THE EFFECT OF DIFFERENT METHODS OF CONTROLLING UROLITHIASIS ON OVINE MINERAL METABOLISM

by

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DECLARATION

I hereby declare that the research in this thesis is of my own investigation and where use was made of the work of others it has been duly acknowledged in the text.

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ABBREVIATIONS

Ca Calcium
Se Selenium
P Phosphorus
Mg Magnesium
K Potassium
Na Sodium
Cu Copper
Zn Zinc
N Nitrogen

NH_4Cl Ammonium chloride
$CaCO_3$ Calcium carbonate (limestone)
H_2CO_3 Carbonic acid
$NaHCO_3$ Sodium bicarbonate
HCl Hydrochloric acid
NaCl Sodium chloride
$CaCl_2$ Calcium chloride

SG Specific gravity
OM Organic matter
ADG Average daily gain
FCE Feed conversion efficiency

HCO_3 Bicarbonate
TCO_2 Total carbon dioxide pressure
pCO_2 Partial carbon dioxide pressure
pO_2 Partial oxygen pressure
BE Base Excess
CO_2 Carbon dioxide
H^+ Hydrogen ions
Hb Haemoglobin

ECF Extra-cellular fluid
ICF Intra-cellular fluid
VFA Volatile fatty acids
DM Dry matter
DCAB Dietary cation-anion balance
NS Non-significant
rpm revolutions per minute

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ABSTRACT

The widespread use of high-energy, low roughage diets among feedlot sheep has lead to the development of several production diseases (Bide *et al.*, 1973). One of the least easily identifiable is urolithiasis, yet it is an important cause of death among feedlot sheep (Emerick, 1988). The primary causative factors of urolithiasis are an alkaline urine and a high urinary P level (Bushman *et al.*, 1965a, 1965b, 1968). The prevention of this disease therefore involves the use of an anionic salt such as NH_4Cl in the diet, to acidify the urine, or the use of a high Ca:P ratio in order to decrease urinary P levels (Bushman *et al.*, 1965a; Robbins *et al.*, 1965). At present NH_4Cl is included in sheep rations with the express purpose of preventing urolithiasis. However, this method has a disadvantage as anionic salts have been shown to cause metabolic acidosis (Harmon & Britton, 1983) and therefore the second means of prevention, that of a high Ca:P ratio, may be the more suitable method. For this reason, an experiment was designed in order to determine whether NH_4Cl or a high Ca:P ratio was the better method of urolithiasis prevention with respect to the animal's performance, mineral metabolism and acid-base status. Furthermore, the effect of Ca and NH_4Cl on Se metabolism was studied as very little work has previously been done on this subject.

With this objective in mind, a growth trial and digestibility study were conducted. For the growth trial, a 3 x 2 x 2 factorial experiment was designed with three levels of NH_4Cl (0, 0.75 and 1.5%) at a high (4:1) and medium (2.5:1) Ca:P ratio. Se was included in the diet at a level of 0 and 0.3mg/kg. The trial extended over a period of 74 days, and during this time weight and feed intake were measured, and blood, urine and faecal samples were collected for mineral and acid-base status analysis. At slaughter, the liver, kidney, heart, pancreas and a portion of the *Longissimus dorsi* muscle were removed for mineral analysis. Fluid from various sections of the digestive tract was sampled for digesta pH determination. The digestibility trial was designed as a 4 x 4 latin square change-over design which was based upon a ten day preliminary period and a five day collection period. Urine volume and pH were measured, and faecal mass and feed intake recorded to allow for the determination of the digestibility of the treatment feeds.

NH_4Cl was found to affect most criteria considered. Increasing levels of NH_4Cl caused performance criteria (mass and feed intake) to decrease, as did blood pH, HCO_3 and BE values. Liver and kidney dry mass, and the urinary excretion of Ca, P and Mg increased. Urine pH and faecal mineral excretion decreased. The effect of 0.75% NH_4Cl on the animal was not significantly different to that of the 0% NH_4Cl diet. However, 1.5% NH_4Cl had a significantly adverse effect on the animal.

The high Ca:P ratio was found to improve mineral retention although absorption decreased as evidenced by an increased faecal mineral excretion. Blood acid-base status was adversely affected

by the higher limestone level as blood pCO_2 levels increased causing blood pH to decrease. Thus, a high limestone level was symptomatic of respiratory acidosis, although blood pCO_2 levels were not sufficiently high to allow for this classification.

The NH_4Cl x Se interaction significantly affected blood acid-base status, urine pH and urinary P excretion. The addition of Se to the diet was found to have a slight alkalizing effect on the animal, as it raised blood acid-base status and urine pH above that of the diet containing no additional Se. The NH_4Cl x Se interaction also caused urinary P excretion to increase, especially at an NH_4Cl level of 1.5%.

The NH_4Cl x Ca interaction produced varied results, as the high Ca x 1.5% NH_4Cl diet had the most detrimental effect on mass and feed criteria and blood BE values, while the most acidic combination according to abomasal and duodenal pH, blood pH, urine volume and urinary mineral excretion was the medium Ca x 1.5% NH_4Cl diet.

From the results of the current investigation, it was concluded that the best method of preventing urolithiasis was through the addition of 0.75% NH_4Cl to the diet, as this resulted in an acidic urine and yet had no significantly adverse effect on the performance, mineral metabolism or acid-base status of the animal.

GENERAL INTRODUCTION

In order to maximise their efficiency, feedlot sheep are fed diets high in energy and low in roughage (Beede & Sanchez, 1989; Staples & Lough, 1989), but these diets have been associated with acidosis (Harmon & Britton, 1983) and several other production diseases (Bide *et al.*, 1973). One of the least easily identifiable diseases is urolithiasis, and yet it is an important cause of death among feedlot sheep (Emerick, 1988). Although calculi may be formed anywhere along the urinary tract, once they move into the ureters or urethra, blockage of the urinary tract may occur. Siliceous calculi are formed under range conditions, but phosphatic calculi occur under feedlot conditions (Emerick, 1988). Factors contributing to the formation of phosphatic uroliths include an alkaline urine, and a high urinary P and low Ca level (Bushman *et al.*, 1965a; 1965b; 1968). As concentrate rations usually contain ingredients which have a relatively high P yet poor Ca content, the Ca:P ratio may often be unbalanced (Emerick & Embry, 1963a), thereby encouraging the formation of phosphatic urinary calculi (Emerick & Embry, 1963a; Bushman *et al.*, 1965a; Hoar *et al.*, 1970a).

The prevention of calculosis can generally follow two routes. One involves the inclusion of an anionic salt in the diet in order to lower urine pH to 6.6, as below this pH, Ca and Mg phosphates (the primary components of phosphatic urinary calculi) do not precipitate out of the urine (Elliot *et al.*, 1961; Carbone, 1965). The second method involves raising the Ca content of the diet, thereby maintaining at all times a high, balanced Ca:P ratio and simultaneously decreasing urinary P concentrations (Bushman *et al.*, 1965a; Robbins *et al.*, 1965).

NH_4Cl has been found to be the most effective anionic salt in the prevention of urolithiasis, and in South Africa is commonly included in sheep rations at a level of 0.5% (Crookshank *et al.*, 1960; Bushman *et al.*, 1968). However, there are disadvantages associated with this method of prevention. Anionic diets have been shown to lower feed intake and weight gains (Huntington, 1983). Furthermore, the addition of NH_4Cl to a concentrate ration aggravates the acidosis induced by high-energy diets, thus lowering acid-base status (Petito & Evans, 1984; Baker *et al.*, 1991). As sheep are foragers, their natural state is an alkaline one, and the reduction of their body pH through the ingestion of either high-grain rations or NH_4Cl will decrease the efficiency of the animal (McDonald *et al.*, 1988). NH_4Cl has also been shown to affect the metabolism of various minerals, especially that of Ca, although little research exists as to its effect on other minerals. Dietary acidity has been found to increase the intestinal absorption of Ca (Vagg & Payne, 1970; Fredeen *et al.*, 1988a) but this was accompanied by a simultaneous increase in bone resorption and urinary Ca excretion (Petito & Evans, 1984; Fredeen *et al.*, 1988b). Thus, although NH_4Cl is effective in controlling urolithiasis, it may not be the best method to use with respect to the mineral metabolism and acid-base status of the animal.

Concentrate rations often have a high P content due to the ingredients used, and as the P level of the diet increases, so too must the Ca:P ratio (Bushman *et al.*, 1965a, Robbins *et al.*, 1965). When limestone is used as the Ca supplement, ratios of greater than 4:1 have been found to decrease animal performance (Lueker & Lofgreen, 1961), and thus the optimal Ca:P ratio for sheep may be 4:1. Not only will such a ratio prevent urolithiasis by ensuring a balanced Ca:P ratio exists within the feed but, if added as limestone, the Ca should have the added benefit of buffering against the acidifying effects of the concentrate ration (Herod *et al.*, 1978).

Limestone is the cheapest and most widely used Ca supplement, and has been shown to have excellent buffering properties (Haaland & Tyrrell, 1982). The rates of animal production expected from high-energy diets have often not been obtained because of depressions in digestibility (Wagner & Loosli, 1967; Wheeler *et al.*, 1975) which may be partly associated with a decrease in pH of the gastro-intestinal tract resulting in unfavourable conditions for nutrient utilization (Wheeler & Noller, 1967). As a buffer, limestone has been found to improve the acid-base status of the animal, thereby improving animal performance (Wise *et al.*, 1965).

Thus, there are two methods of preventing ovine urolithiasis: an anionic salt such as NH_4Cl and a high Ca:P ratio. The one used at present in South Africa (NH_4Cl) has disadvantages, in that although it is not used at a very high level in the diet, concentrate rations have been shown to induce acidosis (Harmon & Britton, 1983) and the addition of NH_4Cl to the feed may exacerbate an already delicate situation. The second method involves raising the Ca:P ratio of the diet through the addition of limestone, which has the added benefit of buffering against the acidifying effects of the high energy diet (Herod *et al.*, 1978).

An experiment was therefore designed to determine whether a high Ca:P ratio was a better method of controlling urolithiasis than NH_4Cl. Furthermore, preliminary experiments showed NH_4Cl to have an effect on the retention of Se in the liver (Van Ryssen, *pers. comm.*), and it was therefore decided to include Se in the diet as a third factor. The effect of NH_4Cl, $CaCO_3$ and their interaction on general performance, mineral metabolism and acid-base status would allow for the determination of the best method for controlling urolithiasis with respect to the animal.

CHAPTER ONE
REVIEW OF LITERATURE

Urolithiasis has been classified as a metabolic disorder, caused either by a disruption of the animal's normal acid-base status, or by an imbalance between various minerals, especially Ca and P (Emerick, 1988). The following review of literature will therefore consider the causative factors of urolithiasis, and what effect the different methods of prevention have on ruminant physiology and nutrient utilization.

1.1 MAINTENANCE OF ACID-BASE STATUS

Extra-cellular fluid (ECF) pH is one of the most vigorously regulated variables of the body, and is a result of the balance between acids and bases (Houpt, 1984). Under normal conditions, acids or bases are added to the body fluids continuously, either through ingestion, or as a result of their production during cellular metabolism (Houpt, 1984). This balance is disturbed when excess acids or alkalis are added to, or removed from the body fluids, resulting in acidosis or alkalosis (Houpt, 1984). Furthermore, the body is able to buffer against acids more successfully than against bases (Block, 1991). To combat these disturbances the body utilizes three basic mechanisms, namely chemical buffering, respiratory adjustment of blood carbonic acid concentration (H_2CO_3), and the excretion of H^+ ions or HCO_3 by the kidneys (Bouda & Jagos, 1991).

1.1.1 Terms Associated with Acid-Base Status

The acid-base balance of the blood is characterized by a number of factors, which are vital when assessing the animal's state. The most important of these are blood pH, pCO_2, HCO_3 and BE (Bouda & Jagos, 1991). The partial carbon dioxide pressure, or pCO_2, is representative of the animal's respiration rate, and lies between 38 and 40mm Hg (Bouda & Jagos, 1991), although some researchers place it as high as 44mm Hg (Beede & Sanchez, 1989). The normal blood pH range for sheep is 7.38 - 7.43, with an average pH of 7.4 (Bouda & Jagos, 1991). Blood bicarbonate (HCO_3) levels usually lie between 20 and 30mmol/l (Beede & Sanchez, 1989). Another useful acid-base index is base excess (BE) which expresses in mmol/l the excess or deficit of titratable base which must be added or subtracted to the blood. Negative BE values indicate excess acid in the animal, and positive values, base excess (Bouda & Jagos, 1991). The normal BE range lies between -0.5 and +3.5mmol/l (Beede & Sanchez, 1989).

1.1.2 Buffer Systems

The principle buffer systems of the blood are the bicarbonate, plasma protein, phosphate and haemoglobin buffers (Houpt, 1984). If a strong acid is added to the blood, 53% of the buffer action is due to bicarbonate, 35% to haemoglobin, 7% to plasma protein and 5% to phosphates (Houpt, 1984). Although the total buffering capacity of the blood is considerable, it is never required to buffer all acid products at one time, as the buffers of the interstitial and intra-cellular fluid (ICF) rapidly assume part of the load, and excretion of the acid by the lungs and kidneys begins immediately in order to reduce the total acid load (Bouda & Jagos, 1991).

1.1.2.1 Bicarbonate buffer system

This is the principle buffer system of the blood and ECF (Tasker, 1980; Bouda & Jagos, 1991). Carbon dioxide (CO_2) the by-product of cellular respiration, reacts with water to form carbonic acid (H_2CO_3), which is quantitatively the most important acid formed in the body (Bouda & Jagos, 1991). The H_2CO_3 then dissociates into HCO_3 and water, as illustrated in the following equation (Houpt, 1984).

$$CO_2 + H_2O <---> H_2CO_3 <---> HCO_3 + H^+$$

Measurement of true H_2CO_3 concentration is difficult and impractical, and therefore in practice the total concentration of dissolved CO_2 (which includes and is proportional to true H_2CO_3) is used instead, and is calculated as $pCO_2 \times 0.03$ (Tasker, 1980). The bicarbonate buffer system is very effective because CO_2 is in plentiful supply in the body, and therefore pCO_2 can be maintained or varied rapidly by changes in the rate at which CO_2 is removed by pulmonary ventilation (Tasker, 1980; Houpt, 1984).

1.1.2.2 Haemoglobin buffer system

The haemoglobin-oxyhemoglobin buffer system is localised in the erythrocytes and is the second most important blood-buffering mechanism (Houpt, 1984). The role of haemoglobin (Hb) is to prevent an increase in the H^+ level of the blood, thereby preventing pH from falling, as illustrated in the following equation (Bouda & Jagos, 1991).

$$H^+ + Hb <---> HHb$$

1.1.2.3 Phosphate buffer system

The phosphate buffer system is of little importance in the ECF owing to its low concentration (Winters *et al.*, 1967), but is an important buffer in the ICF (Robinson, 1967; Wright, 1970) and in the urine (Pitts, 1964).

1.1.2.4 Respiratory buffer system

Blood pCO_2 can be varied extensively and depends upon the exquisite sensitivity of the respiratory control systems to changes in blood pCO_2 and pH (Houpt, 1984). A small increase in blood pCO_2 or decrease in pH stimulates pulmonary ventilation, and the rate of CO_2 expiration increases (Houpt, 1984). When an acid is added to the body fluids, the first reaction is a purely chemical one resulting in the formation of additional carbonic acid and a depletion of bicarbonate. As a result, the HCO_3:H_2CO_3 ratio and pH fall (Bouda & Jagos, 1991). However, the simultaneous increase in pCO_2 and decrease in pH stimulate breathing, causing a rapid expiration of the CO_2 and then, because pH is still below normal, additional CO_2 is slowly expired so that over a period of hours, pCO_2 will decrease to below normal levels (Bouda & Jagos, 1991). As a result, the HCO_3:H_2CO_3 ratio and blood pH are returned to nearly normal values (Tasker, 1980). Although the ratio of base to acid is almost normal, the amounts of each are subnormal (Tasker, 1980). This adjustment of pCO_2 by the respiratory system is compensatory, and full correction of the acid-base abnormality can be affected only by the renal excretion of H^+ ions and the production of bicarbonate (Bouda & Jagos, 1991).

1.1.2.5 Renal buffer system

When acids are added to the body, chemical buffers remove the immediate threat by altering the H^+ concentration of the blood, but a depletion of buffer bases occurs (Houpt, 1984). This problem is solved within the kidney through the formation of hydrogen ions, by a mechanism that produces one bicarbonate ion for every hydrogen ion formed (Houpt, 1984). The hydrogen ions are actively secreted into the tubular fluid of the kidneys in exchange for a cation (usually Na^+), while the bicarbonate ions move into the plasma (Houpt, 1984; Bouda & Jagos, 1991). Thus hydrogen ions, equivalent in amount to those added to the system by the diet are excreted, and the blood bicarbonate level is restored to normal (Bouda & Jagos, 1991).

1.1.3 Acid-Base Balance Disturbances

Disturbances of acid-base balance have been described as respiratory or non-respiratory. Non-respiratory or metabolic acidosis is the most frequent form of acid-base disturbance, and is characterised by a primary decrease of blood bicarbonate levels (Bouda & Jagos, 1991). Respiratory acid-base disturbances are brought about by changes in gaseous exchange between the lungs and

the blood, and thus the primary cause of respiratory acidosis is an increase in the CO_2 levels of the blood (Tasker, 1980). In each type of disturbance the process may be compensated or uncompensated (Bouda & Jagos, 1991).

1.1.3.1 Metabolic acidosis

This disturbance results from the loss of bicarbonate from the ECF which reduces both the HCO_3:H_2CO_3 ratio and blood pH (Houpt, 1984). If the fall in pH persists, it acts as a signal to the respiratory control systems resulting in increased alveolar ventilation and a fall in pCO_2 values (Bouda & Jagos, 1991). This respiratory adjustment of plasma pCO_2 begins within a few minutes of acidosis, but will not be maximally developed for up to 24 hours (Tasker, 1980). Compensation by decreasing pCO_2 values will bring the ratio of base:acid toward normal, but acidemia will persist until the lost bicarbonate is replaced (Tasker, 1980). This requires renal corrective action through the excretion of H^+ ions and the restoration of plasma bicarbonate concentration (Tasker, 1980; Bouda & Jagos, 1991).

1.1.3.2 Respiratory Acidosis

This condition is the result of hypoventilation, as CO_2 is not adequately eliminated from the body and the pCO_2 level of the blood rises (Houpt, 1984). If there is no compensatory reaction, blood pH falls as carbonic acid concentration increases (Houpt, 1984). The inability of the lungs to expire CO_2 at a normal rate may be due to depression of the respiratory centres in the central nervous system, some abnormality of the chest wall or respiratory muscles, or obstruction of gas movement or diffusion within the lung (Bouda & Jagos, 1991). The rise in pCO_2 represents a rise in carbonic acid, and buffer reactions occur with the non-bicarbonate bases (Houpt, 1984). Haemoglobin is the most important of these bases and the reaction will be

$$H_2CO_3 + Hb <---> HCO_3 + HHb$$

This interaction between blood buffers results in an appreciable rise of plasma bicarbonate concentration, and thus the buffer action ameliorates the fall in pH caused by the rise in H_2CO_3 (Tasker, 1980).

The data supplied in Tables 1.1 and 1.2 allows for a comparison of the different effects of metabolic and respiratory acidosis on the various blood parameters.

Table 1.1: The effect of metabolic acidosis on various blood acid-base parameters (Tasker, 1980)

CONDITION	pCO_2	H_2CO_3	HCO_3	HCO_3:H_2CO_3	pH
Normal	40	1.2	24	20:1	7.4
Uncompensated	40	1.2	15	12.5:1	7.2
Partially compensated	32	0.96	15	15.6:1	7.3

Table 1.2: The effect of respiratory acidosis on various blood acid-base parameters (Tasker, 1980)

CONDITION	pCO_2	H_2CO_3	HCO_3	HCO_3:H_2CO_3	pH
Normal	40	1.2	24	20:1	7.4
Uncompensated	90	2.7	24	8.8:1	7.2
Partially Compensated	90	2.7	38	14:1	7.32

1.2 THE OCCURRENCE OF UROLITHIASIS IN SHEEP

1.2.1 Mineral Factors Affecting the Incidence of Urolithiasis

In general, research has shown the occurrence of urinary calculi to be most severe in animals receiving a diet high in P and low in Ca (Emerick & Embry, 1963a; Bushman *et al.*, 1965a; Robbins *et al.*, 1965; Hoar *et al.*, 1970a). The results of various experiments are detailed in Table 1.3 and support the premise, that the more unbalanced the Ca:P ratio, the greater the incidence of urolithiasis. Furthermore, increasing the level of dietary Ca in conjunction with the higher levels of dietary P was usually accompanied by a decrease in the evidence of urolithiasis (Bushman *et al.*, 1965a). However, it was concluded that a Ca:P ratio of at least 2:1 was required for adequate protection against urolithiasis in lambs fed diets high in phosphorus (Bushman *et al.*, 1965a; Robbins *et al.*, 1965).

Table 1.3: The occurrence of urolithiasis in sheep fed diets containing differ

DIETARY PHOSPHORUS LEVEL (%)	BALANCED (Ca > P) Ca:P	% Calculi	UNBALANCED (Ca < P) Ca:P	% Calculi
0.25 - 0.35	1.1:1 1.5:1 2:1	3[$] 0[#] 0[˙]	1:1 1:2	3[α] 13[α]
0.5 - 0.6	1.1:1 1.5:1 2.3:1	7[!] 12.5[˙] 7[$]	1.1:4 1:2 1:4	31[!] 85[!] 86[α]
0.8 - 0.9	1.1:1 1.1:1	25[!] 58[#]	1.1:1 1.1:8	33[!] 73[˙]

[!] Emerick & Embry (1963a) [#] Emerick & Embry (1963b) [˙] Bushman *et al.* (1965b)
[α] Hoar *et al.* (1969) [$] Hoar *et al.* (1970)

From their research, Emerick & Embry (1963a) concluded that the maximum dietary P level that could be tolerated by sheep, while remaining free of calculi was 0.33 - 0.62% of the ration. Although 0.33% P is in excess of the NRC (1985) requirements, they felt that a high-grain ration could easily exceed this level.

Discrepancies have been found to exist between studies in the occurrence of calculi. A study by Hoar *et al.* (1969) found an 85% incidence of urolithiasis to be associated with a dietary P level of 0.55%. This figure was higher than the 50% occurrence observed in previous studies (Bushman *et al.*, 1965a; 1965b; 1967; 1968; Elam *et al.*, 1956). However, the higher incidence of calculi was obtained on an all-concentrate diet, as opposed to a concentrate diet containing roughage in the form of chopped lucerne.

The role of dietary Mg in the formation of urinary calculi remains contentious. The addition of 0.2% Mg to sheep diets in the form of magnesium oxide, resulted in a non-significant decrease in the incidence of urolithiasis (Bushman *et al.*, 1965a), and the added Mg appeared to reduce the incidence of urolithiasis to an extent comparable to the reduction afforded by a similar amount of Ca (Bushman *et al.*, 1965a). However, Robbins *et al.* (1965) found that an increased intake of Mg did not significantly alter the occurrence of urolithiasis. In later research involving Mg, calculi were recovered from the kidney and bladder of sheep receiving only the highest concentration of Mg (Petersson *et al.*, 1988).

Thus, an unbalanced Ca:P ratio, or a low ratio associated with a high level of dietary P appear to be associated with the formation of calculi.

1.2.2 The Effect of Urolithiasis on Blood Mineral Levels

Lindley *et al.* (1953) noted that there was a direct relationship between the Ca:P ratio of the feed and the P content of the blood. This was later confirmed by Emerick & Embry (1963b) who noted that average plasma P values showed some degree of correlation with the level of dietary P, and subsequently with the incidence of calculi. An increasing dietary P level was associated with an increase in serum P concentration and a decrease in serum Ca values (Bushman *et al.*, 1965a; Elam *et al.*, 1959; Hoar *et al.*, 1969; 1970b). Bushman *et al.* (1965a) found that feeding supplementary Mg resulted in a significant increase in serum P levels, and that these increases, whether promoted by the feeding of supplemental P or Mg, were accompanied by an increase in serum Mg. Increasing the level of dietary Ca caused serum Ca values to rise, but had no apparent effect on serum P levels (Hoar *et al.*, 1969). However, earlier studies by Emerick & Embry (1963a), showed that increasing dietary Ca resulted in lower serum P levels. Furthermore, raising dietary Ca resulted in a decrease in serum Mg levels (Bushman *et al.*, 1965a; Hoar *et al.*, 1970a).

