearly complete when the hydrogen-ion concentration is high which would indicate that the protein is being adsorbed as a

cation. The high hydrogen-ion concentration results in an increase in the degree of ionization of the amino or basic groups.

Protein Adsorption, pH

Care should be taken when preparing bentonite-protein complexes that complete mixing in suspension occurs. One way to insure this is to mix an alkaline protein suspension with an alkaline bentonite suspension. In alkaline suspension, both montmorillonite and protein are negatively charged, with the result that the two substances are not mutually attracted to any great extent (Ensminger and Gieseking, 1939). Under these conditions a homogeneous mixture is easily obtained. The final step in the preparation is the acidification of the mixed suspension. The montmorillonite remains negatively charged while the proteins become positively charged when the pH of the suspension becomes lower than its isoelectric pH. Under these final conditions the protein and montmorillonite mutually attract each other and are completely flocculated.

Ensminger and Gieseking (1939) determined the nature of adsorption of proteins by montmorillonite by comparing the adsorption of gelatin treated with nitrous acid to the adsorption of untreated gelatin. Nitrous acid destroys the free amino groups of proteins rendering them non-basic. The treated gelatin was not adsorbed, while the untreated gelatin was.

Montmorillonite was mixed with water and varying amounts of hemoglobin, casein, protamine, pepsin and pancreatin (Ensminger

and Gieseking, 1941). Their results showed that proteins decrease the base-exchange capacity of montmorillonite at low pH values. The different proteins affected the exchange capacity to varying degrees, depending upon the ratio of protein to montmorillonite, the pH of the final suspension and the protein source itself.

Armstrong and Chesters (1964) reported that the closer the final pH of the protein-bentonite suspension is to the isoelectric point of the protein in question, the greater the adsorption of that protein. Proteins are electrically neutral at their isoelectric point but this does not mean that they are inactive. Above the isoelectric point they are primarily negatively charged, while below the isoelectric point they are positively charged. Under both conditions they act as cations. At the isoelectric point the protein molecules neither attract or repell one another. This allows maximum adsorption of the protein molecules within the lattice structure. Cations are adsorbed by the bentonite to satisfy the excess negative charge. Any chemical treatment which would alter the basic or acidic groups of proteins would affect their isoelectric points.

Proteins do not decrease the base-exchange capacity of bentonite in an alkaline medium (Ensminger and Gieseking, 1941). As the pH is lowered, the base-exchange capacity decreases. This indicates that as the acidic properties of the proteins are increased, they are adsorbed as cations.

Protein Adsorption, Organic Substances

Jordan (1949) and Malik et al. (1972) showed that with compounds of up to 8 carbons in the alkyl chain, adsorption in water suspensions is approximately limited to the exchange capacity of the clay. With large organic cations, the adsorption occurs much beyond the exchange capacity. This leads to the conclusion that with short chain compounds, or in partial saturations with longer chain compounds, the chains lie parallel to the clay surface. The chains stand erect to the clay surface with excessive saturation with long chain molecules. This leads to adsorption beyond the exchange capacity of the clay and to a greater expansion of the crystal lattice.

Malik et al. (1972) and Hendricks (1941) stated that the extent to which the organic cation is adsorbed as its size increases is due to the increase in the magnitude of the van der Waal's forces. The adsorption is mostly due to the ionic and van der Waal's forces up to the exchange capacity and due solely to the van der Waal's forces beyond it.

Protein Adsorption, Solvents

Jordan (1949) worked with several different solvents to determine their effect upon the swelling of bentonite. He concluded that maximum expansion occurred in a polar solvent which was highly organic in nature. In liquids with these characteristics a stepwise separation of the platelets was observed as the length of the amine chain attached to the clay was increased. These separation steps

were about 4 A^{O} or approximately the van der Waal's diameter of a methyl group.

Jordan (1949) concluded that the degree of solvation was dependent on three interrelated factors: (1) the nature of the solvating liquid; (2) the degree of saturation of the clay by organic cations prior to solvation; and (3) the extent that organic matter coats the surface area of the clay particles.

Protein Adsorption, Equilibrium Conditions

Armstrong and Chesters (1964) worked with the influence of equilibration conditions on the properties of protein-bentonite complexes. They found that the adsorption of protein by bentonite occurs very rapidly with 90% of the maximum adsorption taking place in the first three minutes of equilibration. Adsorption continues slowly with maximum adsorption occurring after about 12 hours.

Changing the electrolyte concentration of the protein-bentonite suspension affects the adsorption of a protein. Armstrong and Chesters (1964) added .02 M KCl to a lysozyme-bentonite system buffered at pH 7.4. They tripled the adsorption of the protein by the addition of the electrolyte. And, they obtained 2/3 of the adsorption obtained at the protein's isoelectric point. They postulated that the increased adsorption of protein in the presence of an electrolyte may be caused by the anions becoming concentrated around the positively charged protein molecules and effectively neutralizing some of the excess charge of the protein molecule. The bentonite would thus

require a greater number of protein molecules to neutralize its negative charge.

Protein adsorbed at the isoelectric pH of the protein is not appreciably removed by allowing equilibration at a more acid pH (Armstrong and Chesters, 1964). Re-equilibrating at a pH above the isoelectric point causes protein to be desorbed. As the pH is raised above a protein's isoelectric point, molecules of protein are changed from cationic to anionic. The protein is then repelled by the negative charge on the bentonite instead of attracted to it.

X-ray diffraction analysis of protein-bentonite complexes by Armstrong and Chesters (1964) show that expansion of the clay lattice is proportional to the amount of protein adsorbed. Maximum lattice expansion occurred at 64 A^{o} when apparently two layers of protein were adsorbed. They state that more than two layers of protein does not seem likely because the entering third layer of protein would be repelled by the two layers of protein already adsorbed.

Adsorption of Nucleotides

Jacoli (1968) and Jacoli et al. (1973) reported that ribonuclease was inactivated by bentonite when the ratio of bentonite: RNase exceeded 1:1 (w/w) at pH 5. Although the ribonuclease molecules postulated dimensions are too large for the layer spacings of bentonite, with proper orientation the active portion of the molecule will fit into the layer. The inhibition of the enzyme activity follows the expansion of the clay spacings but fails when the layers expand beyond their

maximum capability. This occurs when the ratio of bentonite RNase is below 1:1. It is possible that the inactivation of the enzyme is simply a distortion of the protein molecule entering the interlayers of the clay.

Shaw (1965) tested the inactivation by bentonite of purines and pyrimidines, the products of reactions catalyzed by nucleases. The results showed that adenine, guanine, xanthine and hypoxanthine were almost completely bound at the concentrations used. Of the pyrimidines tested, only cytosine was bound by the bentonite. Nucleotides were bound only to a very small degree. All of these results suggest that the bentonite is acting as a cation exchanges in acid solutions. The degree of binding of compounds follows the relative compound basicities.

Decomposition of Protein: Bentonite Complexes

Ensminger and Gieseking (1942) tested the resistance of bentonite-adsorbed proteins to proteolytic hydrolysis. They used pepsin, active in acid media and pancreatin, active in alkaline media, in order that the rate of hydrolysis could be measured under acid and alkaline conditions. Protein-bentonite complexes were resistant to enzymatic hydrolysis under both acid and alkaline conditions when compared to uncomplexed proteins. They postulated that the bentonite was rendering the active groups inaccessible to the enzymes.

Bentonite has been found to inhibit both pepsin and pancreatine enzymatic activity in the rat (Olesen, 1972). This enzymatic inhibition was also shown to occur in vitro by preventing the lysis of blood clots by gastric juice. The enzymes are apparently adsorbed within the

lattice structure and thus inactivated.

Estermann et al. (1959), Pinck et al. (1954) and Pinck and Allison (1951) studied the digestion of protein montmorillonite complexes by soil microorganisms. All authors concluded that the complexes retarded decomposition by soil microorganisms. All of the organisms used for these experiments secreted exoenzymes which allowed digestion of adsorbed proteins without requiring the microorganisms to enter the clay lattice. The proteolysis of an adsorbed protein on a clay by an enzyme involves simultaneous adsorption of the enzymes. As the substrate is hydrolyzed by the adsorbed enzyme, the split products are desorbed. This permits the adsorption of more enzyme or of more protein on the vacated areas. Estermann et al. (1959) reported that a dried rewetted bentonite-protein complex in soil pastes is much more resistant to action by soil microorganisms than are fresh complexes in suspension. The maximum protective action occurs with low protein-clay ratios and with low moisture levels.

The importance of moisture level is illustrated by the different results reported by Estermann et al. (1959) and Pinck et al. (1954). The former conducted his experiments under high moisture conditions and concluded that bentonite was effective in slowing protein degradation only for approximately one day. Pinck et al. (1954) conducted his tests under dry conditions and his results showed that only 3.0% of the protein had decomposed at the end of 10 days of incubation. Mixing the bentonite and protein before adding microorganisms or enzymes

retards digestion of the proteins more than if all materials are added simultaneously. The former method allows the protein to bind the active bentonite sites before the enzymes offer competition.

Nitrogen Metabolism

Ruminants, just as non-ruminants, have a dietary amino acid requirement. Ruminants, however, possess the ability to convert non-protein nitrogen (NPN) into protein which can be used to meet the animals' amino acid requirement. The conversion of NPN to protein is carried out in the rumen by microorganisms (Annison and Lewis, 1959). These microorganisms allow the ruminant animal to more fully utilize feedstuffs which are of little dietary use to the non-ruminant. The microorganisms can convert a low quality roughage supplemented with NPN into an adequate diet for the ruminant.

There are several possible fates for protein and NPN (together referred to as dietary N) fed to meet the ruminant animal's protein requirement. Some of the dietary N is used for the nourishment of the rumen microorganisms. Ultimately, however, the function of dietary N is tissue synthesis and maintenance. The tissues cannot distinguish between an amino acid from preformed protein and an amino acid from protein synthesized by the microorganisms from NPN. The amino acid patterns from the two sources are different and one may be a more balanced source than the other but lysine is lysine to the tissue.

Tillman (1967) states that ruminants utilize urea by the following four basic steps:

1. Hydrolysis of urea to ammonia and carbon dioxide.
2. The ammonia nitrogen is combined with alpha-keto acids (from carbohydrate fermentation) to synthesize amino acids.
3. The amino acids are converted to microbial protein.
4. The microbial protein is then digested to amino acids in the lower tract and absorbed into the blood stream and utilized by the body tissues for protein synthesis.

