OKLAHOMA STATE UNIVERSITY

IMPACT OF DIETARY SALT AND DIETARY CATION ANION BALANCE ON WATER INTAKE, FEEDLOT PERFORMANCE AND PHYSIOLOGICAL MEASUREMENTS OF FEEDLOT CATTLE

By

ALEJANDRO FRANCISCO LA MANNA

Ingeniero Agronomo

Universidad de la Republica

Montevideo, Uruguay

1987

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, 1999

OKLAHOMA STATE UNIVERSITY

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Thesis Approved:

F. N. Owens

Thesis Adviser

Gerald W. Horn

Donald R Gill

Wayne B. Powell

Dean of the Graduate College

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to my major advisor Dr. Fredric N. Owens for his guidance, counsel and understanding of my graduate program. My appreciation is extended to my graduate committee members Dr. Gerald W. Horn and Dr. Don R. Gill.

I will like to thank for their financial support to the Instituto Nacional de Investigacion Agropecuaria (INIA) and the department of Animal Science of Oklahoma State University. I would like to thank Dr. Jorge Vizcarra for all his help during my first year at O.S.U. I would like to thank also Steve Welty for sharing his experience and for the care he took for all the aspects of my research. I also want to express my gratitude to Kathy Swenson, Joan Summers, Carolyn Lunsford, Afaf Melouk and Donna Perry for their assistance in my research.

A special appreciation to my wife Adriana for her constant sacrifice, support, love and understanding, to my children Ignacio and Federica for their love and for making my life more enjoyable and to my mother Blanca for all her support and love. My appreciation to my fellow graduate students for their friendship. Also my appreciation for my family in Uruguay for their love and support.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

LIST OF FIGURES

Figure Page

CHAPTER II

CHAPTER I

INTRODUCTION

Minerals are critical for most biochemical processes in the body and therefore are essential to animals (Spears, 1996). In the past, feeding recommendations for minerals have been set to maximize animal growth rate, milk yield and pregnancy (Beede, 1998), and in general have not considered their adverse impact on the environment. During the last decades, public concern for environmental issues has led to regulations, codes or laws. Each of these regulations that affect cattle producers have resulted in alterations in management practices (Morse, 1996).

One of the key factors that can reduce the environmental impact from animal operations is to optimize the level at which minerals that are added to diet and minimizing excesses. Data from North Carolina Feed Testing Laboratory (Spears, 1996), showed that the median value of minerals fed to dairy cattle generally exceeded the NRC estimate of dietary requirements. The feeding:requirement ratio for some selected minerals was Ca 1.77; P 1.32; Na 1.78, S 1.20; K 1.34). According to Spears (1996), even though degree of excess of minerals in the diet may vary from state to state, overfeeding of minerals is a common practice in most areas of the United States. By using more accurate models to predict requirements, producers can maintain performance while reducing environmental impacts (NRC, 1996). One model when applied in a dairy reduced nitrogen excretion by 25% while also reducing feed costs (Fox et al., 1995).

One of the new challenges facing animal nutritionists is to curtail adverse environmental impact by reducing nutrient losses and increasing nutrient recovery in edible products from the animal, while maintaining or enhancing productivity and/or economical benefits. To estimate requirements more precisely, it is vital to understand the function, metabolism and interaction of minerals in the animal.

The objective of our research was 1) to test the impact of dietary salt concentration on intake of water and feed, and on ruminal parameters; and in fecal dry matter, urinary and blood (arterial and venous) measurements; and 2) to examine the effects of altering the dietary acid base balance (DCAB) of a feedlot diet on the measurements above.

CHAPTER II

REVIEW OF LITERATURE

Function and Metabolism of the Macrominerals in Ruminants

Based on the quantities present in the body and the amounts required by animals, sodium (Na), chloride (Cl), potassium (K), calcium (Ca), phosphorus (P), magnesium (Mg) and sulfur (S) are considered to be macro mineral elements. All these minerals are involved with body regulation presence or absence of any of them can affect the metabolism. This review will discuss various functions and metabolism of macrominerals in ruminants.

Sodium.

The major cation in extracellular fluid, sodium is involved in maintaining osmotic pressure, controlling water balance and regulating acid-base balance (NRC, 1996). Sodium (Na) also plays a major role in the transmission of nerve impulses and in muscle contraction (Kincaid, 1993; NRC, 1996). In bone, Na represents 30 to 45 % of total body Na, but only the small fraction of Na that is bound to the surface of the bone is part of the active labile Na pool (Ammerman and Goodrich, 1983).

Sodium is needed for the ATP-driven sodium-potassium pump that controls cell volume and drives the active transport of sugars and amino acids (Stryer, 1995).

The regulation of Na body concentration is controlled by aldosterone and antidiuretic hormones in order to maintain a constant Na:K ratio (Mc Dowell, 1992). The

former regulates resorption of Na from the kidney tubules (Mc Dowell, 1992) while the latter is responsive to changes in the osmotic pressure of extracellular fluid (NRC, 1980).

Sodium is absorbed principally from the small intestine through a transport system dependent on a system of passive leaks located in cell membranes (Mc Dowell, 1992). Sodium also is absorbed from the rumen (Ammerman and Goodrich, 1983), with its absorption rate proportional to its concentration in ruminal fluid (Warner and Stacy, 1972), due to simple passive diffusion rate of Na occurs across cell membranes from higher to lower concentrations (Ammerman and Goodrich, 1983). Thereby, Na movement helps to maintain an osmotic balance among plasma, interstitial fluid and cellular fluid in the animal (Carter and Grovum, 1990).

Sodium is excreted mainly in the urine (Guyton and Hall, 1996), with smaller amounts being lost in feces and by perspiration (Mc Dowell, 1992). Under steady state conditions excretion of sodium by the kidneys is proportional to Na intake (Guyton and Hall, 1996), and on a long term basis all sodium ingested must be excreted.

Potassium.

The major cation in intracellular fluid, potassium is involved in the regulation of osmotic pressure, water balance, muscle contraction, acid-base balance, nerve impulse transmission and certain enzymatic reactions (Miller, 1995). Potassium also is important in the transport of oxygen and carbon dioxide through blood, being responsible for at least half of the carbon dioxide capacity of the blood (Mc Dowell, 1992). Potassium helps to maintain the electrical neutrality before buffering of hydrogen ions by hemoglobin in blood, by keeping an ion balance with the carboxyl groups (Reece, 1993a). After the

ionization of the carboxyl groups is suppressed by hydrogen ions, electrical neutrality of K ions is maintained by bicarbonate and chloride ions (Reece, 1993a).

Potassium, as well as sodium, is a component of the ATP-Na-K pump, which maintains a concentration gradient important for the transport of substrates through the cell membrane, and for the regulation of the osmotic pressure (Mc Dowell, 1992).

Potassium is mainly absorbed from the rumen, omasum and the lower gastrointestinal tract (Mc Dowell, 1992). Absorption from the intestine is by simple diffusion (Ammerman and Goodrich, 1983). A large proportion of K in the rumen is derived from the saliva, which is continuously secreted and is rich in K (Mc Dowell, 1992).

Potassium balance depends mainly on the excretion by the kidneys, which adjusts K excretion rapidly and precisely to a wide variation of intake (Guyton and Hall, 1996). High Na intake may increase K urinary excretion (Ammerman and Goodrich, 1983). Adrenal hormones including aldosterones increase potassium secretion by the renal tubules (Mc Dowell, 1992) while increasing Na absorption (Ammerman and Goodrich, 1983). Extracellular fluid potassium concentration is regulated precisely at about 4.2 ± 0.3 mEq/liter (Guyton and Hall, 1996). According to these authors, precise control is necessary because many cellular functions are dependent on extracellular potassium concentration.

Chloride.

Chloride is the major anion in extracellular fluid and plays an important role in maintaining osmotic pressure, normal extracellular fluid volume, blood pH, osmotic pressure and regulating acid-base balance (Neathery, 1980). It is a component of

hydrochloric acid secreted by the abomasum (Neathery, 1980 and Mc Dowell, 1992). Intestinal amylase is activated by chloride (Ammerman and Goodrich, 1983).

Intake plays a minor role in chloride homeostasis since dietary chloride is absorbed almost completely (Neathery, 1980). Except for the abomasum, where there is a net secretion of chloride, net absorption occurs in all other sections of the gastrointestinal tract (Neathery, 1980). Urine is the main route of endogenous chloride secretion (Neathery, 1980; Guyton and Hall, 1996).

Calcium.

Most calcium present in the body mainly exists in teeth and bones (98% - 99%) as calcium phosphate (Yano et al., 1991 and Soares, 1995a). Only 1-2 % of total calcium (Ca) exists in the soft tissues and the extracellular fluid (Yano et al., 1991). Ca is involved in blood clotting, membrane permeability, muscle contraction, transmission of nerve impulses, secretion of certain hormones and activation of certain enzymes (NRC, 1996).

The concentration of Ca in blood ranges between 9-11 mg/dl (Hays and Swenson, 1993 and NRC, 1996). Calcium in plasma is presented in three forms: a) diffusable but unionized calcium (9 %), non diffusable calcium proteinate (41 %) and ionized calcium (Ca^{++}) (50 %) (Guyton and Hall, 1996). Ionized calcium is the calcium form that is most important metabolically (Guyton and Hall, 1996)

Two hormones, parathyroid hormone (PTH) and calcitonin maintain a delicate relationship with dihydroxycalciferol (1,25-$(OH)_2D_3$) to control blood Ca and phosphorus (P) levels within very narrow limits (Mc Dowell, 1992). PTH stimulates the production of 1,25-$(OH)_2D_3$ which increases Ca absorption from the intestine (NRC, 1996) and

stimulates bone resorption and renal tubular resorption of Ca (Fontenot et al., 1989). Dihydroxycalciferol production stimulates transcellular active transport of dietary Ca across the intestinal epithelium (Goff, 1992). In contrast, calcitonin down-regulates high serum levels of Ca by depressing gut absorption, halting bone demineralization and reducing resorption from the kidney (Mc Dowell, 1992).

The absorption of Ca takes place in the duodenum by both active transport (mediated by vitamin D) and a passive process (De Luca, 1974; Braithwaite, 1984). Both absorption systems may be saturable (Yano et al., 1991). Passive diffusion among the intestinal epithelial cells is strictly related to the concentration of Ca ions in the lumen of the gut (Goff, 1992). A low Ca diet may increase the production of 1,25-$(OH)_2D_3$ and enhance Ca absorption (Yano et al., 1991). The amount absorbed depends on the source of Ca, the Ca:P ratio, intestinal pH, lactose intake, and dietary levels of Ca, P, vitamin D, iron, aluminum, manganese and fatty acids (Hays and Swenson, 1993).

Most Ca is excreted in feces with fecal, Ca concentration being a product of unabsorbed dietary Ca and absorbed endogenous Ca (Mc Dowell, 1992).

Phosphorus.

Phosphorus is the major anion of intracellular fluids and the second most abundant mineral found in the animal body. About 80 % of the body phosphorus (P) is in the skeleton with the remaining 20 % in nucleotides such as ATP, nucleic acids, phospholipids and other phosphorylated compounds involved with metabolism (Soares, 1995b). Ruminal microorganisms require P for their growth and cellular metabolism (Ternouth et al., 1985; NRC, 1996).

The levels of inorganic P in plasma are not under strict homeostatic control (Kincaid, 1993). Vitamin D stimulates specific pump mechanisms in the intestine, bone and kidney thereby elevating plasma P (Mc Dowell, 1992). Saliva is an additional source of P for the rumen, since with concentrations ranging from 370-720 mg/liter in mixed saliva, a much higher concentration than that found in plasma (60 mg/liter) (Yano et al., 1991). Thereby, saliva plays an important role in the regulation and homeostasis of P.

The absorption of P takes place, as with Ca, in the duodenum by both active and passive absorption (Wasserman 1981; Braithwaite, 1984; Kincaid, 1993). No matter how P is ingested its absorption will depend on its solubility at the point of contact with the absorbing membranes (Maynard et al., 1979). Phosphorus absorption also is influenced by intestinal pH, animal age, and intake of Ca, iron, aluminum, K and Mg (Mc Dowell, 1992; Hays and Swenson 1993). P is absorbed in the ortho phosphate form (Kincaid, 1993). Phosphate absorption is increased by vitamin D_3 which may change membrane permeability, alter configuration of a phosphate carrier, stimulate pump sites, or by Ca absorption, indirectly increase P absorption by decreasing the degree to which P is insolubilized by Ca. (Kincaid, 1993)

In ruminants, P is excreted mainly in the feces. When plasma P level is high, 2.0 to 2.5 mmol/liter, the kidney will excrete P (Challa and Braithwaite, 1988). When high concentrate diets are fed, more P is excreted in the urine of cattle (Preston, 1977 cited by Mc Dowell, 1992).

Magnesium.

Magnesium is the second most plentiful cation of intracellular fluid. Mg is widely distributed with 65-70 % in bone, 15 % in muscle, 15 % in other soft tissues and 1 % in

extracellular fluid (Mayland, 1993). Mg in the skeleton is important for maintaining the integrity of bone and teeth (Mc Dowell, 1992)

Magnesium is involved in the ATP-Mg complex; thereby, Mg is required for thousands of enzymatic reactions in every major metabolic pathway (Fontenot et al., 1989). Magnesium also is involved in the maintenance of electrical potential across nerve and muscle membranes and for nerve impulse transmission (Henry and Benz, 1995 and NRC, 1996).

There is no endocrine system to maintain Mg concentration in blood there is for Ca, and there is no a strong evidence to indicate that any single hormone or vitamin is related directly with magnesium homeostasis or metabolism (Littledike and Goff, 1987). However, PTH can affect Mg metabolism through decreasing urinary Mg excretion and stimulating bone resorption (Fontenot et al., 1989). During bone resorption 43 Ca ions are released for every Mg ion released (Fontenot et al., 1989).

For ruminants the major site for Mg absorption is the reticulorumen (Tomas and Porter, 1976; Emanuele et al., 1991). The absorption of Mg postruminally is not enough to maintain normal Mg status of the animal (Tomas and Potter, 1976). Increases in ruminal availability and ruminal absorption of dietary Mg due to carbohydrates have been reported (Giduck and Fontenot, 1987) and some authors (Greene et al., 1977; Horn and Smith 1978) have suggested ruminal acidity as one of the main reasons for enhanced this Mg availability. However, Giduck et al. (1988) simulating the acidity that is produced by grains with HCl as one of the treatments suggested that the increased Mg absorption observed with carbohydrates supplementation is not due to alterations in ruminal pH or VFA levels, but perhaps related to effects such as osmotic changes or lactic acid

concentrations in the rumen. The proportion of Mg absorbed decreases as dietary level and the Mg status of the animal increase (Mc Aleese et al., 1961). A number of dietary factors can depress Mg absorption among which dietary potassium level has the most consistent effect (Greene et al., 1983). Urine is the major excretory pathway for absorbed Mg (Mc Dowell, 1992) though most Mg appears in feces because true absorption of Mg typically is very low.