Table 1.4: Serum Ca and P values from sheep with or without phosphatic urinary calculi

SERUM P (mg/100ml)		SERUM Ca (mg/100ml)		REFERENCE
No calculi	Calculi	No calculi	Calculi	
6.56 - 7.52	8.37 -12.07	10.9 - 12.7	7.77 - 10.5	Bushman *et al.* (1965a)
6.9 - 8.2	9.6 - 11.1	11.2 - 11.5	10.1 - 10.7	Bushman *et al.* (1965b)
8.0 - 8.6	9.1 - 9.8	9.8 - 10.2	8.8 - 9.6	Hoar *et al.* (1969)

When serum Ca, P and Mg values are divided according to whether lambs succumbed to urolithiasis or not, it is apparent (Table 1.4), that lambs forming calculi had significantly higher serum P values and lower serum Ca concentrations than those lambs which did not form calculi. Gill *et al.* (1959) concluded that feeding excess Ca with high P rations reduced the incidence of calculi by impairing the intestinal absorption of P.

However, researchers have concluded that serum mineral levels are ineffective in diagnosing urolithiasis due to their variability and that urine mineral levels are a more accurate means of diagnosis.

1.2.3 The Effect of Urolithiasis on Urine Mineral Levels

Research has shown lambs with calculi to have significantly higher urinary P levels and lower Ca concentrations (Table 1.5). Furthermore, urinary P concentrations were directly related to dietary P levels (Hoar *et al.*, 1969). From their data, Packett & Hauschild (1964) concluded that in general, those animals which developed calculi had urinary P concentrations above 20mg/100ml. Martin & Pierce (1934) have reported normal urine P levels to vary between negligible and 18mg/100ml. These figures correspond well with the data presented in Table 1.5.

Table 1.5: Urinary P and Ca concentrations of lambs with or without urolithiasis

URINARY P (mg/100ml)		URINARY Ca (mg/100ml)		REFERENCE
No calculi	Calculi	No calculi	Calculi	
1.45 - 3.7	20.5 - 76.88	5.71 - 8.61	3.04 - 4.56	Bushman *et al.* (1965a)
3.1 - 3.4	27.8 - 47.5	1.1 - 2.0	0.5 - 0.9	Bushman *et al.* (1965b)
19 - 34	93 - 99	3.3 - 3.5	1.8	Hoar *et al.* (1969)

The urinary P values obtained by Hoar *et al.* (1969) as presented in Table 1.5, are notably higher than those of previous experiments (Bushman *et al.*, 1965a; 1965b), but were obtained with lambs fed an all-concentrate ration, while diets fed by Bushman *et al.* (1965a; 1965b) contained roughage in the form of chopped lucerne. Thus, although the no-calculi group showed a high level of urinary P, the group which developed calculi had still higher urinary P concentrations.

Increased levels of urinary P were also found to be associated with a decreased urinary Mg concentration (Lindley *et al.*, 1953; Robbins *et al.*, 1965), and since animals with urolithiasis excreted significantly less Mg and more P in the urine than unaffected lambs, Robbins *et al.* (1965) suggested that the formation of calculi may be associated with the development of metabolic conditions resulting in the retention of Mg. Packett & Hauschild (1964) also concluded that a urinary phosphate-magnesium relationship was important in the development of calculi. Petersson *et al.* (1988) found that increasing dietary Mg from 0.1 to 0.6% increased the total urinary excretion of Mg threefold, but that Ca excretion remained unaffected by Mg concentration. However, urinary P tended to be lower at the highest Mg concentration. Bushman *et al.* (1965a) also found that an increase in dietary Mg contributed towards a significant decrease in urinary P excretion. The reduction in urinary P was most apparent when 0.2% Mg was used in conjunction

with the lower levels of Ca and absent when used with the high Ca level. The failure of diet to exert an effect when used with the highest level of Ca may be due to the relatively low level to which urine P had already been reduced by this level of dietary Ca (Bushman *et al.*, 1965a). However, although 0.2% Mg, when fed with a low level of Ca, reduced urinary P to a larger degree than an equal amount of Ca, it did not exert a correspondingly greater reduction in urolithiasis (Bushman *et al.*, 1965a).

Animals with urolithiasis were found to excrete a significantly larger volume of urine than those without (Lindley *et al.*, 1953), while data from individual animals showed that copious volumes of urine were excreted by animals producing uroliths but not developing acute urolithiasis (Lindley *et al.*, 1953; Robbins *et al.*, 1965). Furthermore, sheep which produced urine with the lowest total solids exhibited the highest incidence of calculosis (Lindley *et al.*, 1953).

Bushman *et al.* (1967; 1968) showed that the calculogenic effects of high urinary P levels in sheep could be overcome by the feeding of acid forming salts, thereby producing a decrease in urine pH. This may indicate an interdependence between the two factors, and the formation of calculi.

1.2.4 The Effect of Dietary Salts on Urolithiasis

Although Udall (1962) and Udall & Chow (1963) reported that a reduction in urine pH did not appear to effect urolith formation, both earlier and later research indicated otherwise. Leoschke & Elvehjem (1954) noted that an alkaline urine appeared more conducive to phosphatic urolithiasis than an acidic urine. Crookshank *et al.* (1960) indicated that both NH_4Cl and phosphoric acid simultaneously increased urine acidity and sharply reduced stone formation, although NH_4Cl appeared to be slightly more effective than phosphoric acid in decreasing the formation of calculi. Bezeau *et al.* (1961) and Bushman *et al.* (1968) also found that the reduction in urinary pH caused by adding NH_4Cl to the diet corresponded with a significant reduction in calculi.

However, research has shown the amount of NH_4Cl included in the diet to be of importance. A level of 0.5% NH_4Cl was found to have no effect on urinary pH (Bushman *et al.*, 1968), while the reduction in urinary pH attributed to the feeding of 1% NH_4Cl was considerably smaller than that obtained by feeding 1.5% NH_4Cl (Bushman *et al.*, 1967).

$CaCl_2$ was also found to be effective in decreasing the number of animals with urolithiasis, but its effectiveness was also dependent upon dietary level (Bushman *et al.*, 1967). The addition of 1.5% $CaCl_2$ to the diet resulted in a significantly lower incidence of calculosis, while a lower level (0.5%) appeared to be ineffective in calculi prevention (Bushman *et al.*, 1967). However, when $CaCl_2$ was compared with a similar level of NH_4Cl (1.5%), urinary pH was found to be in the acidic range only for lambs fed 1.5% NH_4Cl (Bushman *et al.*, 1967). It was concluded that 1.5% NH_4Cl was effective

in preventing the formation of calculi, as it caused urine pH to drop below 6.6-6.8, above which Mg and Ca phosphates (the two primary components of phosphatic calculi) have been shown to precipitate from urine, thereby forming calculi (Elliot *et al.*, 1961; Carbone, 1965).

If acidification of the urine is the only action required, other ammonium salts of strong anions, such as ammonium sulphate and diammonium-phosphate should be equally effective in controlling calculi. In testing this premise, Crookshank (1970) found that the reduction in the total number of cases of urolithiasis was highly significant for both the NH_4Cl and ammonium sulphate treatments. However, only the lambs receiving NH_4Cl showed no clinical cases of urolithiasis. Furthermore, there was a non-significant increase in calculi formation in those animals fed diammonium-phosphate.

If an acidic urine prevents the formation of calculi, an alkaline urine should encourage their development. Hoar *et al.* (1969) reported that the inclusion of 2% $NaHCO_3$ in the diet significantly increased urinary calculi formation, and that the urine was more alkaline when $NaHCO_3$ was present in the diet than when absent (pH of 8.4 versus 7.8). Hoar *et al.* (1970b) found that the addition of 2% $NaHCO_3$ to a 15% lucerne hay diet did not promote stone formation. These results differ from those obtained previously (Hoar *et al.*, 1969) in which an all-concentrate diet was used, but are in agreement with the findings of Crookshank (1966) who discovered no significant increase in the number of phosphatic calculi in response to the addition of 1.4% $NaHCO_3$ to a diet containing 40% cotton seed hulls.

It has been speculated that the effectiveness of various salts in preventing calculi is possibly due, not to the decrease in urine pH, but instead to the presence of certain minerals in the urine, among them Ca, K, Na and Cl.

Feeding 1.5% $CaCl_2$ or NH_4Cl resulted in a significant increase in urinary Ca excretion (Bushman *et al.*, 1967; 1968). Urinary Mg was not effected by the feeding of NH_4Cl (Bushman *et al.*, 1967). They also noted that serum Ca values increased in lambs fed a high (1.5%) level of $CaCl_2$ or a low level of NH_4Cl (0.5%). Gill *et al.* (1959) reported that the urinary excretion of Ca in rats increased when the urine was acidified with NH_4Cl. However $CaCO_3$, although successful in preventing urolithiasis, did not appear to increase urinary Ca levels, but instead appeared to be related to a decrease in urinary P levels (Bushman *et al.*, 1965a). Gill *et al.* (1959) further reported that the protective effect of Ca lactate against phosphatic urolithiasis in rats appeared to involve a decreased intestinal absorption of P, and subsequent reduction in urinary P excretion. Thus, there seemed to be no basis for assigning a role in the prevention of calculi to the increased urinary Ca excretion in the presence of an acidified urine (Bushman *et al.*, 1967).

Bushman *et al.* (1967; 1968) found there to be a trend towards increased urinary P excretion in lambs fed 1 or 1.5% NH_4Cl. The fact that these lambs remained free of calculi, indicated that the

relationship between urinary P and calculi was not upheld under conditions resulting in an acidic urine (Bushman *et al.*, 1967).

Udall & Chow (1963) suggested that the effectiveness of NH_4Cl in preventing urolithiasis was due, not so much to the acidity of the urine, but to the increase in urinary Cl excretion, which protected against calculi through ion competition. However, more recent research has caused this theory to be dismissed. Bushman *et al.* (1967; 1968) observed that the high urinary Cl concentration, due to feeding 4% NaCl was accompanied by only a slight reduction in the number of calculi, while NH_4Cl and $CaCl_2$ resulted in a much lower incidence of calculi without a significant increase in urinary Cl excretion. Thus, it was concluded that an elevated urinary excretion of Cl did not offer protection against urolithiasis (Bushman *et al.*, 1968).

The cations Na and K have also been implicated in the prevention of calculi. K supplements have been seen to afford better protection against the occurrence of calculi than Na supplements (Crookshank, 1966). However, it was noted that the degree of protection was dependent upon the anion associated with the K cation. Monohydrogen phosphate increased the incidence, while chloride reduced the incidence of urolithiasis (Crookshank, 1966). Lambs fed supplementary dietary Ca exhibited higher urinary excretions of Na and K, which was associated with a greater absorption of these minerals (Bushman *et al.*, 1968). The possibility that the higher urinary Na values may contribute in part to the protective effect of supplemental Ca appeared unlikely in view of the small degree of protection provided by NaCl, which resulted in the highest urinary Na level (Bushman *et al.*, 1968).

It can be concluded from these data that variations in the urinary cations Ca, Na and K or the anion Cl, without a concomitant reduction in urinary pH plays no major role in the prevention of phosphatic urolithiasis (Bushman *et al.*, 1968). Furthermore, NH_4Cl appeared to be the most effective anionic salt for this task (Bushman *et al.*, 1967).

1.3 THE EFFECT OF AMMONIUM CHLORIDE ON OVINE PHYSIOLOGY

1.3.1 The Effect of NH_4Cl on Feed Intake and Weight.

The decrease in feed intake, caused by adding an anionic salt to the diet, has been well documented (Upton & L'Estrange, 1977; Oetzel *et al.*, 1991; Jackson *et al.*, 1992). In particular, NH_4Cl and CaCl, when fed at high levels, had an adverse effect on feed intake (Upton & L'Estrange, 1977, Oetzel *et al.*, 1991; Jackson *et al.*, 1992). However, other researches have found the addition of 0.5% NH_4Cl to the diet to have no noticeable effect on feed intake and weight gains and suggested that, if mixed in well, the acid did not render the feed unpalatable (Bushman *et al.*, 1968). The

indecision as to whether increased unpalatability is the cause of the reduced [illegible] animals fed diets containing NH_4Cl, has led to the advancement of other theories. It has been suggested that rumen fluid pH effects voluntary food intake. Tucker *et al.* (1988) noted that a decrease in dietary cation-anion balance (DCAB) caused rumen fluid pH and feed intake to fall. As rumen fluid electrolyte composition and volatile fatty acid profile were largely unaffected by DCAB, they concluded that rumen fluid pH was the factor influencing feed intake.

A high concentrate diet has also been seen to decrease rumen fluid pH and feed intake (Harmon & Britton, 1983). Furthermore, Fulton *et al.* (1979) reported that the intake of high concentrate diets was improved in cattle by the intraruminal infusion of hydroxide.

L'Estrange & Murphy (1972) found that the difference in rumen pH between their control animals and those receiving a diet with a low DCAB was greatest two and four hours after feeding. Furthermore, rumen fluid pH was highest one hour before feeding and lowest two hours after feeding. From their results, L'Estrange & Murphy (1972) suggested that it was not in fact rumen fluid pH which influenced feed intake but that metabolic acidosis, caused by the ingestion of a low DCAB ration, suppressed appetite stimulants, and thus the desire to eat was lessened. Once the animals were returned to a diet with a high DCAB, feed intake increased rapidly.

The reduced weight gains exhibited by animals on acidotic feeds has naturally been ascribed to their reduced intake associated with these diets. Of interest are the findings of Sartorius *et al.* (1949) who noted that humans fed NH_4Cl actually lost 2.2kg during the first five days of the experiment. As there was a concomitant loss of 2*l* of extracellular fluid in the first three days of acidosis, they concluded that the major loss of weight resulted from loss of extracellular fluid.

1.3.2 The Effect of NH_4Cl on Absorption and Retention of Minerals

Acidic diets have been shown to increase apparent Ca absorption (Vagg & Payne, 1970; Braithwaite, 1972; Horst & Jorgensen, 1974; Fredeen *et al.*, 1988a; 1988b), and the size of the exchangeable Ca pool (Vagg & Payne, 1970; Fredeen *et al.*, 1988a; 1988b). Alternately, Lomba *et al.* (1978) observed that acidosis increased apparent Ca absorption only when the animal's Ca balance was positive. Verdaris & Evans (1976) found that acidotic diets elevated Ca absorption when dietary Ca levels were high. Therefore, they concluded that the amount of available dietary Ca relative to requirement, may determine whether or not acid-base status alters Ca absorption.

Apparent P absorption was also enhanced by acidic diets but only in conjunction with an increased apparent Ca absorption (Petito & Evans, 1984). As there appeared to be more efficient utilization of P by calves receiving anionic diets it was suggested by Beighle *et al.* (1988), that a low DCAB may increase availability of P by favouring its intestinal absorption. On the other hand, Sartorius *et al.* (1949) found P absorption to be depressed by metabolic acidosis.

Although [illegible] been shown to increase, mineral retention of Ca, P and Mg was reduced in lambs receiving a diet containing NH_4Cl, but had no effect on body protein (Abu Damir *et al.*, 1990). Conversely, Rajaratne *et al.* (1990) reported a significant increase in the mineral retention of lambs fed diets containing relatively high concentrations of $CaCO_3$.

1.3.3 The Effect of NH_4Cl on Blood Acid-Base Status

Acidic diets have been shown to depress blood pH and HCO_3 levels (Petito & Evans, 1984; Baker *et al.*, 1991). L'Estrange & Murphy (1972) found that blood pH decreased gradually as a result of acidosis, but that during the recovery period, pH returned to normal levels within a day of supplement withdrawal. During recovery from metabolic acidosis, Sartorius *et al.* (1949) noted that there was an over-compensation with respect to pH and HCO_3, before they returned to their original levels.

Controversy exists as to the effect of acidic diets on blood pCO_2 levels. It has been seen to increase in response to a high Cl diet (Oetzel *et al.*, 1991), decrease due to a low DCAB (Baker *et al.*, 1991) or remain unaffected by dietary acidity (L'Estrange & Murphy, 1972; Scott & Buchan, 1981). However, as pH can be represented by the equation, $pH = 6.1 + \log_{10} [HCO_3/10.3 \times pCO_2]$, variations in either blood HCO_3 or pCO_2 will have an immediate effect on pH (Tucker *et al.*, 1988). Respiratory compensation to acidosis is shown by a drop in blood pCO_2 levels (Sartorius *et al.*, 1949). High Cl diets however, appear to produce a non-respiratory acidosis that does not cause blood pCO_2 to decrease (L'Estrange & Murphy, 1972; Scott & Buchan, 1981).

1.3.4 The Effect of NH_4Cl on Blood Mineral Levels

Dietary acidity was found to increase serum Cl levels, while serum Na concentration decreased (Sartorius *et al.*, 1949; Tucker *et al.*, 1988). Plasma Ca and P were slightly lowered, but Mg remained unaffected by increasing dietary acidity (Abu Damir *et al.*, 1990). Beighle *et al.* (1988) however, found that the concentration of plasma P tended to be higher in calves receiving acidogenic diets than those receiving alkaline rations. Once the acid was removed from the diet, Sartorius *et al.* (1949) noted that serum Na and Ca increased during the recovery period, but that there was some degree of over-compensation. However, plasma P and K levels remained low despite positive urinary balances. They attributed this to the replenishment of depleted cellular reserves of K and P from circulating stores.

1.3.5 The Effect of NH_4Cl on Bone Status

Barzel (1969) found that animals undergoing acidosis exhibited bones of normal length and volume but decreased specific gravity and ash content. Furthermore, high urinary Ca concentrations was

observed in acidotic goats in the absence of enhanced Ca absorption indicating possible bone resorption (Fredeen *et al.*, 1988b). Thus, the effect of acidosis on animals was to increase bone resorption as evidenced by decreased bone weight and specific gravity (Petito & Evans, 1984). Barzel (1969) and Barzel & Jowsey (1969) reported a loss of both organic and inorganic substances from bone as a result of NH_4Cl ingestion. For this reason specific gravity is seen as being the best measure of bone substance, as it takes into account both the mineral and organic content of the bone, unlike Ca or ash content (Petito & Evans, 1984).

Plasma acid phosphatase (AP) activity was seen to increase in animals on concentrate diets (Harmon & Britton, 1983). Huntington *et al.* (1981) however, reported no increase in plasma AP of adult sheep (49kg) but a 2.5 fold increase in younger lambs (33kg) while receiving a concentrate diet.

Results from various experiments indicated that metabolic acidosis directly increased Ca mobilization from bone without the action of vitamin D (Petito & Evans, 1984). Even with sustained acid loading of the body, plasma HCO_3 stabilises at a reduced level, indicating that an additional buffer system is brought into play (Petito & Evans, 1984). Lemann *et al.* (1966; 1967) suggested that such additional quantities of buffer could arise from a slow dissolution of bone mineral during chronic metabolic acidosis. Phosphates have been shown to replace carbonates in the bones of acidotic rats, and this is a possible explanation for the enhanced P absorption observed in animals during acidosis (Lemann & Lennon, 1972).

Thus, to prevent the body from using bone mineral as a buffer against the acidifying effects of NH_4Cl, it might be necessary to supply the additional Ca in the diet (Petito & Evans, 1984). However, there may be little merit in increasing skeletal mineralization in slaughter animals unless they are at risk from bone disorders (Abu Damir *et al.*, 1990).

1.3.6 The Effect of NH_4Cl on Urinary and Faecal Mineral Excretion

The most noticeable effect of acid ingestion on the urine was a rapid drop in urine pH (Sartorius *et al.*, 1949; Baker *et al.*, 1991). Sartorius *et al.* (1949) reported that pH decreased from 8 to 6 within 24 hours of acid ingestion and remained at that level throughout the experimental period. Once the acid was removed from the diet urine pH returned to normal over a period of three days. It must however, be remembered that the pH of urine is limited to a minimum of 4.4-4.7 (Houpt, 1984). A decrease in urine pH is accompanied by a decrease in urine HCO_3 concentration (Houpt, 1984). When animals are placed on an alkaline diet the opposite is observed. Urine HCO_3 levels increased with increasing pH from approximately 2mmol/l at a pH of 6 to 100mmol/l at pH 8. Thus, the increase in urine pH can probably be attributed to compensatory renal HCO_3 excretion (Tucker *et al.*, 1988).

ingestion of acid has also been seen to affect the urinary excretion of ammonium. Houpt (1984) found that in ruminants, urinary ammonium levels increased gradually during acidosis, and then remained constant. While studying the effects of acidosis on humans, Sartorius *et al.* (1949) was surprised to observe a prompt increase in the urinary excretion of ammonium. The results of other experiments have stated that the kidney responds early to an increased acid load by excreting acid in a free titratable form, and only after some delay by excreting increased quantities of ammonium. However, Sartorius *et al.* (1949) concluded that ammonium and titratable acid excretion increased at equivalent rates, and suggested that this constitutes a rapid renal compensation to acid ingestion in man.

Acid ingestion markedly effected urinary Ca excretion as an increase in dietary acid caused urinary Ca excretion to increase, sometimes by as much as ten-fold (Petito & Evans, 1984). Tucker *et al.* (1988) noted that the increase in urinary Ca was approximately equal to the increased amount of Ca absorbed from the intestine. Barzel (1969) and Barzel & Jowsey (1969) however, found that increased urinary Ca excretion in rats was derived not from increased Ca absorption, but was rather due to increased bone resorption.

From this it can be seen that the mechanism by which metabolic acidosis causes urinary Ca excretion to increase is not fully understood. One possibility is that the acidosis stimulates the slow dissolution of alkaline bone salts in order to increase the buffering capacity of extracellular fluid, and the resorbed bone Ca is excreted through the urine (Barzel, 1969). A more likely explanation is that Ca excretion is under the control of a renal mechanism which is affected by pH (Braithwaite, 1972). Evidence seems to suggest that acid stress inhibits the reabsorption of Ca from the kidney tubules by a direct effect on the metabolic processes within the renal tubular cells (Lemann *et al.*, 1967; Stacy & Wilson, 1970; Sutton & Dirsk, 1978).

The effect of dietary acidity on the excretion of urinary P is more variable than on Ca. Harmon & Britton (1983) found urinary P to increase as a result of intraruminal lactate infusion, while Tucker *et al.* (1988) noted that P excretion remained unaffected by dietary acidity. On the other hand Sartorius *et al.* (1949) found urinary P excretion to increase. Since the increased excretion was not accompanied by a fall in plasma P concentrations, they concluded that the increased urinary P was derived from either skeletal or intracellular sources. As both urinary Ca and K levels were also elevated, they concluded that P was derived from both sources. They further concluded that acidosis depressed, not only the tubular reabsorption of Ca, but also that of P.

Other minerals are also effected by acidosis. Urinary Mg excretion was seen to increase (Harmon & Britton, 1983) as did Na excretion. The excretion of Cl also increased markedly and Sartorius *et al.* (1949) noted that during the first three days of acidosis there was a net loss of Cl over and above that ingested. They calculated this to represent a loss of 2*l* of extracellular fluid over a three day period.

Information concerning the effect of dietary acidity on faecal Ca excretion is varied as it is seen to increase (Petito & Evans, 1984), decrease (Braithwaite, 1972) or remain unaffected (Wise *et al.*, 1988). However, faecal excretion of P has been shown to increase with increasing dietary acidity (Petito & Evans, 1984).

1.4 THE EFFECT OF LIMESTONE ON RUMINANT PERFORMANCE AND NUTRIENT UTILIZATION

1.4.1 The Effect of Limestone on Animal Performance

The addition of limestone to ruminant diets has been shown by several researchers to have a beneficial effect on animal performance. Wise *et al.* (1965) noted that the inclusion of 5% $CaCO_3$ in an all-concentrate diet improved the performance of steers. Cows fed a 55% concentrate diet, to which had been added 0.01% or 2.7% limestone, either lost 0.27kg/day or gained 0.66kg daily, respectively (Colovos *et al.*, 1958). The inclusion of limestone in concentrate diets has also been shown to increase live mass, carcass mass, feed conversion efficiency and to improve the animal's rate of gain (Wise *et al.*, 1963; Huntington, 1983). Furthermore, Wheeler *et al.* (1981b) showed that the supplementation of concentrate diets with limestone resulted in heavier carcasses with more fat, and which obtained better quality grades.