The ability of the rumen microorganisms to utilize the ammonia from urea is the most important factor in determining its fate in the animal. The balance between rate of production and rate of microbial utilization of ammonia controls the extent to which it accumulates in the rumen and the amount which passes into the bloodstream and down the gut (Schwartz, 1967). The rate of microbial utilization is partially controlled by the quantity of alpha-keto acids available for the synthesis of amino acids (Burroughs et al., 1974).

Natural proteins contained in ruminant rations are attacked to a variable extent by rumen microorganisms, depending upon their solubilities. That protein which is attacked is degraded to ammonia and alpha-keto acids. The ammonia then either is absorbed from the rumen as such or is combined with keto acids for the formation of microbial protein (Annison and Lewis, 1959). That protein which escapes microbial degradation is broken down to amino acids in the lower tract. The amino acids are absorbed into the bloodstream and

utilized by the body tissues for protein synthesis.

Protein By-pass

Natural protein does not need to be digested by the rumen microorganisms in order to be utilized by the ruminant. NPN does need to be converted to be of use to the ruminant. NPN can be used to meet the N needs of the rumen microorganisms which in turn furnish microbial protein to the host animal.

The metabolizable protein concept involves the principle that more efficient use of NPN and high quality natural protein can be obtained than present feeding regimes allow (Burroughs et al., 1974). The NPN and that natural protein which is degraded meet the N needs of the rumen microorganisms. The deficiencies of the microbial protein are then made up by calculating and supplementing an amount of natural protein which will by-pass microbial degradation. The energy content of the ration helps to determine the amount of NPN that can be used.

Several methods have been utilized to increase the quantity of by-passed protein. Sherrod and Tillman (1962) increased nitrogen retention by heating highly soluble soybean meal. Heating caused a reduction in nitrogen solubility and lower levels of ruminal ammonia. Driedger et al. (1969) reported increased average daily gains and decreased feed:gain ratios with steers when tannic acid treated soybean meal was used as the protein supplement. A lamb metabolism trial showed an increase in nitrogen retained as a percent of intake with tannic acid treated soybean meal (Driedger et al., 1969).

Bentonite and Silage Fermentation

Bentonite has been used as an aid in the production of corn silage (Everson et al., 1971). Adding bentonite before ensiling at .5% and 1.0% of the wet weight significantly increased pH, organic acid production, incorporation of ^{15}N urea into microbial protein, decreased free alpha amino-N and eliminated seepage. The authors stated that the elimination of seepage appeared to be one of the most beneficial effects of the bentonite addition. This can be attributed to the water absorption characteristics of bentonite. The increased pH allows the microorganisms more time to utilize NPN for microbial protein synthesis. The neutralizing effect along with the ion exchange properties of bentonite could also be responsible for the increased levels of organic acids. An alternate theory, which could explain the increase in protein N with bentonite addition, is that the bentonite may function to protect endogenous plant protein from extensive degradation during fermentation.

Bentonite in Animal Rations

Little in vitro work has been done with bentonite and rumen microorganisms. Erwin et al. (1957) showed in vitro that nearly a straight line relationship existed between the amount of bentonite present and the amount of carotene that was unavailable to microorganisms. They demonstrated that the carotene was adsorbed and not destroyed. By adding acetone to the experimental medium, the carotene was quantitatively released. Carotene adsorption could be a problem in purified or semipurified diets, but in practical diets other compounds compete for the bentonite binding sites such that most of

the carotene remains unbound (Briggs and Spivey, 1954).

Bentonite can adsorb cations under certain conditions and release them at a later time when conditions change. Martin et al. (1969) demonstrated in vitro that the ammonium ion will be adsorbed when the concentration of the ion is high. And, when the concentration is lowered, the bentonite will release some or all of the ion. This could be a significant effect in the rumen that could result in increasing the utilization of NPN.

Several in vivo beef and sheep finishing trials, using urea supplementation with sodium bentonite, have been inconclusive. Most trials have failed to show a significant increase in average daily gain, feed conversion, or feed intake with sodium bentonite supplementation (Erwin et al., 1957; Martin et al., 1969; Mendel, 1971; Vetter et al., 1968). Vetter (1967) did show an increase in feed intake and an improvement in average daily gain in one trial. Martin et al. (1969) and Erwin et al. (1957) reported that bentonite had no effect upon the digestibility of nitrogen but that it slightly improved nitrogen retention. They also stated that increasing the level of bentonite fed did not decrease organic matter digestibility but did decrease dry matter digestibility in a manner not differing from linearity. Bentonite reduces the retention of calcium, phosphorus and magnesium which suggests that additional mineral supplementation may be required with sodium bentonite supplementation (Rindsig and Schultz, 1970

and Martin et al., 1969).

Sodium bentonite has significantly aided in maintaining milk fat in dairy animals when high concentrate, milk fat depressing diets have been fed (Bringe and Schultz, 1969; Rindsig et al., 1969; Rindsig and Schultz, 1970). Factors related to the reduced milk fat have been decreased acetic acid and increased propionic acid in the rumen, which is characteristic of changing from high to low roughage rations. Reduced plasma acetic acid and reduced acetate uptake by the mammary glands are also related to reduced milk fat. Bringe and Schultz (1969) showed that bentonite addition to fat depressing rations resulted in a greater mammary uptake of acetate. All three sources reported a higher acetate: propionate ratio with bentonite supplementation.

Lactic Acidosis

Lactic acidosis occurs when ruminants are subjected to a rapid change from a low concentrate to a high concentrate ration or when they consume an unaccustomed large amount of grain after adaptation to a high concentrate ration. The two forms of lactic acidosis are acute and sub-acute or chronic acidosis (Mackenzie, 1966). The animal in most cases dies from acute acidosis while performance is lowered to varying degrees with sub-acute acidosis. The external symptoms of acidosis are such that few feedlot animals with acute acidosis are saved from death and few individual cases of sub-acute acidosis are recognized.

Several interrelated changes are taking place in the rumen and in the animal when both forms of acidosis occur. These changes begin with the ingestion of feeds rich in readily fermentable carbohydrates which tend to promote the development of microbial species which usually do not dominate the intraruminal population (Dunlop and Hammond, 1965). The change in microbial population brings about a change in end products of fermentation and also a change in rumen pH. The decrease in pH, along with the change in microbial population and the accumulation of lactic acid, act together in causing the animal problems.

The accumulation of lactic acid, especially D-lactic acid, in the rumen causes the concentration of lactic acid to rise in the blood-stream. D-lactic acid is not metabolized by the animal, while L-lactic acid is (Hinkson et al., 1967). The D-lactic acid overwhelms the blood buffering system in acute lactic acidosis and the animal dies if the blood pH decreases enough (Mackenzie, 1966).

The degree of sub-acute acidosis determines the animal's subsequent performance. The discernible external symptoms include reduced intake, a sluggish appearance and in some cases diarrhea (Tremere et al., 1967). These symptoms are such that the feedlot operator experiences difficulty picking out individual animals suffering from sub-acute acidosis. If an entire pen of animals appear sluggish and have decreased their average daily feed consumption, then the feedlot operator can take steps to alleviate the problem such as increasing ration roughage levels.

If an animal is suffering from a mild case of sub-acute acidosis, he may decrease feed consumption for a few days. In more severe cases of sub-acute acidosis, there is an irritation to the rumen epithelial tissue which eventually causes sloughing of the tissue and bleeding ulcers (Dunlop and Hammond, 1965). This affects metabolism and absorption of nutrients by the rumen wall which consequently lowers animal performance. The resultant liver abscesses may also play a part in reducing performance.

Various methods have been utilized to try to decrease the incidence of lactic acidosis. Considerable research with various alkali supplements has been conducted to attempt to maintain a near normal pH and to improve the performance of feedlot animals receiving high concentrate rations (Wise et al., 1968; Calhoun and Shelton, 1969). Studies by Wise et al. (1965) with the addition of sodium bicarbonate to high concentrate rations showed increased feed intake and rate of gain. These results conflict with those of Kromann and Meyer (1966) who fed sodium bicarbonate at 5 and 12% levels with resulting decreases in feed intake and daily gain. Kissinger (1971) reported an increase in total feed intake with the addition of hydroxide. Burkitt (1972) felt that sodium bentonite helped feedlot animals maintain maximum intake and lessened the effects of sub-acute acidosis. He felt that the alkaline properties helped to maintain rumen pH.

Summary of Literature

The preceding review points out that sodium bentonite is not an inert colloidal clay as it is labeled in many article introductions. Rather, it is an active material which readily complexes with many different substances. The role that sodium bentonite will play in the livestock industry is uncertain. The literature cited has shown inconclusive results in several *in vitro* and *in vivo* trials which used sodium bentonite in some manner. This thesis seeks to clarify the role of sodium bentonite in ruminant rations.

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THE EFFECT OF SODIUM BENTONITE ON IN VITRO AMMONIA RELEASE AND ON NITROGEN UTILIZATION IN LAMBS[1]

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Summary

Four in vitro rumen ammonia (NH_3) release experiments were conducted with combinations of N sources and sodium bentonite (NaB). Twenty mg N were weighed into each in vitro tube for all trials. The 4 hr NH_3 release values for trial 1 were 2.32 mg (SBM), -.10 mg (SBM + NaB), 4.04 mg (casein), 3.36 mg (Casein + NaB), 19.78 mg (urea) and 15.64 mg (urea + NaB). The second and third in vitro trials tested various ratios of SBM:NaB. Four hr NH_3 release values were 2.32 mg (1:0), .68 mg (1:1), .78 mg (2:1), .85 mg (3:1) and 1.79 mg (4:1) for trial 2. The 24 hr NH_3 release values for trial 3 were 18.3 mg (1:0), 13.5 (2:1), 13.6 mg (3:1), 13.5 mg (4:1), 13.7 mg (5:1) and 16.2 mg (10.1). Trial 4 showed that the release of NH_3 from a urea:NaB (1:10) mixture was slowed by mixing with H_2O and drying after 4 hr incubation. A 3 x 2 factorial experiment was conducted (3 N sources, with and

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without NaB) to determine the N utilization of lambs on high roughage rations supplemented with SBM, SBM +NaB, U, U + NaB, 1/2 SBM-N + 1/2 U-N and 1/2 SBM-N + 1/2 U-N +NaB. The NaB treatments had higher N digestibility ($P < .05$). SBM had higher organic matter digestibility, N retained as a % of absorbed and N digestibility (all $P < .01$) than the U supplemented lambs.