Sulfur.

Sulfur is an essential component of proteins and other compounds in the body. Sulfur (S) is a constituent of amino acids and of the disulfide bonds that maintain the tertiary structure of protein molecules (Henry and Ammerman, 1995). Sulfur is a component of methionine and B vitamins (thiamin and biotin) that cannot be synthesized by animal tissue (NRC, 1989). Sulfur constitutes 0.15 % of body tissue (NRC, 1989).

The microbes in the rumen can incorporate S to S-containing amino acids (Shirley, 1992). However, dietary S must be oxidized to sulfate or reduced to sulfide in order to be utilized by the ruminant (Shirley, 1992). The reduction of sulfate to sulfide has its peak at a pH of 6.5 (Kincaid, 1993). Sulfide can be absorbed directly from the rumen and the small intestine whereas sulfate is minimally absorbed from the rumen (Kincaid, 1993). However, when large quantities of sulfide are absorbed from the rumen it can prove toxic (Ammerman and Goodrich, 1983).

Sulfur is excreted in both urine and feces (Shirley, 1992).

Effects of Salt on Ruminal Metabolism and Animal Performance

Salt (NaCl) can be used as a means to limit feed intake of highly palatable feeds such as grain and supplement (Lusby, 1993). When salt is provided ad libitum, ruminants will consume more salt than is required due to their appetite for sodium (NRC, 1996).

According to the NRC (1996) the requirements for sodium of growing and finishing cattle are 0.06 to 0.08%. Daily salt requirements for mature cattle are less than 28 g /head (Rich et al. 1985; Lusby 1993) and although the amount of salt is variable in feedlot rations they amounts usually range between 112 to 224 g/head/day (Matsushima and Phipps 1974) or between 0 and 1% of the diet (Hicks et al. 1988b).

Effects of NaCl on rumen metabolism.

Salt affects the osmotic pressure of fluids. Osmotic pressure is a quantitative measure of the tendency for water to osmose (Reece, 1993b). The osmotic pressure of body fluids serve as a measure of the relationships among electrolytes and the degree to which membranes allow free diffusion of elements. Water diffuses to any area where osmotic pressure is greater. Normally, electrolytes and other compounds in body fluids are maintained at a relative constant osmolality. When rumen or blood osmotic pressure are altered, salivary flow generally is reduced (Church, 1993)

The most important cation affecting osmolality is Na; when given as a chloride salt to ruminants it strongly correlates to osmolality, feed intake, water intake and kinetics of ruminal fluid. Feed intake can be limited if the osmolality of the ruminal fluid is increased during a meal (Carter and Grovum, 1990 and Forbes, 1992). NaCl increases the osmolality of the ruminal fluid; this sensed in the wall of the rumino reticulum and limits feed intake (Carter and Grovum, 1990). Zorrilla Rios et al., (1990) found that

increasing NaCl from 0.5 to 5 % of the DM increased ruminal osmolality from 300 to 344 mOsm/kg.

Type of diet also can affect physiological responses to NaCl. Ruminal osmolality for roughage and silage based diets reaches a maximum between 350 to 400 mOsm/kg (Engelhardt, 1969; Bergen, 1972 and Bennink et al., 1978). However, values for ruminal osmolality for roughages ranges from 240 to 265 mOsm/L with versus 280 to 300mOsm/L for concentrate diets (Garza et al., 1989). Zorrilla Rios et al. (1990) found that kinetics of ruminal fluid is more susceptible to osmotically active substances when added to concentrate than to roughage diets. Forbes et al. (1992) observed an intake reduction of grass silage on dairy cows when they were ruminally infused with NaCl; they concluded that the major mode of action of salt was via the elevation of osmolality of ruminal fluid.

High levels of salt have shown some potential to increase the ruminal bypass of dietary nutrients due in part to an increased water intake (Cheng et al., 1979; Croom et al., 1981; Zorrilla-Rios et al., 1990). Usually high levels of salt increase ruminal liquid dilution rate. Cheng et al. (1979) concluded that adding 4% of NaCl to a concentrate diet increased the rate of flow material of liquid from the rumen and by doing so reduced bloat conditions in the rumen. However, Eliman and Ørskov (1985) found that the increase in water intake with the addition of salts did not increase the fractional outflow of dietary supplements (solids) from the rumen in dairy cows.

Croom et al. (1985) found that the acetate to propionate ratio in the rumen of yearling steers after 62 days on feed was increased from 1.4 to 1.8 when 5% salt was added to the diet. These authors also found differences in molar proportions of acetate

(50.5 vs. 54.6) propionate (38.7 vs. 33.1) and butyrate (7.1 vs. 8.7) when 5% NaCl was added to the diet. However, by day 118 of the trial no effects of added salt were detected for the acetate propionate ratio, or molar proportions of acetate, propionate and butyrate.

Rogers and Davis (1982) working with high grain diets observed increments in the molar proportions of acetate (56.3, 60.3 and 61.7) and butyrate (12.1, 13.5 and 14.2) when Holsteins steers were infused with 8 liters of water, or 8 liters of water plus 200 or 600g of NaCl, respectively.

Effects of NaCl on Animal Performance

Feeding trial data that were reported in journals, experiment station publications and feeder's day report were compiled to examine effects of added salt on average daily gain (ADG), average daily feed intake (ADFI), feed/gain ratio (F/G), gain/feed ratio (G/F), carcass yield (Yield), % of fat (Fat), and dressing percentage (DP). Information was included if 1) there were at least two treatments within trial with percent of salt less than .85; 2) Dietary salt percentage was presented or could be calculated. When some variable was not reported but could be calculated from the other variables reported, it was calculated and included in the database.

Proc GLM (1990) was used to regress the different dependent variables against percentage of salt (linear) and percentage of salt and salt^2 (quadratic) present in the diet. Statistical analyses were weighted by the number of cattle per comparison within each trial. Trial was used as a class variable. This adjustment was used because the precision by which treatment mean is estimated depends on the number of cattle per treatment (Owens et al., 1997). The number of cattle per comparison ranged from 3 to 40.

Effect on ADG. Data from 11 trials were available to calculate the relation between salt and salt and salt squared with ADG.

Figure 1. Relation between ADG (lbs/day) and the percentage of salt in the diet for each of the experiments considered.

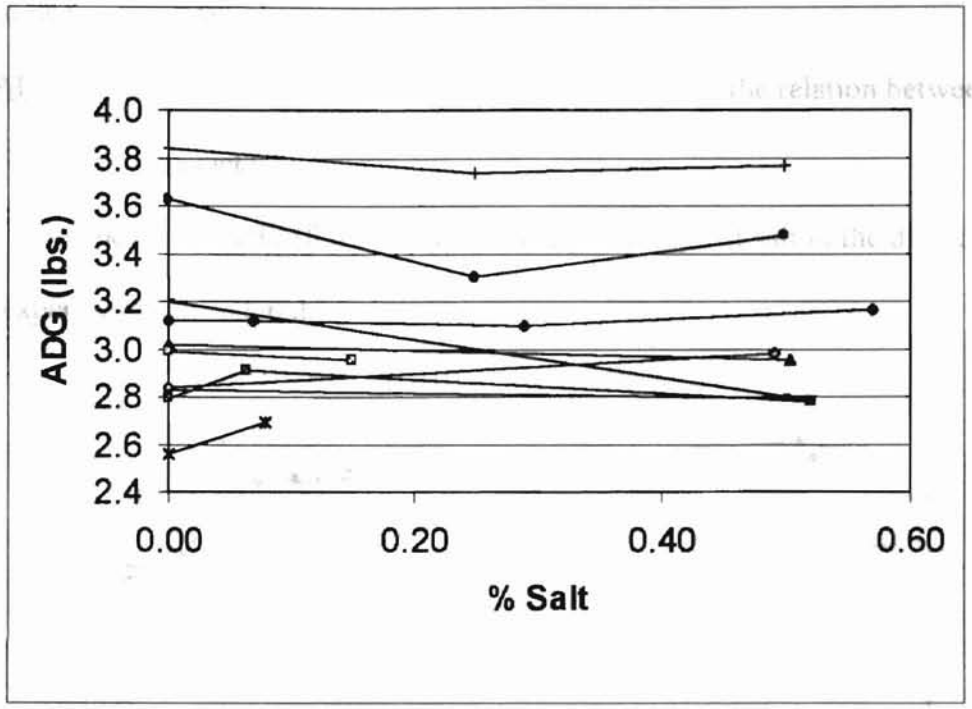
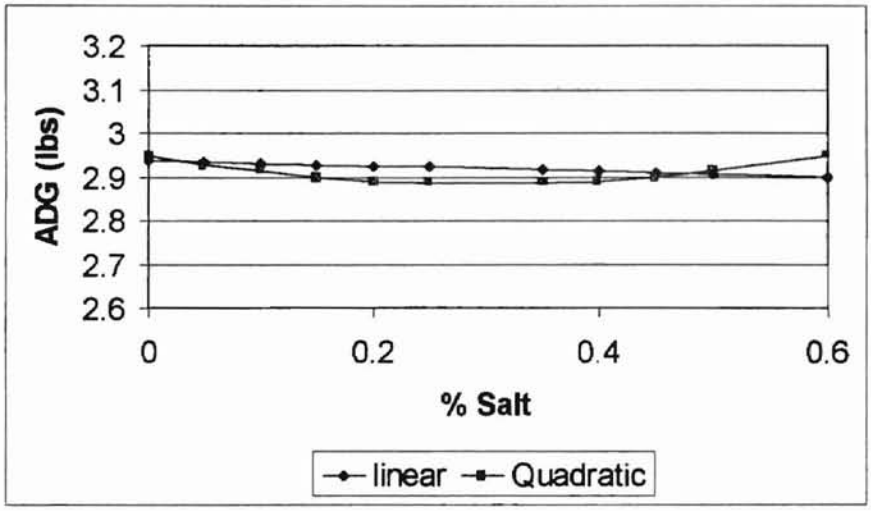


Figure 2. Linear and quadratic relationship between ADG (lbs.) and % of Salt in the diet.



As the percentage of salt in the feed increased from 0 to .6%, average daily gain decreased by .037 lb or 1.3%. Equations for the regressions were: linear ADG (lb/d) = 2.937 - .06289 %salt ± .0717 (P=.3935; r^2=.972) quadratic ADG (lb/d) = 2.9487-.4533% salt ± .3695 + .7488 %salt squared ± .6953 (r^2=.974). Its lower ADG for this set of data was found to be at .3% of salt.

Effects on ADFI. Again data from 11 trials were used to determine the relation between ADFI and % of salt in the diet.

Figure 3. Relation between ADFI (lb.DM/day) and the percentage of salt in the diet for each of the experiments considered.

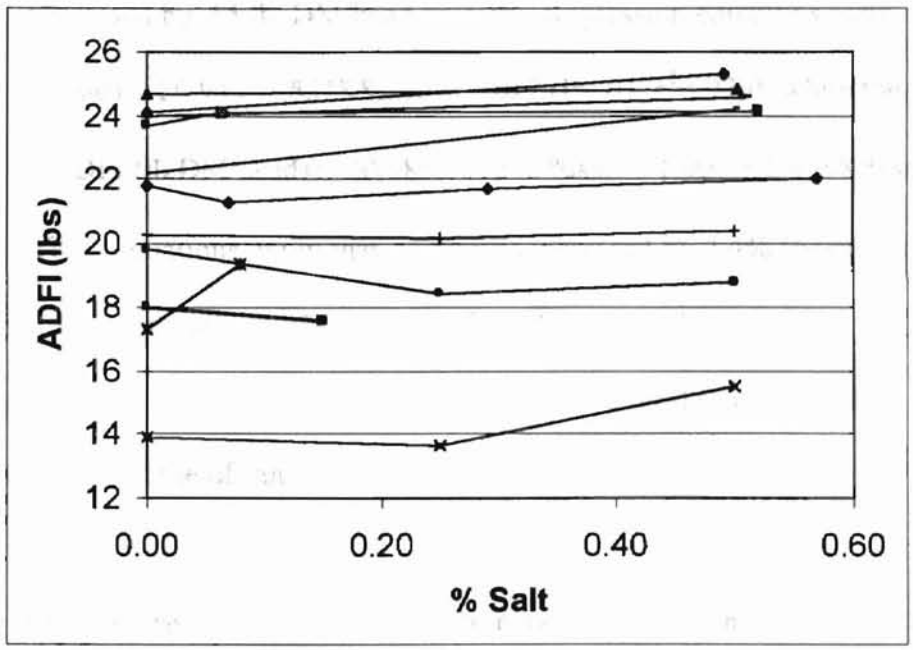
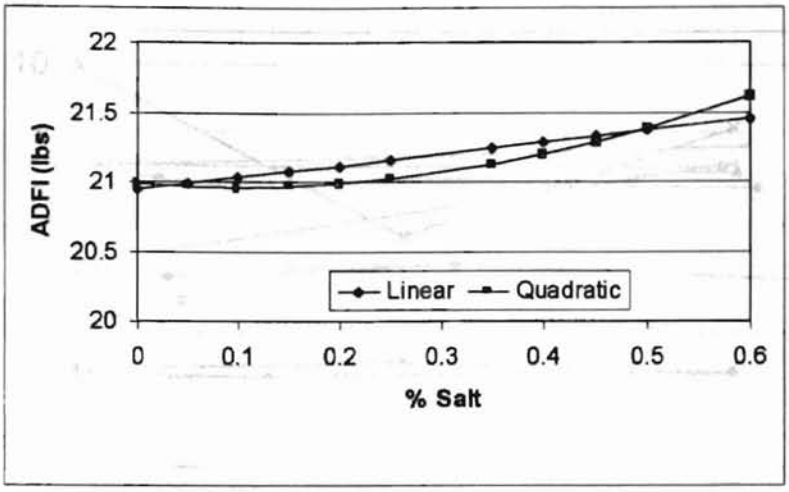


Figure 4. Linear and quadratic relationship between ADFI (lb DM/day) and % of Salt in the diet.