In most instances, the beneficial effects attributed to the addition of limestone to high-grain diets were most pronounced during the first few weeks of supplementation (Embry *et al.*, 1969). However, Wheeler *et al.* (1981a) did not notice an increased buffering effect during the initial period of his trial, but rather during the latter stages.

On the other hand, some researchers have found the inclusion of limestone in beef cattle diets to have an adverse effect on performance, as Dowe *et al.* (1957) reported a reduction in feed intake and weight gain with an increase in dietary limestone levels. Russell *et al.* (1980) also noted that steers fed limestone-supplemented diets had poorer gains and feed conversion efficiency ratios, and a lower feed intake than steers without limestone.

As the response to limestone has been varied, Wheeler *et al.* (1981a) suggested that differences between sources of limestones in rate-of-acid-neutralization could explain some of the inconsistency in animal response to limestone. They reported that steers fed a grain-based diet containing a fast-rate-of-reactivity limestone had a 0.29kg higher ADG than steers receiving a slow-rate-of-reactivity limestone. Thus, although some scientists have found limestone to have a beneficial effect on animal performance, others have found the opposite to be true.

1.4.2 The Effect of Limestone on Nutrient Utilization

It was reported by Davison & Woods (1961) that the addition of limestone to sheep rations counteracted the depressing effect of starch upon OM and cellulose digestibility. Varner & Woods (1972) found that the inclusion of 0.74% $CaCO_3$ in a concentrate diet improved the digestibility of OM, protein, energy and cellulose, and suggested that approximately half the total improvement could be attributed to increased cellulose digestion. Wheeler (1977) demonstrated that the addition of limestone to high energy diets increased the digestibility of both cell walls and starch, while also increasing the availability of dietary energy, which suggested that at least part of the beneficial response of ruminants to limestone may be attributed to an increased availability of dietary nutrients.

Wheeler & Noller (1976) found limestone to increase ration efficiency by improving the utilization of starch in the small intestine, with a corresponding decline in the loss of energy as starch in the faeces. Furthermore, they attributed the effectiveness of $CaCO_3$ in improving ration efficiency to an increased intestinal pH, thereby providing a more favourable environment for various enzymes.

On the other hand, Colovos *et al.* (1953; 1955) reported that a high level of dietary Ca (2%) depressed the digestibility of protein and energy. Dowe *et al.* (1957) observed that gains by calves decreased as the dietary Ca level was raised with supplementary limestone and that the addition of 5.7% $CaCO_3$ to a ground grain ration significantly depressed DM and nitrogen digestibility. However, $CaCO_3$ did not appear to affect the storage of either Ca or P. As the excess Ca did not increase urinary Ca excretion, it was concluded that supplementary Ca was excreted mainly via the faeces (Nicholson *et al.*, 1962). High dietary Ca levels were found to have no effect on Se metabolism in pigs (Buescher *et al.*, 1961; Lowry *et al.*, 1985). However, in ruminants, high Ca levels depressed Se absorption resulting in an increase in faecal Se excretion (Harrison & Conrad, 1984; Alfaro *et al.*, 1987). Furthermore, Alfaro et al. (1987) noted a trend towards decreased urinary Se excretion as dietary Ca levels increased.

Lueker & Lofgreen (1961) performed extensive work on the use of limestone and discovered the following: A Ca:P ratio of greater than 4:1, depressed gains through a detrimental affect of Ca upon nutrients other than P. Furthermore, as Ca intake increased the amount of Ca absorbed increased, resulting in increased Ca retention, while metabolic faecal excretion of Ca remained almost constant. They also noted that the ratio of absorbed Ca:P was approximately 1: 1 although the dietary ratio was 6:1, and that the Ca:P ratio absorbed, varied from 0.13:1 to 1.1:1 even though the dietary ratio varied from 0.8:1 to 6:1. Thus, the lack of absorbtion of either of the elements resulted in quite a different ratio being absorbed than was fed. Therefore, feeding different ratios appeared to have little effect on the amount of Ca or P absorbed, but was instead affected by the amount fed (Lueker & Lofgreen, 1961). Furthermore, P absorbtion appeared to be more efficient with a higher

dietary Ca: P ratio. In agreement with the work of Lueker & Lofgreen (1961), Manston (1967) found that an increase in the dietary Ca level resulted in at least a temporary increase in the absorbtion of that element.

1.4.3 The Effect of Limestone on the Digestive System

Limestone was selected as the primary buffering material for ruminant diets as it was believed to have no influence on rumen pH, and its principle site of action was thought to be further down the digestive tract (Wheeler *et al.*, 1981b). Furthermore, it was felt that Ca was less readily absorbed from the forepart of the gastro-intestinal tract than either Na or K bicarbonates (Wheeler, 1980). The overall Ca content of the small intestine of sheep was actually found to increase owing to endogenous excretion, and the Ca was absorbed mainly in the stomach and large intestine (Georgievskii, 1982). However, experiments with calves showed that absorption took place throughout the intestine and varied with the composition of the ration (Table 1.6). Therefore, the absorption of Ca by ruminants may vary with age and species (Georgievskii, 1982). Thus, it was concluded that the effectiveness of limestone as a buffer was associated with a low rate of absorption from the gastro-intestinal tract which should therefore allow it to influence the lower regions of the digestive tract (Wheeler & Noller, 1977).

Table 1.6: Calcium concentration in the digesta of three month old calves (Georgievskii, 1982)

REGION OF TRACT	CALCIUM CONCENTRATION	
	Concentrates	Concentrates + hay
Rumen	4.4	8.0
Abomasum	8.2	10.4
Duodenum	3.9	5.1
Ileum	2.8	5.6
Appendix	2.1	4.8
Large Intestine	1.8	4.5

Rumen pH is a function of saliva production, VFA production and absorption, level of feed intake and exchange of bicarbonate across the rumen epithelium, and usually remains within the range of 5.5 to 7 due to buffers added via the saliva and across the rumen wall (Wheeler, 1980). Rumen pH has been shown to fluctuate throughout the day, and Tremere *et al.* (1968) found that pH decreased soon after feeding and remained so for up to four hours. The feeding of diets with large

quantities of readily absorbed carbohydrates also resulted in substantial reductions in rumen pH which declined from 6.8 to below 6 as the dietary starch level increased (Wheeler, 1980).

The addition of limestone to ruminant diets has been found to have no effect on rumen or abomasal fluid pH (Wheeler & Noller, 1976; Haaland & Tyrrell, 1982). Haaland *et al.* (1982) investigated the effect of limestone on rumen fluid pH at different dietary protein levels and noted the following: Limestone increased rumen pH on the low (11%) protein diet, but not on the high protein diets (14 and 17%). They suggested that, as limestone has a relatively low solubility at the pH levels encountered in the rumen (Weast & Astle, 1978), this may account for the variation in the response of pH to limestone in the rumen. Furthermore, rumen pH values were higher on the 14 and 17% protein diets, possibly influencing the response of pH to limestone. Furthermore, Haaland *et al.* (1982) noted that buffering capacity (the ability to resist pH change) was increased by limestone at 11% protein but not on the 14 or 17% protein diets.

Wheeler *et al.* (1981b) studied the effect of $CaCO_3$ on rumen VFA concentration. They found that the level of $CaCO_3$ altered the percentage of acetate and propionate in the rumen fluid. Moles acetate/100 moles in steers fed 0.71% $CaCO_3$ ranged from 33.9 to 37.7, concentrations which were lower than the 44.2 to 49.2 moles/100 moles resulting from the lower $CaCO_3$ level. Propionate ranged from 48 to 52.8 moles/100 moles in rumen fluid from steers receiving 0.71% $CaCO_3$ to 36.6 to 41.9 moles for the steers on the lower limestone diet. On the other hand, Varner & Woods (1972) noted that the addition of $CaCO_3$ to the diet caused the molar concentration of propionate to decrease while butyrate concentration increased. They suggested that the function of Ca in the rumen appeared to be in the alteration of microbial metabolites, as judged by the shift in proportions of VFA. Haaland *et al.* (1982) however, found limestone to have no effect on rumen VFA concentration. Thus, it was concluded that the effect of buffers on VFA production is highly variable (VanCampen, 1976).

The effect of limestone on rumen micro-organisms has also been studied. Bryant *et al.* (1959) found that several species of cellulolytic rumen micro-organisms grew better at a pH near neutrality, while rumen protozoa concentration was significantly decreased by the addition of $CaCO_3$ to the diet (Varner & Woods, 1972). Several physiological changes have been attributed to changes in rumen fluid pH. Balch (1958) reported reduced total saliva flow and less total salivary buffer as a result of decreased pH. Rumination was inhibited below a pH of 5.5 (Pearce, 1965) and protozoa and cellulolytic bacteria growth reduced (Slyter *et al.*, 1970).

Hill (1970) reported that digesta in the abomasum of sheep had a pH of 6. Other studies (Ash, 1961a) showed abomasal pH to be in the region of 2 - 3. The secretion of HCl was inhibited once abomasal pH fell below 2 (Ash, 1961a), while the addition of buffered solutions (pH 5.7) did not affect the secretion of HCl (Ash, 1961b). Acidic digesta entering the small intestine of ruminants

s neutralized slowly. Harrison & Hill (1962) found the pH of duodenal digesta in sheep increased slowly from 2.7 to 4 between the proximal duodenum and the pancreatic duct. Low pH values after this, suggests that duodenal secretions have a limited neutralizing capacity (Harrison & Hill, 1962).

Wheeler *et al.* (1975) reported that steers fed all-concentrate rations had considerable quantities of starch in their faeces, and that intestinal pH values were well below neutrality, possibly due to the development of an acidic digesta in the rumen, combined with the limited neutralizing capacity of the small intestine. In contrast, Kern *et al.* (1974) found that steers fed an all-timothy hay diet had intestinal pH values between 7 and 7.3. These observations suggest that decreased starch digestion may be related to reduced activity of pancreatic alpha amylase in the small intestine due to pH values below the optimal of 6.9 (Wheeler & Noller, 1976).

The feeding of $CaCO_3$ to steers was found to increase small intestine, colon and faecal pH values (Wheeler & Noller, 1976; 1977; Wheeler *et al.*, 1981a; Haaland & Tyrrell, 1982; Haaland *et al.*, 1982) and this was associated with a reduction in the loss of starch in the faeces (Wheeler & Noller, 1976; 1977) by as much as 4.95% when 2.71% limestone was included in the diet (Wheeler & Noller, 1976).

Wheeler & Noller (1976) calculated that the correlation coefficient for faecal pH to faecal starch concentration was highly negative (R^2 = -0.94), and they concluded that the addition of limestone increased intestinal pH to a more favourable range for the enzyme pancreatic alpha amylase, and therefore decreased the loss of starch in the faeces.

Wheeler & Noller (1977) proposed that limestone aided in buffering the intestinal tract, thereby creating an increased faecal pH. Galean *et al.* (1979) and Russell *et al.* (1980) reported that buffers allowed for increased ruminal digestion of starch and less to be passed post-ruminally. Less starch in the lower digestive tract would reduce bacterial fermentation post-ruminally and could account for the increased faecal pH. Alternatively, limestone could affect faecal pH by altering the rate of passage of liquid material (Haaland & Tyrrell, 1982). Buffers tend to increase liquid disappearance rate but results are very variable. This would shift more of the digestion from the rumen to the intestine which may decrease apparent digestion, but could increase the efficiency of utilization of absorbed nutrients (Haaland & Tyrrell, 1982). It was therefore concluded that the value of limestone was not dependent only upon an effect in the rumen, but also to a buffering effect in the lower gastro-intestinal tract (Wheeler & Noller, 1976).

The responses in faecal pH and in buffering capacity between pH 4.5 and 5 may suggest that much of the limestone activity occurs postruminally (Haaland *et al.*, 1982). Alternatively, limestone could affect energy digestibility and composition of nutrients absorbed by influencing rumen environment, dilution rate and site of digestion and absorption of nutrients. Furthermore, limestone consistently increased faecal pH but did not consistently improve energy utilization (Haaland *et al.*, 1982).

In situations where buffers do not change pH, their role may be to stabilise pH, allowing for faster digestion (Haaland & Tyrrell, 1982). Changes in blood pH and HCO_3 during the initial weeks of feeding high Ca diets, do suggest effects of dietary Ca on fermentation and digestion patterns in the gut that are conducive to improved performance (Huntington, 1983). Alternatively, the requirement may be for limestone and its beneficial effects on buffering gut contents or on altering rate of passage of digesta from the rumen (Huntington, 1983). Responses to buffers have been variable and unpredictable and therefore seem to indicate a mode of action other than, or in addition to, a change in digesta pH.

The review of literature therefore illustrates that ovine urolithiasis can be prevented through the addition of an anionic salt to the diet, or by ensuring that a balanced dietary Ca:P ratio exists. Although NH_4Cl appears to be the best anionic salt for the prevention of calculi, it has also been shown to have an adverse effect on the animal's acid-base status. Thus, a high, balanced Ca:P ratio may be as effective in preventing urolithiasis as NH_4Cl, without the concomitant detrimental effect of the anionic salt.

CHAPTER TWO
MATERIALS AND METHODS

2.1 GROWTH TRIAL

2.1.1 Experimental Design

A 3 x 2 x 2 factorial design was chosen for the experiment. This statistical approach allowed for the study of both main effects and interactions between the three dietary components viz. NH_4Cl, Ca and Se, which were included in the diet at the following levels:

NH_4Cl	: 0%, 0.75% and 1.5%
Ca	: Medium (0.66 - 0.76%) and High (0.92 - 1.22%)
Se	: 0mg and 0.3mg/kg

2.1.2 Experimental Terrain

The trial was conducted at the University's Research Farm, Ukulinga. The sheep were housed in individual pens in a large, well-ventilated shed, and the floors of the pens were made of wooden slats allowing urine and faeces to fall onto the floor below.

2.1.3 Experimental Animals

Thirty-six, shorn South African Mutton Merino wethers were blocked according to weight and randomly assigned to one of the twelve treatments, resulting in three animals per treatment.

Although the sheep had been dosed for internal parasites prior to the experiment, a severe wireworm infestation broke out among the animals during the first three weeks of the experiment. Although the sheep were dosed repeatedly, the worms appeared to be resistant to all the anthelminthics used. The infestation was eventually brought under control without any of the experimental animals being lost.

2.1 4 Experimental Diet

Two rations, identical in composition, were obtained from Meadow Feeds, Pietermaritzburg. Ground limestone ($CaCO_3$) had been added to the rations, thereby achieving high and medium Ca:P ratios. NH_4Cl and Se, as sodium selenite, (Riedel-deHaen; 45% Se) were incorporated into these diets at Ukulinga Research Farm. The various amounts of NH_4Cl and Se required to obtain the twelve

different treatments were weighed out, added to 20 kilograms of the basal ration and mixed for 20 minutes in a dough mixer. The 20 kilograms of feed was then combined with the remainder of the treatment ration in a large feed mixer for a further 30 minutes.

2.1.5 Experimental Procedure

2.1.5.1 Feeding

Each week, sufficient feed for seven days was weighed into individual bags. From this the sheep were fed twice daily, and at the end of the seven day period, the food remaining in each manger was weighed to determine feed intake. The amount of food received by the sheep was increased over the experimental period as deemed necessary, ensuring that the animals had *ad libitum* access to feed. Water was also available *ad libitum.*

2.1.5.2 Animal mass

The sheep were weighed individually on days 0, 26, 56 and 74 by means of a digital, electronic crate scale. This procedure was carried out in the morning, before feeding.

2.1.5.3 Blood mineral samples

Sheep were bled at the start of the trial (day 0) and again on days 22, 52 and 70. Blood was taken from each sheep before feeding, by jugular venipuncture into three sterile, heparinized blood tubes. The bung was immediately removed from one of the three tubes and replaced with parafilm to prevent Zn present in the bung from contaminating the blood sample. These blood tubes were taken to Allerton Regional Veterinary Laboratory within an hour of sampling, for mineral analysis (Ca, P, Mg, Na, Cu, Zn) using atomic absorption spectrophotometry.

One of the two remaining tubes was refrigerated for later analysis of whole blood Se concentration. The remaining tube was centrifuged at 3000rpm for 20 minutes. The supernatant was aspirated and placed in plastic containers in the refrigerator for later analysis of plasma Se concentration.

2.1.5.4 Blood gas samples

As only six blood samples could be analyzed in a half hour period, bleeding, and therefore feeding had to be staggered. The sheep were divided into six groups, within treatments, and received feed 30 minutes after the previous group had been fed. Each group was bled exactly 90 minutes after feeding.

Blood samples were taken using a 5ml syringe containing 0.01ml Heparin, and a 21 gauge needle. Blood was taken by jugular venipuncture, the needle was folded over and the plastic needle-cap

replaced. The six syringes were placed in ice and taken immediately to the laboratory where the blood-gas parameters were determined using an ABL2 Acid-Base Laboratory analyzer.

2.1.5.5 Urine pH

Urinals were fashioned out of polyurethane sachets and attached to the sheep on day 70. All sheep urinated within 60 minutes of being fed, and urine pH was measured immediately, using a pH meter.

2.1.5.6 Faecal samples

Faecal grab samples were obtained from each sheep on day 74. The samples were placed in aluminium trays in an oven (70°C) for 48 hours. On removal from the oven, they were milled and stored in airtight containers for later analysis.

2.1.6 Slaughter Procedure

The sheep were slaughtered at Ukulinga abattoir. Since the abattoir's facilities are limited, 12 sheep were slaughtered each day, with all treatments being represented on each of the three days. The sheep were fed one hour before slaughter.

The animals were killed by exsanguination. The bladder was removed and any urine present was aspirated using a 20ml syringe and 18 gauge needle. The gastro-intestinal tract was removed from the animal, and digesta samples obtained from the rumen, abomasum, duodenum and ileum for immediate pH determination. Liver, kidneys, heart and pancreas were removed for analysis, and the liver, kidneys and warm carcass were weighed. A portion of the *Longissimus dorsi* muscle at the last rib area, and the fifth rib were removed from the carcass.

2.1.6.1 Urine samples

Urine samples were obtained from 33 of the 36 sheep. They were preserved with concentrated HCl (0.03ml HCl: 1ml urine) and frozen for later analysis. Urine mineral concentrations (Ca, P, Mg, K, Na and creatinine) were determined by Dr Bouwer, Pillay, Morris & Partners (Consulting Pathologists) using atomic absorption spectrophotometry. As urine volume differed between treatments, total urinary excretion of the various minerals was determined using equation 1.

Equation 1 Total mineral excretion (mmol/24 hours) = U_c / U_{Ct}

where U_c = urinary mineral concentration (mmol/liter)

U_{Ct} = urinary creatinine concentration (mmol/liter)

2.1.6.2 Bone preparation

The rib-bones were cleaned of all meat and connective tissue, washed with petroleum ether and placed in a fat-extractor for six hours. The bones were then air-dried for one hour and stored in airtight containers for later analysis.

2.1.6.3 Organ preparation

Samples from the liver and kidney cortex were weighed and dried in an oven, together with the pancreas, muscle and heart muscle (fat removed) for 48 hours. The dried liver and kidney samples were weighed on removal from the oven. All the dried organs were milled and stored for later analysis.

2.2 DIGESTIBILITY TRIAL

As there were only four metabolic crates, four treatments were chosen to participate in the digestibility trial, namely:

T2: H Ca - 0% NH_4Cl - Se
T8: M Ca - 0% NH_4Cl - Se
T6: H Ca - 1.5% NH_4Cl - Se
T12: M Ca - 1.5% NH_4Cl - Se

2.2.1 Experimental Design

The trial was planned as a 4 x 4 latin square change-over design and the design details are illustrated in Table 2.1.

Table 2.1: Details of the 4 x 4 latin square change-over design (A,B,C and D represent the four sheep)

TREATMENT		T2	T6	T12	T8
PERIOD	I	A	B	C	D
	II	D	A	B	C
	III	C	D	A	B
	IV	B	C	D	A

2.2.2 Experimental Procedure

The trial was based on a 10 day preliminary period followed by a five day collection period. During the preliminary period the four wethers were housed in individual pens, together with the sheep participating in the growth trial, but were moved to metabolic crates for the duration of the collection period. Throughout the experimental period they received 750g of feed twice daily and water was available *ad libitum*.

2.2.2.1 Faecal collection

Faecal bags were attached to the sheep at the beginning of the collection period. These were emptied twice a day and the contents placed in aluminium trays. The wet faeces were weighed, placed in an oven (70°C) for 48 hours and weighed again once dry. At the end of the five day collection period the dried faeces from each sheep were mixed thoroughly and a representative sample taken. The representative sample from each sheep was milled and stored for later analysis.

2.2.2.2 Urine collection

Urinals, fashioned from inner tyre tubes, were attached to the sheep for the duration of the collection period. Urine was led from these, via a 1m tube, into a bucket containing 30ml of toluene. A plastic cover was tied over the top of the bucket to minimise urine contamination.

The bucket was emptied every 24 hours, and urine volume measured using a 2*l* measuring cylinder. Urine was made up to 2*l* with distilled water, mixed thoroughly and a 100ml sample taken. To this was added 3ml concentrated HCl, and the sample was placed in the freezer until required. At the end of the collection period, the 5x100ml samples were thawed, mixed together and a 100ml sub-sample taken as being representative of the collection period. This sample was returned to the freezer.

2.2.2.3 Urine pH

Urine pH was measured on the third, fourth and fifth day of the collection period. The sheep were fed and the buckets emptied of the previous days urine. They were rinsed with water and returned to their position beneath the metabolic crates. Toluene was placed in the buckets once a urine sample had been produced for pH measurement. The time taken for each sheep to produce a urine sample varied, but all supplied one within 90 minutes.

2.3 ANALYTICAL PROCEDURES

2.3.1 General

All glassware used for mineral determination was acid-washed, and only distilled-deionised water was used in the analytical procedures.

2.3.2 Mineral Determination

Blood (plasma and whole blood), urine, tissues, faeces and feed were analyzed for Se using the fluorometric method described by Koh and Benson (1983). Tissue Cu concentration was determined by the method of Suttle and Field (1968). The Kjeldahl method (AOAC, 1980) was used to analyze the faeces and feeds for Ca, P and N.

2.3.3 Rib-bone Analyses

The fat-free bones were weighed (M_F) and then volume (V) was determined by placing the whole bone in a measuring cylinder containing a known volume of water. The excess water, produced through displacement, was removed using a 5ml pipette and this volume recorded. Specific gravity of the bones (SG) was calculated using Equation 2.

Equation 2 $$SG = M_F / V_F$$

where M_F = fat-free mass of bone (g)

V_F = fat-free volume of bone (ml)

Following SG determination, the bones were ashed overnight in a muffle furnace at 500°C. On removal from the furnace, the samples were placed in a desiccator and allowed to cool. Once cool they were weighed to determine mass of the ashed bone (M_A). Organic matter (OM) content of the bone was calculated using Equation 3.

Equation 3 $$OM = M_F - M_A$$

where M_F = fat-free mass of bone (g)

M_A = mass of ashed bone (g)

2.4 STATISTICAL ANALYSES

Data from the growth trial was analyzed as a 3 x 2 x 2 factorial using the Genstat 5.13 statistical package (Lawes Agricultural Trust, Rothamsted Experimental Station, 1987), to determine the importance of the main effects and interactions of the three dietary components (viz. NH_4Cl, Ca and Se). Cu and Se content of the various organs was calculated using intake as a co-variate. Data from the metabolism trial was analyzed as a 4 x 4 latin square design using Genstat 5.13. Significant differences were tested using the studentized t-test (Steel & Torrie, 1981).

CHAPTER THREE
EXPERIMENTAL RESULTS

3.1 DIETARY MINERAL COMPOSITION

The Ca, P and Se concentration of the 12 treatment diets is detailed in Table 3.1. Dietary Se concentration was close to the required 0.3 mg/kg. Where no Se had been added, the feed had an average Se concentration of 0.106 mg/kg.

Table 3.1: Mineral concentrations of the 12 treatment diets on a DM basis

DIET	Ca (%)	P (%)	Se (mg/kg)	Ca:P
1	1.06	0.22	0.122	4.8:1
2	1.1	0.24	0.343	4.6:1
3	1.22	0.22	0.137	5.5:1
4	0.92	0.22	0.296	4.2:1
5	1.06	0.23	0.107	4.6:1
6	0.98	0.23	0.308	4.3:1
7	0.7	0.23	0.089	3.0:1
8	0.66	0.22	0.291	3.0:1
9	0.72	0.22	0.092	3.3:1
10	0.76	0.24	0.288	3.2:1
11	0.68	0.21	0.086	3.2:1
12	0.7	0.21	0.302	3.3:1

3.2 ANIMAL MASS AND FEED INTAKE

The effect of various levels of dietary NH_4Cl on body mass over a period of time, is illustrated in Figure 3.1. The slight difference in mass between the sheep receiving no NH_4Cl and those receiving 0.75% was, at all stages of the trial, non-significant. Furthermore, the graph clearly shows that during the first 26 days of the experiment, sheep receiving 1.5% NH_4Cl lost weight instead of gaining.