Key Words: Sodium Bentonite, Ammonia release, Nitrogen Utilization, Soybean meal, urea.

INTRODUCTION

Sodium bentonite is a volcanic colloidal clay containing principally montmorillonite. It has a lattic structure which greatly expands upon absorption of water and cations. Its binding properties are used to an advantage in pelleting.

It has been demonstrated that sodium bentonite will absorb protein as cations (Ensminger and Gieseking, 1941; Pinck et al., 1954; Armstrong and Chesters, 1964). The proteins absorbed are slowly released from the sodium bentonite lattice under certain conditions (Ensminger and Gieseking, 1942; Estermann et al., 1959). Erwin et al. (1957), Martin et al. (1969) and Mendel (1971) conducted in vitro and and in vivo experiments to determine if sodium bentonite would slow the release of nitrogen from urea. The purpose of these experiments was to determine if complexing sodium bentonite with proteins and/or urea would have an effect of N utilization. Various ratios of sodium bentonite to protein were used and different combinations of water and drying were

used to determine the effects on absorption of protein by bentonite.

MATERIALS & METHODS

Trial I

This trial was conducted to determine if in vitro rumen ammonia release could be slowed by adding sodium bentonite (NaB) to casein, soybean meal (SBM) and urea (u). Four different treatments were tested: (1) a control with no NaB added, (2) N source mixed with NaB dry, (3) N source mixed with NaB and an amount of water equal to the weight of the dry mixture, and (4) treatment 3 dried at 65 C and reground through a 20 mesh screen. Sodium bentonite and N sources were mixed so that the NaB:N ratio was constant. Twenty mg N from all treatments were weighed in duplicate into 50 ml plastic centrifuge tubes. Equal volumes of rumen fluid were obtained from two steers fed either alfalfa hay or a 60% ground corncob ration. The rumen fluid was strained through four layers of cheesecloth and transported to the laboratory in a thermos container warmed and gassed with CO_2. Raw rumen fluid and McDougall's buffer (McDougall, 1948) were mixed in equal quantities to serve as inoculum. Thirty ml of inoculum were pipetted into each in vitro tube. The tubes were gassed with CO_2, stoppered and placed in a water bath at 39 C. Fermentation was stopped at 0, 4 and 24 hours of incubation by the addition of 1 ml of 5% $HgCl_2$ to the appropriate tubes. Samples were centrifuged for twenty minutes at 7000 xg. The supernatant was frozen at -20 C for subsequent analysis. Ammonia

was determined in triplicate by the Conway microdiffusion technique (Conway, 1958).

Trials II and III

These two trials were conducted to determine the effect on in vitro rumen ammonia release with different ratios of SBM to NaB. Results in trial I indicated that combining water, NaB and SBM, drying and regrinding was the most effective method used in slowing ammonia release. This method was utilized in Trials II and III to treat the various ratios of SBM to NaB. In Trial II three negative controls were included (Figure 1): (1) SBM heated in the drying oven at 65 C, (2) SBM and water dried at 65 C and (3) SBM neither moistened or heated. Four ratios of SBM:NaB were tested in Trial II (1:1, 3:1, 5:1, and 10:1). The seven ratios tested in Trial III were (Table 3) 1:0, 1:1, 2:1, 3:1, 4:1, 5:1 and 10:1. The rumen in vitro procedures used in Trials II and III were identical to that used in Trial I except Trial II included an 8 and 12 hr sample and Trial III omitted the 4 hr samples.

Trial IV

The purpose of this trial was to determine if sodium bentonite would affect in vitro rumen ammonia release from U in a manner similar to that of SBM. A 10:1 ratio of bentonite to U was used to obtain an end product equal in N content to the U product in Trial I. Four products were used: (1) U, (2) U and NaB mixed dry, (3) U and NaB mixed dry and heated at 65 C for 24 hr and (4) U mixed with NaB and an amount of

water equal to the weight of the dry ingredients, heated at 65 C for 24 hours. Treatment 4 was ground through a 20 mesh screen after drying. The in vitro procedure was identical to that used in Trial I.

Trial V

The results of the previous in vitro trials were used as the basis for a replicated metabolism trial. A 3 x 2 factorial metabolism trial with 24 lambs per replication was conducted to determine the effect of sodium bentonite (NaB) on nitrogen metabolism and organic matter digestibilities in high roughage rations. The rations (Table 1) consisted of corncobs, corn starch, minerals, vitamins, salt and N source. The six N supplements were SBM, U, and U-N and the preceding three complexed with NaB. Starch was used to make the rations isocaloric.

A 3:1 SBM:NaB mixture was prepared by mixing the two dry ingredients and then adding an amount of water equal (w/w) to that of the dry ingredients. The wet mixture was dried at 65 C in a forced air oven and ground through a 2 mm screen before use. A 1:2 U:NaB mixture was similarly prepared. The proper amounts of SBM:NaB and U:NaB dry complexes were mixed to obtain the 1/2 SBM-N:NaB, 1/2 U-N:NaB supplement.

The lambs were individually fed 52 g of ration per $kg^{.75}$ of body weight throughout the trial. The last three days of the ten day preliminary feeding period was done in the metabolism stalls to adjust the lambs to the stalls. The metabolism stalls resembled those described by Briggs and Gallup (1949).

Total urine and fecal collections were made for a seven day period. Urine was collected in a vessel containing 50 ml of 6 N HCl, subsampled daily and stored under refrigeration until analyzed. Feces were frozen daily for subsampling at the end of the collection period.

Nitrogen contents of the urine, dried feces and rations were determined by the Kjeldahl method (A.O.A.C., 1960). Dry matter contents of the feces and rations were determined according to A.O.A.C. (1960).

Following the last day of each seven day collection period, rumen fluid samples were taken by suction strainer (Raun and Burroughs, 1962) at 1, 2 and 4 hours postfeeding. The pH of each sample was immediately determined and 1.0 ml of 5% $HgCl_2$ solution was added to halt fermentation. The samples were then stored at -20 C in plastic bags until analyzed for NH_3-N (Conway, 1958).

Orthogonal comparisons were used to compare treatment means (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

The results from Trial I (Table 2) show that mixing the nitrogen sources with sodium bentonite (NaB) slowed the ammonia release in all treatments at all hours. The ammonia levels from the SBM:NaB mixture after 4 hr of incubation indicated that little N was being released from the SBM:NaB complex or that all N being released was being fixed by the microorganisms. Approximately 10% of the potential free ammonia was released in the SBM control after 4 hours. After 24 hr

of incubation, about 50% of the potential ammonia present in the SBM control had been released while the NaB combinations had released from 7 to 15% of the potential ammonia. The SBM mixed with water and NaB and dried released the least ammonia after incubating 24 hours. This agrees with Estermann et al. (1959) who reported that dried rewetted bentonite protein complexes are much more resistant to microbial action than fresh complexes.

The second most effective treatment was SBM, NaB and water with no drying. This treatment resulted in a product that was an extremely thick paste which would not disperse in the in vitro tube. This was true also with both casein and U products made in this manner. The surface area presented to the rumen microorganisms with these products is small which undoubtedly influenced NH_3-N release.

Casein was affected in a manner similar to SBM but to a lesser extent (Table 2). The ammonia release from the casein:NaB mixture with no added water after 4 and 24 hr of incubation was not greatly lower than the control. The casein in these samples floated to the surface when the rumen fluid was added, while the NaB remained at the bottom. This resulted in a poor casein:NaB complex. In the two treatments where casein was complexed with NaB before being placed in the in vitro tube, NH_3-N release is lower than the control after both 4 and 24 hr of incubation. It thus appears that NH_3-N release from casein can be effectively slowed if the casein is complexed prior to the addition of inoculum.

A possible explanation for only slight inhibition of NH_3-N release from U by NaB is the size of the U molecule itself. With casein and SBM, both long chain molecules, van der Waals forces are acting (Jordon, 1949; Malik et al., 1972). The van der Waals forces aid in binding the absorbed proteins so that they are not as readily exchanged as U. Since the cationic groups of casein and SBM are the amino groups (Ensminger and Gieseking, 1939), the amino acids from these proteins are partially protected from microbial degradation. Steric hindrance may also be involved in the slowed release of NH_3-N from protein: bentonite complexes. When long chain proteins, such as SBM, are absorbed within the NaB lattice structure, it may be difficult for them to be desorbed. It also may be difficult for the proteases from the microorganisms or the microorganisms themselves to be absorbed within the NaB lattice when binding sites are already occupied. If proteins are combined with NaB in some way to protect them from microbial action in the rumen, high quality protein can be bypassed to the abomasum without undergoing degradation and resynthesis. More efficient use of both high quality protein and NPN could then be realized. The rumen microbial N needs could be met with NPN and the deficiencies of microbial protein could be made up with by-passed natural protein.

The purpose of both Trials II and III was to determine the highest ratio of SBM:NaB that could result in significantly slowing the release of NH_3-N from the SBM. Mixing SBM and NaB in a 1:1 ratio

results in approximately a 25% crude protein product on a dry matter basis. This could cause dustiness and palatability problems in some rations. NaB has been added to complete dairy rations at 10% of the diet but no advantage was shown over the 5% level (Rindsig et al., 1969).

Figure 1 and Table 3 show the results of these trials. They agree with Trial I showing that the addition of NaB slows the release of NH_3-N from SBM. Increasing the ratio of SBM:NaB also increased the amount of NH_3-N released. The 1:1 ratio had the greatest inhibiting effect, while the 4:1 (Trial II) and the 10:1 (Trial III) had the least inhibiting effect. Trial II (Figure 1) showed that heating the SBM without NaB and heating SBM and water without NaB had no effect on NH_3-N release.

Trial IV was conducted to determine if U was affected in a manner similar to SBM by NaB, water and heat. The results (Figure 2) indicate that it was affected in a manner resembling SBM:NaB complexes but to a different degree. The release of NH_3-N was affected at all incubation hours but the effect was slight after eight hours. There was a difference among the NaB treatments at 4 hours with the U and NaB mixed with water and dried releasing the least NH_3-N. It can be seen that heat has little effect when urea and NaB are mixed dry. The differences between the NaB treatments largely disappear after the 4 hr incubation with all of the U:NaB complexes releasing nearly the same amounts of NH_3-N after 8, 12 and 24 hr of incubation. Although the effect of NaB has nearly disappeared after 4 hr, the value for the U:NaB

and water, dried is less than half that of the U control at this time. If this would occur in vivo, larger amounts of the urea present might be used. More of the NH_3-N from U treated in this manner might be available for microbial protein synthesis.