There was a trend (P=.0317) for added salt to increase the ADFI from 0 to .6%, average daily gain decreased by .55 lb. DM/head or 2.4%. Regression equations were: linear ADFI (lb DM/head) = 20.943 + .8518 %salt ± .3612 (P=.03; r^2=.979) while the quadratic regression ADFI (lb.DM/head) = 20.985 – .5246 %salt ± 1.901 + 2.6398 %salt^2 ± 3.577 (r^2= .980). These equations imply that ADFI was increased by 2.4% (linear) or 3.0% (quadratic) by increasing salt supplementation from 0 to .6% of the diet.

Effects on feed/gain ratio. Data collected from eleven trials were used to examine the relationship between percentage of salt in the feed and feed/gain ratio.

Figure 5. Relation between feed/gain ratio and the percentage of salt in the diet for each of the experiments considered.

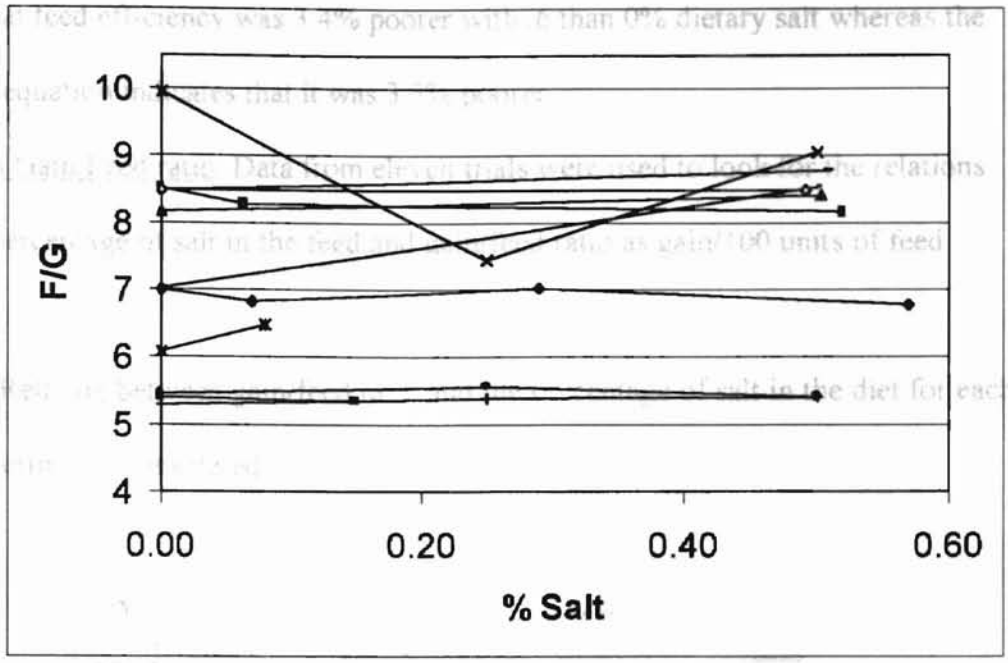
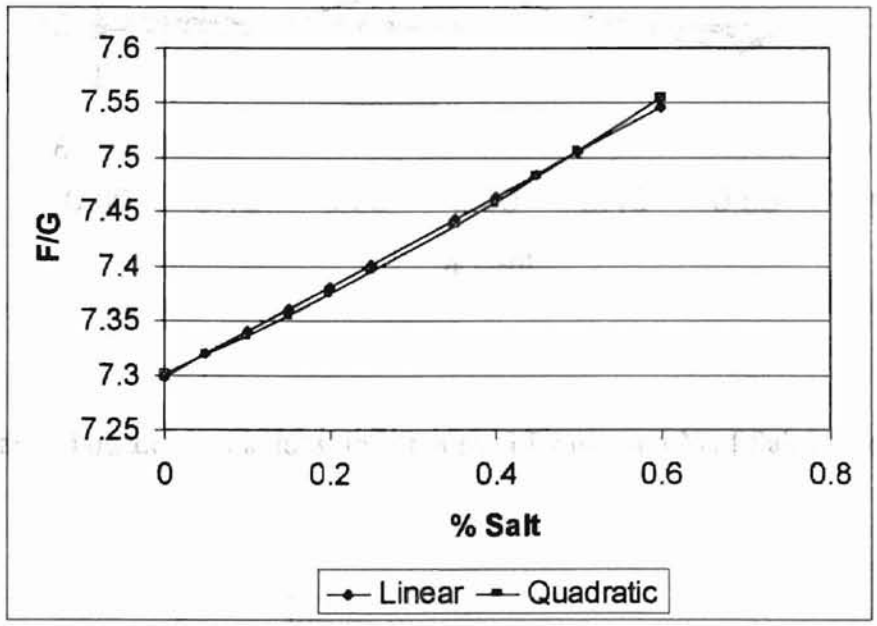


Figure 6. Linear and quadratic relationship between F/G ratio and % of Salt in the diet.



There is a trend (P=.0863) to increase the F/G ratio. For the linear regression F/G= 7.299 + .4125 %salt ± .2257 (P=.086; r^2= .975) while the quadratic regression F/G= 7.301 + .3429 %salt ± 1.207 + .1336 %salt^2 ± 2.711 (r^2= .975). The linear regression

implies that feed efficiency was 3.4% poorer with .6 than 0% dietary salt whereas the quadratic equation indicates that it was 3.5% poorer.

Effects on Gain/Feed ratio. Data from eleven trials were used to look for the relations between percentage of salt in the feed and gain/feed ratio as gain/100 units of feed.

Figure 7. Relation between gain/feed ratio and the percentage of salt in the diet for each of the experiments considered.

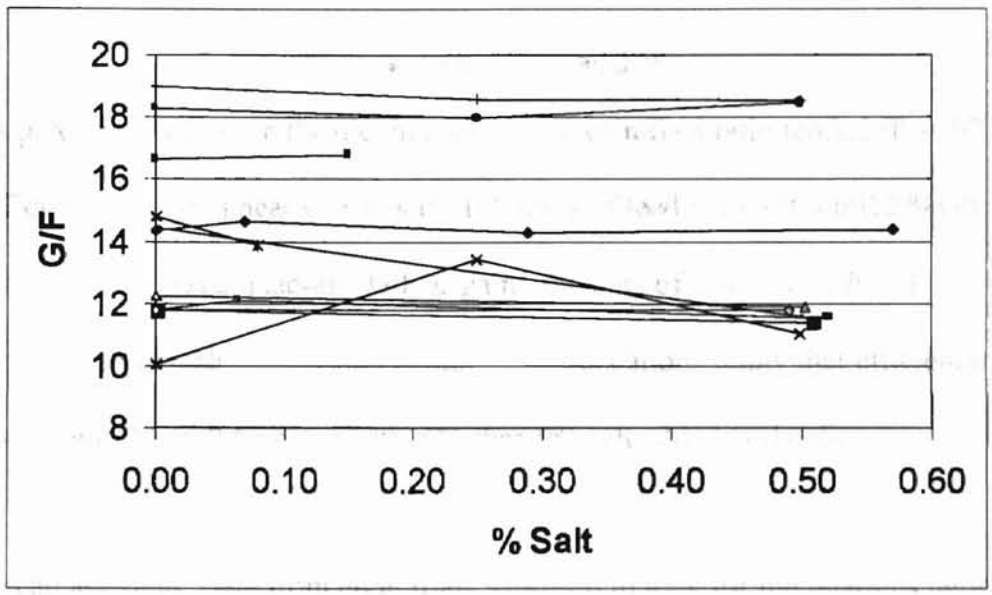
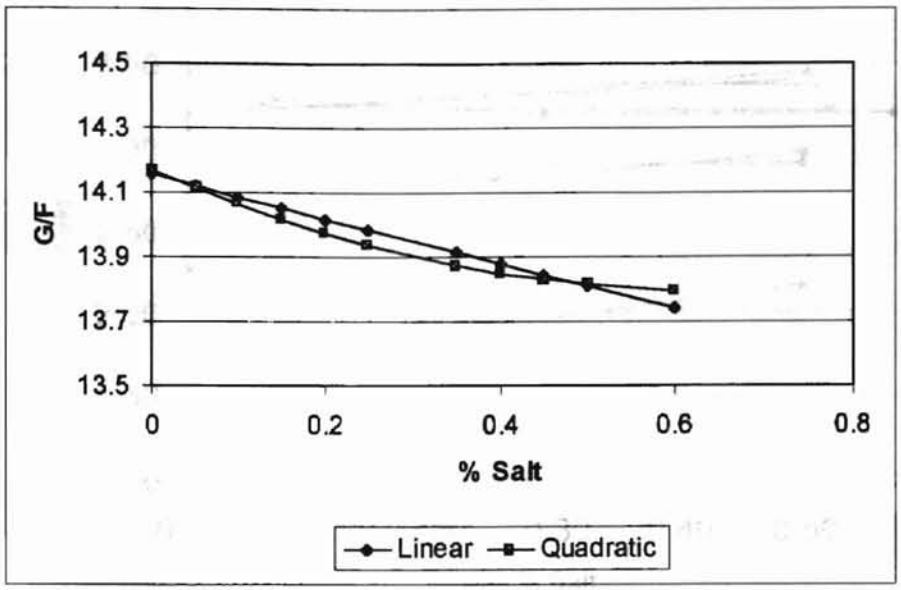


Figure 8. Linear and quadratic relationship between G/F ratio and % of Salt in the diet.



As percentage of salt in the diet increased, and gain/feed ratio tended (P = .071) to decrease. Equations were: linear G/F as gain/100 units of feed = 14.156 -.6922 %salt ± .358 (P=.071; r^2= .986) and quadratic G/F as gain/100 units of feed = 14.170 – 1.161 %salt ± 1.91 + .8985 %salt^2 ± 3.595 (r^2= .986). These equations imply that efficiency of feed use was some 2.9 to 2.6 poorer with .6% than 0% salt added to the diet.

Effects on carcass yield. Data from eight trials was used to look for the relations between percentage of salt in the feed and carcass yield.

Figure 9. Relation between carcass yield and the percentage of salt in the diet for each of the experiments considered.

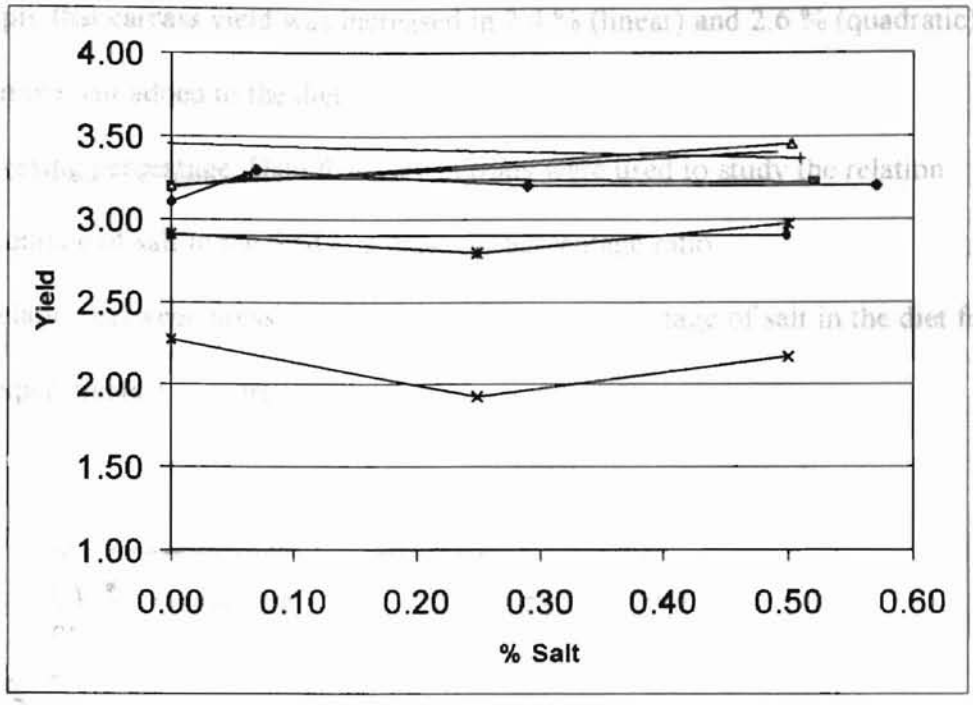
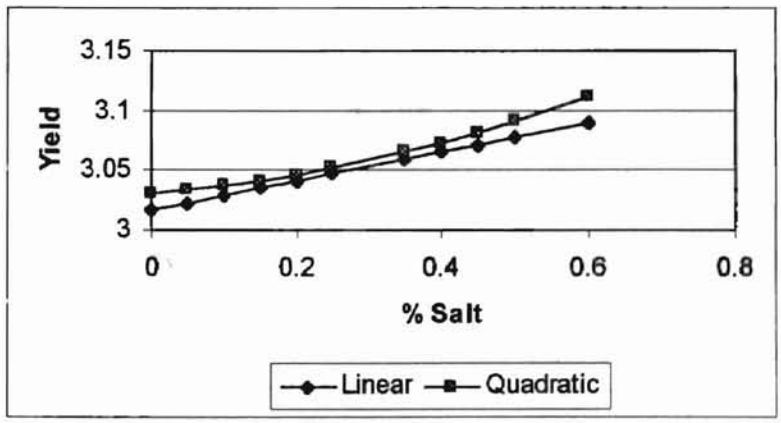


Figure 10. Linear and quadratic relationship between carcass yield and % of Salt in the diet.



The relation between percentage of salt and carcass yield shows a direct relationship, while salt in feed increases the carcass yield increases. Equations were: linear Carcass Yield = 3.016 +.1225 %salt ± .076 (P=.1334; r^2= .927) and quadratic Carcass Yield = 3.031 + .0473 %salt ± .432 + .1437 %salt^2 ± .811 (r^2= .927). These

equations imply that carcass yield was increased in 2.4 % (linear) and 2.6 % (quadratic) with .6% than 0% salt added to the diet.

Effects on dressing percentage. Data from seven trials were used to study the relation between percentage of salt in the feed and dressing percentage ratio.

Figure 11. Relation between dressing percentage and the percentage of salt in the diet for each of the experiments considered.