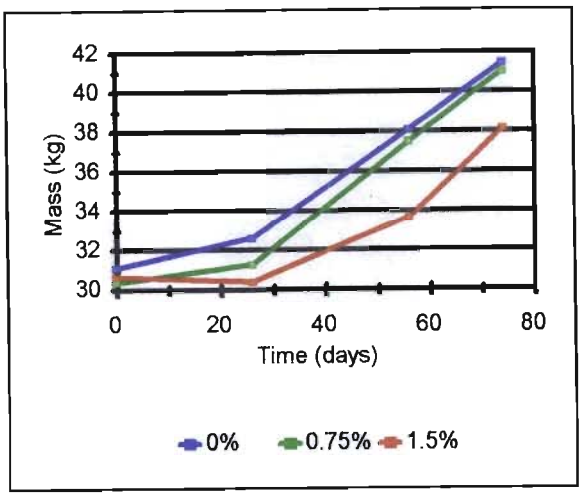


Figure 3.1: The effect of different levels of dietary NH_4Cl on body mass over a period of 74 days

Including NH_4Cl in the diet at a level of 0.75% had little or no detrimental effect on important mass and feed criteria when compared with the performance of sheep on the zero NH_4Cl diet. In fact, average daily gain (ADG) and feed conversion efficiency (FCE) actually improved slightly as a result of including 0.75% NH_4Cl in the diet. However, increasing dietary NH_4Cl further to 1.5% produced a significant decrease in animal performance when compared with the other NH_4Cl treatments (Table 3.2).

Table 3.2: The effect of different levels of dietary NH_4Cl on various performance criteria of the animal

PERFORMANCE CRITERIA	DIETARY NH_4Cl (%) 0	0.75	1.5
Final Mass (kg)	41.46	41.03	38.15**
Carcass Mass (kg)	22.89	22.15	19.24**
Feed Intake (kg)	82.00	81.00	72.00***
ADG (kg)	0.140	0.144	0.089***
FCE	0.126	0.136	0.085*

Within rows, values with an * differ significantly from the values to their left by $P<0.05$, ** by $P<0.01$ and *** by $P<0.005$.

Although dietary Ca level had no effect on animal performance, the interaction between dietary NH_4Cl and Ca had a marked effect on final mass, carcass mass, FCE and feed intake (Figures 3.2 a, b, c, d). Combining a high level of dietary Ca with zero or 0.75% NH_4Cl improved mass and feed criteria over the corresponding medium Ca diets. Yet, when combined with 1.5% NH_4Cl, the high Ca diet caused animal performance to decrease dramatically. Thus, increasing dietary NH_4Cl on the medium Ca diet resulted in a gradual, linear decrease in performance, while the combination of NH_4Cl and a high Ca level produced a curvilinear response.

The highest mass, carcass mass and feed intake were achieved on the 0% NH_4Cl x H Ca x Se diet, while the poorest performance was exhibited by those animals receiving the 1.5% NH_4Cl x H Ca x Se ration.

The results of the digestibility trial revealed that the digestibility of the four diets involved was unaffected by either Ca level (medium or high), or dietary NH_4Cl (0 or 1.5%). The average DM digestibility for the four diets was 67.2%.

3.3 DIGESTA pH

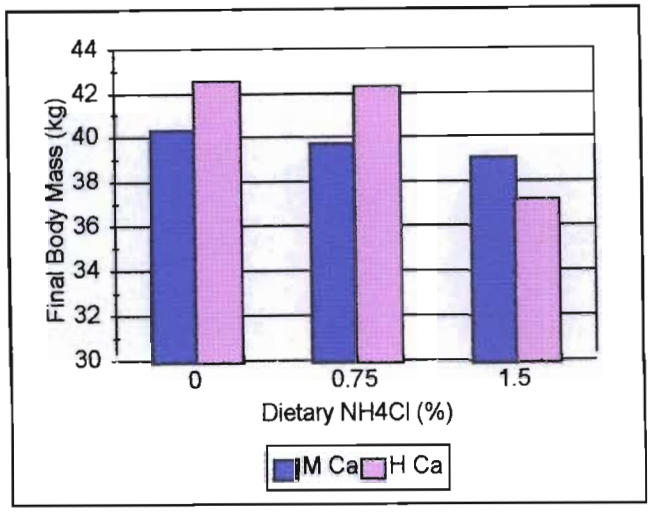
Dietary Ca was the primary factor influencing digesta pH in the rumen, abomasum and duodenum (Table 3.3). Increasing the dietary Ca level from a medium to a high concentration significantly increased digesta pH in these three regions. Ileal pH was unaffected by the level of Ca in the feed and was instead influenced by dietary NH_4Cl (Table 3.4).

Table 3.3: The effect of dietary Ca on digesta pH

DIGESTIVE TRACT REGION	DIGESTA pH	
	Medium Ca	High Ca
Rumen	5.79	5.98***
Abomasum	3.51	4.03*
Duodenum	5.22	5.66*
Ileum	7.36	7.34

Within rows, values with an * differ significantly from the value to their left by $P<0.05$ and *** by $P<0.005$.

The addition of NH_4Cl to the diet effected not only the pH of the ileum, but also that of the duodenum and abomasum, although here its affect was of secondary importance to Ca (Table 3.4).



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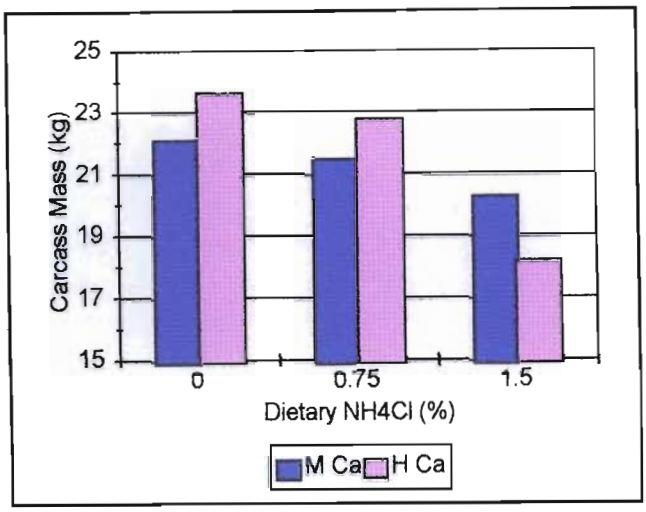

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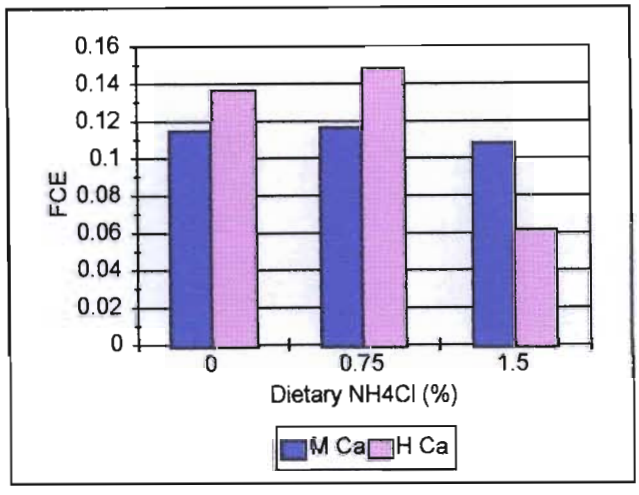

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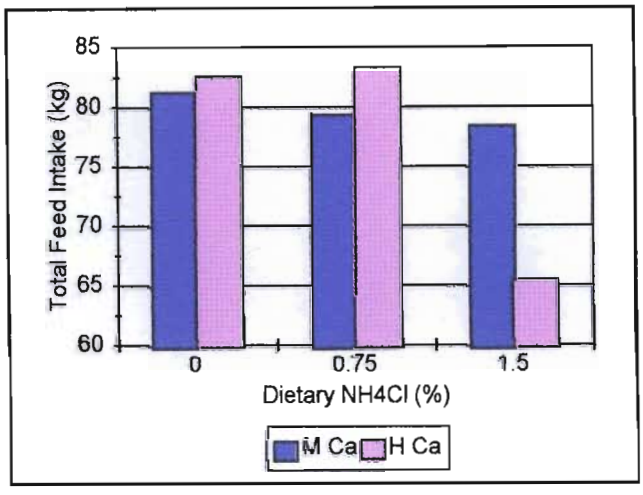


(d)

(a) 1<2 (P<0.05) 3<4 (P<0.025) 2>6 (P<0.005) 4>6 (P<0.005)

(b) 1>5 (P<0.025) 2>6 (P<0.005) 4>6 (P<0.005) 5>6 (P<0.05)

(c) 1<2 (P<0.05) 3<4 (P<0.025) 2>6 (P<0.05) 4>6 (P<0.025)

(d) 3<4 (P<0.025) 2>6 (P<0.005) 4>6 (P<0.005) 5>6 (P<0.01)

Figure 3.2 a, b, c and d: The effect of different combinations of dietary NH_4Cl and Ca on (a) final body mass, (b) carcass mass, (c) feed conversion efficiency and (d) total feed intake.

Increasing dietary NH_4Cl from 0 to 0.75% elevated digesta pH, while increasing still further to 1.5% NH_4Cl resulted in a pronounced decrease in pH. The inclusion of 1.5% NH_4Cl in the diet caused digesta pH of the abomasum, duodenum and ileum to fall to below their original level (0% NH_4Cl), yet pH of the rumen digesta did not drop below the original value of 5.88. However, the increase in pH caused by adding 0.75% NH_4Cl to the diet was evident in all regions of the digestive tract, including the rumen. Abomasal pH appeared to be influenced by dietary Ca and NH_4Cl to a greater extent than were the other regions.

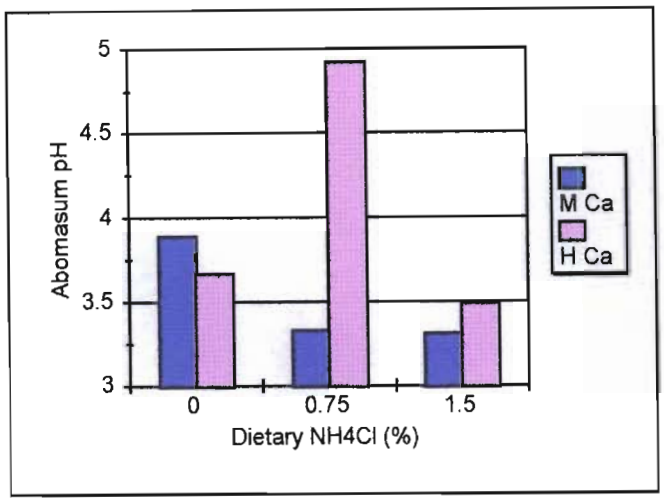
Table 3.4: The effect of dietary NH_4Cl on digesta pH

DIGESTIVE TRACT REGION	DIGESTA pH 0% NH_4Cl	0.75% NH_4Cl	1.5% NH_4Cl
Rumen	5.88	5.91	5.88
Abomasum	3.78	4.12	3.40*
Duodenum	5.49	5.70	5.14*
Ileum	7.33	7.47*	7.25**

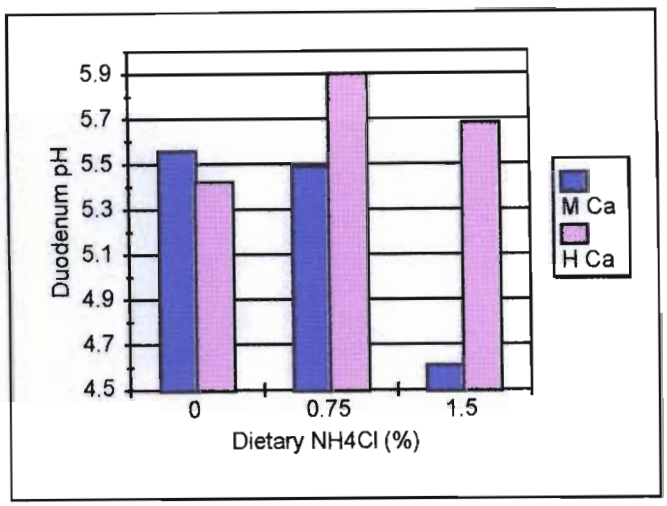
Within rows, values with an * differ significantly from the values to their left by $P<0.05$ and ** by $P<0.01$.

Dietary Ca and NH_4Cl combined to influence digesta pH of the abomasum, duodenum and ileum, although the effect was modified depending on whether Ca or NH_4Cl had the greater influence in that region of the digestive tract (Figures 3.3 a, b, c). In the abomasum and duodenum, where Ca was the primary controlling factor, the combination of a medium Ca concentration and NH_4Cl had an acidifying effect on the digestive tract, and resulted in a decrease in pH. On the other hand, the addition of NH_4Cl to the high Ca diet produced a quadratic effect, as pH first increased and then decreased as the dietary NH_4Cl level increased. Thus, a combination of a high Ca level and 0.75% NH_4Cl produced the highest digesta pH in the abomasum and duodenum. In the ileum, where NH_4Cl was the dominating factor, digesta pH showed a quadratic effect when NH_4Cl was combined with either level of Ca. When no NH_4Cl was present in the diet, the medium Ca diet resulted in a higher digesta pH in the abomasum and duodenum than the high Ca diet. Once NH_4Cl was added to the diet, the high Ca level resulted in a higher digesta pH than the medium Ca level. However, in the ileum the situation was reversed, as the combination of medium Ca and zero NH_4Cl resulted in a higher digesta pH than the corresponding high Ca diet.

Thus, Ca greatly influenced digesta pH. This effect was greatest in the rumen, but lessened as the digesta travelled down the gastro-intestinal tract, and became ineffective once passed the duodenum.



(a)



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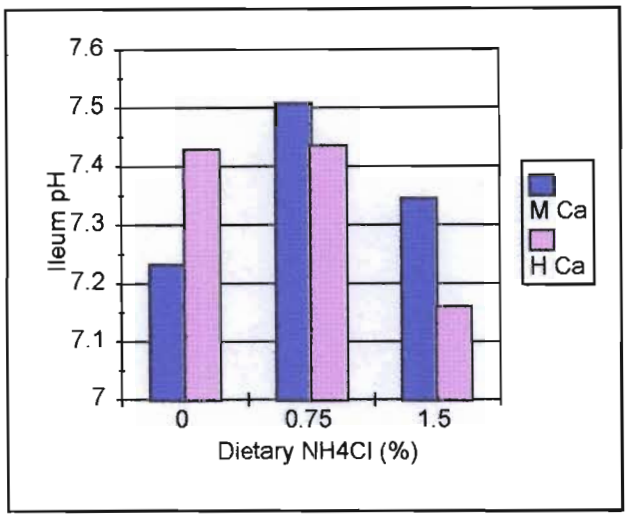


(c)

(a) 2<4 (P<0.005) 4>6 (P<0.005) 3<4 (P<0.0005)

(b) 2<4 (P<0.05) 3>5 (P<0.05) 1>5 (P<0.05) 5<6 (P<0.025)

(c) 1<3 (P<0.025) 2>6 (P<0.025) 4>6 (P<0.05

Figure 3.3 a, b and c: The effect of different combinations of dietary NH_4Cl and Ca on digesta pH of the (a) abomasum, (b) duodenum and (c) ileum.

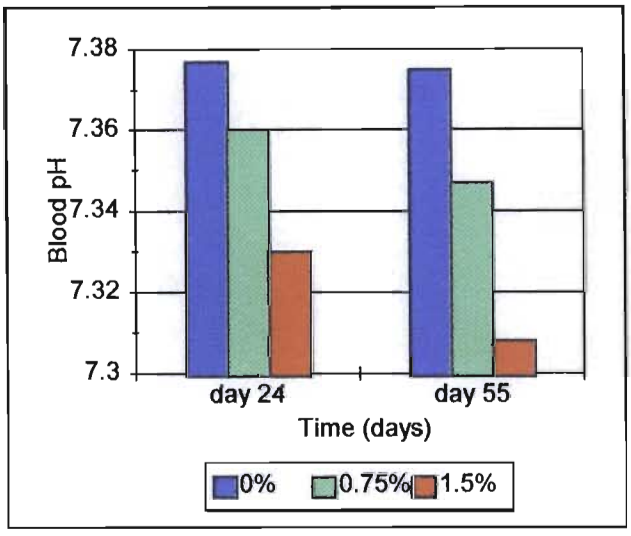
3.4 BLOOD ACID-BASE STATUS

Blood pH, HCO_3, and Base Excess (BE) were influenced by the amount of NH_4Cl in the diet ($P<0.01$), and by the interaction between NH_4Cl and Se. These blood parameters exhibited a decrease with increasing dietary NH_4Cl (Figures 3.4 a, b, c) which was enhanced over time.

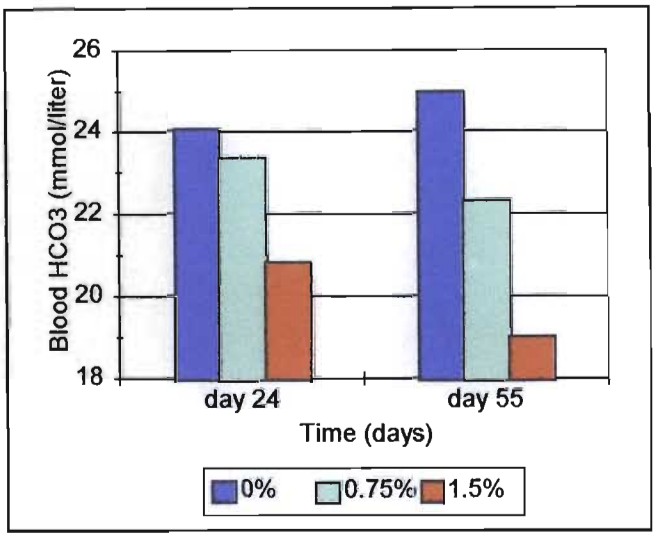
As illustrated in Figures 3.4 a, b, and c, the blood parameters of those animals receiving no NH_4Cl either remained relatively constant or increased slightly between the two sampling periods. However, once NH_4Cl was included in the diet, acidosis continued to develop over time and blood pH, HCO_3, and BE decreased between days 24 and 55 of the trial.

An NH_4Cl x Ca interaction influenced blood pH, pCO_2, HCO_3 and BE by day 24, but this effect was no longer significant by day 55. At all levels of NH_4Cl, the high Ca level resulted in lower blood pH values than did the medium Ca diet (Figure 3.5 a) while blood pCO_2 exhibited the opposite effect, as the high Ca diet resulted in higher pCO_2 values than the medium Ca ration (Figure 3.5 b). At all levels of NH_4Cl, the lower Ca level resulted in higher blood BE values. Furthermore, those animals on the medium Ca x 0% NH_4Cl had a positive blood BE value, while all other diets resulted in a negative BE value (Figure 3.5 c).

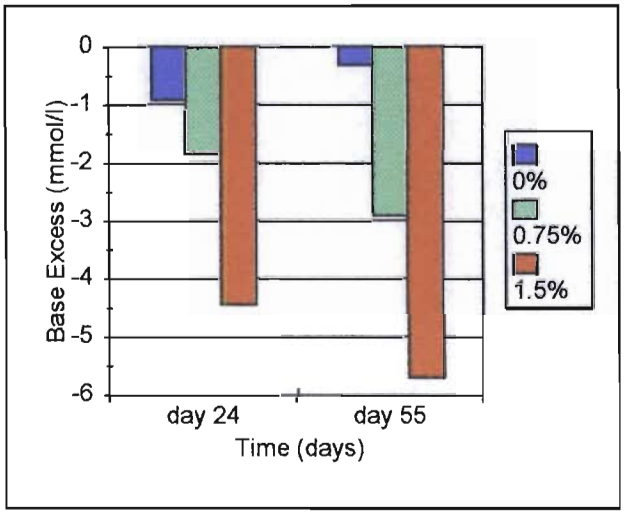
Blood pH, HCO_3, and BE were further influenced by the interaction between dietary NH_4Cl and Se (Figures 3.6, 3.7 and 3.8). The presence of Se in the diet appeared to have an alkalizing effect on these three blood parameters, except when combined with 1.5% NH_4Cl. Blood pH, HCO_3, and BE increased when Se was included in the diet in conjunction with 0 or 0.75% NH_4Cl, but decreased when Se was combined with 1.5% NH_4Cl. Furthermore, the decrease in pH, HCO_3, and BE caused by increasing the dietary NH_4Cl level was more pronounced when Se was present in the diet than when absent. By day 55 the combination of 0% NH_4Cl and 0.3mg Se had produced a slightly positive BE value.



(a)



(b)



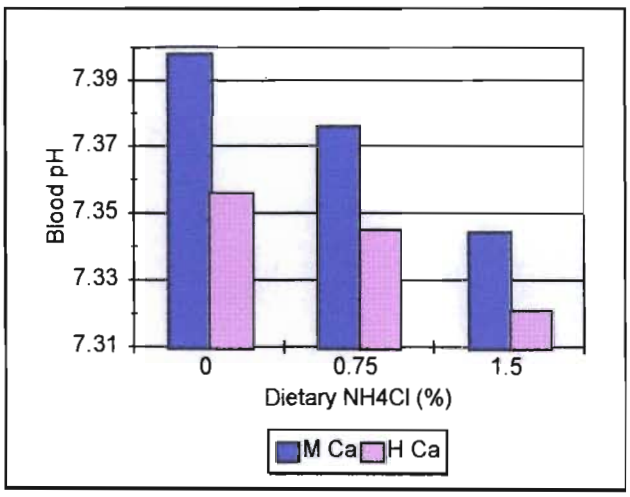
(c)

(a) 1>3 (P<0.005) 2>3 (P<0.05) 4>5 (P<0.05) 4>6 (P<0.0005) 5>6 (P<0.01)

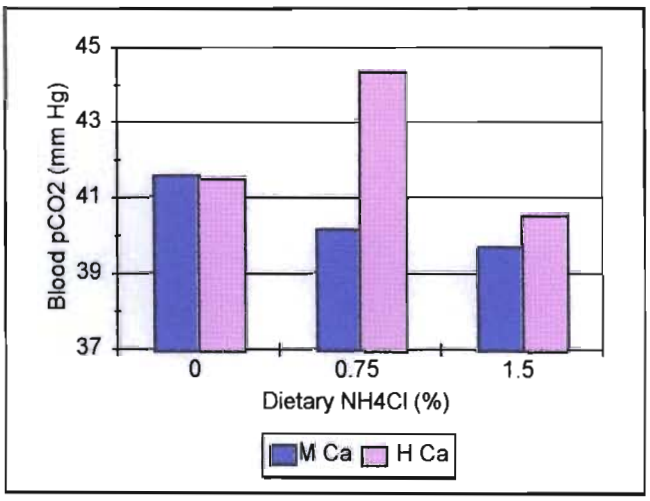
(b) 1>3 (P<0.0005) 2>3 (P<0.0005) 4>5 (P<0.01) 4>6 (P<0.0005) 5>6 (P<0.005)

(c) 1>3 (P<0.005) 2>3 (P<0.005) 4>5 (P<0.01) 4>6 (P<0.0005) 5>6 (P<0.005)

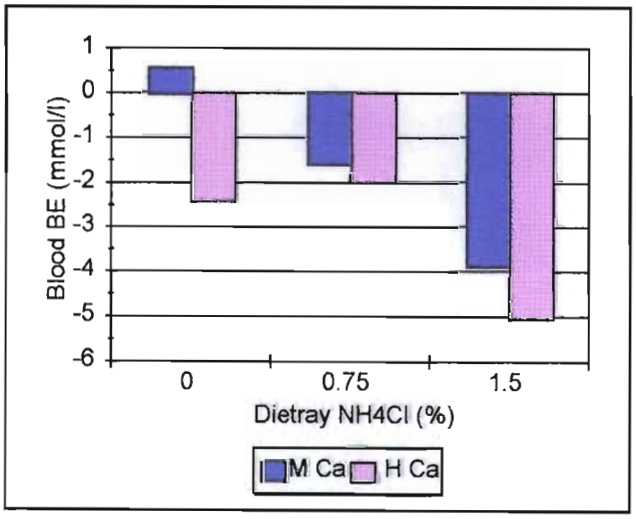
Figure 3.4 a, b and c: The effect of dietary NH_4Cl on blood (a) pH, (b) HCO_3 and (c) BE.