In Trial V, grams of fecal N, urinary N and N retained (Table 4) were divided by the metabolic weights of the respective lambs to adjust for the method of feeding. When this adjustment was made, grams of fecal $N \cdot ^{75}$, urinary $N \cdot ^{75}$ and N retained $\cdot ^{75}$ were higher for the NaB lambs ($P < .01$, $P < .10$ and $P < .01$ respectively). This is a reflection of the greater N content of the NaB rations. There was a significant replication and treatment by replication interaction in nitrogen retained $\cdot ^{75}$ and nitrogen digestibility. It was felt this was due to chance alone since the only difference between replications was time and animals.

Table 4 shows the NaB improved apparent N digestibility, N retained as a % of absorbed and apparent organic matter digestibility when the SBM + NaB treatment was compared to its control. This may show that the NaB is protecting the SBM from rapid microbial degradation, allowing it to be bypassed to and digested in the lower tract. The depression in organic dry matter digestibility with urea and NaB cannot be explained with the available date. The rumen ammonia levels from the U-NaB treatment would indicate that the N was released in the rumen. Since the fecal ash of the NaB fed lambs was much higher than the controls, organic matter digestibilities were calculated.

When the SBM treatments were compared to the U treatments,

organic matter digestibility ($P < .001$), grams N retained/$kg^{.75}$ ($P < .001$), N retained as a percent of absorbed ($P < .001$) and N digestibility ($P < .001$) were higher in the SBM treatments (Table 4).

These results agree with those of Oltjen et al. (1971) who concluded that U is poorly utilized on low energy diets because of insufficient energy and carbon skeletons for microbial synthesis. U is rapidly broken down in the rumen, while the breakdown of SBM is a slower process. The NH_3 from SBM can be more effectively utilized because energy and carbon skeletons from the SBM and the roughage are available when the NH_3 from the SBM is released. The utilization of ammonia present from the release of N from either SBM or U depends upon the amount of energy available for microbial synthesis (Burroughs et al., 1974).

The third orthogonal comparison was made to determine if there was an interaction between SBM and U when 1/2 of the supplemental N came from each. All SBM and U treatments were combined and compared to the combination of both SBM-N + U-N treatments. When this was done the SBM +U was lower ($P < .01$) by 2.25 percentage units in organic dry matter digestibility (Table 4). None of the other parameters were significantly different.

The apparent N digestibility and N retained as a % of absorbed data from this metabolism trial would indicate that SBM is having an effect beyond that of supplying supplemental N. Since all rations were isocaloric, it is not the added energy from the SBM. A possible

explanation would be that a portion of the SBM is bypassed to the abomasum and absorbed. The preformed amino acids thus absorbed may compliment the microbial protein formed from the U and SBM that is degraded in the rumen. Also, the NaB combination with the SBM-N and U-N may have bypassed enough SBM and slowed the release of U enough to enable an increase in organic dry matter digestibility.

The rumen samples obtained on day 18 showed that the effect of NaB was different for each N source (Table 5). As 1 hr intakes were variable, the levels of NH_3-N were adjusted to equal N intakes. Lambs receiving NaB had lower rumen NH_3-N levels at hr 2 ($P < .05$) and 4 ($P < .01$) but not at hour one. The values indicate that all of the SBM-N is not tied up at the 3:1 ratio. NH_3-N release might be lower at hour 1 if the SBM:NaB ratio was decreased. The microorganisms utilize the SBM-N that is not bound first and then utilize the NaB bound SBM-N which is less available. The 2 and 4 hr NH_3-N levels with the NaB treatments are an indication of this. The lowered NH_3-N levels indicate that the N source is being protected from degradation in the rumen. This could also mean that more high quality protein is being bypassed to the abomasum for absorption.

When the SBM treatments were compared to the U treatments, the rumen ammonia levels were lower for the SBM lambs at hour 1 ($P < .001$), 2 ($P < .001$) and 4 ($P < .01$) (Table 5). The rumen ammonia levels at all hours were not significantly different when the SBM and U treatments were compared against the SBM-N + U-N

treatments. These data would indicate there is no interaction between SBM and U that affects the release of N in the rumen.

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TABLE 1. COMPOSITION OF RATIONS FOR METABOLISM TRIAL

Ingredients	IRN[a]	Treatments					
		Control			Sodium Bentonite		
		SBM	U	SBM + U	SBM	U	SBM + U
Corn cobs	1-02-782	77.25 kg	77.25 kg	77.25 kg	72.02 kg	72.02 kg	72.02 kg
SBM	5-04-612	14.75	-	7.38	14.99	-	7.49
Urea	-	-	2.43	1.21	-	2.47	1.23
Corn starch	4-02-889	-	12.60	6.30	-	12.74	6.37
Na bentonite	-	-	-	-	5.00	5.00	5.00
Dicalcium phosphate	6-01-080	1.33	1.94	1.63	1.11	1.94	1.63
Molasses	4-04-696	6.00	6.00	6.00	6.00	6.00	6.00
Salt	-	.30	.30	.30	.30	.30	.30
Trace minerals[b]	-	.03	.03	.03	.03	.03	.03
Percent N		1.84	1.61	1.59	1.92	1.51	1.95
Percent ash		7.48	5.77	7.48	11.19	10.51	10.15

[a]International Reference Number.

[b]Premix contained 10% Mn, 10% Fe, 10% Zn, 1% Cu, .3% I and .1% Co.

TABLE 2. IN VITRO NH_3-N RELEASE AS AFFECTED BY SODIUM BENTONITE, WATER AND DRYING

N source	Treatment[a]			
Hr of incubation	Control	Na bentonite	Na bentonite water[b]	Na bentonite water[b],dried[c]
		mg of NH_3-N released[d]		
Soybean meal				
4 hr	2.32[e]	-.10	.17	-.04
24 hr	10.79	3.59	3.82	1.51
Casein				
4 hr	4.04	3.36	1.93	0.56
24 hr	16.78	12.42	5.42	5.28
Urea				
4 hr	19.78	15.64	14.72	11.59
24 hr	17.17	15.43	14.47	15.41

[a]Ratios of N source to sodium bentonite; SBM (1:1), casein (1:2), urea (1:10).

[b]Water added w/w to dry ingredients.

[c]Dried at 65 C for 24 hours.

[d]Twenty total mg nitrogen added per sample.

[e]Average of duplicate incubations replicated on different days with the appropriate hour inoculum ammonia levels subtracted from the treatments.

FIGURE 1. IN VITRO RUMEN NH_3-N RELEASE AS AFFECTED BY DIFFERENT RATIOS OF SBM:NAB[a]

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[a]All SBM, NaB samples mixed with H_2O (w/w) and dried at 65 C for 24 hours.

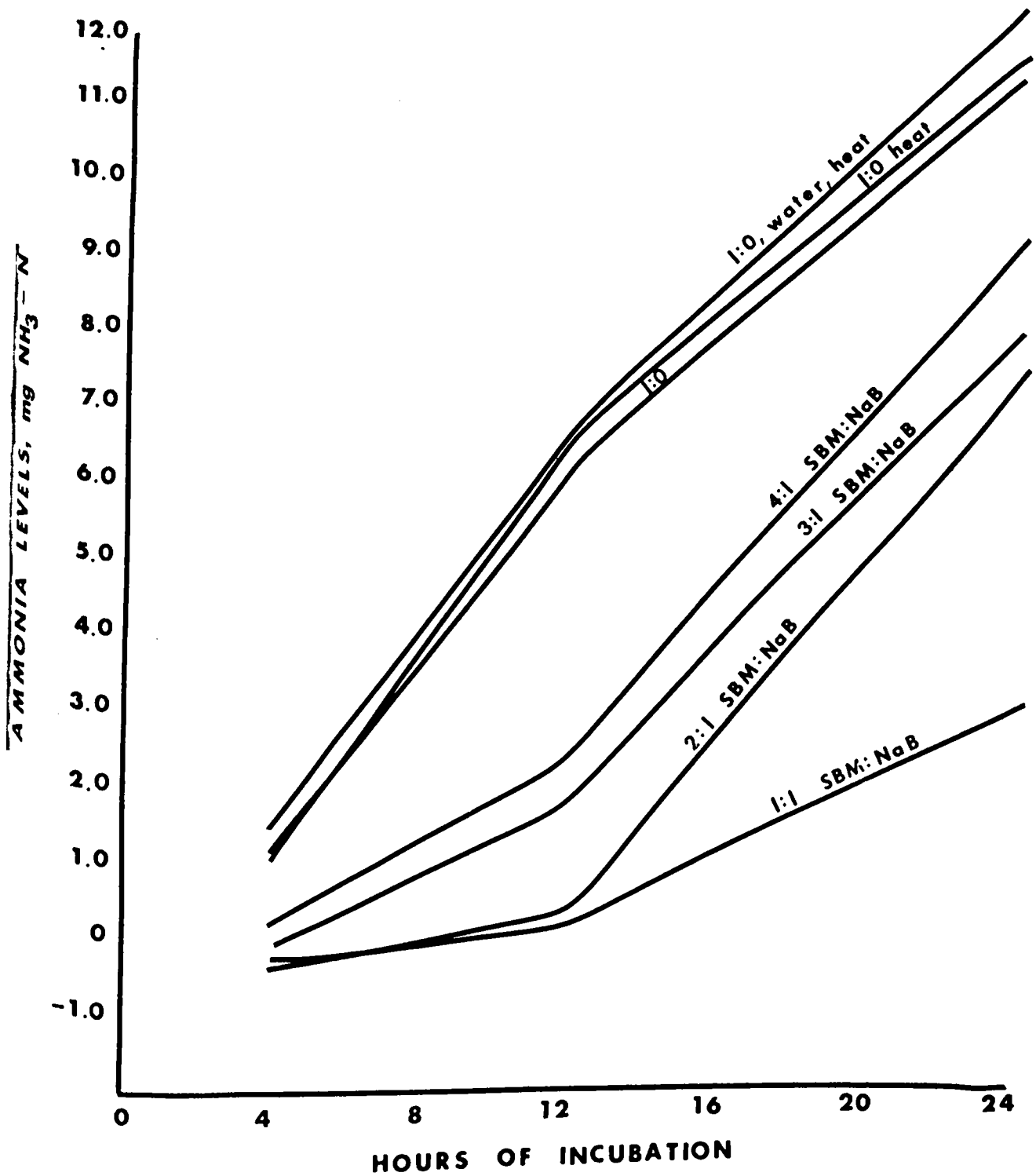
AMMONIA LEVELS, mg NH_3-N
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
-1.0
0
4
8
12
16
20
24
HOURS OF INCUBATION
1:0, water, heat
1:0 heat
1:0
4:1 SBM:NaB
3:1 SBM:NaB
2:1 SBM:NaB
1:1 SBM:: NaB

