# CHAPTER II

# BACKGROUND INFORMATION

## Sodium content in body fluid

The atomic number of Na is 11, and its atomic weight is 22.99. The animals body contain approximately 0.2% of Na. Distribution of Na in various parts of animal body is 25% of total in bones, 22% in hide, 16% in muscles, 20% in blood and lymph and 17% in other tissue (McDowell, 1995).

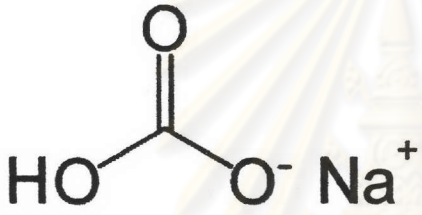
The Na content of the body of adult cattle (BW = 500 kg) has been estimated to be about 1.3 g/kg BW (ARC, 1980). Roughly, 45% of the body store of Na is found in the extracellular fluid (ECF), while only 10% is found within intracellular fluid (ICF). Typical Na concentrations in ECF are 142-146 mmol/l and 142-145 mmol/l; in plasma and interstitial fluid, respectively, while Na concentration in ICF is rather low; i.e. 12-14 mmol/l (Houpt, 1993; Harper et al., 1997; Rose, 2001).

## Dietary sources of sodium

Most plant and plant products contain relatively small amounts of Na in comparison to animal products. Most grains and vegetable protein concentrates are fairly low in Na, containing 0.01 to 0.06%. Forages usually range from 0.007 to 0.12% Na (McDowell, 1995). Sodium in the animal diet is mainly supplied through feeds of animal origin and mineral supplements. Sodium has been supplied to ruminant in the various forms; such as NaCl, $NaHCO_3$ and $Na_2CO_3$ (Clive et al., 2000, West et al., 1992; Belibasakis and Triantos, 1991). The soluble in water of NaCl, $NaHCO_3$ and $Na_2CO_3$ are 36.0, 9.6, 30.0 g/100 ml (at 20°C). Sodium salt from these sources is readily soluble and rapidly absorbed by the gastro-intestinal tract (McDowell, 1995; Wikipedia, 2006).

Sodium chloride (NaCl)

Sodium bicarbonate ($NaHCO_3$)



Sodium carbonate ($Na_2CO_3$)

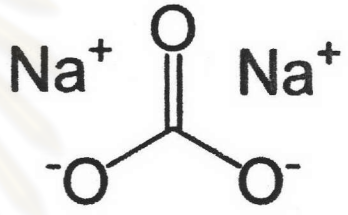


Figure 1 Chemical structures of NaCl, $NaHCO_3$, and $Na_2CO_3$ (Wikipedia, 2006)

The atomic weight of NaCl, $NaHCO_3$ and $Na_2CO_3$ is 58.44, 84.01, and 105.99, respectively. The amount of Na in NaCl, $NaHCO_3$ and $Na_2CO_3$ is approximately 39%, 27%, and 43%, respectively. The chemical structures of NaCl, $NaHCO_3$ and $Na_2CO_3$ were shown in Figure 1 (Wikipedia, 2006).

**Function of sodium**

Sodium and chloride along with potassium function to maintain osmotic pressure, regulate acid-base equilibrium. These electrolytes in body fluids are specifically involved at the cellular level in water metabolism, nutrient uptake (acid-base, glucose and for amino acid transport) and transmission of nerve impulses. Sodium has a major role in the

transmission of nerve impulses, and in maintaining proper muscle and heart contractions (McDowell, 1995).

Sodium and Chloride considerably control the passage of nutrients into the cells and transport waste products out. Sodium ion must be presented in the lumen of the small intestine and acts as media for sugars and amino acids absorption. Insufficient Na lowers the utilization of digested protein and energy. Water absorption in the intestine may also by closely link to Na ion transport (McDowell, 1995).