(a)



(b)



(c)

(a) all differences non-significant

(b) 3<4 (P<0.025) 2<4 (P<0.05) 6<4 (P<0.05)

(c) 1>2 (P<0.025) 1>3 (P<0.025) 1>5 (P<0.005) 3>5 (P<0.025) 2>6 (P<0.05)
4>6 (P<0.025)

Figure 3.5 a, b and c: The effect of different combinations of dietary NH_4Cl and Ca on blood (a) pH, (b) pCO_2 and (c) BE by day 24.

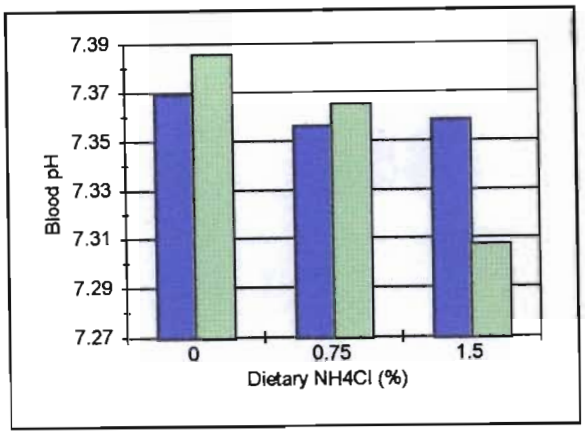


Figure 3.6 a

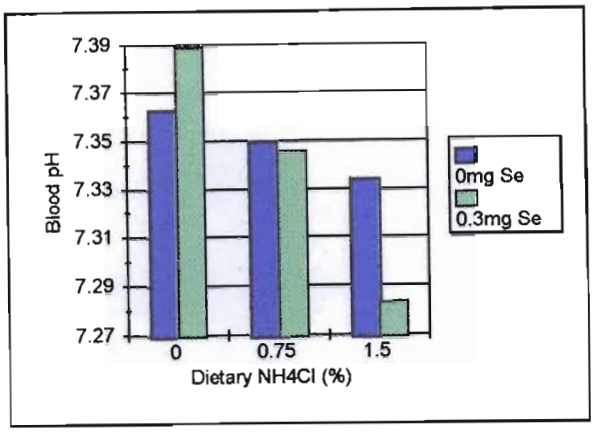


Figure 3.6 b

(a) 5>6 (P<0.005) 4>6 (P<0.025) 2>6 (P<0.005)
(b) 5>6 (P<0.005) 2>4 (P<0.025) 4>6 (P<0.005) 2>6 (P<0.0005)

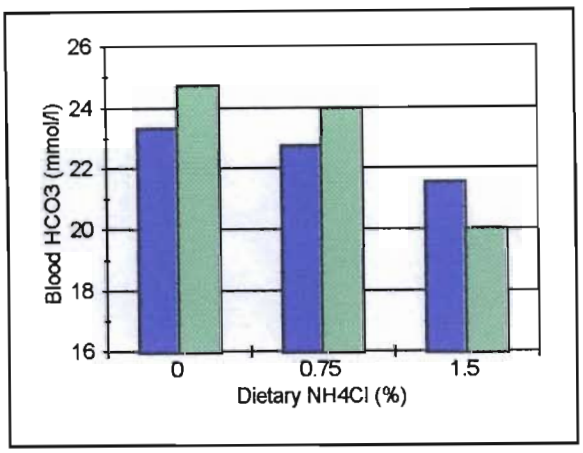


Figure 3.7 a

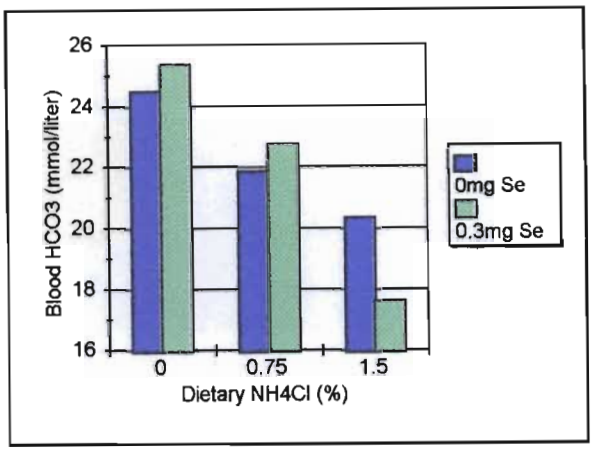


Figure 3.7 b

(a) 4>6 (P<0.005) 2>6 (P<0.0005)
(b) 5>6 (P<0.05) 1>3 (P<0.05) 1>5 (P<0.01) 4>6 (P<0.0005) 2>6 (P<0.0005)

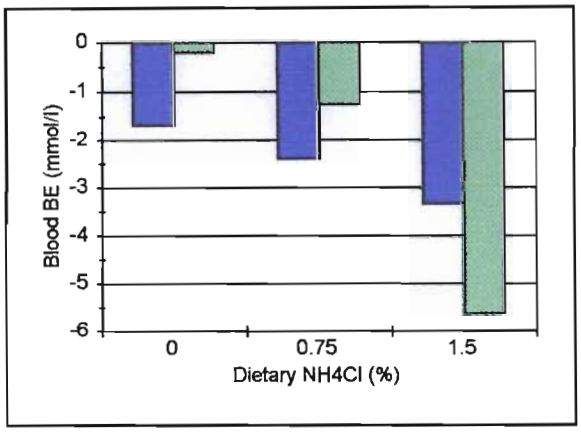


Figure 3.8 a

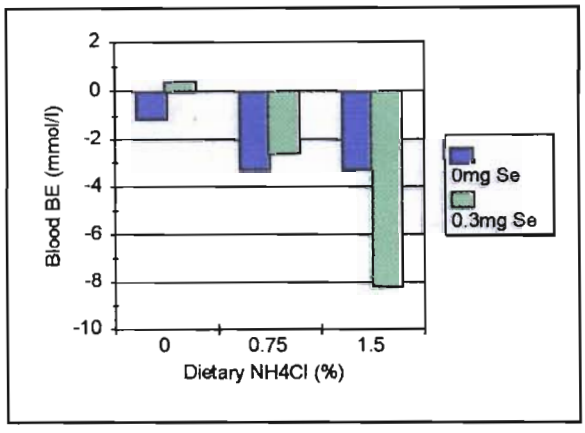
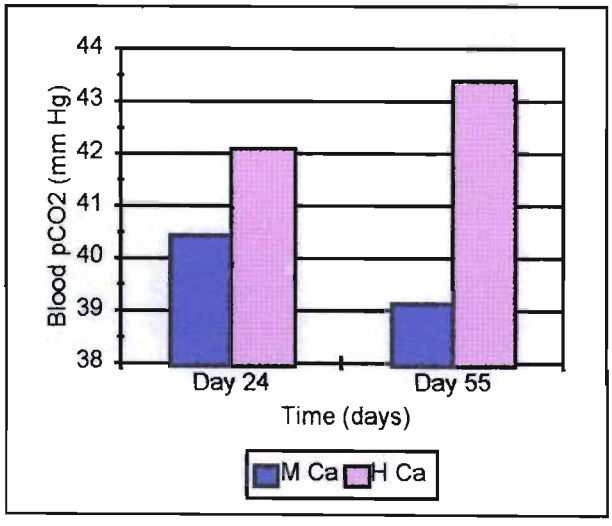


Figure 3.8 b

(a) 2>6 (P<0.005) 2>4 (P<0.005) 5>6 (P<0.05)
(b) 1>3 (P<0.05) 1>5 (P<0.05) 2>4 (P<0.05) 2>6 (P<0.0005) 4>6 (P<0.005)
5>6 (P<0.025)

Figures 3.6, 3.7 and 3.8 a and b: The effect of different combinations of dietary NH_4Cl and Se on blood pH, HCO_3 and BE by days (a) 24 and (b) 55.

Blood pCO_2, although influenced by NH_4Cl (day 55) and the NH_4Cl x Ca interaction (day 24), was effected primarily by the level of Ca in the diet. The high level of dietary Ca resulted in a higher blood pCO_2 level (Figure 3.9) than the medium Ca diet and as before, the difference in pCO_2 level due to the different levels of Ca increased during the trial period. Furthermore, the blood pCO_2 level of those animals receiving the medium Ca diet decreased over time, while the blood pCO_2 level of the animals eating the high Ca diet increased. By day 55, NH_4Cl markedly influenced blood pCO_2 level, as increasing the NH_4Cl content of the ration caused blood pCO_2 to decrease from 43.27 mm Hg on the zero NH_4Cl diet to 38.74 mm Hg ($P<0.01$) on the 1.5% NH_4Cl ration. Blood pH was also influenced by dietary Ca level as the higher level of Ca resulted in a lower pH by day 24 (7.373 versus 7.341; $P<0.01$) and 55 (7.352 versus 7.337; NS). Blood pO_2 level was unaffected by any of the treatments.



1 < 2 ($P<0.05$)

3 < 4 ($P<0.005$)

Figure 3.9: The effect of dietary Ca level on blood pCO_2 values

Of interest were the correlation coefficients between various blood parameters (Table 3.5). Most of the parameters were poorly correlated with each other, although a few showed a relatively high correlation. An almost perfect correlation occurred between blood HCO_3 and TCO_2 throughout the experimental period. This correlation is not altogether surprising, as examination of the raw data revealed that blood TCO_2 was always approximately 1 unit higher than blood HCO_3.

Table 3.5: Correlation coefficients between various blood parameters

BLOOD PARAMETER	CORRELATION COEFFICIENT Day 24	Day 55
pH - HCO_3	0.812	0.687
pH - TCO_2	0.790	0.667
pCO_2 - HCO_3	0.277	0.727
pCO_2 - TCO_2	0.310	0.745
HCO_3 - TCO_2	0.999	0.999

Table 3.6 details the effect of the NH_4Cl x Ca interaction on the blood HCO_3:H_2CO_3 ratio, and illustrates that increasing NH_4Cl levels, when combined with the high Ca diet caused the ratio to decrease at both the 0.75 and 1.5% levels. However, when included in the medium Ca ration, the ratio decreased only when NH_4Cl was raised to a level of 1.5%.

Table 3.6: The effect of different combinations of dietary NH_4Cl and Ca on the blood HCO_3:H_2CO_3 ratio

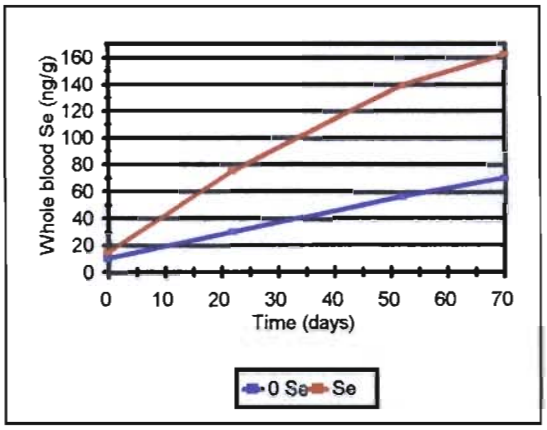
DIETARY CALCIUM	HCO_3:H_2CO_3 RATIO[$] 0% NH_4Cl	0.75% NH_4Cl	1.5% NH_4Cl
Medium Ca	19.6:1	19:1	16:1
High Ca	18.8:1	16.9:1	16.7:1

[$] $H_2CO_3 = 0.03 \times pCO_2$ (Houpt, 1984).

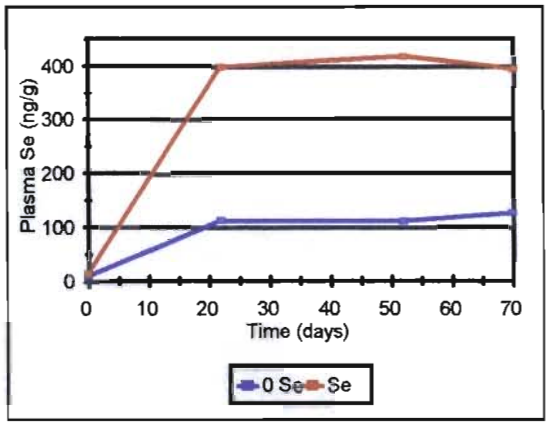
The diet producing the lowest blood pH, HCO_3 and BE level was initially 1.5% NH_4Cl x H Ca x Se, but by day 55 this had changed to the 1.5% NH_4Cl x M Ca x Se ration. Thus, over time it appears that the combination of 1.5% NH_4Cl x M Ca x Se is more acidifying to the system than a similar ration with a high Ca content.

3.5 BLOOD MINERAL CONCENTRATION

Blood mineral concentrations varied, but remained unaffected by any of the three dietary constituents throughout the trial period. The exception to this was Se which was significantly ($P < 0.01$) effected by dietary Se level. Whole-blood Se increased linearly during the trial period while plasma Se increased linearly until day 22 and then reached a plateau (Figures 3.10 a and b).



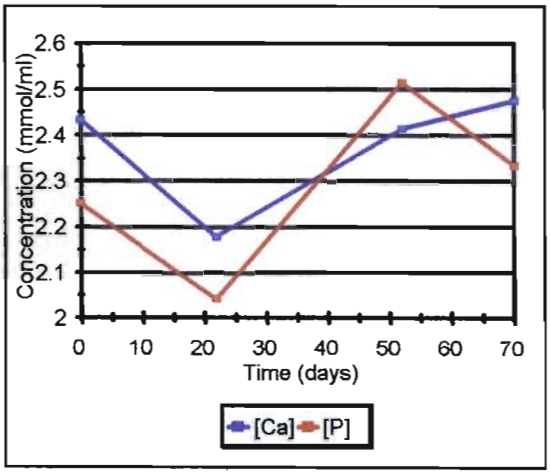
(a)



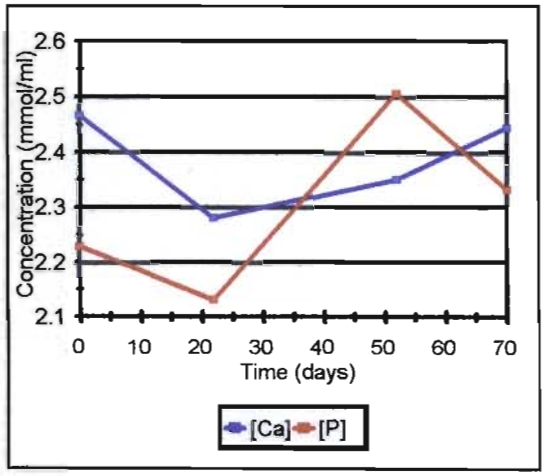
(b)

Figure 3.10: The concentration of (a) whole blood and (b) plasma Se over time

The average plasma Ca:P ratio of the medium and high Ca diets was 1.02 and 1.07 respectively, except when serum P concentration rose above that of Ca, causing the ratio of those animals on the medium Ca diet to decrease to 0.97, and to 0.94 for those animals receiving the high Ca diet (Figures 3.11 a and b).



(a)



(b)

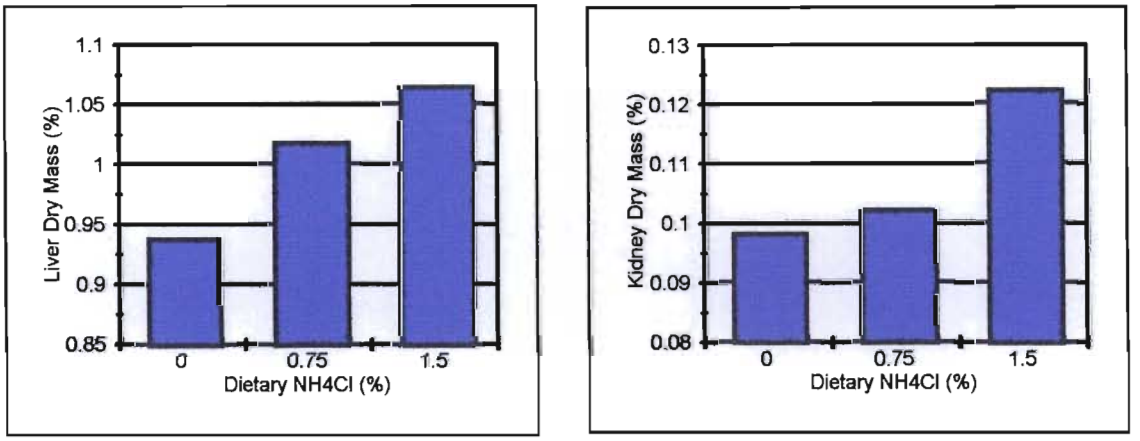
Figure 3.11: Variations in serum Ca and P concentration over time for the (a) medium and (b) high Ca diets

Although the other serum mineral concentrations (Mg, Na, Cu and Zn) fluctuated during the trial period, they were all within the normal range for sheep.

3.6 MINERAL RETENTION

3.6.1 Organ Mass

As carcass mass differed between treatments, liver and kidney dry mass was calculated as a percentage of carcass mass. These results revealed that organ mass was significantly influenced by dietary NH_4Cl ($P<0.01$), as increasing amounts of NH_4Cl caused liver and kidney dry mass to increase (Figures 3.12 a and b).



(a) (b)

(a) 1<2 ($P<0.05$) 1<3 ($P<0.025$)

(b) 2<3 ($P<0.005$) 1<3 ($P<0.005$)

Figure 3.12: The effect of dietary NH_4Cl on (a) liver and (b) kidney dry mass expressed as a percentage of carcass mass

3.6.2 Selenium Concentration of Various Organs

The Se content of the organs was determined using Se intake as a co-variate revealing that the Se concentration of the organs analyzed was significantly ($P<0.01$) affected by dietary Se. As seen from Table 3.7, including Se in the diet caused organ Se concentration to increase significantly.

Table 3.7: The effect of dietary Se level on tissue Se concentration

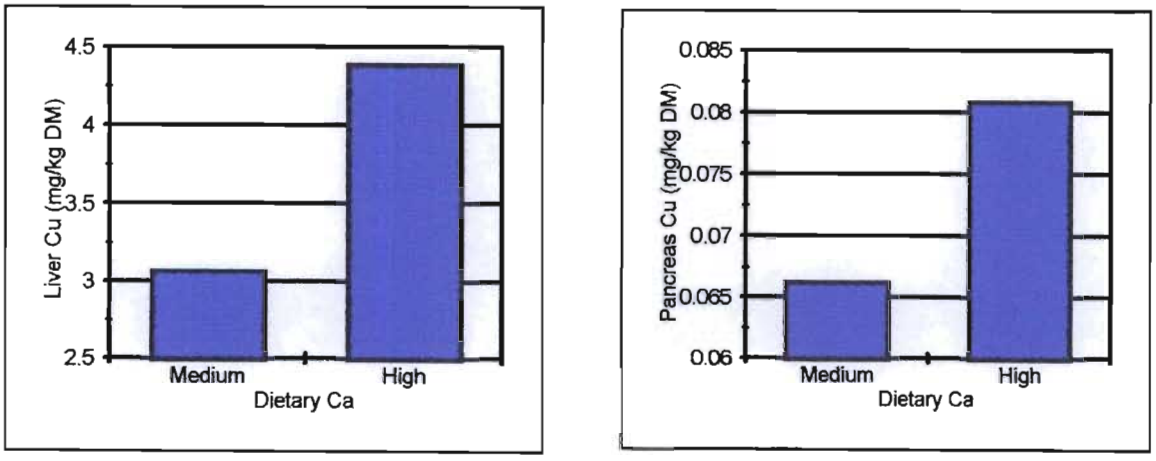
TISSUE	Se CONCENTRATION (mg/kg DM) 0 mg Se	0.3mg Se
Kidney	0.040	0.866***
Heart	0.008	0.165***
Liver	0.038	0.160***
Pancreas	0.007	0.153***
Muscle	0.003	0.035***

Within rows, values with *** differ significantly from the value to their left by $P<0.005$.

Dietary Ca level had no effect on organ Se concentration. However, increasing NH_4Cl from 0 to 0.75% caused organ Se concentration to increase slightly, while raising the NH_4Cl level still further caused Se concentration to decrease. At no time were these differences significant.

3.6.3 Copper Concentration of Various Organs

Liver and pancreas Cu concentration were significantly ($P<0.01$) influenced by the Ca level of the diet (Figures 3.13 a and b) as the higher level of dietary Ca resulted in a greater organ Cu concentration.



(a) (b)

(a) 1 < 2 ($P<0.0005$)

(b) 1 < 2 ($P<0.005$)

Figure 3.13: The effect of different levels of dietary Ca on (a) liver and (b) pancreas Cu concentration.

Kidney Cu concentration was affected by dietary NH_4Cl level. Increased amounts of NH_4Cl caused kidney Cu concentration to increase from 0.25mg/kg at 0% NH_4Cl to 0.32mg/kg at 1.5% NH_4Cl ($P<0.01$). Muscle and heart Cu concentration remained unaffected by any of the treatments.

3.7 BONE COMPOSITION

The parameters used to measure bone status (ash %, ash:volume, OM:volume and ash:OM) were all influenced by dietary NH_4Cl.

Table 3.8: The effect of dietary NH_4Cl on bone status.

BONE PARAMETERS	DIETARY NH_4Cl (%) 0%	0.75%	1.5%
Ash %	55.5	55.06	53.7
Ash:volume	0.77	0.79	0.70
OM:volume	0.62	0.64	0.51
Ash:OM	1.24	1.22	1.17*

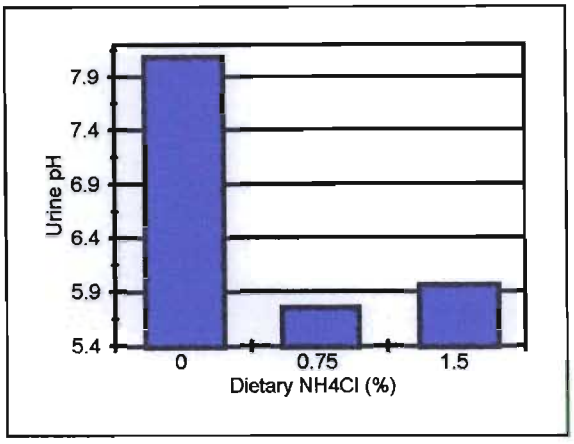
Within rows, values with an * differ significantly from the values to their left by $P<0.05$.

Bones parameters decreased due to the inclusion of 1.5% NH_4Cl in the diet, whereas 0.75% NH_4Cl had no affect (Table 3.8). Those animals which received the high Ca diet produced bones with a higher ash percentage (55.58 versus 50.9; $P<0.05$) and a high ash:OM ratio (1.25 versus 1.17; $P<0.025$) than those on the medium Ca diet.

3.8 URINARY EXCRETION

3.8.1 Urine pH

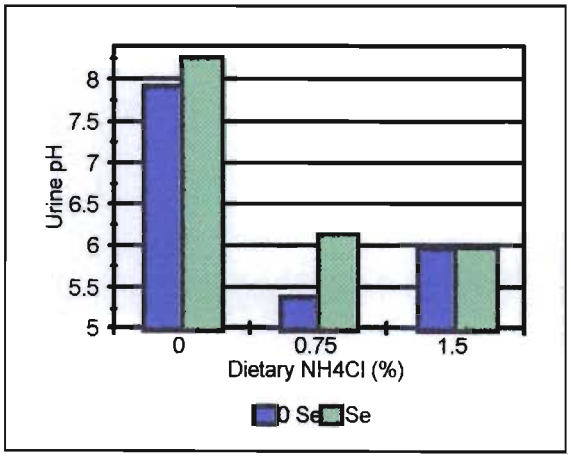
As illustrated in Figure 3.14, the level of dietary NH_4Cl had a significant effect on urine pH ($P<0.01$). The addition of 0.75% NH_4Cl to the diet lowered urine pH from 8.09 to 5.75. However, increasing the NH_4Cl level from 0.75 to 1.5% resulted in a slightly less acidic urine, although this difference was non-significant.