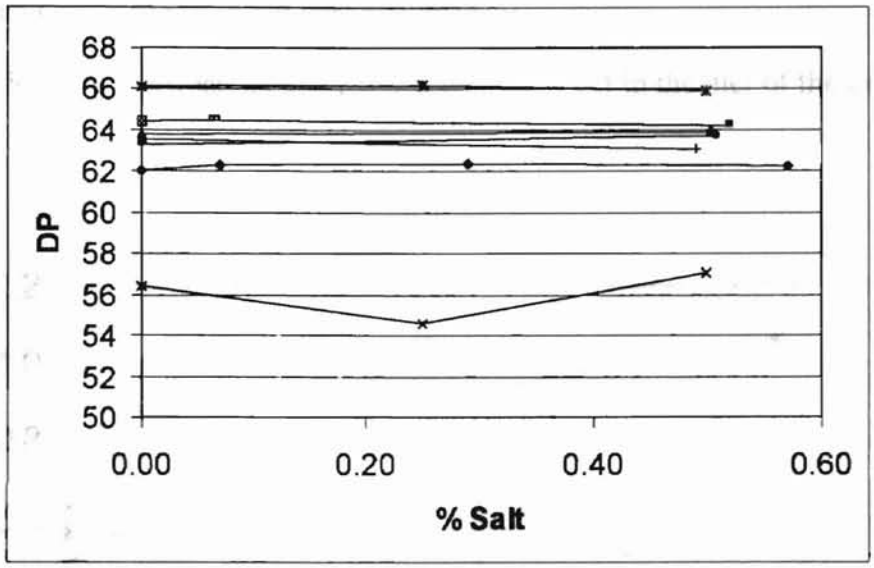
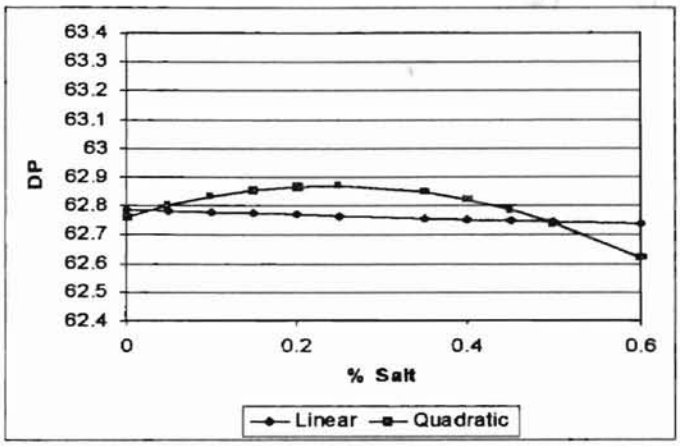


Figure 12. Linear and quadratic relationship between dressing percentage and % of salt in the diet.



Equations were: linear DP = 63.672 -.0859 %salt ± .2989 (P=.779; r^2= .980) and quadratic DP = 62.758 + .933 %salt ± 1.644 – 1.942 %salt^2 ± 3.078 (r^2= .981). These equations imply that dressing percentage was decreased in .08 % (linear) and 0.2 % (quadratic) with .6% than 0% salt added to the diet. This change might be expected if added salt increase the quantity of fluid in the digestive tract or in non-carcass tissues.

Effects on fat. Data from six different trials were pool together to study the relation between percentage of salt in the feed and fat.

Figure 13. Relation between fat and the percentage of salt in the diet of the experiments considered.

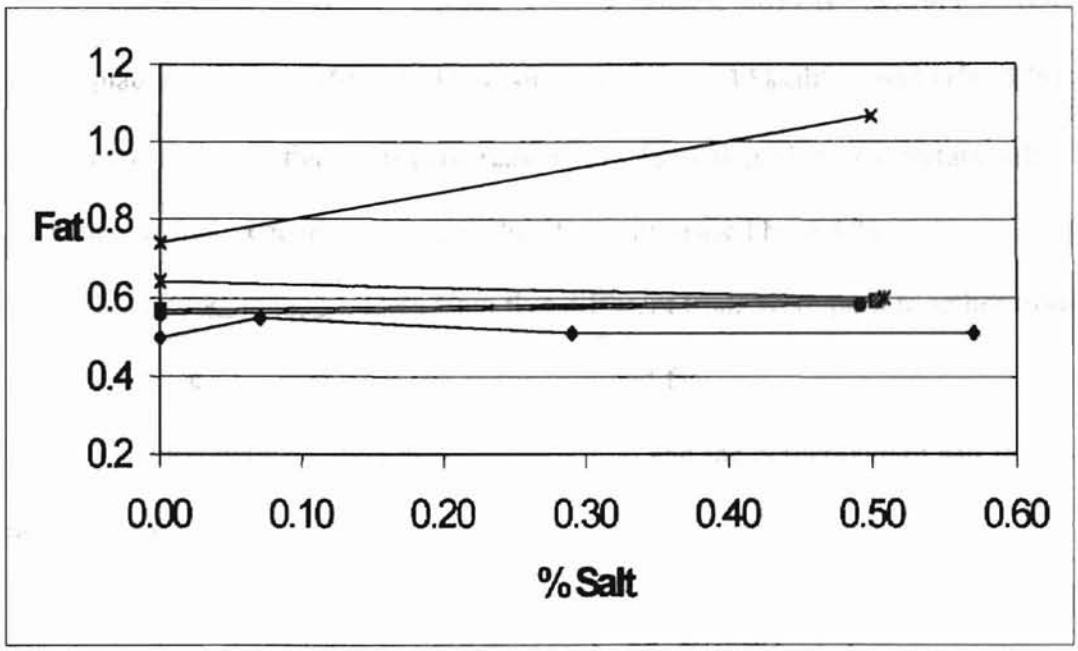
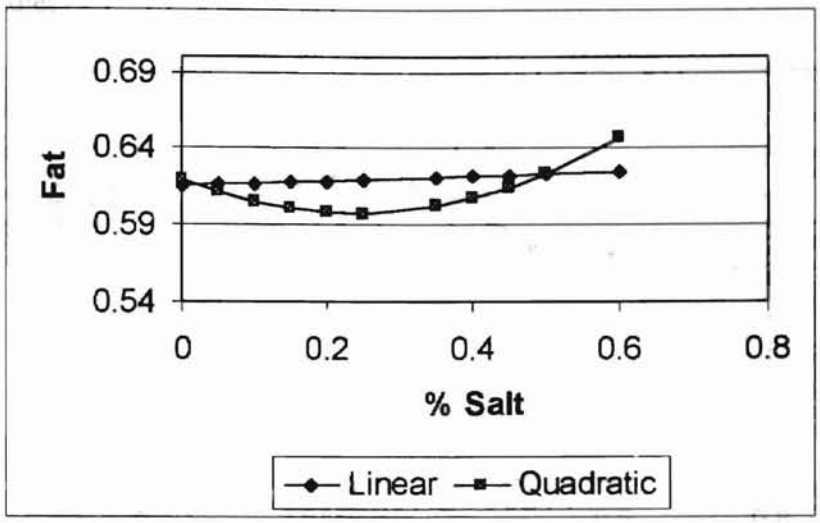


Figure 14. Linear and quadratic relationship between fat and % of salt in the diet.



Equations were: linear Fat = .6157 + .0149 %salt ± .0677 (P=.8318; r^2= .764) while the quadratic Fat = .6196 - .1855 %salt ± .361 + .3853 %salt^2 ± .681 (r^2= .775). The linear regression implies that fat was increase by 1.4% with .6 than 0% dietary salt whereas the quadratic equation indicates that it was increased by 4.4 %.

Effects on liver condemnation. Data from five different trials were pool together to study the relation between percentage of salt in the feed and fat.

Figure 15. Relation between liver condemnation (%) and the percentage of salt in the diet for each of the experiments considered.

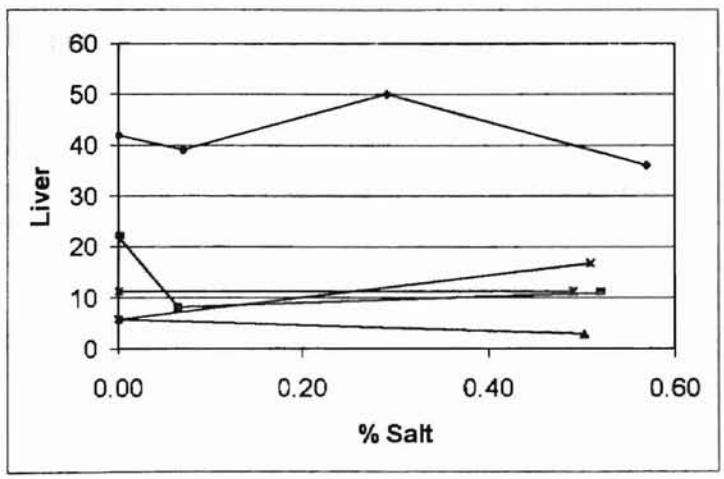
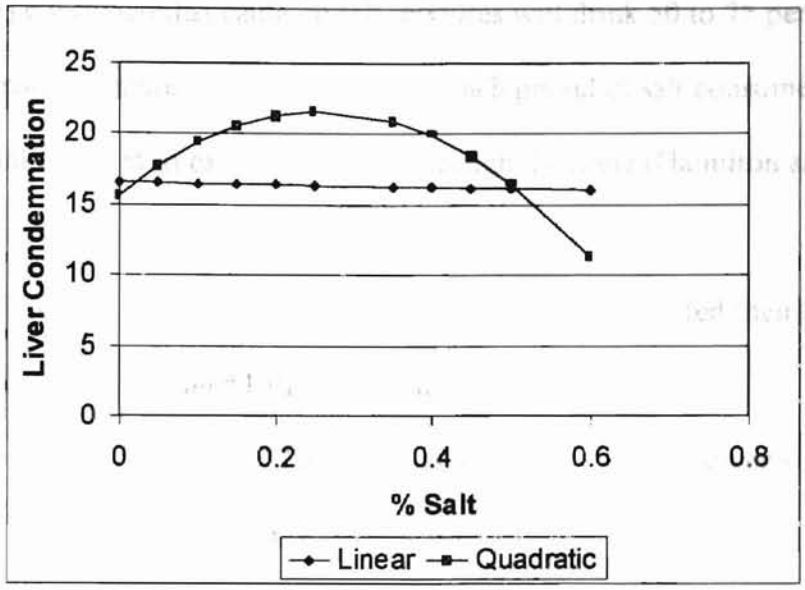


Figure 16. Linear and quadratic relationship between liver condemnation (%) and % of salt in the diet.



Equations were: linear Liver Condemnation (%) = 16.607 - .9919 %salt ± 6.99 (P=.8912; r^2= .919) while the quadratic Liver Condemnation (%) = 15.659 – 46.352 %salt ± 41.9 – 89.367 %salt^2 ± 78.029 (r^2= .934). The linear regression implies that the percentage of liver condemnation was decreased by 3.7% with .6 than 0% dietary salt whereas the quadratic equation indicates that it was increased by 38.6 %.

Effect of salt on water intake.

Several reviews have addressed the importance of water intake (Winchester and Morris 1956; ARC, 1980; NRC, 1981; Squires, 1993). According to Shirley (1986) the primary factor affecting water intake is dry matter intake. However, physiological conditions, water availability, stage of growth of the animal, temperature of water, and ambient temperature also can affect water intake (NRC, 1981).

High intakes of NaCl in concentrate diets will increase the intake of water (Bell and Sly 1979; Linn et al., 1987; and Zorrilla-Rios et al., 1990). As a practical rule, Rich et al. (1993) concluded that cattle on salt mixtures will drink 50 to 75 percent more water or approximately 5 more gallons of water for each pound of salt consumed. This increase in water intake is used to excrete the NaCl through the urine (Hamilton and Webster, 1987).

Matsushima and Phipps (1974b) reported that the steers fed their highest level of salt (6 oz. per day) consumed half a gallon more of water than the control steers. Water consumption was 6.99, 6.94, 7.22 and 7.39 gallons/day for cattle given 0, 2, 4 and 6 oz. daily salt (or approximately 0, .5, 1.04, and 1.52 % salt) respectively. Zorrilla-Rios et al., (1990) also found that when salt was increased from .5 to 5% of the diet, water intake increased from 5.81 to 8.98 gallons per day.

In contrast, Hicks et al.(1988a) indicated that increasing dietary salt level from 0 to .5% decreased feed intake by about 5% and also tended to decrease water intake by 8%). Whether the reduced water intake can be ascribed to a lowered dry matter intake rather than to salt intake alone is not clear.

Effect of Dietary Cation Anion Balance on Animal Physiology and Animal Performance

The concept of dietary cation anion balance (DCAB) is an empirical hypothesis and not a physiological mechanism (Ramberg et al., 1996). This concept of balancing rations for cations and anions has been explained for poultry by Mongin (1981) and reviewed for ruminants by Wheeler (1981). Most of the studies on DCAB in ruminants involved dairy cattle, mainly preparturient cows; little research has been conducted with

in other ruminants type of livestock. Dietary CAB can be calculated as $(Na^+ + K^+) - Cl^-$ with all minerals expressed as mEq /day or (per kg. of DM. This equation uses the concept of fixed ions (those bioavailable ions which are not further metabolized in the body) that determine acid base balance in biological fluids (Stewart, 1978). Block (1991) included sulfur in the DCAB equation even though S is not a fixed ion, in this equation in order to consider situations when sulfates are deliberately added to or protein is oversupplied in the diet. Tucker et. al., (1991) found that dietary sulfur and chloride had similar effects on acid base status. In spite of their results, these same authors recommended, that it may be necessary, when more research become available, to include a modifying coefficient for S to adjust for differences in acid-generating potential when comparing Cl and S. When S is included, the dietary cation anion balance equation containing S (DCAB:S) will be $(Na^+ + K^+) - (Cl^- + S^{2-})$ mEq /day or kg. of DM. (Note sulfur is double minus, so per ion, sulfur is given only half as much weight as other ions). Some other authors (Owens et al., 1998) have speculated that ammonia should be included with Na and K due to its effect on increasing the base load. On the other hand, chloride and sulfur increase the acid load. These anions and cations indirectly affect the hydrogen ion concentration in the body via buffer systems, kidney function and cellular respiration (Block, 1991); these are the primary systems that regulate the hydrogen ion concentration in body fluids (Guyton and Hall, 1996). However, a number of variables will contribute to the variation of the effect of the diet on the acid base; these include bioavailability of the minerals to the animal, stage of animal production (lactation, growing, etc.) and the animal's capacity to buffer any lack of adjustment of these anions and cations in the diet.

Effect of DCAB on blood.

The acid-base homeostatic mechanism in the animal helps to maintain blood pH within very narrow limits. Almost all enzymes in the body are influenced by hydrogen ion concentration. A large alteration of pH (out of its normal limits 7.31-7.53) can kill cells, while less severe alterations can affect cellular enzyme activity and the structure of hormone receptors and thereby affect animal performance (Goff, 1992).