Sodium is the principal cation in ECF. Therefore, it is the major determinant of the osmotic pressure of ECF and is of great importance in maintaining water balance in animals. The typical intra- and extracellular distribution of Na and K is regulated by Na/K-pumps, which are vital for the generation (and maintenance) of membrane potentials. Furthermore, Na also plays an important role in the regulation of acid-base balance in body fluids, although this is secondary to its role in maintaining osmotic pressure of ECF. Apart from the physiological functions of Na mentioned above, Na is also required for the absorption of glucose and galactose, most of the peptides and amino acids and bile acids from the gastro-intestinal tract (Hays and Swenson, 1993).

**Sodium absorption**

Sodium is readily absorbed, principally from the upper small intestine. The transport of Na across intestinal epithelium appears to be dependent on a system of pumps and passive leaks located in cell membrane (McDowell, 1995). Sodium uptake from the gut lumen is achieved by coupling to glucose, amino acid, and volatile fatty acid uptake via cotransporter and also by exchange with hydrogen ions ($H^+$) via an Na-H antiporter, intracellular $H^+$ being generated by carbonic anhydrase in the enterocytes of the gut mucosa (Henry, 1995; Harper et al., 1997). In animals approximately 80% of the Na entering the gastrointestinal tract arises from internal secretions such as saliva, gastric fluids, bile, and pancreatic juice (NRC, 1980).

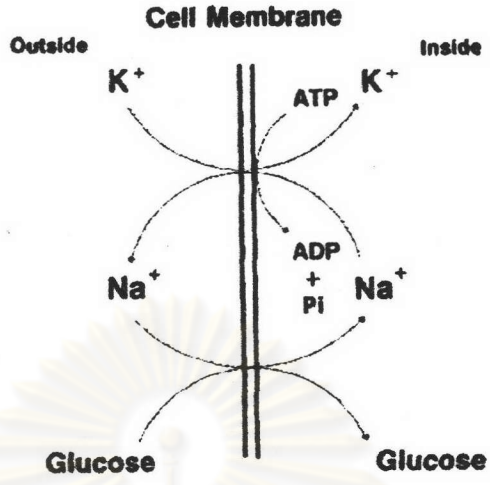



Figure 2 The $Na^+/K^+$ ATP-dependant pump mechanism in the entry of glucose to cells (Block, 1994)

Sodium is highly labile in the animal body. At the cellular level, the continuous exchange of $Na^+$ and $K^+$ via ATP-dependent $Na^+$-$K^+$ pump provides the basis for glucose (Figure 1) and amino acid uptake (by cotransport), maintaining high intracellular K concentrations but requiring about 50% of the cell's maintenance need for energy (Milligan and Summers, 1986). Sodium transport across membranes is also achieved by a wide variety of complementary mechanisms: by Na-H exchanges; by electroneutral Na-K-2Cl cotransporters; by Na-Cl and Na-magnesium (Mg) exchangers; and by voltage-gated $Na^+$ channels.

**Mechanism of sodium absorption**

The first pathway of sodium absorption is via sodium co-transport proteins (Figure 3a), as previously discussed. This secondary active transport pathway is not only the mechanism for glucose and amino acid absorption but also a major of sodium absorption. The second sodium absorption mechanism is via the Na/H exchanger (Figure 2 and 3a), which was mentioned previously as an example of an ion exchanger or antiport (Cunningham, 2002).