1 > 2 (P < 0.0005) 1 > 3 (P < 0.0005)

Figure 3.14: The effect of dietary NH_4Cl on urine pH

The interaction between NH_4Cl and Se also influenced urine pH (Figure 3.15). Although the quadratic trend produced was the same, the acidifying effect of NH_4Cl on the urine was less severe when Se was present in the diet. This was most marked at the level of 0.75% NH_4Cl, where the difference in urine pH due to the inclusion or omission of dietary Se was significant (P < 0.0005). Although Se appeared to have a slight alkalizing effect on urine pH, the presence of NH_4Cl (0.75 and 1.5%) was sufficient to lower urine pH into the acidic range.



3 < 4 (P < 0.0005) 1 > 3 (P < 0.0005) 3 < 5 (P < 0.0005) 1 > 5 (P < 0.0005)
2 > 4 (P < 0.0005) 2 > 6 (P < 0.0005)

Figure 3.15 The effect of different combinations of dietary NH_4Cl and Se on urine pH

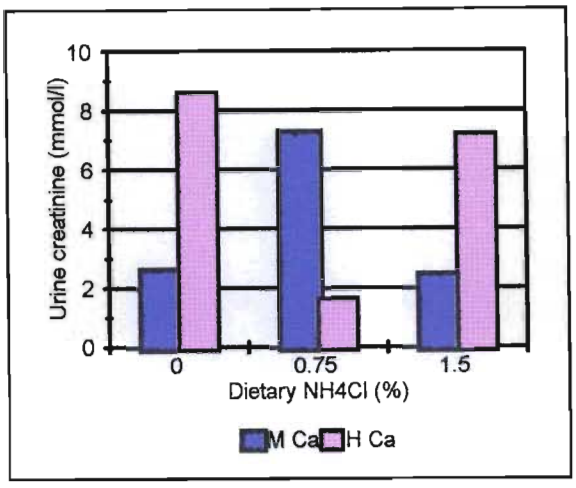
3.8.2 Urine volume

Urine volume was measured directly from the sheep participating in the digestibility trial, and therefore this information was only available for the four diets involved in this trial. The results are presented in Table 3.9.

Table 3.9: The effect of different treatments on average urine volume

SHEEP No.	AVERAGE URINE VOLUME (ml/day)			
	MEDIUM Ca		HIGH Ca	
	0% NH_4Cl (T8)	1.5% NH_4Cl (T12)	0% NH_4Cl (T2)	1.5% NH_4Cl (T6)
No. 134	493	813	297	412
No. 141	1601	2630	603	1480
No. 58	1120	1354	901	980
No. 60	2869	3430	1445	2087
AVERAGE	1519	2057	812	1240

As total urine creatinine excretion per day remains constant per animal (De Groot & Aafjes, 1960), this value can be used as an indicator of urine volume, and more importantly as an indirect measure of the volume of water taken in by the animal. From Figure 3.16 it can be seen that the high Ca diet, at 0 and 1.5% NH_4Cl, resulted in the production of concentrated urine and therefore these animals appear to have consumed very little water. The medium Ca diet, at 0 and 1.5% NH_4Cl, caused the animals to produce dilute urine, and therefore it is assumed that they had a high water intake. However, at an NH_4Cl level of 0.75% the situation was reversed, and the high Ca diet was productive of a dilute urine, while those animals on the medium Ca diet exhibited a very concentrated urine.



1<3 (P<0.05) 4<6 (P<0.025) 3>4 (P<0.01) 5<6 (P<0.05)

Figure 3.16: The effect of different combinations of NH_4Cl and Ca on urinary creatinine concentration as urine creatinine dilution provides an indicator of urine volume and therefore of water intake

3.8.3 Total Urinary Mineral Excretion

As urine volume varied considerably between treatments, urine mineral concentration was corrected for this, using urinary creatinine concentration to determine total urinary mineral excretion (Equation 1, page 26). The effect of dietary NH_4Cl level on the urinary excretion of various minerals is presented in Table 3.10. Increasing the NH_4Cl content of the diet caused urinary Ca, P and Mg to increase and K to decrease, while urinary Na and Se exhibited a quadratic response.

Table 3.10: The effect of dietary NH_4Cl on total urinary mineral excretion (corrected for creatinine)

MINERAL	TOTAL URINARY EXCRETION (mmol/day)		
	0% NH_4Cl	0.75% NH_4Cl	1.5% NH_4Cl
Ca	0.61	1.61***	3.34***
P	0.28	0.55	2.00***
Se	70.7	56.0	83.4
Mg	2.70	3.46	3.75
K	46.0	33.0*	30.3*
Na	15.9	20.7	18.4

Within rows, values with an * differ significantly from the values to their left by P<0.05 and *** by P<0.005.

The excretion of Ca, P, Se, Mg and Na was also influenced by the level of dietary Ca (Table 3.11), as the higher level of Ca caused less of each of these minerals to be excreted in the urine, although this difference was only significant for P and Na ($P<0.05$).

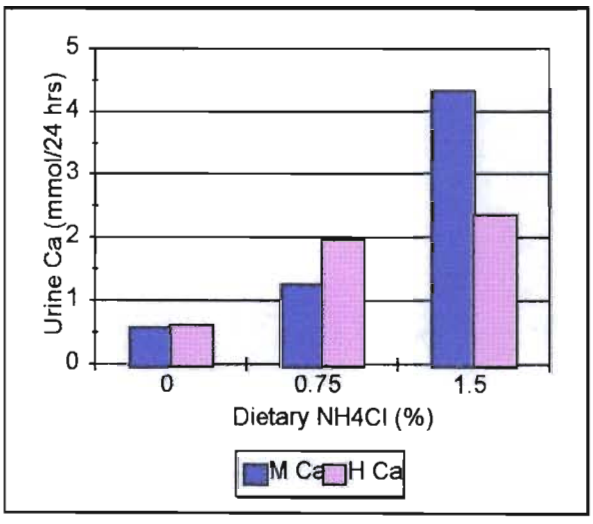
Table 3.11: The effect of dietary Ca level on the urinary excretion of various minerals (corrected for creatinine)

MINERAL	TOTAL EXCRETION (mmol/24 hrs)	
	Medium Ca	High Ca
Ca	2.05	1.65
P	1.77	0.26*
Se	71.1	69.0
Mg	3.67	2.94
Na	22.4	14.3*

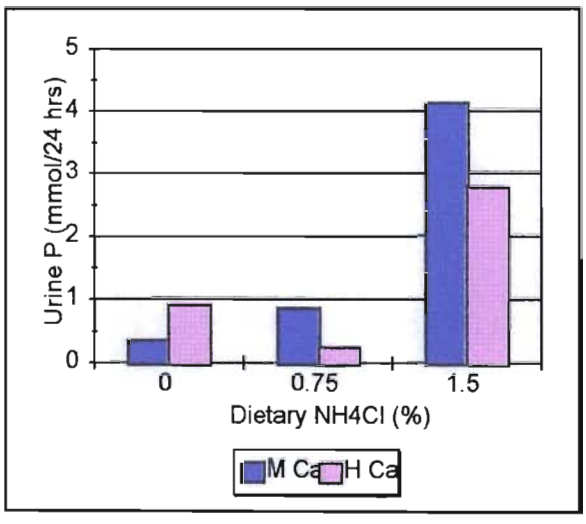
Within rows, values with an * differ significantly from the value to their left by $P<0.05$.

The most common interaction affecting total urinary mineral excretion was that of NH_4Cl and Ca (Figures 3.17 a, b and c) and influenced the excretion of Ca, P and Mg. Increasing dietary NH_4Cl caused urinary Ca excretion to increase at both levels of dietary Ca. However, the combination of medium Ca and 1.5% NH_4Cl was productive of a marked increase in urinary Ca excretion ($P<0.005$). Increasing the NH_4Cl content of the medium Ca diet from 0 to 0.75% caused urinary P excretion to increase, but when combined with the high Ca diet, increasing amounts of NH_4Cl caused urinary P excretion to decrease. Urinary Mg excretion responded slightly differently as the high Ca diet caused a positive quadratic response while the low Ca diet resulted in a negative quadratic response to dietary NH_4Cl. However, for all three minerals, the medium Ca x 1.5% NH_4Cl diet resulted in a greater excretion of the mineral than the corresponding high Ca diet.

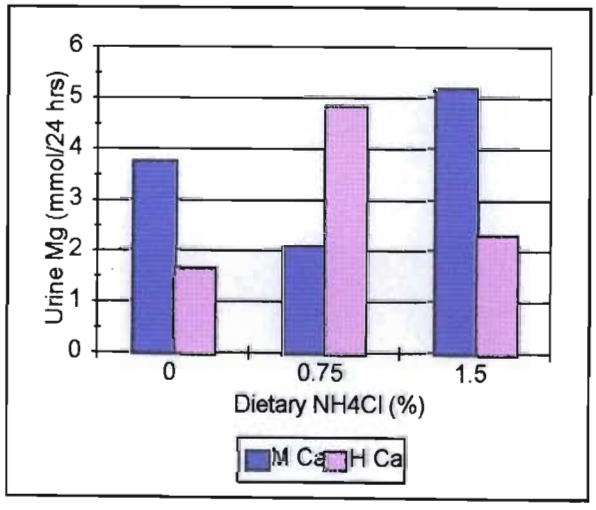
Urinary P excretion was also influenced significantly ($P<0.01$) by the NH_4Cl x Se interaction, as the addition of Se to the diet caused urinary P excretion to increase, especially at an NH_4Cl level of 1.5% (Figure 3.18).



(a)



(b)



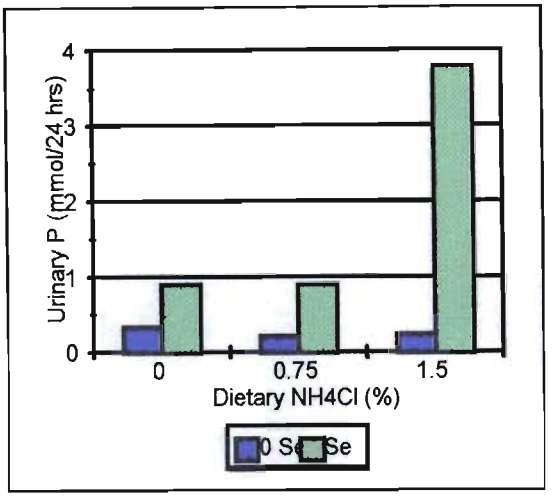
(c)

(a) 3<5 (P<0.005)

(b) 1<5 (P<0.005) 3<5 (P<0.025)

(c) 3<5 (P<0.005) 5>6 (P<0.025)

Figure 3.18 a, b and c: The effect of different combinations of dietary NH_4Cl and Ca on urinary (a) Ca, (b) P and (c) Mg (corrected for creatinine).



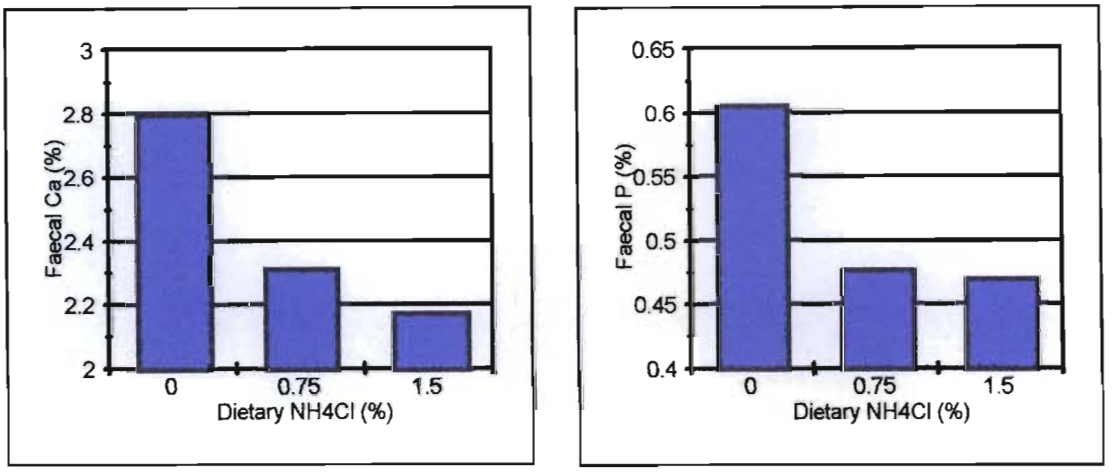
4 < 6 ($P < 0.05$) 2 < 6 ($P < 0.05$)

Figure 3.18: The effect of different combinations of dietary NH_4Cl and Se on urinary P excretion (corrected for creatinine)

The urine mineral and creatinine concentrations obtained from the digestibility trial supported the urine results collected from the animals participating in the growth trial.

3.9 FAECAL EXCRETION

As the DM digestibility of the feed was unaffected by any of the treatments, comparisons could be made concerning faecal mineral concentrations. The effect of dietary NH_4Cl on faecal Ca and P excretion is illustrated in Figures 3.19 a and b. Increasing the amount of NH_4Cl in the diet resulted in less Ca and P being excreted in the faeces. It is interesting to note that the ratio between the amount of Ca and P excreted in the faeces remained constant at 4.6, despite the NH_4Cl content of the diet. Thus, it appears that Ca and P are excreted in conjunction in the faeces.



(a) (b)

(a) 1 > 3 ($P < 0.025$)

(b) 1 > 2 ($P < 0.005$) 1 > 3 ($P < 0.005$)

Figure 3.19: The effect of dietary NH_4Cl on faecal (a) Ca and (b) P excretion

Dietary Ca level affected the amount of Ca, P, and Se excreted in the faeces, as the higher Ca level resulted in a greater amount of the mineral being excreted through the faeces (Table 3.12). Faecal Se showed a similar response to dietary Se, as the addition of Se to the diet caused faecal Se excretion to increase significantly from 0.398 to 5.068 mg/kg ($P < 0.0005$).

Table 3.12: The effect of dietary Ca on the faecal excretion of Ca, P and Se.

MINERAL	FAECAL EXCRETION (mg/kg DM)	
	Medium Ca	High Ca
Ca	1.812	3.038***
P	0.515	0.518
Se	2.446	3.020

Within rows, values with an *** differ significantly from the value to their left by $P < 0.005$

Faecal protein was unaffected by any of the three dietary components. However, there was a slight increase in the amount of protein excreted in the faeces (14.54 versus 15.23%) when dietary Ca was decreased from a high to a medium concentration. The faecal mineral results from the digestibility trial compared well with those of the growth trial.

CHAPTER FOUR
DISCUSSION OF RESULTS

4.1 THE EFFECT OF AMMONIUM CHLORIDE ON OVINE PHYSIOLOGY

4.1.1 *Animal Performance*

Neither final mass, carcass mass, nor feed intake were affected by the raising of dietary NH_4Cl from 0 to 0.75%. However, increasing dietary acidity still further through the addition of 1.5% NH_4Cl resulted in a significant ($P<0.01$) decrease in mass and feed intake (Table 3.2). These results agree with those of other researchers, who found that an NH_4Cl level of 1% was sufficient to significantly lower animal performance (Bushman *et al.*, 1967). The decreased feed intake resulting from diets with a high level of acidity, has been attributed to a decrease in rumen fluid pH (Tucker *et al.*, 1988). However, L'Estrange & Murphy (1972) suggested that metabolic acidosis, evidenced by a lowered blood pH, suppressed appetite stimulants, thereby decreasing feed intake. Table 4.1 shows the effect of increasing amounts of dietary NH_4Cl on feed intake, rumen fluid pH and blood pH and illustrates that 0.75% NH_4Cl caused rumen pH to increase, and yet feed intake did not show a concomitant increase. Furthermore, an NH_4Cl level of 1.5% did not cause rumen fluid pH to fall below the original value of 5.88, but feed intake decreased significantly at this point ($P<0.005$). Blood pH however decreased with increasing amounts of NH_4Cl. Thus, the results from the current investigation support the theory of L'Estrange & Murphy (1972), that the degree of metabolic acidosis, and not rumen fluid pH, determines feed intake.

Table 4.1: The effect of dietary NH_4Cl on feed intake, rumen fluid pH and blood pH

CRITERIA	DIETARY NH_4Cl LEVEL (%)		
	0	0.75	1.5
Feed Intake (kg)	82.0	81.3	72.0
Rumen Fluid pH	5.88	5.91	5.88
Blood pH	7.37	7.35	7.31

The loss of weight exhibited by those animals on the 1.5% NH_4Cl diet (Figure 3.1) during the first 22 days of the trial concurs with the findings of Sartorius *et al.* (1949) who attributed this weight loss during the initial stages of extreme acidosis to loss of extracellular fluid. After this initial period, the animals involved began to gain weight, although their ADG was significantly ($P<0.005$) less than that of the animals on the zero or 0.75% NH_4Cl diets (Table 3.2)

Thus, the addition of 0.75% NH_4Cl to the concentrate ration did not affect animal performance and even caused animal ADG and FCE to increase slightly. However, the inclusion of 1.5% NH_4Cl in the ration was very detrimental to animal performance as it significantly lowered animal mass and feed intake ($P<0.01$).

4.1.2 Digesta pH

Raising the level of dietary NH_4Cl from 0 to 1.5% did not alter rumen fluid pH, yet caused digesta pH of the abomasum, duodenum and ileum to decrease significantly ($P<0.05$), as seen in Table 3.4. This is in agreement with the findings of (Tucker *et al.*, 1988). However, increasing the NH_4Cl level from 0 to 0.75% caused digesta pH to rise in all four sections of the gastro-intestinal tract, although this increase was only significant in the ileum ($P<0.05$). Why 0.75% NH_4Cl should cause digesta pH to increase is unclear. It may be that the addition of this level of NH_4Cl to the diet stimulated the buffering ability of the rumen, causing rumen HCO_3 and saliva secretions to increase, thus raising pH. This increase in pH was maintained throughout the digestive tract due to the poor neutralizing ability of the upper regions of the gastro-intestinal tract (Harrison & Hill, 1962). This increase, due to the inclusion of 0.75% NH_4Cl was especially marked in the abomasum where pH rose to 4.12. Other studies (Ash, 1961a) showed abomasal pH to be in the region of 2 to 3 and this raises the question as to whether abomasal enzymes are able to function efficiently at a pH of 4.12.

4.1.3 Blood Acid-Base Status

Blood acid-base status was significantly affected by dietary NH_4Cl ($P<0.01$). The inclusion of 0.75% NH_4Cl in the diet caused blood pH, HCO_3 and BE values to decrease (Figure 3.4 a, b, c) although they remained just within the acceptable range (Beede & Sanchez, 1989). However, an NH_4Cl level of 1.5% lowered blood acid-base status significantly ($P<0.01$) and by day 55 pH, HCO_3 and BE values had fallen below acceptable levels, as blood pH decreased to 7.31, blood HCO_3 fell to 19mmol/liter and BE values decreased to -5.65. Thus, an NH_4Cl level of 0.75% lowered blood acid-base status, yet pH and HCO_3 values remained within an acceptable range. Increasing the NH_4Cl level to 1.5% of DM resulted in severe acidosis by day 55 as blood pH and HCO_3 values decreased significantly. Furthermore, there appeared to be respiratory compensation to the acidosis as evidenced by the significant decrease in blood pCO_2 levels. The work of others (L'Estrange & Murphy, 1972; Scott & Buchan, 1981) has led to the assumption that a high Cl diet causes metabolic, as opposed to respiratory, acidosis and thus blood pCO_2 values should remain steady (Sartorious *et al.*, 1949). However, the results from the current investigation indicate that by day 55, NH_4Cl significantly affected blood pCO_2, causing it to decrease from 43.27 to 38.74 mm Hg ($P<0.01$). Thus in this instance, the acidosis induced by increasing amounts of NH_4Cl appeared to be partially compensated by an increased respiration rate. Blood BE values were at all times negative, even when no NH_4Cl was included in the diet, indicating that concentrate diets, as

opposed to hay-based rations, induce a mild form of acidosis in the animal. However, at the zero NH_4Cl level the acidotic effect of the concentrate ration lessened between days 24 and 55, and therefore the animal appeared to be able to buffer against the acidifying effect of concentrate diets. Whether the animal's buffer systems would enable it to obtain a positive BE value while on a concentrate ration is not known.

4.1.4 Organ Mass and Mineral Concentration

Increasing the dietary NH_4Cl level caused liver and kidney dry mass (expressed as a percentage of carcass mass) to increase significantly ($P<0.05$) (Figure 3.12 a and b). The reason for this is not understood. Furthermore, increasing amounts of NH_4Cl caused the Cu concentration of various organs to increase, although this was only significant in the kidney ($P<0.01$). The addition of 0.75% NH_4Cl to the diet caused organ Se concentration to increase slightly, yet 1.5% NH_4Cl caused Se concentration to decrease. At no stage were these differences significant. Abu Damir *et al.* (1990) however, found the retention of Ca, P and Mg decreased due to the inclusion of NH_4Cl in the diet.

4.1.5 Bone Parameters

Dietary NH_4Cl influenced the bone parameters measured (Table 3.8). Raising dietary acidity to 0.75% had no affect on bone status, yet 1.5% NH_4Cl resulted in a decrease in all bone parameters but especially in the ash:OM ratio ($P<0.05$). This concurs with previous research, where acidosis has been shown to decrease both organic and inorganic bone substances (Barzel, 1969; Barzel & Jowsey, 1969). However, Barzel (1969) reported that acidotic animals produced bones with reduced substance yet normal volume, but the current investigation found that both bone substance and volume decreased with increasing levels of NH_4Cl. Thus, it appears that 1.5% NH_4Cl encouraged bone resorption.

4.1.6 Urine pH

The addition of acid to the diet (0.75 and 1.5% NH_4Cl) resulted in a marked decrease in urine pH into the acidic range (Figure 3.14). When no NH_4Cl was present in the diet, urine pH was 8.09 but once NH_4Cl was added to the ration urine pH decreased to 5.75 ($P<0.0005$). However, urine pH of those animals on the 1.5% NH_4Cl diet was slightly higher than the pH of those receiving the 0.75% NH_4Cl diet, although not significantly so. However, as increasing amounts of NH_4Cl caused blood pCO_2 to decrease indicating possible respiratory compensation to the acidosis, the level of HCO_3 in the urine may have increased slightly in response to this (Houpt, 1984), thereby accounting for this small rise in urine pH.

versus 79kg). However, previous research has shown that when limestone was used as the Ca supplement, Ca:P ratios greater than 4:1 decreased animal performance (Lueker & Lofgreen, 1961).

4.2.2 Digesta pH

Research has shown limestone to have its greatest effect on digesta pH in the small intestine, colon and faeces (Wheeler & Noller, 1976; 1977; Wheeler *et al.* 1981a) and to have no effect on rumen or abomasal fluid pH (Wheeler & Noller, 1976; Haaland & Tyrrell, 1982). The results of the current investigation showed the buffering effect of limestone to be greatest in the rumen and abomasum and then lessened as the digesta passed down the gastro-intestinal tract (Table 3.3). The Ca concentration of the digesta has been shown to fluctuate throughout the digestive tract (Georgievskii, 1982). Furthermore, the data in Table 1.6 shows that the Ca concentration is greatest in the abomasum, followed by the rumen, duodenum and ileum. The results of the current investigation revealed that the high dietary Ca level had the greatest effect on abomasal pH (3.51 to 4.03), and then effected the digesta in the duodenum and rumen. Ileal pH was barely effected by dietary Ca level. Thus, digesta pH appears to be a function of the Ca concentration in the digestive tract, and therefore the high Ca diet resulted in a greater concentration of Ca in the digestive tract, thereby having a positive influence on digesta pH. Furthermore, the region where Ca concentration was highest (abomasum) according to Georgievskii (1982) was the area most affected by dietary Ca level.

4.2.3 Blood Acid-Base Status

Blood acid-base indicators, apart from pCO_2, were unaffected by the amount of limestone in the diet. The high Ca diet increased the blood pCO_2 level above that of the medium Ca diet (Figure 3.9). If $CaCO_3$ is converted to CaO and CO_2 in the body, it is not surprising that the higher level of $CaCO_3$ resulted in a higher blood pCO_2 value. Over time, the pCO_2 value resulting from the lower level of $CaCO_3$ decreased, thereby indicating that respiration increased, allowing the animals to eliminate the extra CO_2 from the body. However, at the higher limestone level, pCO_2 increased as the trial progressed. Thus, the body appeared to be unable to eliminate the CO_2 resulting from the high level of $CaCO_3$. However, by day 55 the higher Ca diet had also caused blood HCO_3 levels to rise above those of the medium Ca diet, indicating partial metabolic compensation to the increasing pCO_2 levels (Bouda & Jagos, 1991). Thus, although the difference in pCO_2 levels brought about by the different $CaCO_3$ levels was significant by day 55, the difference in pH was non-significant due to the partial metabolic compensation. Thus, instead of decreasing acidosis, a high level of limestone increased the acidotic state of the animal as evidenced by the increased pCO_2 values and decreased blood pH.