FIGURE 2. IN VITRO RUMEN NH_3-N RELEASE FROM 1:10 UREA:SODIUM BENTONITE MIXTURES AS AFFECTED BY WATER AND DRYING

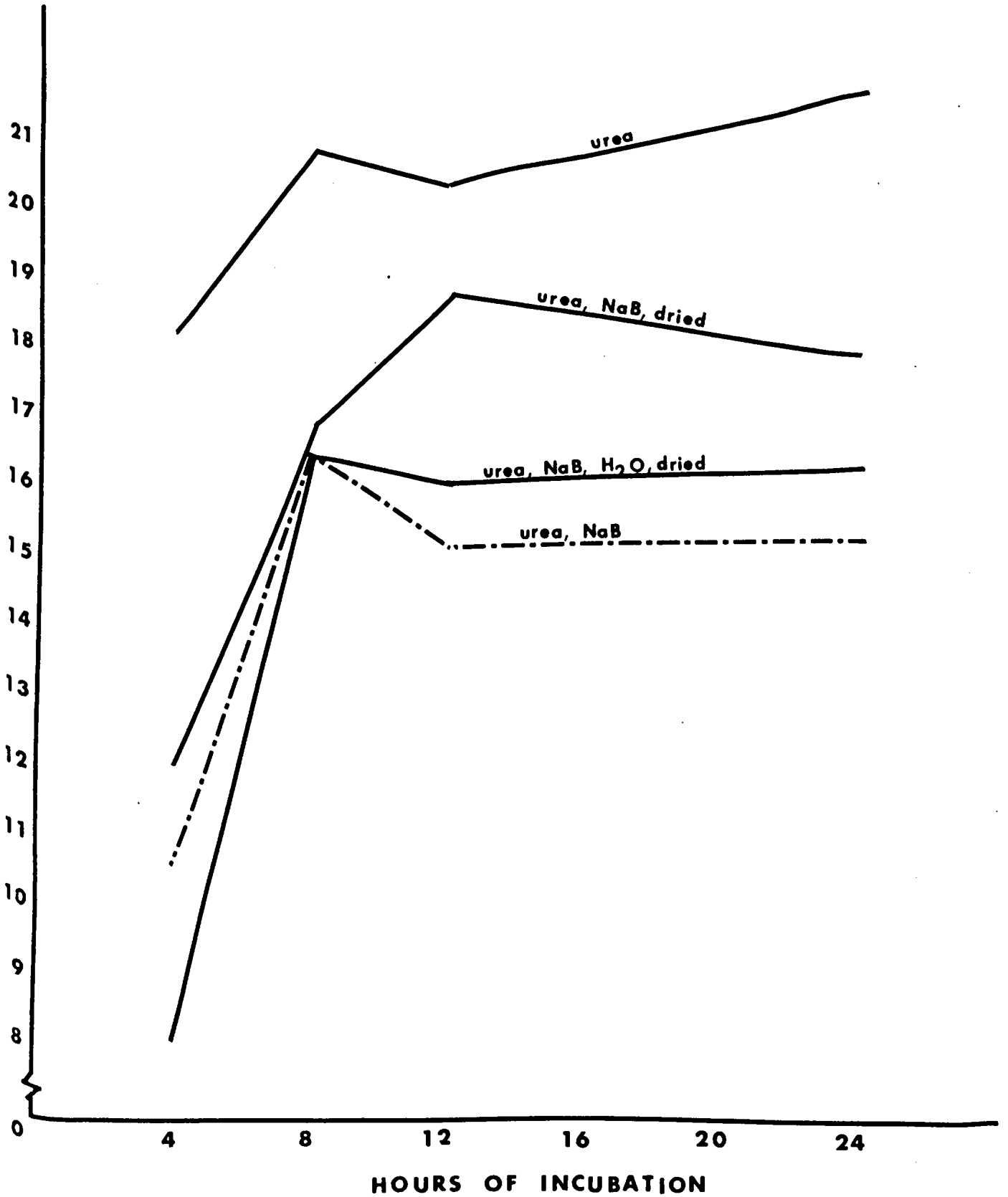
21
20
19
18
17
16
15
14
13
12
11
10
9
8
0
urea
urea, NaB, dried
urea, NaB, H_2O, dried
urea, NaB
4
8
12
16
20
24
HOURS OF INCUBATION

TABLE 3. IN VITRO RUMEN NH_3-N RELEASE AS AFFECTED BY DIFFERENT RATIOS OF SBM:NaB[a,b,c]

Ratio SBM:Na bentonite	Hours of incubation	
	0	24
	mg NH_3-N	mg NH_3-N
1:0	4.67	18.29
1:1	4.24	10.85
2:1	4.56	13.50
3:1	4.75	13.63
4:1	4.34	13.50
5:1	4.61	13.67
10:1	5.09	16.24

[a]Water added w/w to dry ingredients.

[b]All products dried at 65 C for 24 hours.

[c]Twenty mg N added to each in vitro tube.

TABLE 4. EFFECTS OF DIFFERENT N SOURCES AND NaB ON N UTILIZATION IN LAMBS

Treatment	N intake	N retained	Apparent N dig	N retained % absorbed	Apparent organic matter dig
	$g/kg^{.75}$	$g/kg^{.75}$	%	%	%
SBM	5.30[a]	1.40	64.60	38.87	63.79
SBM + NaB	6.41	2.05	70.89	45.03	66.19
U	4.31	.30	61.43	8.76	62.49
U + NaB	4.87	.36	61.44	10.93	58.75
SBM + U	5.15	.73	65.40	21.59	64.62
SBM + U + NaB	5.68	1.25	65.34	31.16	65.47
SE		±.144	±1.06	±4.49	±.94
[b]Standard errors are computed with 36 degrees of freedom.					
NaB vs no NaB		P<.01	P<.05	NS	NS
S + U vs 1/2 S + 1/2 U		NS	NS	NS	P .01
Soy vs U		P<.001	P<.001	P<.001	P<.001
Interaction: NaB by level of N source		NS	NS	NS	NS
Interaction NaB by N source		NS	P<.01	NS	P<.01

[a]Data are the average of 8 animals per treatment.

TABLE 5. RUMEN AMMONIA LEVELS OF LAMBS FED DIFFERENT N SOURCES[b]

Treatments	Hours[a] 1	2	3
	mg N/ml/g N[d]	mg N/ml/g N	mg N/ml/g N
SBM	18.36	22.57	14.73
SBM + NaB	21.23	17.70	12.99
U	89.20	73.04	26.55
U + NaB	108.54	67.58	22.44
SBM + U	75.78	53.45	28.59
SBM + U + NaB	50.91	33.66	12.06
SE[c]	±7.00	±4.38	±3.28

[a]Hours post feeding.

[b]Average of 8 animals per treatment.

[c]Standard errors are computed with 36 degrees of freedom.

[d]mg N/ml/g N consumed after 1 hours.

Orthogonal comparisons			
NaB vs no NaB	NS	$P<.05$	$P<.01$
S + U vs 1/2 S + 1/2 U	NS	NS	NS
Soy vs U	$P<.001$	$P<.001$	$P<.01$
NaB by level of N source	$P<.01$	NS	$P<.05$
NaB by N source	NS	NS	NS

THE EFFECT OF SODIUM BENTONITE ON VFA PATTERNS, LACTATE LEVELS AND PH WITH THE ADAPTATION OF LAMBS TO HIGH CONCENTRATE RATIONS WITH EITHER DRY CORN, WHEAT OR HIGH MOISTURE CORN[1]

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Summary

A 3 x 2 factorial experiment was conducted to study the effects of grain sources and sodium bentonite (NaB) in adapting lambs to 90% concentrate rations. The rations were fed ad lib at 35, 55 and 75% concentrate with each concentrate level fed for 5 days. The 90% concentrate was fed for 15 days. A total of 30 mixed breed wether lambs, which averaged 38 kg, were randomly allotted to the following treatments: dry corn (DC), DC + NaB, wheat (W), W + NaB, high moisture corn (HMC) and HMC + NaB. The NaB was fed at 2.5% of ration DM replacing an equal amount of corncobs. Rumen samples were obtained at 2, 3 and 5 hr postfeeding on the first day of each concentrate level and also at days 10 and 15 of the 90% rations. Rumen samples were analyzed for VFA's, lactate and H+ concentration. Average daily gain and feed/gain were calculated according to equal carcass dress. As the concentrated level increased, the acetate (moles/100 g) decreased and propionate

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(moles/100 g) increased. NaB supplementation resulted in increases (P .05) in acetate and butyrate (moles/100 g) and a decrease (P .01) in propionate (moles/100 g). NaB had no effect on H^+ concentration. Lambs fed W had greater H^+ concentrations than DC. NaB had no significant effect on lactate levels. Daily DM intake and daily gain for the 30 day trial were 1.16, .09; 1.35, .17; 1.07, .04; 1.11, .08; 1.18, .13; and 1.20, .15 kg for DC, DC + NaB, W, W +NaB, HMC and HMC + NaB, respectively.

INTRODUCTION

Sodium bentonite, a colloidal clay of volcanic origin, absorbs water many times its own weight. And, under the proper conditions of moisture and pH, it will absorb certain cations. It is used in industry to improve pelleting characteristics of feeds.

Sodium bentonite has been used to maintain fat test in dairy cows fed fat depressing diets (Bringe and Schultz, 1969; Rindsig et al., 1969). It was shown that sodium bentonite acted by decreasing propionate and increasing acetate levels in the rumen. (Erwin et al., 1957; Martin et al., 1969; Mendel 1971). Erwin et al. (1957) and Vetter (1967) reported an improvement in average daily gain and feed/gain ratio and observed that animals appear to "stay on feed" better with sodium bentonite supplementation. Martin et al. (1969) showed negative effects on performance with high concentrate rations but reported positive effects on high roughage rations when sodium bentonite was included in the rations. Mendel (1971) and Vetter (1968) reported that supplementing sodium bentonite decreased both average daily gain and feed/gain ratio.