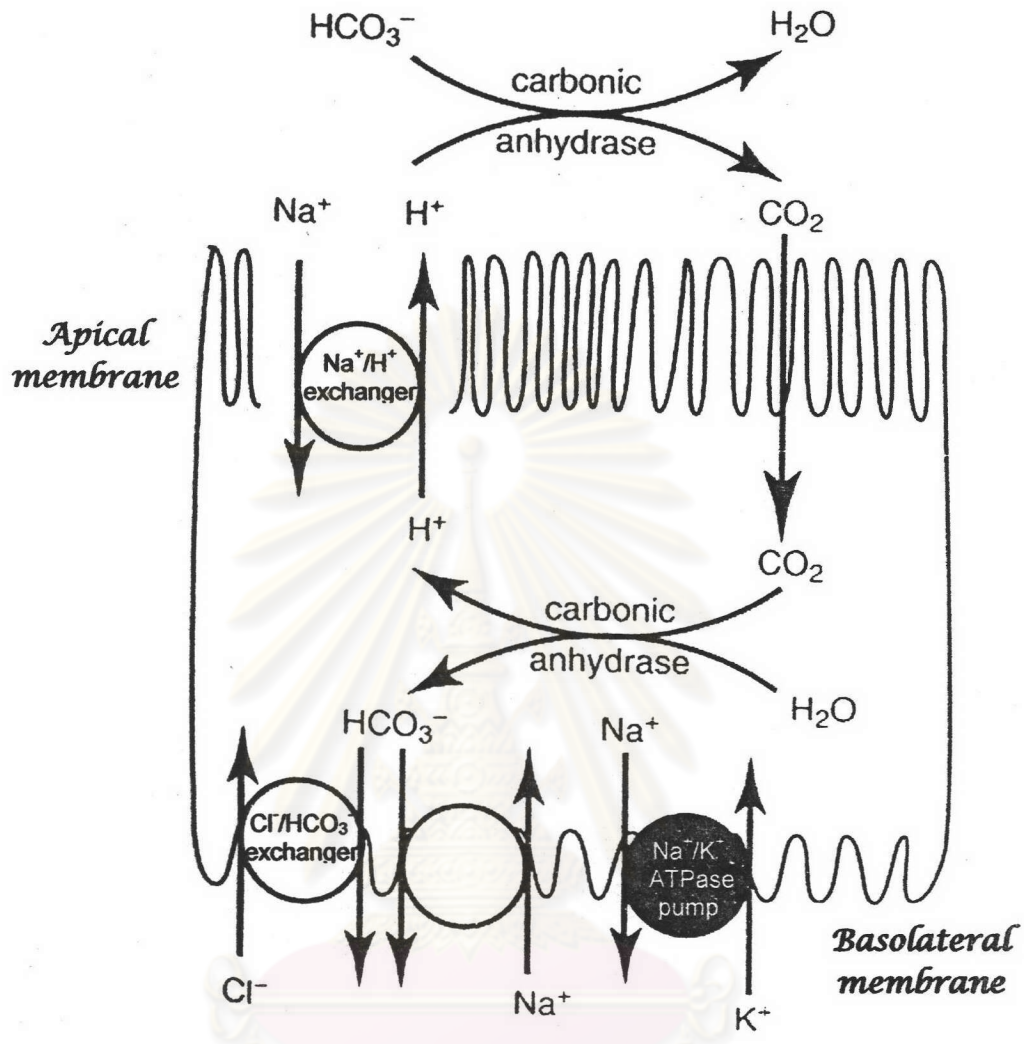



Figure 3 The $Na^+/H^+$ exchanger, $Cl^-/HCO_3^-$ exchanger, and $Na^+/K^+$ ATPase pump (Cunningham, 2002)

Via this mechanism, intracellular $H^+$ is exchanged for luminal $Na^+$ across the apical membrane. The $H^+$ for this exchange is formed by the action of carbonic anhydrase, which generates $HCO_3^-$ as well as $H^+$. As $H^+$ is exchanged for $Na^+$ (at $Na^+/H^+$ exchanger), $HCO_3^-$ concentrations build up in the cell. The resulting transcellular $HCO_3^-$ gradient drives the action of the $Cl^-/HCO^-$ exchanger, which results in the exchange of intracellular $HCO_3^-$ for luminal $Cl^-$. Because of the close connection between $Na^+$ and $Cl^-$ absorption by this pathway, this transport mechanism is often called coupled sodium chloride transport, as illustrated in Figure 3b (Cunningham, 2002).