4.2.4 Organ Cu Status

Liver and pancreatic Cu concentration was significantly increased ($P<0.01$) by the higher level of dietary limestone. As increasing amounts of NH_4Cl had the same effect, the increased Cu concentration does not appear to be due to the buffering effect of Ca but rather to some interaction between Ca and Cu. Rajaratne *et al.* (1990) reported significantly higher mineral retention levels in lambs fed high levels of $CaCO_3$, therefore Cu appears to be one of those minerals favourably affected by a high dietary Ca level.

4.2.5 Bone Parameters

Dietary Ca level significantly affected the bone ash percentage and the ash:OM ratio, as the animals receiving the high Ca diet had bones with a greater ash content than the animals on the medium Ca ration. This is in agreement with Newell & Beauchene (1975) and Van Ryssen (1993) who found that the higher the level of Ca in the diet, the greater the amount of Ca deposited in the bones.

4.2.6 Urinary and Faecal Mineral Excretion

Urinary Ca, P, Se, Mg and Na excretion decreased as a result of the increased dietary Ca level (Table 3.11), while faecal Ca, P and Se excretion increased (Table 3.12). Thus, the high level of Ca interfered with Ca, P and Se absorption, especially Ca as shown by the increased faecal excretion of these minerals. This concurs with the results of other researchers (McDonald *et al.*, 1988). High Ca levels have been found to decrease both the absorption and urinary excretion of Se (Harrison & Conrad, 1984; Alfaro *et al.*, 1987), while increasing faecal Se excretion (Alfaro *et al.*, 1987). Thus, the results of the current investigation agree with the limited information available as to the effect of Ca on Se metabolism. Abu Damir *et al.* (1990) found mineral retention increased due to high levels of dietary Ca. Thus, although absorption may have been interfered with as evidenced by the increased mineral faecal excretion, the high Ca diet may have improved mineral retention resulting in lower urinary excretions than the medium Ca diet.

4.3 THE EFFECT OF DIETARY SELENIUM ON OVINE PHYSIOLOGY

4.3.1 Blood and Organ Se

At the onset of the trial the average plasma (10.08 ng/g) and whole blood Se concentration (39.28 ng/g) revealed that the sheep were deficient in Se (Puls, 1988). Plasma Se levels increased linearly, reaching a plateau approximately 50 days into the trial while whole blood Se increased linearly throughout the trial period (Figure 3.10). These findings are in agreement with other reports (Puls, 1988; Hartmann, 1994). Final plasma Se concentration of those sheep receiving additional dietary

Se was significantly higher ($P<0.005$) than that of the sheep receiving no added Se (400 ng/g versus 130 ng/g; $P<0.005$). The difference in final Se concentration of whole blood between the two treatment groups was not as great (160 ng/g versus 70 ng/g; $P<0.005$). Even though no additional Se had been added to some of the treatments, resulting in a dietary Se level of 0.106 mg/kg, all the animals in the experiment had final plasma Se levels within the normal range of 0.08 - 0.5 mg/kg. The whole blood Se level of those animals receiving additional Se was in the normal range, while those animals who received the diet to which no Se had been added showed final whole-blood Se values in the upper region of the marginal range (Puls, 1988).

The data in Table 3.7 shows that the addition of Se to the ration caused organ Se concentration to increase significantly ($P<0.0005$) in accordance with the results of others (Echevarria *et al.*, 1988). At both levels of dietary Se, the kidney exhibited the highest Se concentration.

4.3.2 Urinary and Faecal Excretion of Se

The addition of Se to the diet caused both urinary and faecal Se to increase significantly ($P<0.0005$). Faecal Se increased from 398 to 5068 ng/g as a result of adding Se to the ration, while urinary Se increased from 8.2 to 131.9 ng/g. This is in agreement with the results of Langlands *et al.* (1986).

4.4 THE EFFECT OF THE NH_4Cl x Ca INTERACTION ON OVINE PHYSIOLOGY

4.4.1 Animal Performance

Figures 3.2 a, b, c and d illustrate that the high Ca diet significantly improved animal performance ($P<0.05$) over that of the medium Ca ration at the 0 and 0.75% NH_4Cl levels. However, when combined with 1.5% NH_4Cl, the high Ca diet resulted in a significantly poorer performance than the medium Ca diet ($P<0.005$). The improved performance of those animals on the high Ca diet is expected, due to the buffering effect of limestone (Herod *et al.*1978), yet the combination of high Ca and 1.5% NH_4Cl proved more detrimental to animal performance than the medium Ca x 1.5% NH_4Cl diet. Thus, the diet which resulted in the highest final mass, feed intake and feed conversion efficiency contained a high level of Ca and 0.75% NH_4Cl, while the high Ca x 1.5% NH_4Cl ration resulted in the worst performance.

4.4.2 Digesta pH

Digesta pH exhibited a slightly different response to the NH_4Cl x Ca interaction than did the performance criteria. Rumen fluid pH was unaffected by this interaction, yet the remaining three regions of the digestive tract exhibited noteworthy responses. Figures 3.3 a and b show that the

addition of a high level of limestone to the zero NH_4Cl ration caused abomasal and duodenal pH to decrease below that of the medium Ca diet, yet when included with 0.75 and 1.5% NH_4Cl, the higher limestone level caused the pH of these two regions to increase above that resulting from the lower level of limestone. Why the high Ca level caused abomasal and duodenal pH to decrease at 0% NH_4Cl, but increase once NH_4Cl was included in the ration is not understood. In the ileum (Figure 3.3 c), the situation was reversed as the high level of limestone improved ileal pH over that of the medium Ca diet when no NH_4Cl was present but once NH_4Cl was included in the feed, those animals on the high Ca diet exhibited lower ileal pH values than those receiving the medium Ca diet.

4.4.3 Blood Acid-Base Status

Figures 3.5 a, b and c illustrate the effect of the NH_4Cl x Ca interaction on blood pH, pCO_2 and BE values. The higher level of limestone caused blood pCO_2 values to increase above those of the medium Ca diet. This occurred at all levels of NH_4Cl, but the difference between the pCO_2 values of the medium and high Ca diets was only significant at the 0.75% NH_4Cl level. Blood pH and BE values showed the opposite effect as the high Ca diet resulted in lower pH values than the medium Ca diet. Thus, the higher level of $CaCO_3$ appears to result in the formation of more carbonic acid, thus causing blood pH and BE values to decrease. Furthermore, the medium Ca x 0% NH_4Cl diet resulted in a positive BE value indicating an alkaline body state. The HCO_3:H_2CO_3 ratios indicate that partial compensation to metabolic acidosis occurred in those animals on the medium Ca x 1.5% NH_4Cl, and both high Ca diets containing NH_4Cl (Table 3.6). The other three diets had HCO_3:H_2CO_3 ratios near normal (Tasker, 1980). Thus, irrespective of the NH_4Cl level, the high Ca diet does not appear to be the more alkaline as was expected, but is instead more acidic than the medium Ca diet, as evidenced by the decreased blood pH and BE values, increased pCO_2 levels and decreased HCO_3:H_2CO_3 ratios.

4.4.4 Urine Volume

Urinary creatinine concentration provides an indirect measure of urine volume, as a low creatinine concentration indicates a dilute urine and vice versa (De Groot & Aafjes, 1960). The influence of the NH_4Cl x Ca interaction on urine volume produced some noteworthy results (Figure 3.16). At an NH_4Cl level of 0 and 1.5%, the high Ca diet resulted in the production of a concentrated urine while the animals on the medium Ca diet produced a dilute urine. This could be attributed to the buffering effect of the higher limestone level on the concentrate diet, thereby decreasing dietary acidity with a corresponding decrease in the need for water. Thus, the animals on the high Ca diet had less need of water and therefore produced a more concentrated urine than the animals receiving the medium Ca diet. However, at an NH_4Cl level of 0.75% the situation was reversed as the high Ca x 0.75% NH_4Cl ration resulted in a dilute urine. This diet has been shown to result in a very high blood pCO_2 level, and in accordance with the blood buffering system, CO_2 binds with water to form H_2CO_3

(Houpt, 1984). Thus, those animals receiving the high Ca x 0.75% NH_4Cl diet required large amounts of water due to the high blood CO_2 levels, and therefore produced a more dilute urine than the corresponding medium Ca diet. The indirect method of measuring urine volume through urine creatinine concentration corresponded well with the direct method used in the digestibility trial. It was however, unfortunate that due to insufficient metabolic crates it was not possible to measure urine volume of those animals on the 0.75% NH_4Cl diet.

4.4.5 Urine Minerals

The excretion of Ca through the urine appeared to be controlled by either the dietary Ca or NH_4Cl level, at different levels of NH_4Cl (Figure 3.18a). At an NH_4Cl level of 0 or 0.75%, the high Ca diet resulted in significantly more Ca being excreted in the urine than the medium Ca diet. This conflicts with the results of (Nicholson *et al.*, 1962) who found that supplementary Ca in the form of $CaCO_3$ did not increase urinary Ca excretion. However, at the 1.5% NH_4Cl level the situation changed as those animals on the medium Ca diet exhibited significantly ($P<0.05$) higher urinary Ca levels than those animals on the high Ca ration. This change could be attributed to the increased acidity of the diet. Braithwaite (1972) and Sutton & Dirsk (1978) stated that acidic diets caused urinary Ca excretion to increase due to decreased tubular reabsorption of Ca in the kidneys. As the medium Ca x 1.5% NH_4Cl diet was less well buffered by Ca than the corresponding high Ca diet, it resulted in a greater urinary excretion of Ca. Thus, the excretion of Ca through the urine appeared to be governed at the lower levels of NH_4Cl, by dietary Ca, and by dietary acidity at the highest NH_4Cl level.

Urinary P showed a similar patten to that of Ca (Figure 3.18b). At the zero NH_4Cl level the high Ca diet caused more P to be excreted through the urine than the medium Ca diet. However, once acid was added to the diet, the medium Ca diet resulted in more P being excreted in the urine than the high Ca diet. This has again been attributed to the decreased tubular reabsorption of P resulting from increased dietary acidity (Sartorius *et al.*, 1949). Whereas urinary Ca excretion was affected by dietary acidity at only 1.5% NH_4Cl, P excretion increased at both the 0.75 and 1.5% NH_4Cl levels.

Mg excretion has been shown to be related to urinary P (Lindley *et al.*, 1953; Robbins *et al.*, 1965) as a high level of P in the urine results in a low level of Mg and vice versa. The results of the current investigation support this as, at an NH_4Cl level of 0 and 0.75% urinary Mg excretion was opposite to that of P (Figure 3.18c). Once NH_4Cl was raised to 1.5% urinary Mg displayed the same response as that of Ca and P, as the least buffered diet (medium Ca x 1.5% NH_4Cl) caused urinary Mg to increase significantly ($P<0.025$).

In summary, the addition of 1.5% NH_4Cl to the diet appeared to have an all inclusive effect on the excretion of Ca, P and Mg, as the medium Ca diet, being the most acidic, resulted in the excretion of greater amounts of these three minerals than did the high Ca diet, whereas at lower levels of NH_4Cl mineral excretion varied.

4.5 THE EFFECT OF THE NH_4Cl x Se INTERACTION ON OVINE PHYSIOLOGY

4.5.1 Blood Acid-Base Status

Selenium, when considered alone, caused blood pH to decrease slightly. This was to be expected as Se is an anion, and a similar result was obtained by Hartmann (1994). However, when the effect of the NH_4Cl x Se interaction on blood acid-base parameters was studied, the presence of selenium in the diet was found to have a slight alkalizing effect on blood acid-base status at the zero and 0.75% NH_4Cl levels (Figures 3.6, 3.7, 3.8). As Se, when considered alone lowered acid-base parameters, the alkalizing effect of Se at 0 and 0.75% NH_4Cl appears to be an indirect, rather than a direct one. One way Se could indirectly improve blood acid-base status would be to increase the mobilization of bone minerals. However, the addition of Se to the diet resulted in bones with slightly higher ash, OM and SG values and therefore this indirect method of buffering against acidosis seems unlikely. A more plausible reason may lie in the fact that Se is found to a large extent in the erythrocytes and, more specifically, the presence of the seleno-enzyme glutathione peroxidase in haemoglobin (Georgieivskii, 1982). GSH-Px acts as a scavenger of peroxide, and in this way may decrease the amount of avaliable H^+ ions, thereby raising body acid-base status.

4.5.2 Urine pH and Mineral Excretion

As with blood acid-base status, the NH_4Cl x Se interaction effected urine pH, as the addition of Se to the diet caused urine pH to increase at all levels of NH_4Cl (Figure 3.15). The reason for this may lie in the significant effect of the NH_4Cl x Se interaction on urinary P excretion (Figure 3.18). At each level of NH_4Cl, the addition of Se to the diet caused urinary P excretion to increase, especially at an NH_4Cl level of 1.5%. As the phosphate buffer system plays an important role in controlling urine pH (Pitts, 1964), the increase in urinary P excretion due to the addition of Se to the diet may have assisted in raising urine pH. From Figures 3.6, 3.7 and 3.8 it can be seen that, although the presence of Se in the diet was able to raise blood acid-base status at 0 and 0.75% NH_4Cl, it was unable to overcome the effect of 1.5% NH_4Cl. However, the addition of Se to the 1.5% NH_4Cl diet caused urinary P excretion to increase from 0.23 mmol/24 hours to 3.78 mmol/hours. This significant increase in urinary phosphates may have been sufficient to overcome the acidifying effect of 1.5% NH_4Cl, thereby allowing Se to indirectly increase urine pH at all levels of NH_4Cl, including 1.5%. However, the mechanism by which Se increased urinary P excretion is not known.

Neither animal performance, mineral metabolism or acid-base status were influenced by the Ca x Se interaction or the NH_4Cl x Ca x Se interaction.

CHAPTER FIVE

GENERAL DISCUSSION AND CONCLUSION

The formation of urinary calculi in feedlot sheep can be controlled in one of two ways. Either an anionic salt, such as NH_4Cl can be included in the ration with the express purpose of lowering urine pH into the acidic range (Crookshank *et al.*, 1960; Bushman *et al.*, 1968), or a high, balanced Ca:P ration can be used to decrease the amount of P excreted in the urine (Bushman *et al.*, 1965a; Robbins *et al.*, 1965). Either method is successful, yet each affects the animal differently. In order to determine whether NH_4Cl or a high Ca:P ratio of 4:1 was the better method of preventing urolithiasis with respect to animal performance, mineral metabolism and acid-base status, the current research was instigated and some interesting results were obtained.

At present in South Africa, an NH_4Cl level of 0.5% is included in concentrate sheep rations with the express purpose of preventing urolithiasis. Research by Bushman *et al.* (1968) has shown an NH_4Cl level of 0.5% to be insufficient to lower urine pH into the acidic range, and therefore it appears that the level of NH_4Cl included in sheep rations is too low. The results of the current investigation revealed that an NH_4Cl level of 0.75% lowered urine pH to 5.75, well below the pH of 6.6 - 6.8, above which Ca and Mg phosphates precipitate out of the urine forming the basis of phosphatic calculi (Elliot *et al.*, 1961; Carbone, 1965). As a urine pH of only 6.6 is required, and 0.75% NH_4Cl causes the pH to fall as low as 5.75, further research may be justified in order to determine an NH_4Cl level between 0.5 and 0.75% which results in the desired pH.

The inclusion of 0.75% NH_4Cl in a concentrate ration leads to the question, as to what effect this level of dietary acid has on the animal. The findings of the current investigation revealed that those animals receiving a concentrate diet containing no NH_4Cl had negative blood BE values, indicating that concentrate, as opposed to forage-based rations, are acidifying. Thus the addition of NH_4Cl to the diet might be expected to lower acid-base status still further. Although 0.75% NH_4Cl caused blood pH, HCO_3 and BE values to decrease below that due to the zero NH_4Cl diet, this difference was significant only by day 55 of the trial. Furthermore, blood pH did not fall below 7.34 and HCO_3 levels remained above 22mmol/liter.

The 0.75% NH_4Cl level had little significant effect on mineral metabolism. Faecal Ca and P excretion decreased significantly ($P<0.01$) indicating increased intestinal absorption of these two minerals. Bone parameters decreased slightly, thus this level of dietary acidity did not cause a significant increase in bone resorption. Urinary Ca excretion increased significantly ($P<0.01$), yet urinary P rose only slightly. Urinary Se excretion decreased and as this was accompanied by a slight increase in organ Se concentration, it appeared that this level of NH_4Cl improved the retention of Se. Thus, 0.75% NH_4Cl appeared to have a beneficial effect on some aspects of mineral metabolism, namely

Ca and P absorption, and Se organ concentration. Although it did not have a beneficial effect on bone status and urinary mineral excretion, neither did it have a significantly detrimental effect.

Other researchers have found 1% NH_4Cl to significantly effect the animal performance (Bushman *et al.*, 1967), and the results of the current investigation indicated that 1.5% NH_4Cl was very detrimental to the animal. Performance criteria were significantly lowered, as were blood pH, and HCO_3 values. Urine pH however was slightly higher than that resulting from the 0.75% NH_4Cl diet. The reason for this may lie in the respiratory compensation to acidosis evidenced by the decreased pCO_2 levels. This would cause urinary HCO_3 levels to rise slightly resulting in a less acidic urine (Houpt, 1984).

Furthermore, 1.5% NH_4Cl had a significant effect on mineral metabolism. Faecal Ca and P levels were not significantly different to those resulting from the 0.75% NH_4Cl diet. Thus, increasing dietary acidity still further did not appear to significantly increase the intestinal absorption of Ca and P. Bone SG, volume, ash and OM values were significantly decreased ($P<0.01$) when compared with the lower NH_4Cl levels, especially the bone ash content ($P<0.005$), indicating increased bone resorption. Urinary Ca, P and Se were significantly increased due to 1.5% NH_4Cl. Thus, although intestinal absorption of Ca and P increased, the high level of NH_4Cl also increased their rate of excretion. The increased urinary Se was associated with a decrease in organ Se concentration, and therefore a high level of dietary acidity would appear to interfere with Se retention.

Thus, an NH_4Cl level of 0.75% appears to be an acceptable means of controlling urolithiasis, as it lowers urine pH, yet has no significantly detrimental effect on the animal beyond that induced by a concentrate ration. However, increasing dietary acidity any further appears to be detrimental to the animal with respect to performance, acid-base status and mineral metabolism.

Limestone was used to raise the Ca:P ratio to 4:1. This Ca supplement was chosen as being the cheapest and most widely used, and has the added advantage of being a good buffer (Herod *et al.*, 1978). The high Ca:P ratio was therefore expected to, not only prevent urolithiasis, but to also improve the productivity of the animal by buffering against the effects of the concentrate ration. It did indeed improve digesta pH, and increase bone ash and organ Cu content, yet it had a very surprising influence on acid-base status. If $CaCO_3$ is catabolized to CaO and CO_2 in the body, this will account for the dual response exhibited by the animals to the high Ca diet. As the high limestone level resulted in the formation of more CaO, the Ca concentration of the digesta was raised resulting in higher pH values, especially in the abomasum where Ca concentration was apparently at its highest (Table 1.6). Furthermore, the higher bone ash and organ Cu content could also be attributed to the beneficial effect of a greater amount of CaO on mineral retention. On the other hand, the high level of $CaCO_3$ also caused the blood pCO_2 level to increase above that of the medium Ca diet. Furthermore, the blood pCO_2 level of those animals on the high $CaCO_3$ diet

increased over time instead of decreasing. This increase in blood pCO_2 was accompanied by a decrease in blood pH and BE values. This trend of blood acid-base status (increasing pCO_2 and decreasing pH) is symptomatic of respiratory acidosis (Houpt, 1984; Bouda & Jagos, 1991).

This usually occurs when the respiratory centres of the central nervous system have been depressed, or respiratory organs have been damaged (Bouda & Jagos, 1991). Although blood pCO_2 levels were above the norm of 40mm Hg, they were still within the acceptable range of 39 - 44mm Hg (Beede & Sanchez, 1989). According to Tasker (1988), animals with respiratory acidosis exhibit pCO_2 levels of approximately 90mm Hg and a pH of 7.3 or below (Table 1.2). Thus, the high $CaCO_3$ level produced blood acid-base trends symptomatic of respiratory acidosis, yet blood pCO_2 values were not sufficiently high to allow for the classification of this form of acidosis. It therefore appears that the symptoms of respiratory acidosis can be produced nutritionally through the feeding of a carbonate, supplement as Hartmann (1994) found that sheep supplemented with 4% $NaHCO_3$ also developed symptoms of respiratory acidosis.

The formation of CO_2 from the medium $CaCO_3$ level did not appear to have an adverse effect on the animal, as their pCO_2 levels were approximately 40mm Hg and decreased over, time indicating that respiration rate was enhanced allowing for the elimination of excess CO_2. Thus, instead of improving the animal's acid-base status, the high level of limestone increased blood pCO_2 levels thereby causing blood pH to decrease. Therefore, the high limestone level was more acidifying that the medium level.

Apart from adversely affecting the acid-base status of the animal, the high limestone level also had a detrimental effect on mineral metabolism, causing faecal Ca, P and Se to increase while urinary Ca, P, Se, Mg and Na decreased. Thus, a high level of $CaCO_3$ interfered with the intestinal absorption of various minerals although, as bone ash and organ Cu concentration increased as a result of the higher Ca level, the decreased urinary mineral excretion of these minerals may be due to their improved retention by the body. Thus, a high Ca level appeared to improve the efficiency of mineral utilization. However, Gill *et al.* (1959) attributed the effectiveness of a high Ca level in lowering urine P excretion to the decreased intestinal absorption of P. Although the higher Ca:P ratio had the desired effect of decreasing urinary P excretion and thereby preventing urolithiasis, it had an adverse effect on the acid-base status of the animal, yet it had a dual effect on mineral metabolism as absorption decreased but retention increased.

Thus, when limestone is used as the supplement, a Ca:P ratio of 4:1 appeared to be too high, as it had an adverse effect on the animal. If a different Ca supplement, other than a carbonate, was used it may be possible to raise the Ca:P ratio to 4:1, but this would require further research.

The most effective way of preventing urolithiasis therefore appears to be through an NH_4Cl level

of 0.75%. However, as sheep rations usually contain a Ca:P ratio of approximately 2:1 the interaction between 0.75% NH_4Cl and a medium Ca level is of interest. At the medium Ca level, increasing amounts of NH_4Cl caused a gradual linear decrease in most of the characteristics under consideration, yet the difference between the zero and 0.75% NH_4Cl diet was never significant. The most acidic combination according to abomasal and duodenal pH, blood pH, urine volume and urinary mineral excretion, was the medium Ca x 1.5% NH_4Cl diet. However, the high Ca x 1.5% NH_4Cl ration had the most detrimental effect on mass and feed criteria, ileal pH, blood BE values and bone ash and OM content. The reason for this is not understood and will require further investigation as to the precise mechanism of the NH_4Cl x Ca interaction.

Various conclusions can be drawn from the results of the current investigation as to the effect of the different methods of controlling urolithiasis on acid-base status and mineral metabolism.

(1) An NH_4Cl level of 0.75% was sufficient to lower urine pH into the acidic range (5.75) thereby preventing the formation of calculi. Furthermore, this level of NH_4Cl had no significantly adverse effect on either animal performance, mineral metabolism or acid-base status when compared with those animals on the zero NH_4Cl diet. However, 1.5% NH_4Cl had a detrimental effect on animal productivity. Performance was lowered, urinary mineral excretion (Ca, P and Se) increased and blood pH and HCO_3 values fell below the acceptable range.

(2) The high Ca:P ratio (4:1) resulting from supplementation with limestone proved to be detrimental to the animal, as the high $CaCO_3$ level caused blood pCO_2 levels to increase and pH to decrease resulting in an acidic state. Furthermore, the high Ca level interfered with the absorption of various minerals as evidenced by the increased faecal excretion of Ca, P and Se, yet mineral retention improved, as evidenced by the increased bone ash and organ Cu content. Thus, the high level of limestone had an adverse effect on the acid-base status and mineral metabolism of the animal.