It was postulated that the alkaline properties of sodium bentonite might aid in the prevention of subacute acidosis and thus show an improvement in performance. Dunlop and Hammond (1965) report that a possible contributing factor in subclinical acidosis is the rapid

consumption of feed upon the initial presentation of an increased concentrate level. The following experiment was conducted to further study the effects of sodium bentonite in adjusting ruminant to high energy diets.

MATERIALS & METHODS

A 3 x 2 factorial experiment was conducted to study the effects of 3 grain sources and NaB[1] in adapting lambs to 90% concentrate rations. A total of 30 mixed breed wether lambs averaging 38 kg were blocked according to weight and randomly allotted within blocks to one of six treatments. The trial was replicated, with the first replication consisting of 3 animals per treatment and the second replication consisting of 2 animals per treatment. The grain sources were corn grain, ensiled (HMC), ground corn grain (C), and hard red winter wheat (W). The HMC was harvested at approximately 72% dry matter and was ground through a hammer mill and water added to decrease the dry matter to 70%. The HMC was then sealed in plastic lined 242 liter steel drums and allowed to ensile for 21 days. The ensiled HMC was frozen at -20 C until required for the experimental rations. The thawing procedure allowed the barrel to thaw for 24 hours at room temperature. The barrel was then placed in a cooler and maintained at 4 C until needed.

The rations were fed ad libitum at 35, 55 and 75% concentrate with each concentrate level fed for 5 days. The 90% concentrate level

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[1]Volclay 90, American Colloid Company, Skokie, Illinois.

was fed for 15 days. Ration concentrate levels were increased at 5 day intervals to simulate adaptation to a high concentrate diet. Under feedlot conditions a feeding regime such as this might cause subclinical lactic acidosis. This trial was designed to cause and study subclinical acidosis.

The ration compositions for the 35, 55, 75, and 90% concentrate rations are shown in Table 1. Each grain source was fed with and without NaB. The NaB was fed at 2.5% of ration dry matter replacing an equal percent of corncobs. Rations were balanced to 12% crude protein on a dry matter basis with SBM. The wheat ration with no protein supplementation exceeded 12% crude protein at the 90% concentrate level.

A ration consisting of 80% cobs and 20% SBM was fed for 10 days prior to initial feeding of the 35% concentrate rations. The weight at the end of the 10 day prefeeding period was used as the initial weight. Initial weights were obtained after withholding feed and water for 16 hours. Final live weights were obtained by adjusting carcass weights to an equal dressing percent. Rations were individually fed to the lambs with refusals weighed and discarded daily to maintain fresh feed at all times. The lambs were tethered to their respective individual feed bunks inside a building maintained at 25 C.

Rumen samples were obtained via stomach tube (Raun and Burroughs, 1962) at 2, 3 and 5 hours postfeeding on the first day of each concentrate level and at days 10 and 15 of the 90% concentrate

feeding. To obtain rapid feed consumption upon initial presentation of an increased percent concentrate ration, feed was removed 14-16 hours prior to sample day feeding. Lambs were allowed 2 hours free access to feed and water prior to the first rumen sample. Feed was removed from the lambs immediately before the 2 hour postfeeding rumen sample and 2 hour consumption determined. The feed refused was returned to the respective lambs after the 5 hour postfeeding sample was obtained.

The pH of the rumen fluid was determined immediately after sampling with a pH meter equipped with a glass-calomel electrode. After pH determination, microbial activity was halted by adding 1 ml of 5% mercuric chloride solution to the 30 ml sample.

The samples were placed in small plastic bags and frozen at -20 C until needed for further analyses.

The rumen samples were thawed and total lactate determined by the calorimetric procedure of Barker and Summerson (1941).

Rumen samples were prepared for VFA analysis by adding 1 ml of rumen fluid to 5 ml of 25% metaphosphoric acid (Erwin et al., 1961). Samples were frozen at -20 C until analyzed. The analyses of the 3 hour postfeeding rumen fluid samples were for moles/100 g of acetic, propionic, butyric and total other acids. The analyses were done with a Barber-Coleman Series 5000 gas chromatagraph. The 1.8 m x 8 mm (outside diameter) coiled glass column was packed with 10% supelco-1200/1% H_3PO_4 on 80/100 chromosorb W-AW. The carrier gas was nitrogen at a flow rate of 73 ml per minute. Temperatures

for the column, injection port, and detector were 125 C, 165 C, and 185 C respectively.

The data were analyzed according to a split-plot design using Harvey's least squares analysis (Snedecor and Cochran, 1967) with unequal subclass numbers. For the VFA data, the main plot factors were groups, treatments, group by treatment interaction and animals within group-treatment subclasses; the latter was the error term. The subplot factors for the VFA data were concentrate levels, groups by concentrate level interaction, treatment by concentrate level interaction and the remainder or error term. Lactate, H+/intake and pH data were analyzed using the same main plot factors as the VFA data. In the subplot of the lactate and pH analysis, the factors were concentrate levels, sampling hours, concentrate level by sampling hour interaction, concentrate level by group interaction, concentrate level by treatment interaction, sampling hour by group interaction, sampling hour by treatment interaction and the remainder or error term. Lactate values were transformed to log values for the analysis due to the large amount of variability in the raw data.

RESULTS AND DISCUSSION

Orthogonal comparison showed no significant differences between treatments in average daily intake, average daily gain and feed/gain ratio (Table 2). These results agree with those of Vetter (1967, 1968) in average daily gain and feed/gain ratio but not in

average daily intake. Vetter showed approximately a one kg increase in average daily intake in 1967 and showed a significant increase in average daily intake in 1968 with sodium bentonite included in the ration. There was a consistent trend in this experiment with the sodium bentonite (NaB) supplemented lambs having higher average daily intake and lower feed/gain ratio. The greatest differences were in the DC and W rations. There was only a small effect with NaB supplementation in the HMC rations. The performance data cannot be heavily weighted due to the short duration and the numerous times the lambs were handled and rumen sampled.

Rumen pH is dependent upon type of substrate, amount of substrate consumed, rate of consumption, microbial population, rumination and previous treatment of the animal. Amount of substrate consumed, type of substrate and previous treatment were factors which could be measured or controlled in this experiment. Previous treatment was identical for all lambs and substrate was the subject of the experiment. Because the NaB supplemented lambs had significantly higher two hour intake, it was felt that pH without some adjustment for the unequal intakes, would not be a fair evaluation of the treatments. The pH values were changed to hydrogen ion concentrations and then divided by the two hour intake. Although pH values were lower ($P < .05$) with NaB treatments, there were no significant differences between control treatments and NaB treatments in hydrogen ion concentration divided by intake (H+/IN).

The pH values obtained in this experiment ranged from 4.50 to 6.30. The lowest pH's were obtained on the high concentrate rations with most pH's in the low to mid 5 range. These values are comparable to those reported by Fulton (1972) when he fed high concentrate control rations of wheat or corn. These pH's were not low enough to be a manifestation of acute acidosis. Dunlop and Hammond (1965) report that in acute lactic acidosis pH values range from 4.0 to 4.5 and in severe cases may drop as low as 3.88.

There was an effect on H+/IN ($P < .001$) with increasing the percent concentrate in the ration (Figure 1). As concentrate level increased H+/IN increased. The DC treatments had lower ($P < .05$) H+/IN values than the W treatments (13.6 and 26.9) respectively. This is a reflection of the faster fermentation rate of wheat. There were no significant differences in the H+/IN values between rumen samples obtained at 2, 3 and 5 hours postfeeding at any concentrate level.

There was an effect ($P < .001$) on H+/IN with time after reaching the 90% concentrate level with the H+/IN values increasing with time (Figure 2). There was a concentrate by treatment interaction ($P < .01$) resulting from H+/IN decreasing after 15 days with NaB treatments (Figure 3). There was no significant effect due to NaB supplementation over the three 90% concentrate samplings. These data might indicate that even after animals become accustomed to high concentrate rations, it is possible to lower rumen pH by rapid grain consumption such as was produced by withdrawing feed in this

experiment. This also shows the importance of having feed available at all times after the animal has begun receiving a high concentrate ration.

Different gain sources require different degrees of management. W has the potential to cause more problems than DC (Mackenzie, 1966; Fulton, 1972). W had higher (P .001) H+/IN values than DC (88.8 and 30.4 respectively) after reaching the 90% concentrate rations. The rumen microorganisms are able to ferment the carbohydrates contained in a given quantity of W more rapidly than that contained in the same quantity of DC. The subsequent lactic acid and VFA production results in greater lowering of rumen pH with W than with DC.

VFA analysis showed that as concentrate level increased, acetate (moles/100 g) decreased (P .01) and propionate (moles/100 g) increased (P .01) (Table 3). These results agree with Church (1969) who states that high levels of acetate are usually seen with high roughage rations. He also reports that a survey of the literature showed that as roughage levels decrease and concentrate levels increase, acetate (moles/100 g) decreases and propionate (moles/100 g) increases.

Since there was no significant treatment by concentrate level interaction, VFA data from all concentrate levels were pooled (Table 4) for analysis. NaB supplementation resulted in increases in acetate (moles/100 g) (P .05) and butyrate (moles/100 g) (P .05) and a decrease in propionate (mole/100 g) (P .01). This agrees with Rindsig et al. (1969) who showed that NaB supplementation raised

acetate (moles/100 g) and lowered propionate (moles/100 g) with milk fat depressing rations. Their fat depressing ration, a high-grain ration, resulted in 40.3 moles/100 g of acetate, 36.8 moles/100 g of propionate and 14.6 moles/100 g of butyrate. The inclusion of 5% NaB in the fat depressing ration resulted in 54.8 moles/100 g acetate, 23.0 moles/100 g propionate and 15.2 moles/100 g butyrate. VFA values obtained on their normal diets, 50% concentrate, were 58.8 moles/100 g of acetate, 20.1 moles/100 g of propionate, and 16.2 moles/100 g of butyrate. The inclusion of NaB resulted in a shift of VFA patterns such that on a milk fat depressing diet the VFA pattern is nearly the same as that obtained on a normal dairy ration. The inclusion of NaB in a high concentrate milk fat depressing ration results in the maintenance of near normal levels of milk fat.

Vetter (1967, 1968) reported increased intake with steers whose rations included NaB. There was also a trend toward higher dry matter intake in this experiment. If propionate is the satiety signal in high concentrate rations (Jones, 1972) then the shift from propionate to acetate may be responsible for the increased intake with NaB supplementation.