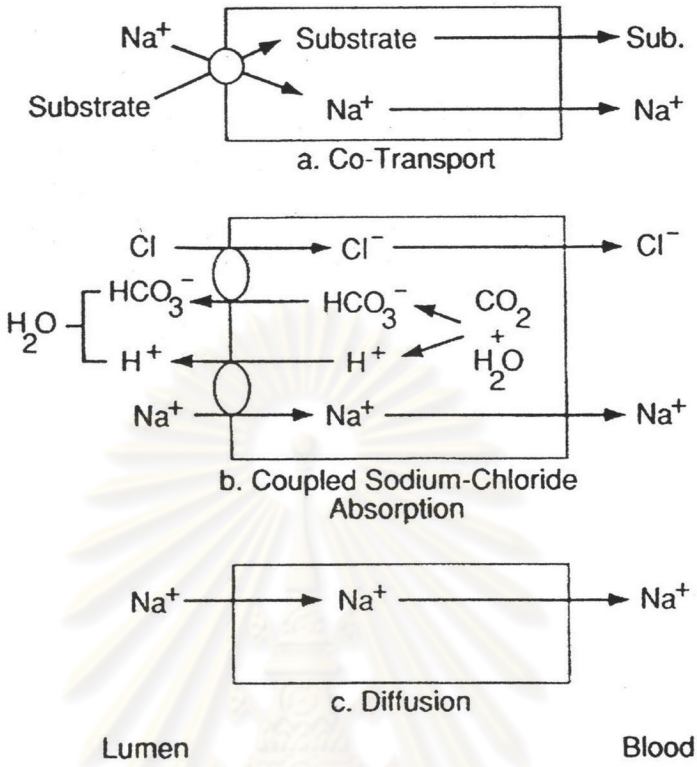



Figure 4 Three mechanisms of sodium absorption (Cunningham, 2002)

(a) Sodium co-transport with organic molecules is a major mean of sodium uptake during active digestion and absorption.

(b) Chloride-coupled sodium absorption is also and important means of sodium absorption and requires the action membrane of bicarbonate-chloride and sodium-hydrogen exchange mechanisms.

(c) Simple diffusion of sodium across the apical membrane may occur because of the large favorable concentration gradient, but it is relatively minor means of sodium absorption.

One must appreciate, however, that it is only the intracellular balance of $H^+$ and $HCO_3^-$ that couples the two exchange pathways. There are instances in which the intracellular pH is such that $Na^+/H^+$ exchange occurs without $Cl^-/HCO_3^-$ exchange, and vice versa. Coupled sodium chloride absorption is usually most active in the ileum and colon, where the sodium concentration in the gut is usually relatively low compared with that in the duodenum and jejunum. As usual, sodium entering the enterocytes is

transported across the basolateral membrane to the lateral spaces via the action of the $Na^+/K^+$ATPase pump. Chloride, however, remains in the enterocyte until its concentration is high enough to promote the diffusion of chloride through special channels in the basolateral membranes. The rate of absorption of $Na^+$ and $Cl^-$ by the coupled mechanism appears to depend on the permeability of the $Cl^-$ channels; when the permeability is high, $Cl^-$ passes rapidly out of the enterocyte, allowing continued $Cl^-$ absorption. Conversely, when $Cl^-$ channel is relative closed, the intracellular $Cl^-$ concentration rises, diminishing $Cl^-$ absorption by the creation of an unfavorable concentration gradient.

The third mechanism of $Na^+$ absorption is via simple diffusion through ion channels in the apical membrane (Figure 3c). The large electrochemical gradient that can exist for $Na^+$ across the enterocyte apical membrane allows direct, uncoupled movement of $Na^+$ across the membrane when the ion channels are open. Although some $Na^+$ absorption probably occurs by this mechanism, its overall importance in body $Na^+$ homeostasis is probably not large (Cunningham, 2002).

**Secretion and excretion**

Much of the Na that enters the gastrointestinal tract from saliva, particularly in ruminants, which daily secrete about 0.31 $kg^{-1}$ live weight (LW) containing Na (150 mmol/l) as the major cation. The rumen can contain 50% of the available body Na, providing an important reserve (Bell, 1995), but on K-rich diet is displaced by K.