As DCAB or DCAB:S in the diet was increased, blood pH increased linearly in dairy calves (Jackson et al., 1992) growing angus and crossbred angus steers (Ross et al., 1994a) and dairy cows (Tucker et al., 1988) and quadratically in growing and finishing angus and crossbred angus steers (Ross et al., 1994ab). However, no trend was seen with dairy cows by Tucker et al., (1991) or finishing steers after 84 days on trial (Ross et al., 1994b).

Blood bicarbonate (HCO_3^-) was reduced by anion supplementation with chloride or sulfur (Tucker et al., 1991). Blood HCO_3^- also was reduced from 23.2 to 19.3 mEq/liter when DCAB was reduced from +20 to –10 (Tucker et al., 1988). Likewise, blood HCO_3^- increased linearly when DCAB was increased from 0 to 450 meq/kg. of DM (Ross et al., 1994a) and from 0 to 520 meq/kg. of DM (Jackson et al., 1992) or quadratically when DCAB was increased from 0 to 45 meq/100 g. of DM (Ross et al., 1994b).

Blood partial pressure of carbon dioxide (pCO_2) also increased as DCAB:S increased (Tucker et al., 1988) and DCAB increased (Den Hartog et al., 1989; Jackson et al., 1992 and Ross et al., 1994b). However, no change was detected for pCO_2 in growing steers (Ross et al., 1994a).

Effect of DCAB on mineral metabolism.

The changes in pH of body fluids from feeding diets with more cations than anions or vice versa may produce and may affect cellular enzyme activity and the structure of hormone receptors; thereby, some changes in mineral metabolism can be expected.

The acidifying effect of anionic diets would be expected to affect calcium metabolism similarly to metabolic acidosis (Ramberg et al., 1996); this should stimulate Ca mobilization from the bone and elevate rate of Ca absorption from the lower gut (Block, 1984; Freeden et al., 1988 and Takagi and Block, 1991). Vagg et al., (1970) suggest that 5 to 6 g. more Ca can be mobilized daily from the bones in cows when fed high anionic salts.

Leclerc and Block (1989) reported, when using diets in which DCAB ranged from 400 to -50 mEq/kg. DM, a negative correlation (r = - .51) of DCAB to total plasma calcium. These authors explained that the higher total plasma Ca when DCAB decreased was due to an increase in bone mobilization as verified by increased plasma concentrations of hydroxyproline.

Under Ca stress, ruminants fed high anionic diets were capable of mobilizing more Ca from the bones than those fed high cationic diets (Vagg and Payne, 1970 and Block, 1984). Wang and Beede (1992) also found that ionized calcium (Ca^{++}) in blood increased from 4.68 to 4.88 mg/dl DCAB:S was decreased from 69 to –428 meq/kg of DM. However, plasma Ca increased linearly from 10.10 to 10.72 mg/100ml when DCAB was increased from 0 to 520 meq/kg of DM. (Jackson et al., 1992).

Ross et al. (1994a) found a quadratic response in Ca++ after 84 days of treatment feeding a higher DCAB diet (450 vs 0 meq/kg). However, Ross et al., (1994b) with finishing steers did not find any significant effect of DCAB on Ca^{++} .

Goff et al., (1992b) reported that as DCAB decreases, the responsiveness of target tissue receptors to 1,25-$(OH)_2D_3$ increases. According to Wheeler (1981) metabolic acidosis can impair the metabolism of vitamin D. Gaynor et al (1989) measured 1,25-dihydroxyvitamin D in blood and reported that cows fed a high anionic salt had elevated concentrations of these vitamin 3 days prepartum. They cited experiments with dogs and rats that show that tissues are refractory to PTH during metabolic alkalosis (high DCAB diets); thereby 1,25dihydroxyvitamin D production is reduced.

Jackson et al. (1992) found that plasma Mg and Cl decreased linearly as DCAB increased. Oetzel et al., (1988) and Gaynor et al., (1989) reported that serum Mg was higher for cows receiving anionic diets than for those cows receiving cationic diets, but serum P was not affected in either study. Tucker et al., (1988) detected no effect of dietary supplementation with S or Cl on plasma concentrations of Mg and P. However, Block (1984) reported that an anionic diet, serum P of peripartum cows increased. According to Tucker et al., (1988) if lowering DCAB increases PTH, P would be released from the bone, but because the threshold for reabsorption in the kidney would be reduced, more P would be excreted in the urine; thereby, plasma P concentration would not be affected.

Chloride decreased linearly with the increase of DCAB in growing steers (Ross et al., 1994a) and dairy cows (Tucker et al., 1988) but no change was found in finishing steers after 84 days on diet (Ross et al., 1994b). Den Hartog et al. (1989) observed an

increase of the blood chloride for their lowest DCAB treatment (64 mEq/kg DM) when compared to the other treatments (15.7, 25.0, 34.4 and 43.8 mEq/kg DM).

Effects of DCAB on animal performance.

Altering DCAB can alter weight gain by chicks (Mongin, 1981), milk yield of dairy cows (Tucker et al., 1988), weight gain in steers (Wheeler, 1981), average daily gain of steers (ADG, Ross et al., 1994ab) and gain:feed of growing steers (Ross et al., 1994a).

Wheeler (1981) summarized 5 experiments in which weight gain was increased when DCAB was increased from 100 to near 500 mEq/kg of DM. However, this same author found little difference in weight gain from increasing DCAB from 200 to 500 mEq/kg diet. Diets containing DCAB balances 777 to 1181 mEq/kg of DM resulted in either no improvement or reduction in animal performance when compared to steers fed control diets.

Ross et al (1994a) reported that average daily feed intake increased linearly from 7.07 to 7.81 kg. when DCAB was increased from 0 to 450 meq/kg. and that ADG and gain:feed increased quadratically. These same authors (Ross et al., 1994b) using finishing steers, found quadratic responses in dry matter intake, ADG and marbling score when DCAB was increased from 0 to 450 meq/kg. However, no differences were detected in gain:feed, yield grade, ribeye area, hot carcass weight, kidney, pelvic and heart fat nor backfat thickness. (Ross et al., 1994b).

Den Hartog et al., (1989) reported that ADG by veal calves was a significantly increased when DCAB was increased from 64, to either 157, 250, and 344 mEq/kg of DM (1.189 vs. 1.275, 1.293, and 1.298 kg respectively). However, no differences on

ADG were observed between diets with DCAB of 64 vs. 438 mEq/kg of DM. No statistical differences were found in carcass weight by these same authors, although carcass weights for calves fed diets with DCAB of 157, 250 and 344 mEq/kg of DM were 7 to 10 kg greater than for calves fed the diet with a DCAB of 64 mEq/kg of DM.

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CHAPTER III

IMPACT OF DIETARY SALT INTAKE CONCENTRATION ON WATER INTAKE, AND PHYSIOLOGICAL MEASUREMENTS OF FEEDLOT CATTLE

Abstract

Nine ruminally cannulated heifers (510 kg) in a triplicated 3 by 3 Latin square were given ad libitum access to 85% concentrate feedlot diets based on cracked corn with one of three levels of supplemental salt (0, .25, .50% of DM). Effects of salt level on intake and blood and ruminal measurements were monitored. The period of each latin square included two weeks for diet adaptation and one week for sampling and measurement. Although water intake was not significantly (P = .27) increased by added salt, water intakes averaged 14 and 30% more with the .25 and .5% salt levels than without added salt. Daily dry matter intakes also tended to increase with added salt (8.9, 10.2 and 10.4 kg with 0, .25, .50% salt, respectively). The water to dry matter intake ratio was not altered (P = .55) significantly (4.45, 4.15 and 4.53 kg water/kg dry matter consumed with 0, .25, .50% salt, respectively). Arterial blood pH tended to respond quadratically (P =.09). Arterial partial pressure of oxygen increased (P =.08), while carbon dioxide decreased (P = .07) linearly with added salt. Added salt linearly decreased arterial blood potassium (P =.06). None of the serum macrominerals were altered by added salt. Addition of salt linearly increased (P<.01) the ruminal concentration of sodium and linearly decreased the concentration of potassium (P=0.2). Magnesium and chloride responded quadratically to increasing levels of salt (P<.05 and P=.02,

respectively). Although total VFA concentrations were not altered, the butyrate percentage was lower ($P < .05$) with the 0% salt diet than the diets with .25 and .5% added salt (8.5 vs.10.8 and 11.2%). Added salt linearly increased percentages of butyrate ($P = .03$) and isobutyrate ($P = .05$) leading to an increased ($P < .05$) energy charge of ruminal VFA. Effects of dietary salt concentration on ruminal pH, evacuated weights of ruminal liquid and solids, and urinary pH were not significant.

Keywords: Salt, Feedlot Performance, Intake, Beef Cattle

Introduction

Dietary salt (NaCl) has been used for hundreds of years as an important mineral supplement even before it's composition was known. Its low price, convenience and availability has made it the preferred way of supplementing sodium and chloride. Salt is used frequently to limit feed intake of highly palatable feeds such as grain and supplement (Lusby, 1993).

In the past, feeding recommendations for minerals have been set to maximize animal growth rate, milk yield and pregnancy (Beede, 1998). However, high levels of dietary salt increase sodium and chloride concentrations in urine and feces. These salt nutrients in animal waste can limit its application to soils mainly in low rainfall or irrigated areas due to increased salinity of the soil (Van Horn et al., 1994; Eghball and Power, 1994).

One of the challenges for animal nutritionists is to reduce the environmental impact due to precise diet formulation while maintaining or enhancing productivity

and/or economical benefits. Hence, we need to understand function, metabolism and interaction of minerals in the animal.

The objectives of this experiment were 1) to test the impact of dietary salt concentration on intake of water and feed by feedlot cattle 2) to examine the impact of dietary salt on ruminal parameters and mineral concentrations and 3) to measure physiological responses in fecal dry matter, urinary and blood (venous and arterial) to dietary salt level.

Materials and Methods

Animals and treatments

Nine 510 kg ruminally cannulated heifers were allocated randomly to individual pens. The animals were given ad libitum access to a concentrate diet (Table 1) with fresh feed added daily (0830) in each 21 day period of each Latin square (14 days adaptation, 7 days to sample and measure different factors). The treatments that were superimposed to the diets were the addition of 0%, .25% and .50 % of Kansas rock salt # 4 (Cargill) with at least 96 % NaCl. The analyzed chemical composition of the diet is shown in table 2.

Total amount of feed provided as well as refused was weighted daily. Water was provided free choice in large barrels. Daily water intake was measured by reading water to the barrel to a specified level through a water meter; residual water was measured with a ruler and transformed to liters.

Marker Preparation and dosing

Chromic oxide (Cr_2O_3) was used as indigestible external marker to estimate fecal output. Chromic oxide (15 g/dose in two doses each day at 0700 and 1800) was dosed directly in the rumen for a seven-day preliminary period and three-day collection period.

Sample collection and Ruminal evacuation.

The first 14 days of each period were used for adaptation to the diets. On day 18 of each period blood samples were collected for each animal. A 10 ml. sample of arterial blood was collected from an artery in the ear with a lithium heparinized syringe (Gas Lyte, Marquest Medical Products Inc. Englewood CO). The syringe was immediately put on ice with a rubber stopper in the needle and analyzed within two hours of being drawn. A 30 ml blood sample was also collected via jugular venipuncture, using vacuum containers for serum collection with no additives. Samples were immediately put on ice, and were centrifuged and frozen within two hours.

Urine samples were collected twice a day for three consecutive days using a procedure of stimulating urination by massaging the ventral commissure of the vulva. A sample of 200ml was obtained each time and pH was measured immediately with a pH meter (Digi-sense, Cole Parmer Instrument Company, Chicago IL.). Urine samples were then frozen immediately for later analysis.

Fecal samples were collected twice daily (0700 and 1900), for 3 consecutive days and pH (Digi-sense, Cole Parmer Instrument Company, Chicago Il) was measured immediately. Fecal samples were then frozen for later analysis.

On day 21 total ruminal contents were removed mechanically using a vacuum device. Ruminal contents were screened twice (.63 x .63 and .31 x .31 cm square pore

mesh) manually to separate ruminal particulates from liquid contents. Ruminal fluid pH was measured with a pH meter (Digi-sense, Cole Parmer Instrument Company, Chicago Il). Each phase was weighed, mixed thoroughly and sampled. Samples were immediately frozen. After sampling the remaining ruminal contents were returned into the rumen. At the same time a 1 L. subsample of the liquid phase was used to determine the density and pH of ruminal liquid. Approximately 25 minutes per animal were used for the entire ruminal evacuation-replacement procedure.

Laboratory analysis

Feed and feces contents were thawed, dried at 55 °C for 48 h, air equilibrated and ground using a Wiley mill equipped with a 2 mm screen. Two 1 gram sub-samples were dried for 24 h. at 90°C to determine DM. Two subsamples of ruminal fluid and two of ruminal solids from each heifer and period were dried at 100 °C. Total DM in the rumen was calculated by adding the DM present in the liquid phase to that in the solid phase. Therefore, total ruminal DM includes solids from both the liquid and the solid phases, and total ruminal liquid includes both imbibed and free liquid .

Arterial blood was analyzed within two hours after being drawn in a Critical Blood Analyte (CBA Data Mate, Ciba Corning) for pH, partial pressure of carbon dioxide (pCO_2), partial pressure of oxygen (pO_2), total hemoglobin (thb), bicarbonate (HCO_3), total carbon dioxide (tCO_2), oxygen saturation (O_2sat), base excess (BE), sodium (Na^+) and potassium (K^+). Later, serum blood samples were thawed, and then analyzed for Ca, Mg, P, Na, Cl and K using the Blood Chemistry Automated Analyzer (Roche-Cobas Mira, Roche Diagnostics Systems Inc, Montclair, NJ.)

Ruminal fluid samples were prepared for VFA analysis by adding .05 g. of meta-phosphoric acid to 5 ml aliquots of ruminal fluid for initial deproteinazation and then centrifuged at 10,000 x g for 20 minutes. The ruminal fluid samples obtained were later analyzed for VFA concentrations using a Perkin-Elmer Autosystem gas chromatograph (Perkin-Elmer 9000 model series, Norwalk, CN) with 2-ethylbutyric acid added as an internal standard. High purity helium was used as the carrier gas, with a 8 ml/minute flow rate and the column used was Megabore phase DB-FFAP (J&W Scientific). Energy charge (EC) was calculated as (2 x butyrate)/acetate. The non glucogenic ratio (NGR) was calculated as (acetate + 2 x butyrate)/propionic.