(3) Although increasing the Ca:P ratio in sheep rations to 4:1 through the addition of limestone appear to be an effective way of controlling urolithiasis, this method was detrimental to the animal and therefore the Ca:P ratio should remain at a lower level of between 2.5-3:1. Instead, the level of NH_4Cl should be increased from 0.5 to 0.75% of the diet, as this results in an acidic urine, thereby preventing the formation of calculi and at the same time has no significantly adverse effect on the animal.

Thus, the formation of urinary calculi in sheep will be prevented and the method used will not have a significantly detrimental effect on the general performance, mineral metabolism or acid-base status of the animal, as shown by the results of the current investigation.

REFERENCES

Abu Damir, H., Scott, D., Thomson, J.K., Topps, J.H., Buchan, W. and Pennie, K. 1990. The effect of a change in blood acid-base status on body composition and mineral retention in growing lambs. Anim. Prod. **51**:527.

Alfaro, E., Neathery, M.W., Miller, W.J., Gentry, R.P., Crowe, C.T., Fielding, A.S., Etheridge, R.E., Pugh, D.G. and Blackmon, D.M. 1987. Effects of varying the amounts of dietary calcium on selenium metabolism in dairy calves. J. Dairy Sci. **70**:831.

Ash, R.W. 1961a. Acid secretion of the abomasum and its relation to the flow of food material in the sheep. J.Physiol. **156**:93.

Ash, R.W. 1961b. Stimuli influencing the secretion of acid by the abomasum of sheep. J. Physiol. **157**:185.

Association of Official Analytical Chemists. 1980. *Official Methods of Analysis.* 13th Ed. AOAC, Washington DC.

Baker, L.A., Topliff, D.R., Freeman, D.W., Teeter, R.G. and Breazile, J.W. 1991. Effect of dietary cation-anion balance on acid-base status in horses. Anim. Sci. Res. Rep. pp279.

Balch, C.C. 1958. Observations on the act of eating in cattle. Brit. J. Nutr. **12**:330.

Barzel, U.S. 1969. The effect of excessive acid feeding on bone. Calc. Tiss. Res. **4**:94.

Barzel, U.S. and Jowsey, J. 1969. The effects of chronic acid and alkali administration on bone turnover in acidic rats. Clin. Sci. **36**:517 (cited by Petito & Evans, 1984).

Beede, D.K. and Sanchez, W.K. 1989. Macromineral nutrition and electrolyte balance of lactating dairy cattle. In: *50th Anniversary Minnesota Nutrition Conference and Heartland Lysine Technical Symposium.* Bloomington, Minnesota. pp 81.

Beighle, D.E., Tucker, W.B. and Hemken, R.W. 1988. Interactions of dietary cation-anion balance and phosphorus: effects on growth and serum inorganic phosphorus in dairy calves. J. Dairy Sci. **71**:3362.

Bezeau, L.M., Bailey, C.B. and Slen, S.B. 1961. Urine pH and the formation of uroliths in cattle. J. Anim. Sci. **20**:924 (abstr).

Bide, R.W., Dorward, W.J. and Tumbleson, M.E. 1973. Clinical chemistry of grain fed cattle I. A preliminary study of a basic biomedical profile. Can. J. Anim. Sci. **53**:697.

Block, E. 1991. Anion-cation balance and its effect on the performance of ruminants. In: *Recent Advances in Animal Nutrition.* (Ed. by Haresign and D.J.A. Cole), Butterworth-Heinermann Ltd, United Kingdom.

Bouda, J. and Jagos, P. 1991. Disorders in the acid-base balance. In: *Metabolic Disorders and their Prevention in Farm Animals.* (Ed. by L. Vrzgula), Elsevier, New York.

Braithwaite, G.D. 1972. The effect of ammonium chloride on calcium metabolism in sheep. Br. J. Nutr. **27**:201.

Bryant, M.D., Robinson, I.M. and Chu, H. 1959. Observations on the nutrition of *Bacteroides succinogenes* - a ruminal cellulolytic bacterium. J. Dairy Sci. **42**:1831.

Buescher, R.G., Bell, M.C. and Berry, R.K. 1961. The effect of excessive calcium on selenium-75 in swine. J. Anim. Sci. **20**:368.

Bushman, D.H., Embry, L.B. and Emerick, R.J. 1967. Efficacy of various chlorides and calcium carbonate in the prevention of urinary calculi. J. Anim. Sci. **26**:1199.

Bushman, D.H., Emerick, R.J. and Embry, L.B. 1965a. Experimentally induced ovine phosphatic urolithiasis: relationships involving dietary calcium, phosphorus and magnesium. J.Nutr. 87:499.

Bushman, D.H., Emerick, R.J. and Embry, L.B. 1965b. Incidence of urinary calculi in sheep as affected by various dietary phosphates. J. Anim. Sci. **24**:671.

Bushman, D.H., Emerick, R.J. and Embry, L.B. 1968. Effect of various chlorides and calcium carbonate on calcium, phosphorus, sodium, potassium and chloride balance and their relationship to urinary calculi in lambs. J. Anim. Sci. **27**:490.

Carbone, M.G. 1965. Phosphocrystalluria and urethral obstruction in the cat. J. Am. Vet. Med. Ass. **147**:1195.

Colovos, N.F., Keener, H.A. and Davis, H.A. 1955. The effects of pulverised limestone and dicalcium phosphate on the nutritive value of dairy cattle feed. J. Dairy Sci. **38**:627.

Colovos, N.F., Keener, H.A. and Davis, H.A. 1958. Effect of pulverized limestone and dicalcium phosphate on the nutritive value of dairy cattle feed. J. Dairy Sci. **41**:676.

Colovos, N.F., Keener, H.A., Davis, H.A. and Teeri, A.E. 1953. Too much limestone lowers digestibility of both protein and energy in silage. Rpt of the Director of the New Hampshire Exp. Sta. Bul. 402 (cited by Dowe *et al.,* 1957).

Crookshank, H.R. 1966. Effect of sodium or potassium on ovine calculi. J. Anim. Sci. **25**:1005.

Crookshank, H.R. 1970. Effect of ammonium salts on the production of ovine urinary calculi. J. Anim. Sci. **30**:1002.

Crookshank, H.R., Keating, F.E., Burnett, E., Jones, J.H. and Davis, R.E. 1960. The effect of chemical and enzymatic agents on the formation of urinary calculi in fattening steers. J. Anim. Sci. **19**:595.

Davison, K.L. and Woods, W. 1961. Calcium and corn oil interrelationships as influencing ration utilization by lambs. J. Anim. Sci. **20**:532.

De Groot, T.H. and Aafjes, J.H. 1960. On the constancy of creatinine excretion in the urine of the dairy cow. Br. vet. J. **116**:409.

Dowe, T.W., Matsushima, J. and Arthaud, V.H. 1957. The effects of adequate and excessive calcium when fed with adequate phosphorus in growing rations for beef calves. J. Anim. Sci. **16**:811.

Echevarria, M.G., Henry, P.R., Ammerman, C.B. and Rao, P.V. 1988. Effects of time and dietary selenium concentration as sodium selenite on tissue selenium uptake by sheep. J. Anim. Sci. **66**:2299.

Elam, C.J., Ham, W.E. and Dyer, I.A. 1959. An attempt to induce urinary calculi formation in steers with the effects on certain blood and urine constituents. J. Anim. Sci. **18**:383.

Elam, C.J., Schneider, B.H. and Ham, W.E. 1956. Experimentally produced urinary calculi in sheep. J. Anim. Sci. **15**:800.

Elliot, J.S., Todd, H.E. and Lewis, L. 1961. Some aspects of calcium phosphate solubility. J. Urol. **85**:428.

Embry, L.B., Dye, L.B., Luther, R.M. and Gastler, G.F. 1969. Alkali supplements and chlortetracycline with corn silage rations for growing and finishing beef cattle. South Dakota State Univ. A.S. Series 69-6.

Emerick, R.J. 1988. Urinary calculi. In: *The Ruminant Animal: Digestive Physiology and Nutrition.* (Ed. by D.C. Church). Prentice Hall, New York.

Emerick, R.J. and Embry, L.B. 1963a. Calcium and phosphorus levels related to the development of phosphate urinary calculi in sheep. J. Anim. Sci. **22**:510.

Emerick, R.J. and Embry, L.B. 1963b. Effects of calcium and phosphorus levels and diethylstilbestrol on urinary calculi incidence and feedlot performance of lambs. J. Anim. Sci. **23**:1079.

Fredeen, A.H., DePeters, E.J. and Baldwin, R.L. 1988a. Characterisation of acid-base disturbances and effects on calcium and phosphorus balances of dietary fixed ions in pregnant or lactating does. J. Anim. Sci. **66**:159.

Fredeen, A.H., DePeters, E.J. and Baldwin, R.L. 1988b. Effects of acid-base disturbances caused by differences in dietary fixed ion balance on kinetics of calcium metabolism in ruminants with high calcium demand. J. Anim. Sci. **66**:174.

Fulton, W.R., Klopfenstein, T.J. and Britton, R.A. 1979. Adaptation to high-concentrate diets by beef cattle II. Effect of ruminal pH alteration on rumen fermentation and voluntary intake of wheat diets. J. Anim. Sci. **49**:785.

Galyean, M.L., Wagner, D.G. and Owens, F.N. 1979. Level of feed intake and site and extent of digestion of high concentrate diets by steers. J. Anim. Sci. **49**:199.

Georgievskii, V.I. 1982. The physiological role of macroelements. In: *Mineral Nutrition of Animals.* (Ed. by V.I. Georgievskii). Butterworths, London.

Gill, W.B., Finlayson, B. and Vermeulen, C.W. 1959. Prevention of urinary calculi in rats by adding calcium to the diet. J. Urol. **82**:256.

Haaland, G.L. and Tyrrell, H.F. 1982. Effects of limestone and sodium bicarbonate buffers on rumen measurements and rate of passage in cattle. J. Anim. Sci. **55**:935.

Haaland, G.L., Tyrrell, H.F., Moe, P.W. and Wheeler, W.E. 1982. Effect of crude protein level and limestone buffer in diets fed at two levels of intake on rumen pH, ammonia-nitrogen, buffering capacity and volatile fatty acid concentration of cattle. J. Anim. Sci. **55**:943.

Harmon, D.L. and Britton, R.A. 1983. Balance and urinary excretion of calcium, magnesium and phosphorus in response to high concentrate feeding and lactate infusion in lambs. J. Anim. Sci. **57**:1306.

Harrison, J.H. and Conrad, H.R. 1984. Effect of dietary calcium on selenium absorption by the nonlactating dairy cow. J. Dairy Sci. **67**:1860.

Harrison, F.A. and Hill, K.J. 1962. Digestive secretions and the flow of digesta along the duodenum of the sheep. J.Physiol. **162**:225.

Hartmann, F.D. 1994. The effect of dietary sodium bicarbonate supplementation on the metabolism of selenium and copper in sheep. MSc. Thesis, University of Natal.

Herod, E.L., Bechtle, R.M., Bartley, E.E. and Dayton, A.D. 1978. Buffering ability of several compounds *in vitro* and the effect of a selected buffer combination on ruminal acid production *in vivo*. J. Dairy Sci. 61:1114.

Hill, K.J. 1970. Digestion in the small intestine. In: *Duke's Physiology of Domestic Animals.* (Ed. by M.J. Swenson) Cornell University Press, Ithaca, New York,

Hoar, D.W., Emerick, R.J. and Embry, L.B. 1969. Ovine phosphatic urolithiasis as related to the phosphorus and calcium contents and acid-base-forming effects of all-concentrate diets. J. Anim. Sci. **29**:647.

Hoar, D.W, Emerick, R.J. and Embry, L.B. 1970a. Potassium, phosphorus and calcium interrelationships influencing feedlot performance and phosphatic urolithiasis in lambs. J. Anim. Sci. **30**:597.

Hoar, D.W., Emerick, R.J. and Embry, L.B. 1970b. Influence of calcium source, phosphorus level and acid-base-forming effects of the diet on feedlot performance and urinary calculi formation in lambs. J. Anim. Sci. **31**:118.

Horst, R.L. and Jorgensen, N.A. 1974. Effect of ammonium chloride on nitrogen and mineral balance in lactating and nonlactating goats. J. Dairy Sci. **57**:683.

Houpt, T.R. 1984. Water, electrolytes and acid-base balance. In: *Duke's Physiology of Domestic Animals.* 10th Edition. (Ed. by M.J. Svensen) Comstock Publishing Associates, London.

Huntington, G.B. 1983. Feedlot performance, blood metabolic profile and calcium status of steers fed high concentrate diets containing several levels of calcium. J. Anim. Sci. **56**:1003.

Huntington, G.B., Britton, R.A. and Prior, R.L. 1981. Feed intake, rumen fluid volume and turnover, nitrogen and mineral balance and acid-base status of wethers changed from low to high concentrate diets. J. Anim. Sci. **52**:1376.

Jackson, J.A., Hopkins, D.M., Xin, Z. and Hemken, R.W. 1992. Influence of cation-anion balance on feed intake, body weight gain and humeral response of dairy calves. J. Dairy Sci. **75**:1281.

Kern, D.L., Slyter, L.L., Leffel, E.C., Weaver, J.M. and Oltjen, R.R. 1974. Ponies vs steers: microbial and chemical characteristics of intestinal ingesta. J. Anim. Sci. **38**:559.

Koh, T.S. and Benson, T.H. 1983. Critical re-appraisal of fluorometric method for determination of selenium in biological materials. J. Assoc. Off. Anal. Chem. **66**. 918.

Langlands, J.P., Bowles, J.E., Donald, G.E. and Smith, A.J. 1986. Selenium excretion in sheep. Aust. J. agric. Res. **37**:201.

Lemann, J. and Lennon, E.J. 1972. Role of diet, gastrointestinal tract and bone in acid-base status. Kid. Int. **1**:275.

Lemann, J., Litzow, J.R. and Lennon, E.J. 1966. The effects of chronic acid loads in normal man: further evidence for the participation of bone mineral in the defence against chronic acidosis. J. Clin. Invest. **45**:1608.

Lemann, J., Litzow, J.R. and Lennon, E.J. 1967. Studies of the mechanism by which chronic metabolic acidosis augments urinary calcium excretion in man. J. Clin. Invest. **46**:1318.

Leoschke, W.L. and Elvehjem, C.A. 1954. Prevention of urinary calculi formation in mink by the alteration of urinary pH. Proc. Soc. Exp. Biol. Med. **85**:42 (cited by Crookshank, 1970).

L'Estrange, J.L. and Murphy, F. 1972. Effects of dietary mineral acids on voluntary food intake, digestion, mineral metabolism and acid-base balance of sheep. Br. J. Nutr. **28**:1.

Lindley, C.E., Taysom, E.D., Ham, W.E. and Schneider, B.H. 1953. Urinary calculi in sheep. J. Anim. Sci. **12**:704.

Lomba, F., Chauvaux, G., Teller, E., Lengele, L. and Bienfet, V. 1978. Calcium digestibility in cows as influenced by the excess of alkaline ions over stable acid ions in their diets. Br. J. Nutr. **39**:425.

Lowry, K.R., Mahan, D.C. and Corley, J.R. 1985. Effect of dietary calcium on selenium retention in postweaning swine. J. Anim. Sci. **60**:1429.

Lueker, C.E. and Lofgreen, G.P. 1961. Effects of intake and calcium to phosphorus ratio on absorption of these elements by sheep. J. Nutr. **74**:233.

Manston, R. 1967. The influence of dietary calcium and phosphorus concentration on their absorption by the cow. J. agric. Sci., Camb. **68**:263

Martin, C.J. and Pierce, A.W. (1934). Studies on the phosphorus requirements of sheep I. The effect on young merino sheep of a diet deficient in phosphorus but containing digestible proteins and vitamins. Aust. Council Sci. Ind. Res. Bull. 77 (Cited by Packett & Hauschild, 1964).

McDonald, P., Edwards, R.A. and Greenhalgh, J.F.D. 1988. *Animal Nutrition.* 4th edition. Longman Scientific and Technical.

Newell, G.K. and Beauchene, R.E. 1975. Effects of dietary calcium level, acid stress, and age on renal, serum and bone responses of rats. J. Nutr. **105**:1039.

Nicholson, J.W.G., Cunningham, H.M. and Friend, D.W. 1962. The addition of buffers to ruminant rations III. The effect of additions of sodium bicarbonate, sodium propionate, limestone and cod liver oil on apparent digestibility and nitrogen retention of an all-concentrate ration. Can. J. Anim. Sci. **42**:82.

NRC, 1985. *Nutrient Requirements of Sheep.* 6th Rev. Ed. National Academy Press, Washington D.C.

Oetzel, G.R., Fettman, M.J., Hamar, D.W. and Olson, J.D. 1991. Screening of anionic salts for palatability, effects on acid-base status, and urinary calcium excretion in dairy cows. J. Dairy Sci. **74**:965.

Packett, L.V. and Hauschild, J.P. 1964. Phosphorus, calcium and magnesium relationships in ovine urolithiasis. J. Nutr. **84**:185.

Pearce, G.R. 1965. Rumination in sheep II. The circadian pattern of rumination. Austr. J. Ag. Res. **16**:635.

Petersson, K.H., Warner, R.G., Kallfelz, F.A. and Crosetti, C.F. 1988. Influence of magnesium, water and sodium chloride on urolithiasis in veal calves. J. Dairy Sci. **71**:3369.

Petito, S.L. and Evans, J.L. 1984. Calcium status of the growing rat as affected by diet acidity from ammonium chloride, phosphate and protein. J. Nutr. **114**:1049.

Pitts, R.F. 1964. *Physiology of the Kidney and Body Fluids.* Year Book Medical Publishers, Chicargo.

Puls, R. 1988. *Mineral Levels in Animal Health.* Trinity Western University Press, Canada.

Rajaratne, A.A.J., Scott, D., Thompson, J.K., Buchan, W. and Pennie, K. 1990. The effect of variation in dietary calcium supply on the phosphorus requirements of growing lambs. Anim. Prod. **51**:135.

Robbins, J.D., Kunkel, H.O. and Crookshank, H.R. 1965. Relationship of dietary mineral intake to urinary mineral excretion and the incidence of calculi in lambs. J. Anim. Sci. **24**:76.

Robinson, J.R. 1967. *Fundamentals of Acid-base Regulation.* Oxford Press (cited by Bouda & Jagos,1991).

Russell, J.R., Young, A.W. and Jorgensen, N.A. 1980. Effect of sodium bicarbonate and limestone additions to high grain diets on feedlot performance and ruminal and faecal parameters in finishing steers. J. Anim. Sci. **51**:996.

Sartorius, O.W., Roemmelt, J.C., Pitts, R.F., Calhoon, D. and Miner, P. 1949. The renal regulation of acid-base balance in man IV. The nature of the renal compensation in ammonium chloride acidosis. J. Clin. Invest. **28**:423.

Scott, D. and Buchan, W. 1981. Changes in blood composition and urinary mineral excretion in the sheep in response to acute acid-base disturbance. Res. Vet. Sci. **31**:43.

Slyter, L.L., Oltjen, R.R., Kern, D.L. and Blank, F.C. 1970. Influence of type and level of grain and diethylstilbestrol on the rumen microbial population of steers fed all-concentrate diets. J. Anim. Sci. **31**:996.

Stacy, B.D. and Wilson, B.W. 1970. Acidosis and hypercalciuria: renal mechanisms affecting calcium, magnesium and sodium excretion in the sheep. J. Physiol. **210**:549.

Staples, C.R. and Lough, D.S. 1989. Efficacy of supplemental dietary neutralising agents for lactating dairy cows: a review. Anim. Feed Sci. Technol. **23**:277.

Steele, R.G.D. and Torrie, J.H. 1981. Principles and procedures of statistics. McGraw-Hill, New York.

Suttle, N.F. and Field, A.C. 1968. Effect of intake of copper, molybdenum and sulphate on copper metabolism in sheep I. Clinical condition and distribution of copper in the blood of the pregnant ewe. J. Comp. Path. **78**:351.

Sutton, R.A.L. and Dirsk, J.H. 1978. Renal handling of calcium. Fed. Proc. **37**:2112 (cited by Harmon & Britton, 1983).

Tasker, J.B. 1980. Fluids, electrolytes and acid-base balance. In: *Clinical Biochemistry of Domestic Animals*. (Ed. by J.J. Kaneko), Academic Press, New York.

Tremere, A.W., Merrill, W.G. and Loosli, J.K. 1968. Adaptation to high concentration feeding as related to acidosis and digestive disturbances in dairy heifers. J. Dairy Sci. **51**:1065.

Tucker, W.B., Harrison, G.A. and Hemken, R.W. 1988. Influence of dietary cation-anion balance on milk, blood, urine and rumen fluid in lactating dairy cattle. J. Dairy Sci. **71**:346.

Udall, R.H. 1962. Studies in urolithiasis V. The effects of urinary pH and dietary sodium chloride on the urinary excretion of proteins and the incidence of calculosis. Am. J. vet. Res. **23**:1241.

Udall, R.H. and Chow, F.H.C. 1963. Ion competition and the formation of phosphatic uroliths. Ann. N.Y. Acad. Sci. **104**:612 (cited by Bushman et al., 1968).

Upton, P.K. and L'Estrange, J.L. 1977. Effects of chronic hydrochloric and lactic acid administrations on food intake, blood acid-base balance and bone composition of the rat. Q. J. Exp. Physiol. **62**:223.

Vagg, M.J. and Payne, J.M. 1970. The effect of ammonium chloride induced acidosis on calcium metabolism in ruminants. Br. Vet. J. **126**:531.

VanCampen, D. 1976. Effects of buffers on ruminal acids. In: *Buffers in Ruminant Physiology and Metabolism.* (Ed. by L. Weinberg and A.L. Sheffner), Church and Dwight Co., Inc., New York.

Van Ryssen, J.B.J. 1993. Suitability of a lime source high in manganese as a feed ingredient for sheep. S. Afr. J. Anim. Sci. **23**:115.

Varner, L.W. and Woods, W. 1972. Effect of calcium and starch additions upon ration digestibility by steers. J. Anim. Sci. **35**:410.

Verdaris, J.N. and Evans, J.L. 1976. Diet calcium and pH versus mineral balance in holstein cows 84 days pre- to 2 days post partum. J. Dairy Sci. **59**:1271.

Wagner, D.G. and Loosli, J.K. 1967. Studies on the energy requirements of high producing dairy cows. Cornell Univ. Agr. Exp. Sta. Memoir 400.

Weast, R.C. and Astle, M.J. 1978. *CRC Handbook of Chemistry and Physics* (59th Ed). CRC Press Inc., West Palm Beach, Florida.

Wheeler, W.E. 1977. Effect of limestone buffers on the digestion of complete mixed rations by dairy cows. Annu. Meet. Amer. Dairy Sci. Assoc. Ames, IA, p117.

Wheeler, W.E. 1980. Gastrointestinal tract pH environment and the influence of buffering materials on the performance of ruminants. J. Anim. Sci. **51**:224.

Wheeler, W.E. and Noller, C.H. 1976. Limestone buffers in complete mixed rations for dairy cattle. J. Dairy Sci. **59:** 1788.

Wheeler, W.E. and Noller, C.H. 1977. Gastrointestinal tract pH and starch in faeces of ruminants. J. Anim. Sci. **44**:131.

Wheeler, W.E, Noller, C.H. and Coppock, C.E. 1975. Effect of forage-to-concentrate ratio in complete feeds and feed intake on digestion of starch by dairy cows. J. Dairy Sci. **58**:1902.

Wheeler, W.E, Noller, C.H. and White, J.L. 1981a. Effect of level of calcium addition and rate of reactivity on response to calcitic limestones in high concentrate diets by beef steers. J. Anim. Sci. **52**:882.

Wheeler, W.E., Noller, C.H. and White, J.L. 1981b. Effect of level of calcium and sodium bicarbonate in high concentrate diets on performance and nutrient utilization by beef steers. J. Anim. Sci. **53**:499.

Winters, R.W., Engel, K., Dell, R.B. and Berkson, R.P. 1967. *Acid-base Physiology in Medicine.* Westlake, Copenhagen (cited by Bouda & Jagos, 1991).

Wise, M.B., Ordoveza, A.L. and Barrick, E.R. 1963. Influence of variations of dietary calcium:phosphorus ratio on performance and blood constituents of calves. J. Nutr. **79**:79.

Wise, M.B., Blumer, T.N., Craig, H.B. and Barrick, E.R. 1965. Influences of rumen buffering agents and hay on performance and carcass characteristics of steers fed all-concentrate diets. J. Anim. Sci. **24**:83.