In Na deficiency, salivary Na is replaced on a molar basis by K to conserve Na, in an adaptation modulated by aldosterone, a hormone secreted by the adrenal gland. All three elements (Na, Cl, and K) are lost via skin secretions, but there are major differences between species. In non-ruminants, Na is the major cation in sweat, but the high loss of Na balance the loss of water and provides a defense against hypernatraemia. Sodium concentrations in milk (normally 17 mmol/l) decline only slightly during Na depletion and there are no compensatory changes in milk K to compare with those seen in saliva.

Sodium is excreted principally in the urine as salt, with smaller amounts lost in feces and perspiration. Loss of salt through perspiration is a major excretion route for some species. Considerable quantities of Na may also be lost via secretion in milk (McDowell, 1995). Fecal excretion is not an integral part of Na or chlorine regulation in ruminants; thus, measurement of apparent absorption yields values very similar to those representing true absorption. Urinary Na excretion increases with increasing Na intake (Morris, 1980).

Sodium balance in ruminants may be affected by a loss of saliva. In cattle, where 50 to 100 liters of alkaline parotid gland saliva may be produced per day, most Na is salvaged by its reabsorption from the digestive tract. However, animals slobber considerably during elevated environment temperature, losing NaCl in salivary secretions (McDowell, 1995).

**Regulation of body content**

The regulation of Na balance is closely related to that of water balance. As mentioned previously, Na is the main cation of the ECF and together with its associated anions it accounts for more than 90% of the osmotic pressure of the ECF. The Na concentration of the ECF is the primary subject of regulation. An increase of the Na concentration of the ECF results in an increase of the water consumption and triggers the release of anti-diuretic hormone (ADH), which increases the re-absorption of water from the tubular fluid of the nephron. These two actions will counteract the initial increase of the Na concentration of the ECF. However, at this stage the total amount of Na and volume of the ECF are raised. This rise of ECF volume includes a rise of blood pressure, which enhances glomerular filtration rate (GFR).

The excess of Na and water are excreted by the kidneys, thereby restoring ECF volume to its normal level. Furthermore, at increased rates of filtered Na, tubular re-absorption of Na is less efficient causing increased rates of Na excretion by the kidney.

A deficit of Na in the ECF hampers the release of ADH, which causes a rapid excretion of excess water so as to increase the Na concentration of the ECF. When blood pressure becomes depressed, GFR is lowered and less Na is flitered out from the

blood. Furthermore, secondary to the stimulation of the renin-angiotensin system (restore blood pressure), the release of aldosterone is stimulated, which enhances the tubular re-absorption of Na. Aldosterone is only important during Na deficiency. The combination of these mechanisms can cause a drop in urinary Na excretion so that virtually no Na is excreted by the kidneys during prolonged Na deficiency. Furthermore, it should be stressed that apart from the kidneys, especially the large intestine play a very important role in reducing the faecal Na losses to almost zero, during Na deficiency (Renkema et al., 1962).

When Na intake is inadequate, the body has a remarkable capacity to conserve it, excreting extremely low levels in the urine. High Na intake triggers greater excretion of Na by the kidney, and water needs increase (McDowell, 1995). Regulation of Na body concentrations is controlled by hormones acting to maintain a constant Na:K ratio in the extracellular fluid. Aldosterone, secreted from the adrenal cortex, regulates the reabsorption of Na from the proximal tubule of the kidney. The antidiuretic hormone of the posterior pituitary is responsive to changes in osmotic pressure of the extracellular fluid; both hormones maintain a constant Na:K ratio. (NRC, 1980; McDowell, 1995).

The rennin-angiotensin-aldosterone (RAA) system is known to adjust distal tubular Na reabsorption in the kidney, and hence excretion, to balance the Na needs of the body. Renin is a proteolytic enzyme that breaks the leucine-leucine bond of angiotensinogen. The rate of production of rennin substrate appears to be substantially stimulated during Na deficiency. Angiotensin II appears to be the major factor for secretion and release of aldosterone by the adrenal cortex. Feedback control cuts off further stimulus to the RAA mechanism when the increased reabsorption of Na restores Na homeostasis (McDowell, 1995).