Lactic acid production in the rumen is associated with acidosis (Mackenzie, 1966) and rumen samples were obtained to estimate the effects of NaB on lactate production. The lactate data was transformed to $\log_{10}$ due to the large range of values within treatments (5 to 3000 mg/ml). There were no significant treatment effects although there

was a concentrate level effect (P .05).

Since there were no significant treatment by group or treatment by concentrate interactions, the data were pooled for analysis (Table 5). The data (Table 6) show that lactate values vary inconsistently from concentrate level to concentrate level with no consistent trend upward or downward as concentrate level increases. The highest lactate values were obtained at the 35% concentrate level, while the second highest lactate values were obtained at the 90% concentrate level after 15 days. The greatest 2 hour intake occurred with the 75% concentrate rations, so lactate production is not entirely a function of amount of intake. The rate of consumption could account for the variation in response to the different concentrate levels. It was expected that as concentrate level increased, lactate production would increase. As concentrate level increases, the percent of readily fermentable carbohydrate in the rations increases. Lactate production increases as the amount of readily fermentable carbohydrates in the ration increases (Mackenzie, 1966). The data (Table 5) shows that the lowest lactate values were obtained on DC rations and the highest on the HMC rations. The carbohydrates in HMC are partially fermented during the ensiling process (Heath, et al., 1973). The microorganisms are able to use the HMC carbohydrates for lactate production without a lag period because of the preliminary enigymatic degradation. W is fermented more rapidly than DC which is reflected in the pooled date (Table 5).

The lactate values for the NaB treatments were in most cases

higher than their respective controls. This is a reflection of the increased 2 hour intake obtained with NaB supplementation (Table 7).

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TABLE 1. RATION COMPOSITION[a] (%)

Ingredients	IRN[b]	Ration Number					
		35[d]			55[d]		
		1,1[c]	2,2[c]	3,3[c]	1,1[c]	2,2[c]	3,3[c]
Corn	4-07-911	15.35	-	-	38.90	-	-
Wheat	4-05-268	-	17.75	-	-	44.60	-
High Moisture Corn	3-07-739	-	-	15.27	-	-	38.80
Corn Cobs	1-02-782	65.00	65.00	65.00	45.00	45.00	45.00
Cane Molasses	4-04-696	3.00	3.00	3.00	3.00	3.00	3.00
Soybean Meal	5-04-604	15.56	13.11	15.58	12.00	6.40	12.1
Dicalcium Phosphate	6-01-080	.96	.96	.96	.65	.65	.65
Limestone	6-01-069	.16	.17	.16	.38	.38	.38
Trace Minerals[e]		.03	.03	.03	.03	.03	.03

[a]100% dry matter.

[b]International reference number.

[c]Treatment rations which were the same as the corresponding control except 2.5% sodium bentonite replaced 2.5% of the corn cobs.

[d]Percent concentrate contained in ration.

[e]Premix contained 10% Mn, 10% Fe, 10% Zn, 1% Cu, 3% I, and .1% Co.

TABLE 1. RATION COMPOSITION[a] (%) -Cont'd.

Ingredients	IRN[b]	Ration Number					
		75[d]			95[d]		
		1, 1[c]	2, 2[c]	3, 3[c]	1, 1[c]	2, 2[c]	3, 3[c]
Corn	4-07-911	63.30	-	-	80.30	-	-
Wheat	4-05-268	-	71.10	-	-	86.00	-
High Moisture Corn	3-07-739	-	-	62.30	-	-	80.10
Corn Cobs	1-02-782	25.00	25.00	25.00	10.00	10.00	10.00
Cane Molasses	4-04-696	3.00	3.00	3.00	3.00	3.00	3.00
Soybean Meal	5-04-604	8.4	-	8.70	5.80	-	6.00
Dicalcium Phosphate	6-01-080	.35	.35	.35	.12	.12	.12
Limestone	6-01-069	.57	.57	.57	.75	.75	.75
Trace Minerals[e]		.03	.03	.03	.03	.03	.03

[a]100% dry matter.

[b]International reference number.

[c]Treatment rations which were the same as the corresponding control except 2.5% sodium bentonite replaced 2.5% of the corn cobs.

[d]Percent concentrate contained in ration.

[e]Premix contained 10% Mn, 10% Fe, 10% Zn, 1% Cu, 3% I, and .1% Co.

TABLE 2. EFFECT OF SODIUM BENTONITE ON ANIMAL PERFORMANCE

Treatment	Average daily intake[a]	Average daily gains	Feed/gain
Corn	1.16 kg	.09 kg	12.58
Corn + Na bentonite	1.35	.17	8.39
Wheat	1.07	.04	24.68
Wheat + Na bentonite	1.11	.08	14.37
High moisture corn	1.18	.13	9.03
High moisture corn + Na bentonite	1.20	.15	7.78
SE	±.31	±.05	±1.00

Orthogonal comparisons[b]

1. Dry corn vs wheat
2. H.M. corn[c] vs dry corn
3. Bentonite vs no bentonite
4. Bentonite x H.M. corn vs dry corn
5. Bentonite x dry corn vs wheat

[a]100% dry matter.

[b]No significant differences.

[c]High moisture corn.

TABLE 3. VFA LEVELS AS AFFECTED BY TREATMENT AND CONCENTRATE LEVEL OF THE RATION

Treatment	Acetate[a]	Propionate[a]	Butyrate[a]	Other acids[a]
	35% concentrate			
Corn	72.68	17.76	9.15	.41
Corn + Na bentonite	73.11	16.51	9.94	.44
Wheat	73.62	19.46	6.52	.40
Wheat + Na bentonite	72.13	20.63	6.81	.43
H.M. corn	72.47	19.62	7.45	.46
H.M. corn +Na bentonite	71.61	17.34	10.63	.42
S.E.	±3.55	±4.08	±1.62	
	55% concentrate			
Corn	72.34	21.80	5.41	.45
Corn +Na bentonite	74.67	17.85	7.13	.35
Wheat	65.15	28.70	5.61	.33
Wheat + Na bentonite	74.99	21.88	2.76	.37
H.M. corn	70.56	19.11	9.80	.53
H.M. corn +Na bentonite	68.57	21.36	9.38	.69
S.E.	±3.55	±4.08	±1.62	

See footnotes at end of Table

TABLE 3. VFA LEVELS AS AFFECTED BY TREATMENT AND CONCENTRATE LEVEL OF THE RATION (Cont'd)

Treatment	Acetate[a]	Propionate[a]	Butyrate[a]	Other acids[a]
	75% concentrate			
Corn	69.19	17.84	12.67	.31
Corn + Na bentonite	68.50	22.41	8.77	.32
Wheat	62.14	29.81	7.74	.58
Wheat + Na bentonite	68.44	22.32	8.93	.31
H.M. corn	65.77	24.35	9.52	.36
H.M. corn + Na bentonite	65.73	26.72	7.15	.40
S.E.	±3.55	+4.08	+1.62	
	90% concentrate			
Corn	54.27	37.91	7.48	.34
Corn + Na bentonite	60.55	27.04	12.06	.35
Wheat	57.26	36.04	6.28	.42
Wheat + Na bentonite	55.87	37.77	5.94	.42
H.M. corn	44.69	47.16	7.65	.50
H.M. corn + Na bentonite	67.53	23.72	8.44	.31
S.E.	±3.55	±4.08	±1.62	

See footnotes at end of table

TABLE 3. VFA LEVELS AS AFFECTED BY TREATMENT AND CONCENTRATE LEVEL OF THE RATION (Cont'd)

Treatment	Acetate[a]	Propionate[a]	Butyrate[a]	Other acids[a]
	90% concentrate (10 days)[b]			
Corn	46.12	45.23	8.32	.33
Corn +Na bentonite	52.30	35.15	12.18	.37
Wheat	47.97	46.89	4.93	.21
Wheat + Na bentonite	54.41	37.92	7.25	.42
H.M. corn	59.96	33.53	6.15	.36
H.M. corn +Na bentonite	57.98	29.73	11.79	.50
S.E.	±3.55	±4.08	±1.62	
	90% concentrate (15 days)[b]			
Corn	53.79	37.75	8.12	.34
Corn +Na bentonite	57.10	33.78	8.77	.20
Wheat	52.11	42.90	4.73	.36
Wheat + Na bentonite	51.28	43.06	5.30	.36
H.M. corn	62.99	32.44	4.23	.34
H.M. corn +Na bentonite	62.17	27.45	9.92	.46
S.E.	±3.55	±4.08	±1.62	

[a]Moles/100 grams.

[b]Days after initial presentation of 90% concentrate rations.

TABLE 4. POOLED VFA DATA AS AFFECTED BY TREATMENT

Treatment	Acetate[a]	Propionate[a]	Butyrate[a]	Other acids[a]
Corn	61.40	29.72	8.53	.35
Corn + Na bentonite	64.47	25.36	9.81	.36
Wheat	59.71	34.00	5.97	.32
Wheat + Na bentonite	62.85	30.60	6.17	.38
H.M. Corn	62.74	29.37	7.47	.42
H.M. Corn + Na bentonite	65.60	24.39	9.55	.46
S.E.	± .55	± .63	± .25	
		Orthogonal Comparisons		
1. Dry Corn vs. Wheat	N. S.	P .05	P .001	N. S.
2. H.M. Corn vs. Dry Corn	N. S.	N. S.	N. S.	N. S.
3. Bentonite vs. no Bentonite	P .05	P .01	P .05	N. S.
4. Bentonite x H.M. Corn vs. Dry Corn	N. S.	N. S.	N. S.	N. S.
5. Bentonite x Dry Corn vs. Wheat	N. S.	N. S.	N. S.	N. S.

[a]Moles/100 g

TABLE 5. POOLED LOGS OF LACTATE LEVELS AS AFFECTED BY TREATMENT, CONCENTRATE LEVELS AND TIME

Treatment	Concentrate level (%) 35, 55, 75, 90	90, 90(10)[a], 90(15)[a]
Corn	1.288	1.202
Corn + Na Bentonite	1.111	1.131
Wheat	1.388	1.239
Wheat + Na Bentonite	1.422	1.693
H. M. Corn	1.650	1.601
H. M. Corn + Na Bentonite	1.805	1.949
S. E.	±.082	±.087

Orthogonal comparisons[b]

1. Dry Corn vs Wheat
2. H. M. Corn vs Dry Corn
3. Bentonite vs Na Bentonite
4. Bentonite x H. M. Corn vs. Dry Corn
5. Bentonite x Dry Corn vs Wheat

[a]Days after initial presentation of 90% concentrate rations.