Rumen fluid samples were centrifuged for 30 minutes at 20,000 x g. The resulting pellet was discarded, with the supernatant fluid being separated in two subsamples. One subsample was analyzed for Ca, Mg, P, Na, S and K using an Inductively Coupled Plasma Spectrometer (Spectroflame FTM-08, Spectro Analytical Instruments, Fitchburg, Ma.). The second subsample of 30ml of rumen fluid was dried at 100 °C and then ashed at 600 °C for 8 hours. Then 30ml of deionized water was added and the resulting solution was analyzed for chloride in a Flow Injector analyzer (Model Quick Chem 8000, Lachat Instruments Mn.).

Ten ml of nitric acid were added to one gram samples of feces and one g samples of feed that were digested in a Microwave (model MDS 2000 CEM Corporation, Matthews MN.). Then, 20 ml of deionized water was added and this sample was analyzed for Ca, Mg, P, Na, S and K using also the same Inductively Coupled Plasma Spectrometer. Another 1 gram sample of feed and feces were dried at 100 °C and then ashed at 600 °C for 8 hours. Thirty ml of deionized water was added and the resulting

solution was analyzed for chloride in a Flow Injector analyzer (Model Quick Chem 8000, Lachat Instruments Mn.).

Statistical analysis

The nine ruminally cannulated heifers were stratified by weight and assigned to three 3 x 3 latin squares. The three latin squares included both of the possible arrangement of treatments in order to balance for potential carry-over effects. Period, animal, and treatment were used as a source of variation and the statistical analyses were performed using the GLM procedure of SAS (1990). Linear and quadratic effects of salt were tested using contrast statements.

Results and Discussion

Dry matter and water intake

Average DMI (Table 3.) was not significantly (P=.37) altered by dietary salt concentration. Dry matter intake tended to increase 8.94, 10.25 and 10.42 kg/head/day as level of dietary salt was increased. The increase in DMI from 0 to .5 % of salt was of 16 %. This 16% increase in DMI, although numerically higher, follows the same trend of higher DMI with an increased level of dietary salt from 0 to .5 % of DM, that detected both linear (2.0 %) and quadratic (1.9 %) regressions as discussed in the literature review. Neither linear nor quadratic effects in this trial were significant.

Although water intake (WI) tended to increase with dietary salt concentration (36, 42, and 48 L/d for 0, .25%, and .5% dietary salt, water intake (WI) was not altered (P=.27) altered by dietary salt concentration, and neither linear nor quadratic effects were

found significant. Differences in WI between 0 and .50 % of salt, although non significant (P=.11) were about 30.5%. Despite lack of statistical significance, this magnitude of difference, nevertheless, may be physiologically important from the standpoint of amounts of fluid that animals must excrete and dilution of urinary components that may cause urinary calculi. Matsushima and Phipps (1974a and b) found smaller increments (1 and 8.9% for their two studies, respectively) on WI from increasing salt level from 0 to near .5% of salt. However, Hicks et al. (1988) found a decrease on WI of about 8 % from increasing the dietary salt level from 0 to .5 %. However, DMI tended to be lower in their study with the higher dietary salt concentration, and one might expect a correlation between WI and DMI.

The ratio WI/DMI did not detect either significant treatment effects (P=.55) or linear or quadratic responses. The WI/DMI was 8-9 % less for the .25% of salt treatment (4.15) when compared to the 0 and .50% treatments (4.45 and 4.53% respectively). Therefore, less water per kg. of DM was consumed by the animals on the .25% treatment. To examine this relationship more closely, WI was regressed on DMI; the regression equation after removing the effects of animal and period was WI(lts)= .075 + 4.234x(kg of DM) ± 1.15 ($P<.01$; $r^2=.86$). Thus, there was a close relationship between DMI and WI in this experiment. This suggests that the response of WI to DMI is closer than the response of WI to the level of dietary salt. Presumably with these levels of salt, which are not high enough to reduce feed intake, ruminants may show a preference consuming more salt due to their appetite for sodium (NRC, 1996). Hicks et al. (1988) also speculated that the reduction in water intake seen in their .5% salt treatment when compared with the control (0%) could be in part due to a decrease in the DMI. Murphy et

al. (1983) determined the amount of water consumed during the first 16 weeks of lactation by dairy cows fed a 40% corn silage and 60% concentrate diet. In their study dry matter intake explained more variation than any other variable although DMI was closely correlated with milk production. The other parameters that were included after a stepwise regression were minimum temperature, production, and sodium intake being the theirs contribution to the increment of r^2 of .154, .040 and .006 respectively.

Ruminal parameters

Total volatile fatty acids (VFA) and their molar proportions are presented in Table 4. Neither total VFA's, acetate, propionate nor acetate to propionate ratio (A/P) was affected by the treatments. These results differ with Croom et al. (1985) who reported a significant on day 62 ($p<.05$) from adding 5 % salt in fattening diets with an increase in the molar proportion of acetate and also a significant ($p<.05$) decrease in propionate though they detected no significant differences on day 118 of that same study. However, the maximum level of salt in their experiment was 10 times greater than in ours.

The molar proportion of butyrate was lower ($p<.05$) for the control (8.47) than for the .25 and .50 % treatments (10.81 and 11.17 respectively). There was a linear trend for butyrate and isobutyrate to increased ($P < .03$; $P < .06$) with level of dietary salt. This change in the butyrate molar proportion led to a linear increase ($P<.05$) in the energy charge (EC; butyrate to acetate ratio) of ruminal VFA. No differences were found either in valerate, isovalerate and NGR (table 4).

Ruminal pH, amounts and proportion of total ruminal contents in liquid and solid phases were not affected by the (dietary salt level (Table 4). Zorrilla Rios et al. (1990)

found that rumen contents (solids and liquids) were decreased when salt was increased from .5 to 5% of the diet.

In Table 5 the least square means for rumen fluid mineral concentration are presented. Ruminal fluid is very important since it is the biological active and soluble fraction (Owens and Goetsch, 1993). The soluble concentrations of macrominerals in the fluid fraction can affect ruminal characteristics such as rate of passage, osmolality, biological activity and buffering capacity (Durand and Kawashima, 1980). The sodium concentration increased linearly ($P<.01$) with level of dietary salt. Levels of sodium were significantly higher ($P<.01$) for .25% and .50% salt treatments (2402 and 2390 ppm) when compared with the 0% treatment (1162 ppm). This difference in Na concentration between the 0% and the .25 and .50% treatments levels can be explained by the difference in sodium intake. No Na concentration differences were found between the .25% and .50% treatments. Sodium may be absorbed from the rumen (Ammerman and Goodrich, 1983), with its absorption rate proportional to its concentration (Warner and Stacy, 1972); therefore increased absorption of sodium in the .50% treatment may explain why there was no difference between .25 and .5% dietary salt.

Potassium decreased ($P<.05$) as level of dietary salt increased. If the dietary supply of Na is inadequate, K replaces Na in the parotid saliva in order to reduce the Na fecal loss (Bailey and Balch, 1961; Morris and Gartner, 1971). This increase in the ratio of K to Na in saliva would increase the potassium concentration in the rumen fluid since more of 70 % of the water entering the rumen does so via saliva (Church, 1993). Furthermore, an increase in the concentration of one of these ions in the rumen is

accompanied by a decrease in the other (Scott, 1967) resulting in an almost constant concentration of the sum of K and Na (Martens and Blume, 1987).

Chloride concentrations responded quadratically to increasing level of salt ($P<.05$). Chloride concentration with 0 % salt (432.0 ppm) was significantly greater ($P<.01$ and $P<.05$) than with .25 % (272.9 ppm) and .50% (320.9 ppm) dietary salt, respectively. Martens and Blume (1987) found that Na and Cl transport are somehow coupled and they demonstrated that the transport of one of the ions depends on the presence of the other. These authors found using an isolated rumen of sheep that replacing Na by lithium and leaving K constant, resulted in a negative net absorption of Cl. Likewise, Dobson (1959) showed that when the concentration of potassium in the rumen was high and that of sodium low, chloride moved from the blood into the rumen against the electrochemical gradient. Although two mechanisms have been proposed for Na and Cl coupling transport during the absorption, these have not been demonstrated for the rumen. However, based on the literature, it is accepted that the absorption of Cl in the rumen is concurrent with Na absorption. A low concentration of sodium and the high K concentration in the rumen fluid of the 0 % treatment presumably would decrease the absorption of Cl and might even increase its secretion into the rumen. If so, this could explain the higher Cl concentrations in the rumen in the 0% treatment even when the Cl intake were low.

An increase in the dietary concentration of K has been shown to decrease absorption of Mg and to increase ruminal Mg concentrations (Tomas and Porter 1976; Greene et al., 1983; Fontenot et al., 1989). The site of this depression in absorption apparently is the rumen (Tomas and Porter 1976; Wylie et al., 1985). Hence, the increase

in K concentration in rumen fluid in this study can readily explain the increased ruminal Mg we detected. Magnesium concentration was significantly (P<.05) higher in the 0 % treatment (223 ppm) than in the .25 and .50% treatments (132.1 and 165.5 ppm, respectively), presumably because high ruminal K concentrations with the 0% treatment decreased Mg absorption which thereby increased its concentration in ruminal fluid. The ratio Na/K in the rumen showed a linear (P<.05) trend. The control treatment was significantly (P<.05) different from the .25% and .5% salt treatments. However, no differences were found in serum Na concentration. There were no effects (P>.10) on Ca, P and S concentrations in the rumen fluid.

Arterial blood parameters.

The arterial pH responded quadratically (P<.10) to the level of dietary salt (Table 6). Although differences from 7.42 to 7.44 are of little physiological importantce since its normal range is 7.35 to 7.45 (Stanton and Koeppen, 1998), these pH changes were consistent through the trial. Base excess refers to an empirical expression which approximates the amount of acid or base which would be needed to titrate one liter of blood back to a normal pH of 7.40. Base excess also tended to respond quadratically (P=.10) to dietary salt level. No changes on blood pH or BE were expected since the addition of NaCl to the diet has no effect on the dietary H^+ concentration, because the number of cations and anions added are the same (Goff, 1992). However, when the amount of salt in the diet is increased, sodium concentration in the body, that is in close homeostatic control (Guyton and Hall, 1996) may increase within limits. If Na increases continuously, this will trigger the kidney control mechanisms to increase Na excretion in order to keep blood Na levels within its normal range. On the other hand, dietary Cl that

is absorbed almost completely (Neathery, 1980) is not subjected to as strict control as sodium. Hence, chloride tends to increase in the blood in essence, increasing the acid load of the organism. This increase in Cl while Na is strictly maintained within limits could explain the differences between treatments in pH and BE. For control of acid excess, if pH tends to decrease, the animal's primary alteration is to reduce HCO_3 concentration in blood (Stanton and Koeppen, 1998). Following this decrease in HCO_3 concentration in blood, additional defense mechanisms will are triggered in the animal that do may not correct the acid-base disturbance but instead are designed to minimize the change in pH imposed by the disturbance (Stanton and Koeppen, 1998). Such defense mechanisms include an increase of intra and extracellular buffers, hyperventilation, which results in an increase of pO_2 and in a reduction of pCO_2; however, if the acid base disorder is excessive, it will increase a renal acid excretion (Guyton and Hall, 1996; Stanton and Koeppen, 1998). In this trial, the partial pressure of carbon dioxide (pCO2) decreased linearly (P=.08) while partial pressure of oxygen (pO2) increase linearly (P=.07) with increasing level of salt (Table 6). However, because no differences in urine pH were detected (Table 6), homeostatic mechanisms were adequate to maintain blood pH within the normal range.

Potassium concentration of blood decreased linearly (P=.06) as dietary salt level was increased (Table 6). Potassium in blood is strictly controlled for avoiding excess concentrations; however, decreases in K concentration are not controlled (Mc Dowel, 1992). Considering the statistical design used each heifer served as its own control. If we also conclude that, WI equals total urine output during this trial, then we can calculate K output. On that basis, the total urine output was higher with increasing levels of salt and

this would increase the total potassium output (Stanton and Koeppen, 1998). High levels of Na intake can also increase the K urinary excretion (Ammerman and Goodrich, 1983). Aldosterones increase potassium secretion by the renal tubules (Mac Dowell, 1992) and Na resorption through increasing the K output in the urine (Ammerman and Goodrich, 1983).

Serum minerals

No differences were observed in serum macrominerals with various salt intakes (Table 7.) These results agree with Croom et al. (1985) who found no significant differences in Na and K either on day 62 or day 118 of the experiment. Sodium in blood is maintained constant because of close regulation mechanisms (Guyton and Hall, 1996) and was not affected by differences in dietary Na (Morris and Gartner, 1971; Morris and Murphy, 1972).

Implications

The addition of salt to the diet increased ruminal fluid concentration of sodium but reduced the ruminal concentrations of potassium, magnesium and chloride. Addition of salt to the diet also linearly decreased arterial potassium and tended to acidify blood. The molar proportion of butyrate was increased by the addition of salt to the diet and led to an increased energy charge for the VFA's in the rumen.

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Table 1. Composition of concentrate diet (dry matter basis)

Ingredient	%
Dry rolled corn	63.40
Dehydrated alfalfa pellets	6.17
Cottonseed hulls	14.76
Soybean meals (44 %)	10.19
Cane molasses	4.25
Ground limestone	0.57
Dicalcium phosphate	0.55
Urea (46%)	0.11
Total	100.00

Table 2. Analyzed chemical composition of the diet (Dry basis)

Mineral	%
Sodium	0.02
Potassium	0.83
Chloride	0.13
Calcium	0.55
Phosphorus	0.32
Magnesium	0.13
Sulfur	0.15

Table 3. Dry matter intake, water intake and ratio WI/DMI in heifers receiving different levels of salt.

	Treatments (% salt)			Probability	
	0	0.25	0.50	Linear	Quadratic
DMI, kg.	8.94	10.25	10.47	.20	.56
WI, L.	36.46	41.60	47.58	.11	.94
WI/DMI, L/kg	4.45	4.15	4.52	.83	.29

Table 4. Least square means of ruminal VFA, pH and liquid and solid contents from heifers receiving 0, .25 and .50 % salt per kg of DM.