Mediation is achieved by active transport and changes in membrane permeability. Sodium reabsorption in the distal tubule can be impaired by excess K but enhance and by aldosterone, so that urinary losses become negligible when Na intakes are low. Regulation is highly complex and beyond the scope of this chapter (Harper et al., 1997).

**Requirement for sodium**

The Na requirement of dairy cattle has been investigated in a wide range of conditions; in tropical and temperate climates, on good and heavily fertilized pastures and poor natural grazing and at low and high rates of growth and of milk production. All of these variables may influence requirements, so that single minimum dietary intakes are limited use. For example requirements increase with temperature and humidity. In unacclimatized cattle, the dribbling of saliva can result in daily losses of up to 1.4 g Na and 0.9 g Cl 100 $kg^{-1}$LW (Aitken, 1976). Substantially higher Na requirements of 0.18%DM have been given for lactating dairy cows (NRC, 1989), but factorial estimates by ARC (1980) gave a maximum need which was 30% lower (Table 1). Although cow's milk averages about 0.5 g Na, 1.2 g Cl and 1.5 g K $l^{-1}$, the concentration of Na required in the diet increase only slightly with a rise in milk yield, provide that there is an increase in feed consumption to meet demands for more energy and protein.

Table 1 Estimates of the minimum dietary Na concentration required by cattle at the given dry matter intakes

| | Live weight | Growth or | DMI | Gross Na requirement | |
|---|---|---|---|---|---|
| | (kg) | product | (kg $day^{-1}$) | g $day^{-1}$ | g $kg^{-1}$ DM |
| Cow (milking) | 600 | 10 kg $day^{-1}$ | 9.4 | 10.3 | 1.1 |
| | | 20 kg $day^{-1}$ | 14.0 | 16.8 | 1.2 |
| | | 30 kg $day^{-1}$ | 18.8 | 22.6 | 1.2 |

Reference: ARC, 1980

The new dairy cattle NRC (2001) estimates the requirements for absorbed mineral based on needs for maintenance and production (growth, lactation, pregnancy). The requirement of Na for lactating cow is 0.22%. Beede (1994) shows a greater need for Na than suggested in the NRC update, especially under heat stress conditions. As a result of the Florida Studies, they recommend the total diet dry matter contain 0.3 to 0.4% Na under normal Florida conditions and 0.5 to 0.6% under heat stress conditions.

**Deficiency of sodium**

Sodium deficiency is more likely to occur in cattle grazing tropical pasture species, as these plants generally accumulate less Na than do temperate species (Morris, 1980; McDowell, 1995). Natural forages low in Na has been reported in numerous tropical countries throughout the world (McDowell, 1985; 1995). The first sign of Na deficiency in ruminant is a pica or craving for salt, manifested by keen licking of wood, soil, or sweat of other animals.

Other signs are weight loss, in appetence, increased water consumption, reduced milk yield not accompanied by a decrease in milk Na concentration, and decreased Na concentration in feces, urine and saliva in ruminant (Underwood, 1981). Na deficiency will result in decreased milk production (Fettman et al., 1984; Link and Olson, 1985)

**Toxicity of sodium**

Sodium toxicosis is characterized by increases in water consumption, anorexia, weight loss, edema, nervousness, paralysis, and a variety of signs that are dependent on the animal species involved. The maximum tolerable levels of NaCl and Na in animal diet are shown in Table 2.

Table 2 Maximum tolerable levels of NaCl and Na in animal diets

| | Na Cl in total diet (%) | Na in total diet (%) |
|---|---|---|
| Cattle : Lactating | 4 | 1.57 |
| Non-lactating | 9 | 3.54 |

Reference: Underwood, 1981

**Determination of the absorption of minerals in various sections of the gastrointestinal tract using markers**

The inert label (marker) method greatly simplifies determination of the apparent absorption, by eliminating the need for a full collection of excreta. It is based on the use

of poorly assimilatable substances such as chromic oxide ($Cr_2O_3$), polyethylene glycol, radioactive cesium and yttrium, etc. The magnitude of the absorption is determined from the ratio between the element and label in the feed and in the feces. Calculations are carried out using the formula:

$$\% \text{ apparent absorption} = 100 \times 1 - \frac{\text{Concentration of marker in feces}}{\text{Concentration of marker in feed}}$$