[b]No significant differences.

TABLE 6. LOGS OF LACTATE LEVELS AS AFFECTED BY TREATMENT, CONCENTRATE LEVEL AND TIME

Treatment	35%	55%	75%	90%	90% (10)[a]	90% (15)[a]
Corn	2.286	0.841	1.290	0.971	1.003	0.875
Corn + Na	1.722	0.804	0.716	1.304	0.910	1.156
Wheat	1.699	1.091	1.403	1.403	0.891	1.394
Wheat + Na	1.385	0.928	1.573	1.723	1.633	1.662
H. M. Corn	2.194	1.491	1.595	1.237	1.429	2.288
H. M. Corn + Na	1.854	1.574	1.494	2.255	1.680	1.929
S. E.	±.193	±.152	±.152	±.152	±.152	±.152

Orthogonal comparisons[b]

1. Dry Corn vs. Wheat - P .05
2. H. M. Corn vs. Dry Corn - N. S.
3. Bentonite vs. no Bentonite - P .05
4. Bentonite x H. M. Corn vs. Dry Corn - N. S.
5. Bentonite x Dry Corn vs. Wheat - N. S.

[a]Days after initial presentation of 90% concentrate rations.

[b]No significant differences.

TABLE 7. TWO HOUR INTAKES AS AFFECTED BY CONCENTRATE LEVEL AND TREATMENT

Treatment	Concentrate level (%)					
	35	55	75	90	90 (15)[a]	90 (15)[a]
Corn	803[b]	578[b]	1035[b]	647[b]	570[b]	638[b]
Corn + Na bentonite	772	737	999	1173	619	681
Wheat	571	554	648	438	463	607
Wheat + Na bentonite	685	570	943	604	662	614
H. M. corn	722	990	872	587	560	555
H. M. corn + Na bentonite	696	1091	854	875	473	602
S. E.	±49.9	±81.8	±48.8	±48.8	±48.8	±48.8

Orthogonal comparisons of pooled data

1. Dry corn vs wheat - $P < .05$
2. H. M. corn vs dry corn - N. S.
3. Bentonite vs no bentonite - $P < .05$
4. Bentonite x H. M. corn vs dry corn - N. S.
5. Bentonite x dry corn vs wheat - N. S.

[a]Days after initial presentation of 90% concentrate rations.

[b]Grams of dry matter.

FIGURE 1. EFFECT OF INCREASING PERCENT CONCENTRATE IN THE RATION ON H+/INTAKE.

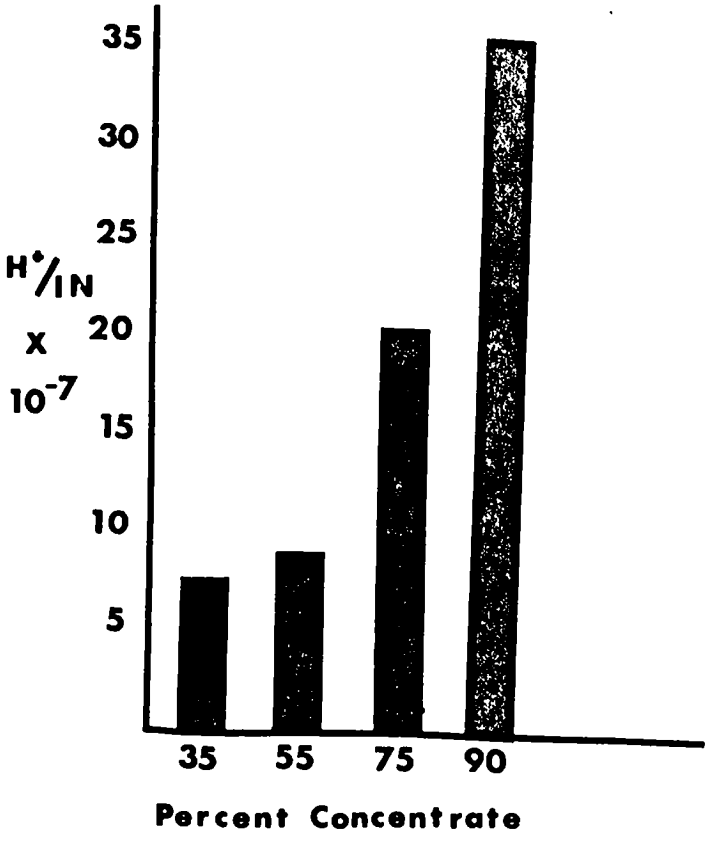

35
30
25
H+/IN
X
10-7
20
15
10
5
35
55
75
90
Percent Concentrate

FIGURE 2. EFFECT OF TIME WITH 90 PERCENT CONCENTRATE RATIONS ON H+/INTAKE.

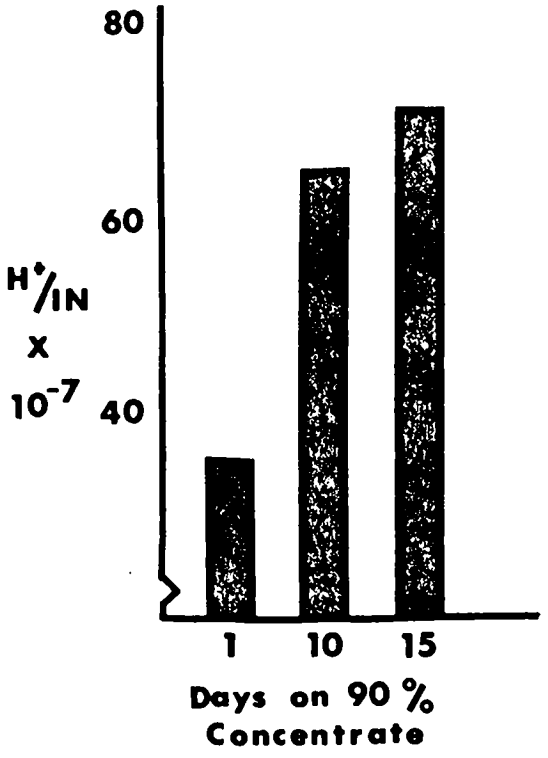
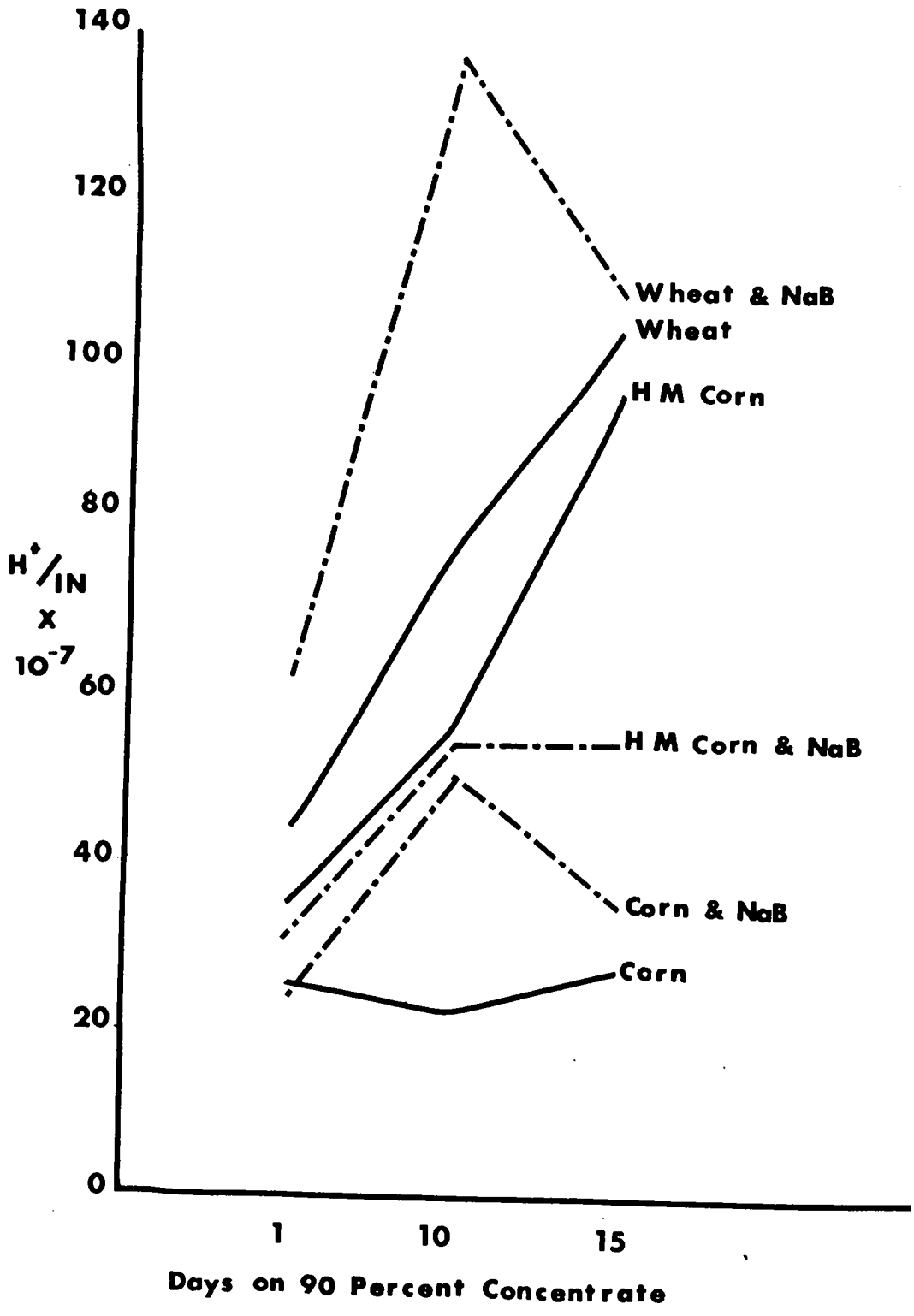

80
60
40
H+/IN
X
10-7
1
10
15
Days on 90 %
Concentrate

FIGURE 3. CONCENTRATE BY TREATMENT INTERACTION OF H+/INTAKE AS AFFECTED BY TIME ON 90% CONCENTRATE RATIONS.


140
120
100
80
60
40
20
0
$H^+/IN \times 10^{-7}$
Wheat & NaB
Wheat
H M Corn
H M Corn & NaB
Corn & NaB
Corn
1
10
15
Days on 90 Percent Concentrate