	Treatment (%salt)			Probability	
	0	0.25	0.50	Linear	Quadratic
VFA (molar %)					
Acetate	57.71	57.15	55.09	.33	.74
Propionate	28.32	26.40	27.89	.89	.53
Butyrate	8.47[b]	10.81[a]	11.17[a]	.03	.31
Isobutyrate	1.19[d]	1.28[cd]	1.33[c]	.06	.70
Isovalerate	2.86	3.029	3.04	.80	.90
Valerate	1.44	1.32	1.47	.84	.31
A/P	2.18	2.42	2.11	.86	.43
Total VFA, mmol/L	117.0	122.9	123.4	.54	.77
NGR	2.81	3.32	2.95	.80	.34
EC	0.30[bd]	0.38[abc]	0.41[a]	.03	.49
Rumen pH	5.78	5.96	5.81	.86	.24
Rumen Contents					
Total kg.	36.24	40.76	37.04	.85	.29
Solid %	17.6	15.9	17.6	.99	.14
Liquid %	82.4	84.1	82.4	.99	.14

[ab] Means with different superscripts within row P<.05
[cd] Means with different superscripts within row P<.10

Table 5. Least square means of mineral concentration centrifuged ruminal fluid from heifers receiving 0, .25 and .50 % salt per kg of DM.

	Treatment (%salt)			Probability	
	0	0.25	0.50	Linear	Quadratic
Ruminal fluid					
Na, ppm	1392.6[a]	2125.0[b]	2131.5[b]	.01	.11
K, ppm	3410.6[c]	2520.0[d]	2420.3[d]	.02	.26
Cl, ppm	432.0[ac]	272.9[b]	320.9[abd]	.03	.02
Ca, ppm	97.5	85.5	111.4	.59	.40
P, ppm	1092.1	1110.6	1174.4	.39	.78
Mg, ppm	210.8[c]	156.2[d]	181.8[cd]	.19	.05
S, ppm	64.1	71.6	73.2	.38	.74
Ratio Na/K, ppm/ppm	0.54[c]	1.00[d]	1.00[d]	.02	.08

[ab] Means with different superscripts within row P<.01
[cd] Means with different superscripts within row P<.05
[ef] Means with different superscripts within row P<.10

Table 6. Arterial blood parameters from heifers receiving 0, .25 and .50 % salt of DM.

	Treatments			Probability	
	0	.25	.5	Linear	Quadratic
pH	7.422	7.440	7.421	.94	.08
PCO_2 mmHg	38.56[c]	37.62[cd]	36.83[d]	.08	.55
pO_2 mmHg	92.23[c]	96.13[cd]	98.36[d]	.06	.74
Na mmol/L	139.1	139.7	139.2	.86	.50
K mmol/L	4.30[c]	4.08[cd]	3.99[d]	.06	.65
HCO_3 mmol/L	25.11[c]	25.61[cd]	23.93[d]	.17	.14
BE mmol/L	1.48[cd]	2.33[c]	0.48[d]	.27	.10

[cd] Means with different superscripts within row P<.10

Table 7. Serum macrominerals from heifers receiving 0, .25 and .50 % salt of DM.

	Treatments (% salt)			Probability	
	0	.25	.5	Linear	Quadratic
Cl mmol/L	106.2	107.5	107.9	.30	.76
Na mmol/L	146.3	148.3	147.9	.36	.50
K mmol/L	4.32	4.36	4.36	.69	.83
Ca mg/dl	9.09	8.96	9.05	.83	.45
P mg/dl	7.17	6.77	6.55	.21	.85
Mg meq/L	2.12	2.22	2.08	.68	.22

Table 8. Urine and fecal pH from heifers receiving 0, .25 and .50 % salt of DM.

	Treatments (% salt)			Probability	
	0	.25	.5	Linear	Quadratic
Urine pH	6.71	6.55	6.75	.90	.52
Fecal pH	6.16	5.84	5.91	.32	.38

Chapter IV

IMPACT OF DIETARY CATION ANION BALANCE ON WATER INTAKE AND PHYSIOLOGICAL MEASUREMENTS OF FEEDLOT CATTLE

Abstract

Nine ruminally cannulated heifers (525 kg) in a triplicated 3 by 3 Latin square were given ad libitum access to 85% concentrate feedlot diets based on cracked corn. Diets were modified only by addition of either.46 % ammonium chloride (NH_4Cl), .50% plain salt (SALT) or .61 % sodium sulfate (Na_2SO_4) to achieve dietary cation-anion balances (DCAB) of 98, 186 and 270 mEq/kg DM for NH_4Cl, SALT and Na_2SO_4 respectively. Although water intake was not significantly ($P = .49$) altered by any of the treatments, the ratio of water intakes to dry matter intake linearly increased DCAB was increased being 28% ($P<.05$) and 24 % ($P<.10$) greater for NH_4Cl than SALT and Na_2SO_4, respectively. Increasing DCAB linearly increased partial pressure of carbon dioxide ($P<.05$) and bicarbonate ($P<.10$) in arterial blood; with NH_4Cl being significantly different ($P < .10$) from Na_2SO_4 for all of these measurements. Although Serum chloride decreased linearly ($P<.05$) as DCAB was increased, none of the other macrominerals in serum were not affected by DCAB. Increasing DCAB linearly increased ($P<.05$) urine pH. NH_4Cl addition to the diet increased ($P<.05$; $P<.10$) ruminal fluid concentrations of chloride and potassium when compared to the other two treatments. Sodium concentration in ruminal fluid was increased linearly ($P<.05$) by the addition of SALT or Na_2SO_4 ($P<.05$) when compared to addition of NH_4Cl to the diet. Total weight and liquid weight of ruminal contents were increased ($P<.10$) by the SALT treatment when

compared to the other two treatments. Total ruminal concentrations of VFA and molar proportions of acetate, propionate and butyrate were not affected by DCAB.

Keywords: DCAB, Intake, Minerals, Beef cattle

Introduction

Altering the dietary cation anion balance (DCAB) has been shown to alter weight gain of chicks (Mongin, 1981), milk yield of dairy cows (Tucker et al., 1988a), average daily gain of steers (Wheeler, 1981; Ross et al., 1994a and b) and swine (Patience et al. 1987), and gain:feed ratio of growing steers (Ross et al., 1994a).

Presumably, modifying the dietary cation anion balance has an effect on animals through altering blood acid base balance and mineral status. Most of the studies on DCAB in ruminants have involved dairy cattle, mainly preparturient cows. Research has been limited with other ruminants type of livestock.

The objectives of this experiment were 1) to test the impact of DCAB concentration on intake of water and feed by feedlot cattle 2) to examine the impact of DCAB on ruminal parameters and mineral concentrations and 3) to measure physiological responses in fecal dry matter, urinary and blood (venous and arterial) to DCAB.

Materials and Methods

Animals and treatments

Nine 525 kg ruminally cannulated heifers were allocated randomly to individual pens. The animals were given ad libitum access to a concentrate diet (Table 1) with fresh feed added daily (0830) during each 21 day period of each latin square (14 days for adaptation,; 7 days for sampling and measurements. The dietary modifications included addition of either .46% ammonium chloride (NH_4Cl), .61% of sodium sulfate (Na_2SO_4) .50% of Kansas rock salt # 4 (Cargill) with at least 96 % NaCl (SALT). The first two diets provided equal amounts of dietary chloride while the second and third diets provided equal amounts of dietary sodium. Analyzed chemical compositions of these diets are shown in Table 2.

Daily water and feed intake were recorded as it was described in chapter 3.

Marker preparation and dosing

Chromic oxide (Cr_2O_3) was prepared and dosing of heifers was done as described in the first trial. Complexes of Co-EDTA were prepared as specified by Uden et al. (1980). Animals were dosed with Co-EDTA at 0800 on day 20, and ruminal contents were sampled at 2, 5, 7 and 24 h later using a vacuum pump. These samples obtained were frozen immediately.

Sample collection and ruminal evacuation

Arterial and venous blood, urine and fecal samples and rumen evacuation and sampling were done as described in the first trial.

Laboratory analysis

Feed, feces, arterial blood, serum, ruminal VFA and mineral concentration lab procedures were done as described in the first trial.

Ruminal samples were thawed and centrifuged at 10,000 x g for 30 minutes. The supernatant fluid was analyzed for Co concentration using an Atomic Absorption spectrophotometer (Model 4000, Perkin Elmer, Norwalk CN) with samples being diluted with water to ensure that the marker concentration remained in the detection range of the spectrophotometer. Calculations for dilution rate were done according to Galyean (1997).

Statistical analysis

The nine ruminally cannulated heifers were stratified by weight and assigned to three 3 x 3 latin squares. The three latin squares included both of the possible arrangements to avoid biasing results by potential carry-over effects. Period, animal and treatment were used included as class variables and the statistical analyses were performed using the GLM procedure of SAS (1990) as it was for trial 1. Treatments were assumed to be equally spaced so that linear and quadratic effects of DCAB could be tested by using contrast statements.

Results and Discussion

Dry matter and water intake

Treatments did not significantly (P=.32) alter DMI. Neither linear nor quadratic effects of DCAB were significant (Table 3). Although not significant, DMI for heifers fed the NH_4Cl diet (7.9 kg.) tended to be lower than for heifers fed the SALT and Na_2SO_4 diets (9.0 and 8.5 kg respectively). Ross et al. (1994 a and b) found a linear increase in DMI with growing steers and a quadratic response peaking at the 150 mEq/kg with finishing steers, respectively, on DMI when they increased DCAB from 0 to 450 mEq/kg DM. Jackson et al. (1992) also found a quadratic response peaking at the150

mEq/kg treatment, in DMI when they increased DCAB from 0 to 520 mEq/kg DM. Takagi and Block (1991) reported non significant lower intakes in wethers with their lowest DCABS treatment (63 mEq/kg DM) when compared to their higher DCABS treatments (218 and 343 mEq/kg DM).

Water intake (WI) was not significantly different (P=.48) among treatments. Neither linear nor quadratic effects were significant (Table 3).

The ratio WI/DMI was significantly (P<.05 and P<.10) higher for the NH_4Cl diet (5.00 L/kg) than for the SALT (3.91 L/kg) and Na_2SO_4 (4.03 L/kg) diets. This was detected as a linear decrease (P<.10) as DCAB was increased. On the average, animals fed the NH_4Cl diet drank 24 to 28 % more water per kg of DM.

Ruminal parameters

Total volatile fatty acids and their molar proportions are presented in Table 4. Neither total VFA's, acetate, propionate, butyrate nor acetate to propionate ratio (A/P) was affected by the treatments. Similarly, Ross et al. (1992a) detected no effect of increasing DCAB on the molar proportions of acetate, propionate and butyrate on day 28 in growing steers. However, On day 84 these same authors found that increasing DCAB resulted in a linear (P<.05) increase in the molar proportion of propionate and a linear (P<.10) decrease in butyrate, but no effect was found on acetate. This contrasts with results of Ross et al. (1992b) who found a linear in the molar proportion of acetate while increasing DCAB from 0 to 450 mEq/kg DM on day 42. These authors found no effect of DCAB on propionate, butyrate, or A/P ratio on days 42 and 84, and no effect on acetate on day 84 didn't.

The molar proportion of valerate responded quadratically ($P<.05$) to increasing level of DCAB. The molar proportion of valerate was higher ($P<.05$) for NH_4Cl than for SALT. This contrast with results of Ross et al. (1992a) who on day 28 found a linear increase of valerate with increasing DCAB, for growing steers fed a corn silage diet. However, Ross et al. (1992b) found no significant differences in the molar proportion of valerate with finishing steers.

Ruminal pH was not affected by the treatments ($P=.99$). This is in agreement with Ross et al (1992a) who didn't find any trend on day 84 and Ross et al (1992b). However, Ross et al (1992a) observed a linear increase on day 28, in ruminal pH with increasing level of DCAB. Tucker et al. (1988) also found that ruminal pH increased when DCAB was increased from –100 mEq/kg DM (6.45) to 0 (6.63) and 100 (6.73) mEq/kg DM. They suggested that this was a result of the acidogenic properties of the diets. Likewise, Freeden et al. (1988) observed that anionic diets increased H^+ concentrations in the rumen. However, in our experiment neither linear nor quadratic effects on ruminal pH were detected as being significant (Table 4).

Total ruminal contents and liquid ruminal contents responded quadratically to increasing DCAB ($P=.05$; $P<.05$ respectively; Table 4). Total weight of ruminal contents (kg) was higher ($P<.10$) for the SALT treatment (35.65) when compared with NH_4Cl (29.69) and Na_2SO_4 (28.81). The higher liquid content that the SALT treatment exhibit when compared with the other two explains this larger amount of rumen contents in the SALT treatment. According to Owens and Goetsch (1993) as level of feed intake increases, ruminal volume increases as well. Though the response was not significant, dilution rates tended to decrease as DCAB increased (Table 4).

Least square means of the rumen soluble minerals for the three treatments are presented in Table 5. Sodium increased linearly ($P<.05$) with increasing level of DCAB as a result of sodium concentration in the rumen being lower ($P<.05$) in the NH_4Cl treatment than in the SALT and Na_2SO_4 treatments. This response may simply be a result of greater sodium intake being greater with the SALT and Na_2SO_4 diets. Ross et al. (1992a) found a linear increment in ruminal sodium concentration with increasing DCAB. However, again in their experiment sodium intake increased as DCAB was increased. On day 84 they detected no linear trend but instead noted that ruminal Na was lower with the 0 and 150 mEq/kg (.07 and .08% sodium in the diet) but no differences between 300 and 450 mEq/kg (.48 and .74% sodium in the diet).

Ruminal potassium concentration was lower ($P<.05$) for the NH_4Cl treatment when compared to the other two treatments. Neither linear nor quadratic responses were observed (Table 5). Tucker et al. (1988a) reported that ruminal Na and K were inversely related. A similar trend was reported by Scott (1967). Martens and Blume (1987) reported that the sum of K and Na in the rumen remained surprisingly constant. As discussed in trial 1, if the dietary supply of sodium is inadequate is replaced by potassium in the parotid saliva in order to reduce the Na fecal loss (Bailey and Balch, 1961; Morris and Gartner, 1971). This would increase the potassium concentration in the rumen fluid since at least 70 % of the water entering the rumen does so via saliva (Church, 1993).

The ruminal concentration of chloride was lower ($P<.05$) in the SALT (279.8 ppm) treatment than in the NH_4Cl (358.4 ppm) treatment. Neither linear nor quadratic responses were observed (Table 5). No differences were found between Na_2SO_4 and the other two treatments (Table 5). Ross et al (1992a) found that chloride concentration in the

rumen tended to decrease with increasing DCAB. Likewise, Tucker et al. (1988) found that Cl tended to decrease with increasing DCAB. Martens and Blume (1987) suggested that Na and Cl transport and absorption are coupled. Dobson (1959) showed that when the concentration of potassium in the rumen was high and that of sodium was low, chloride moved from the blood into the rumen against the electrochemical gradient. If this is so, one would expect to find a higher ruminal absorption of chloride in the SALT treatment, decreasing its concentration of chloride. The higher concentration of potassium and the higher chloride intake with low sodium might explain the higher value in chloride ruminal concentration obtained by the NH_4Cl treatment. Although the concentration of dietary Na was similar for Na_2SO_4 and SALT diets, the higher concentration of potassium in the rumen, could be partially responsible for the slight increase in the ruminal chloride concentration with the SALT treatment.