Apparent absorption of Na in feedstuffs measured in cattle range from about 70 to 95% with an average of 85% apparent absorbed. The ARC (1980) estimates absorption to be 91% for Na and 85% for chlorine for cattle. Dairy cows fed fresh herbage have apparent absorption of Na range from 77 to 95% and averaged 85%. Cows fed hay plus oatmeal had apparent Na absorption values of 81% and values for cows fed hay, corn, and beet pulp averaged 69% (Kemp, 1964). Martz et al. (1988) fed dairy heifers either semi-purified (solka floc or corn cobs as the main fiber source) or conventional (corn silage) diets and measured apparent absorption and retention of Na. Apparent Na absorption was 86, 91 and 74% for solka, corn cob, and corn silage diets, respectively. Urinary Na excretion averaged 6.1, 7.8, and 4.4% of intake, respectively.



**Compositions of blood electrolytes**



If blood drawn from the portal vein is analyzed at various stages of digestion, and the data obtained are compared with the corresponding parameters of peripheral blood, information can be obtained on the dynamics of mineral absorption in the stomach and intestine. Serum Na concentrations tend to remain constant regardless of dietary Na concentration; however, serum Cl decrease with decreasing intake and may be a useful indicator of availability.

Table 3 Suggested marginal bands for Na and Cl in serum, urine and diet as a guide to the diagnosis of dietary deprivation (D) or excess (E) of each element for livestock

| | | | Serum (mmol/l) | Urine (mmol/l) | Diet (%) |
|---|---|---|---|---|---|
| Na | Cattle | D | 124-135 | <3 | 0.05-0.10 |
| | | E | 140-150 | 40-60 | 3.00-6.00 |
| | Sheep | D | 140-145 | 1-3 | 0.05–0.10 |
| | | E | 150-160 | 40-60 | 3.00-6.00 |
| Cl | Cattle | D | 70-85 | 2-5 | 0.10-0.30 |
| | | E | >150 | >100 | >8.00 |
| | Sheep | D | 70-85 | 2-5 | 0.10-0.30 |
| | | E | >150 | >100 | >5 |

Reference: Fettman et al. (1984)

**Effects of Na supplementation**

Clive et al. (2000) reported about Na dietary was increased from 0.1% to either 0.6% or 1.1% DM (by adding NaCl 0, 200, and 400 g/d), milk yield was increased (8.9, 8.9, and 10.1 kg/d, respectively) when the Na supplement was include at the high rate ($P$<0.01). But milk composition such as fat and protein; the difference was not significant. Blood electrolyte such as plasma Na tended to decrease. Fecal Na concentration tended to increase but fecal K concentration was reduced for cows receiving high level of Na supplement ($P$<0.01). Fecal K was decreased (0.94%, 0.61% and 0.69%, respectively) when the Na supplementation was increased (0.1%, 0.6% and 1.1%, respectively).

In the study of Tucker and Hogue (1990) the Na content of the TMR diet was increased from 0.20% to 0.67% DM (as NaCl; from 0.38% to 1.55%). DMI was decreased ($P$<0.001) when the Na supplement was included in TMR diet. MY and MC were not different. Plasma Na and plasma Cl was not significant difference, but plasma K was lower for the diets with supplemental Na than for basal diet ($P$<0.05). Urine mineral excretion reflected dietary mineral concentration, urinary Na:creatinine ratio was increased ($P$<0.001), but urinary K:creatinine ratio was decreased ($P$<0.001).

West et al. (1992) reported that the Na content in the diet was increased from 0.53% to 0.87% (as $NaHCO_3$; from 0.98% to 1.9%) increased linearly significant ($P<0.05$) in DMI, DMI/%BW, urinary K:creatinine and urinary Cl:creatinine ratio. DMI and DMI/%BW were improved 4% and 4.5% respectively. Urinary K:creatinine ratio increased from 2.34 to 2.67, while urinary Cl:creatinine ratio increased from 0.08 to 0.27. There were no effect on BW, MY, 3.5%FCM, plasma electrolyte, and urinary Na:creatinine ratio.

Vicini et al. (1988) found that the effects of $NaHCO_3$ supplementation on lactational performance, were significantly increased DMI ($P<0.05$). DMI were improved 12.1%. MY, 4%FCM, and MC were not affected by increasing Na supplement from 0.2% to 0.47% (as 0% to 1% $NaHCO_3$) in the diet.

West et al. (1987) observed that the Na content in the diet increase from 0.2% to 0.4% by 0 to 1.5% $NaHCO_3$ supplementation did not affect DMI, MY, MC, DDM, DADF, DNDF, rumen pH and the concentration of VFA.

Roger et al. (1985) found the result of their research confirm that $NaHCO_3$ improved DMI and MY when cows were fed 1.2% $NaHCO_3$ supplementation diet (Na in the diet increased from 0.2% to 0.52%). Electrolytes plasma and urinary were various effects from $NaHCO_3$ supplement; urinary K and Cl excretion, plasma Na and Cl did not significantly difference. Urinary Na:cratinine ratio was increased ($P<0.001$) by increasing Na supplementation. Plasma K and rumen pH decreased ($P<0.05$) approximately 6.3% and 2.2%. But the concentration of VFA and digestibility of DM, ADF were unaffected by Na supplementation in the diet.

Kilmer and Muller (1980) studied on the effects of Na supplementation in the diet, when used different Na form; 0.48% NaCl compare with 0.72% $NaHCO_3$. There were affected on DMI, DMI/%BW, MY, and 4%FCM, when added $NaHCO_3$ to the diet. DMI, DMI/%BW, MY, and 4%FCM were greater for cows fed $NaHCO_3$ (21.4, 3.51, 32.5, and 31.9, respectively) than for cows fed NaCl (18.9, 3.04, 29.7, and 28.8, respectively). The concentrate of milk fat, protein, VFA in rumen fluid, rumen pH, and plasma Na were unaffected by $NaHCO_3$ supplementation.

Sanchez and Beede (1997) observed that the effects of Na content in TMR diet, which increased from 0.32% to 0.64%; i.e. from 0.15% NaCl to 0.15% NaCl plus 1% $NaHCO_3$. Cows that were fed the $NaHCO_3$ were unaffected on DMI, MY, 3.5% FCM, MF, MP, milk Na, milk Cl, and plasma electrolytes. With the exception of milk K had a significant impact on lactational performance ($P<0.01$). It was increased from 35.52 to 36.60 mmol/l, when Na dietary increased.

Belibasakis and Triantos (1991) studied on the effects of Na (as $Na_2CO_3$) on MY, MC, plasma metabolites Na and K in early lactation. Diets were TMR containing either 0% or 0.42% $Na_2CO_3$ (as fed); dietary Na are 0.13%, 0.46%DM, respectively. DMI, MY, milk protein yield, the concentrate of milk protein, lactose and SNF were not significantly affected. Compared with the control diet, the $Na_2CO_3$ treatment increased in the milk compositions. The concentration of fat was increased ($P<0.01$) from 3.53% to 3.98%, 4%FCM, the fat yield and the concentration of TS were increased ($P<0.05$) from 28.2 kg/d, 1.07% and 12.04% to 30.9 kg/d and 1.23%, 12.47%, respectively. No significant differences were observed in plasma concentrations of Na, or K when $Na_2CO_3$ was added to diets for early lactating cows.

Several studies on the effects of Na supplementation from the difference sources in diets indicated that high level of Na could affect DMI and milk yield, but milk compositions and electrolytes of plasma, urinal, and fecal were variable. No study on the high level of Na supplementation from $Na_2CO_3$ has been done. Therefore, the objectives of this study were to investigate the effects of high level of Na supplementation from $Na_2CO_3$ on DMI, nutrient digestibility, milk yield, milk compositions, and electrolytes of plasma, urinal and fecal.