There were no effects ($P>.10$) of dietary alteration on ruminal concentrations of Ca, P, S and Mg. Neither linear nor quadratic responses were observed (Table 5). Ross et al. (1992a), on day 84, didn't find any trend of increasing DCAB on the ruminal concentrations of Ca and Mg, however, they detected a linear trend for ruminal concentration of P to decrease as DCAB was increased. The ratio Na:K tend to increase linearly with increasing DCAB ($P<.10$).

Arterial blood parameters

Arterial blood pH increased as DCAB was increased. However, neither linear nor quadratic effects were significant (table 6). Tucker et al. (1988 b) found that blood pH was not affected by DCAB and DCABS, although both S and Cl supplementation tended to lower pH. Tucker et al. (1988a) observed a significantly lower blood pH with a

lower DCAB diet. Likewise, Den Hartog et al. (1989) also found a significantly lower pH in the 64 mEq/kg DM treatment when compared with the 157, 250, 344 and 438 mEq/kg DM treatments. Jackson et al. (1992) found that blood pH increased when DCAB was increased from 0 to 520 mEq/kg DM. Likewise, as DCAB was increased, blood pH increased linearly in growing angus and crossbred angus steers (Ross et al., 1994a) and quadratically in growing and finishing angus and crossbred angus steers (Ross et al., 1994ab). However, no pH response to DCAB was detected with dairy cows by Tucker et al., (1988b) or finishing steers after 84 days on trial (Ross et al., 1994b).

Base excess tended to increase linearly (P=.12) as DCAB was increased (Table 6). Den Hartog et al. (1989) also observed an increase in base excess when DCAB was increased. These increase in base excess would be expected due to the reduced acidogenic characteristics of the diets with higher DCAB.

When challenged with an acid load, the animal will minimize the change in blood pH by increasing intra and extracellular buffers, hyperventilation (to increase pO_2 and decrease pCO_2 under acid conditions) and can increasing the renal acid excretion (Stanton and Koeppen, 1998). As could be expected, pO_2 tended to decrease (P =.47) with increasing DCAB while pCO_2 increased linearly (P=.05) with increasing DCAB. Partial pressure of carbon dioxide was significantly lower (P<.10) in the NH_4Cl treatment when compared to the highest DCAB treatment Na_2SO_4. Blood concentration of HCO_3 also increased linearly (P<.10) with increasing DCAB. These results are in agreement with Den Hartog et al. (1989), Jackson et al. (1992) and Tucker et al. (1988b). Ross et al. (1994b) found a linear increase on day 84 in pCO_2 and HCO_3 from increasing DCAB but they detected no trends in pO_2 and pCO_2 in their experiment.

Urinary pH tended to increase with increasing DCAB (Table 8). The heifers excreted their acid excess and via urine, urinary pH is considered to be a reliable index of the effectiveness of DCAB programs (Sanchez et al. 1997). Urine pH decreased linearly ($P<.05$) with increasing DCAB. The urine pH in the NH_4Cl treatment (5.95) was significant different ($P<.05$) than the Na_2SO_4 treatment (6.91). Tucker et al. (1988a and 1991) and Jackson et al. (1992) also reported that increasing DCAB increased urine pH.

No difference ($P>.10$) in arterial concentrations of Na and K were detected (table 6). If we assume that, WI equals total urine output during this trial and that the urine sample was representative of total urine excreted, then we can calculate K output. On that basis, K concentration excretion via urine was slightly smaller in the NH_4Cl treatment perhaps due to a slightly higher water intake than in the other two treatments.

No differences were found in ionic calcium (Ca^{++}) among treatments. Ross et al., (1994b) with finishing steers similarly did not find a significant effect of DCAB on Ca^{++}. In contrast, Ross et al. (1994a) found a quadratic in Ca++ after 84 days of treatment being its peak around 300 mEq/kg. However, Wang and Beede (1992) found that ionized calcium (Ca^{++}) in blood increased from 4.68 to 4.88 mg/dl as DCAB:S was decreased from 69 to –428 mEq/kg of DM.

Serum minerals

Concentration of Cl in the serum decreased with increasing level of DCAB (table 7). Although, neither linear ($P=.18$) nor quadratic ($P=.12$) responses were significant (Table 7), the serum Cl was higher ($P < .05$) with the NH_4Cl treatment than the Na_2SO_4 treatment. Linear decreases of chloride concentration in blood with increasing levels of DCAB had been observed by Ross et al. (1992 a and b), Jackson et al. (1992) and Tucker

et al. (1988a). An increased intake of chloride typically will increase serum Cl (Coppock et al., 1979; Neathery et al., 1981; Fettman et al., 1984 a and b). Fettman (1984b) reported a significant decline in serum chloride while reducing intake chloride concentration from .42 to .10 %. Differences in chloride concentrations in blood serum among treatments in our experiment can be explained by DCAB and chloride intake.

No differences were detected in serum Na, K, Mg, Ca, and P among treatments (Table 7.) and no linear or quadratic effects were observed. Similarly, Tucker et al (1988a) found no differences in serum in Na and K concentrations as DCAB was altered. As discussed in trial 1, sodium in blood remains constant because of homeostatic regulation mechanisms (Guyton and Hall, 1996) and was not affected by differences in dietary Na (Morris and Gartner, 1971; Morris and Murphy, 1972).

Implications

The ratio of water intake dry matter ratio decreased linearly as DCAB was increased. Additions of ammonium chloride increased the this ratio by 24 to 28 % when compared to the other treatments. Lower DCAB tended to acidify arterial blood and linearly decreased bicarbonate and partial pressure of oxygen. Chloride concentration on venous serum decreased linearly as DCAB was increased. Addition of ammonium chloride to the diet to decrease DCAB increased ruminal concentrations of both potassium and chloride. Increasing DCAB by replacing ammonium chloride with salt or sodium sulfate linearly increased with ruminal concentrations of sodium.

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Table 1. Composition of concentrate fed (dry matter basis)

Ingredient	%
Dry rolled corn	63.40
Dehydrated alfalfa pellets	6.17
Cottonseed hulls	14.76
Soybean meals (44 %)	10.19
Cane molasses	4.25
Ground limestone	0.57
Dicalcium phosphate	0.55
Urea (42%)	0.11
Total	100.00

Table 2. Chemical composition analysis of the diets (%) (Dry basis)

	Treatments		
	NH_4Cl	SALT	Na_2SO_4
Sodium	0.02	0.22	0.22
Potassium	0.83	0.83	0.82
Chloride	0.43	0.43	0.13
Calcium	0.55	0.55	0.55
Phosphorus	0.32	0.32	0.32
Magnesium	0.13	0.13	0.13
Sulfur	0.15	0.15	0.29
DCAB mEq/Kg DM[1]	98.2	185.8	270.2
DCABS mEq/Kg DM[2]	6.5	92.5	90.9

[1]DCAB mEq/Kg DM = mEq(Na + K)-mEqCl

[2]DCABS mEq/Kg DM = mEq(Na + K)- mEq(Cl + S)

Table 3. Dry matter intake, water intake and ratio WI/DMI in heifers receiving ammonium chloride (NH_4Cl), salt and sodium sulfate (Na_2SO_4).

	Treatments			Probability		
	NH_4Cl	SALT	Na_2SO_4	Linear	Quadratic	
DMI, kg.	7.90	8.99	8.51	.42	.22	
WI, lt.	35.97	33.19	33.09	.31	.57	
WI/DMI	4.95^{bd}	3.88^{a}	4.00^{abc}	.09	.20	Na**

[ab] Means with different superscripts within row P<.05
[cd] Means with different superscripts within row P<.10
Na= (salt and Na_2SO_4) vs NH_4Cl ; Cl= (salt and NH_4Cl) vs Na_2SO_4;** P<.05

Table 4. Least square means of ruminal VFA, pH and liquid and solid contents in heifers receiving ammonium chloride (NH_4Cl), salt and sodium sulfate (Na_2SO_4).

	Treatments			Probabilty		
	NH_4Cl	SALT	Na_2SO_4	Linear	Quad.	
VFA (molar %)						
Acetate	51.02	54.67	53.30	.51	.40	
Propionate	31.64	28.33	29.73	.64	.49	
Butyrate	12.54	12.60	12.57	.98	.97	
Isovalerate	2.49	2.86	2.58	.77	.23	
Valerate	2.31^{a}	1.53^{b}	1.82^{ab}	.11	.04	Na**
A/P	1.81	2.01	2.04	.58	.81	
Total VFA mmol/L	118.3	117.9	129.3	.33	.52	
NGR	2.66	2.92	3.02	.55	.88	
EC	0.51	0.46	0.47	.54	.62	
Methane	23.87	26.55	25.51	.59	.49	
Rumen pH	5.82	5.81	5.79	.91	.98	
Rumen Contents						
Total kg.	29.69^{d}	35.65^{c}	28.81^{d}	.81	.05	
Solids kg	4.65	5.35	4.24	.68	.23	
Liquids kg	25.04^{d}	30.30^{c}	24.57^{d}	.87	.04	
Solid %	15.6	14.9	14.8	.66	.87	
Liquid %	84.4	85.1	85.2	.66	.87	
Co-EDTA Dilution rate	3.93	3.62	3.35	.43	.98	

[ab] Means with different superscripts within row P<.05
[cd] Means with different superscripts within row P<.10
Na= (salt and Na_2SO_4) vs NH_4Cl ; Cl= (salt and NH_4Cl) vs Na_2SO_4;** P<.05

Table 5. Centrifuged ruminal fluid parameters in heifers receiving ammonium chloride (NH_4Cl), salt and sodium sulfate (Na_2SO_4).

	Treatment			Probability		
	NH_4Cl	SALT	Na_2SO_4	Linear	Quad.	
Ruminal fluid						
Na, ppm	1128.5 [a]	1723.3 [b]	1638.2 [b]	.05	.10	Na**
K, ppm	3052.5c	2237d	2355.8d	.10	.18	Na**
Cl, ppm	358.4 [a]	279.8 [b]	305.8 [ab]	.18	.12	Na*
Ca, ppm	81.2	86.02	110.2	.46	.77	
P, ppm	972.19	1059.8	984.6	.89	.29	
Mg, ppm	152.9	158.7	164.6	.67	.99	
S, ppm	60.4	66.6	60.9	.98	.32	
Ratio Na/K, ppm/ppm	0.45 [ac]	0.99 [bd]	0.92 [d]			Na**

[ab] Means with different superscripts within row P<.05
[cd] Means with different superscripts within row P<.10
Na= (salt and Na_2SO_4) vs NH_4Cl ; Cl= (salt and NH_4Cl) vs Na_2SO_4;** P<.05; *P<.10

Table 6. Arterial blood parameters in heifers receiving ammonium chloride (NH_4Cl), salt and sodium sulfate (Na_2SO_4).

	Treatments			Probability		
	NH_4Cl	SALT	Na_2SO_4	Linear	Quad.	
pH	7.409	7.422	7.430	.32	.92	
pCO_2 mmHg	34.57	35.91	37.63	.05	.88	Cl*
pO_2 mmHg	106.7	104.5	102.8	.47	.95	
Ca^{++}	4.80	4.88	4.74	.67	.34	
Na mmol/L	139.39	140.10	140.38	.27	.71	
K mmol/L	4.24	4.30	4.30	.55	.69	
HCO_3 mmol/L	22.00	23.43	24.99	.09	.96	
BE mmol/L	-1.33	0.12	1.58	.13	.99	

Na= (salt and Na_2SO_4) vs NH_4Cl ; Cl= (salt and NH_4Cl) vs Na_2SO_4;* P<.10

Table 7. Serum macrominerals in heifers receiving ammonium chloride (NH_4Cl), salt and sodium sulfate (Na_2SO_4).

	Treatments			Probability		
	NH_4CL	SALT	Na_2SO_4	Linear	Quad.	
Cl mmol/L	113.1	111.5	109.0	.04	.73	Cl*
Na mmol/L	145.3	146.4	144.7	.73	.28	
K mmol/L	4.18	4.32	4.29	.41	.43	
Ca mg/dl	9.26	9.14	9.20	.74	.62	
P mg/dl	5.65	5.83	5.71	.91	.76	
Mg meq/L	2.03	2.07	1.98	.55	.42	

Na= (salt and Na_2SO_4) vs NH_4Cl ; Cl= (salt and NH_4Cl) vs Na_2SO_4;* P<.10

Table 8. Urine and fecal pH in heifers receiving ammonium chloride (NH_4Cl), salt and sodium sulfate (Na_2SO_4).

	Treatments			Probability		
	NH_4Cl	SALT	Na_2SO_4	Linear	Quad.	
Urine pH	5.95^{a}	6.53^{ab}	6.91^{b}	.04	.76	Na**
Fecal pH	5.67	5.91	5.73	.87	.44	

[ab] Means with different superscripts within row P<.05

Na= (salt and Na_2SO_4) vs NH_4Cl ; Cl= (salt and NH_4Cl) vs Na_2SO_4;** P<.05

VITA

Alejandro Francisco La Manna

Candidate for the Degree of

Master of Science

Thesis: IMPACT OF DIETARY SALT AND DIETARY CATION ANION BALANCE ON WATER INTAKE, FEEDLOT PERFORMANCE AND PHYSIOLOGICAL MEASUREMENTS OF FEEDLOT CATTLE

Major Field: Animal Science

Biographical:

Personal data: Born in Montevideo, Uruguay on January 20 1963

Education: Graduated from the Universidad de la Republica, Facultad de Agronomia Montevideo Uruguay in October 1987. Completed the requirements for the Master of Science degree with a major in Animal Science at Oklahoma State University in May, 1999.

Experience: Employed by the Instituto Nacional de Investigacion Agropecuaria (I.N.I.A.), La Estanzuela Experimental Station as research assistant, from 1991 to present. Employed by Oklahoma State University as graduate research assistant 1997 to May 1999. Published several papers, abstracts and extension publications. More than 100 presentations for producers or professionals in Uruguay. Consultant to the livestock industry in Uruguay, 1987 to 1997.

Professional Memberships: Uruguayan Society of Agricultural Engineers. The Honor Society of Phi Kappa Phi (April, 1999).