Razi University
Faculty of Agriculture
Department of Animal Science

M. Sc. Thesis

Effects of variuos levels of dietary electrolyte balance and crude protein on laying hens' performance, blood physiological and biochemical parameters and egg quality characteristic

Supervisor:
Dr.Mehran Torki

By:
Rohollah Ghasemi

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Abstract

The purpose of this project is to evaluate the nature of the interaction between acid-base balance and dietary protein level on laying hens performance. In this trial, two electrolyte balances and two protein levels were tested on LSL laying hens at the first stage of oviposition to investigate its effect on productive performance, egg traits and blood parameters.

The total number of 144 LSL- lohman laying hens. After production peak was randomly distributed between 24 cages. A 2*2 factorial arrangement including dietary crude protein level (13.87 and 15.42%) and electrolyte balance (165 and 250) was assigned laying hens in a completely randomized design with 6 replicates per each treatment. The composition of four iso-caloric experimental diets formulated to meet the hens.

The production performance of hens including egg production, egg weight, daily feed intake, feed conversion ratio and egg mass were measured during 42 days. Egg quality traits and blood samples hens per treatment were obtained at 35 day to determine metabolites in the serum of the hens using appropriate commercial laboratory kits. The results indicated that different levels dietary electrolyte balance no effect significantly on performance factors ($P>0.05$) but levels crud protein affect on egg mass ($P<0.05$) and egg weight ($P<0.01$). There was no significant effect of CP and DEB on egg quality characteristic except for egg shell thickness and weight. No interaction between dietary CP and DEB on egg quality trait was observed. Serum levels of Na^{+}, K^{+}, Ca^{2+} and Cl^{-} were not affected by CP ($P>0.05$). Serum levels of $K^{+}$and Ca^{2+} were not affected by DEB ($P>0.05$); however, higher level of DEB (250 mEq/kg) increased serum level of Na^{+} ($P=0.03$) and decreased serum level of CL ($P=0.04$). No interaction between dietary CP and DEB on blood electrolytes was observed. Dietary treatment did not have significant effect on blood biochemical parameters ($P>0.05$) except glucose ($P=0.02$) which was higher in hens fed on low protein diet (CP=13.87 %) comparing to control diet (CP=15.42 %). Dietary treatment did not have significant effect on lymphocyte counts, except for heterophils which was significantly higher in hens fed on low protein diet comparing with control diet ($P=0.04$). In addition feeding low protein diet causes increased the stress indicator (ratio of heterophile to lymphocyte). There was no significant interaction between DEB and CP on measured feces ash and pH ($P>0.05$). However, feeding low protein diet and low DEB (165 mEq/kg) decreased feces moisture comparing with control diet and diet with high DEB, respectively ($P<0.05$).

Key Words: Dietary electrolyte balance, crude protein, Production performance, Blood parameters.

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1-1. Introduction

Meeting the nutritional requirements for growing birds constitutes the majority of costs associated with poultry production (May *et al*., 1998), accounting for around 75 percent of the expense (Nakaue and Arscott, 1991), and certainly is becoming an issue of even greater significance as the prices of feed ingredients continue to rise. A large portion of that cost involves meeting the protein requirement of the birds (Corzo *et al.,* 2004; Firman and Boling, 1998; Eits *et al.,* 2005). By reducing the level of crude protein in the diet, it is possible to achieve significant cost savings. Firman and Boling (1998) reported that it is possible to save five dollars per ton of feed by reducing the protein level in the diet of turkeys by one percent. In addition to reducing feed costs, the ability to lower crude protein in the diet can result in decreased nitrogen excretion (Kidd *et al*., 1996; Ferguson *et al*., 1998; Nahm, 2002; Namroud *et al*., 2008), improved ability to cope with heat stress, and allow for the use of a greater variety of feedstuffs (Kidd *et al.,* 1996), which can be valuable in itself as a method to increase flexibility in the choice of locally available feedstuffs, potentially decreasing transportation costs.

Developing feeding programs that utilize concepts such as ideal protein, formulation programs that calculate the ingredient combinations that will closely meet the birds' nutritional requirements at the least possible cost, digestible amino acid values, and crystalline amino acid supplementation has allowed the poultry industry to reduce dietary crude protein to decrease excess amounts of amino acids and the cost of rations (Kidd *et al.*, 1996). However, the lowest level to which crude protein can be reduced with amino acid supplementation in broiler diets without reducing bird performance is still controversial, and additional research on the subject could yield significantly greater cost savings in the future.

Amino acid metabolism influences, and is influenced by, the acid-base balance of an animal (Murakami *et al.*, 2003). Interactions, in terms of biochemistry and physiology, are established clearly. From a practical perspective, however, both qualitative and quantitative considerations remain unclear. As we learn more about the impact of acid-base balance on animal production, it becomes more than a peripheral subject, and it requires considerably more attention. The fact that acid-base balance can influence growth and appetite (Patience *et al*., 1987), structural soundness (Sauveur, 1984), the response to thermal stress in poultry (Teeter *et al*., 1985) and the incidence of milk fever in dairy cattle **(**Block**,** 1984) as well as the metabolism of certain nutrients such as amino acids minerals (Lutz, 1984) and vitamins demonstrates clearly the need for a more thorough understanding of this subject.

The dietary electrolyte balance (dEB, dEB=$Na^+ + K^+ - Cl^-$, mEq/100 g) depends on the ratio among sodium, potassium and chloride. Ingredients commonly used in poultry feed formulation, excluded fish meal, generally present low sodium content but high potassium and chloride level. Such diets result inadequate in the mineral content and electrolyte balance, and they need adjustments to permit the animals to achieve good productive performance.

Several studies are carried out on the dEB effect on productive and physiological response in meat chicken and turkey (Johnson and Karunajeeva, 1985; Oviedo-Rondon *et al*., 2001).

In laying hens the dietary electrolyte balance (changing in the sodium, potassium and chloride ratio in the diet) can affect the body weight and the feed conversion ratio, the oviposition percentage (Gongruttananum *et al*., 1999; Kuchinski *et al*., 1999) and the quality of the eggs (Keshavarz and Austic, 1990; Roberts and Balnave, 1992; Leeson and Caston, 1997). Otherwise the results obtained are sometimes controversial because of not adopting homogeneous experimental conditions. In fact, resulting from research work available, the salts tested and the quantity of electrolytes employed to adjust the dEB value are different (Gongruttananun *et al*., 1999), the diet composition, and the rearing thermal and hygrometric conditions the age of the hen and the stage of oviposition (Leeson and Caston, 1997; Gongruttananun *et al*., 1999).

The purpose of this project is to evaluate the nature of the interaction between acid-base balance and dietary protein level on laying hens performance, biochemical parameters blood and egg quality traits. In this trial, two electrolyte balances and two protein levels were tested on LSL laying hens at the first stage of oviposition.

2-1. Acids and bases

An acid is any substance capable of donating a proton (H^+) and a base is any substance capable of accepting a proton. Movement of protons between acids and bases is reversible:

$HA \rightarrow \quad H^+ \quad + \quad A^-$

acid proton base

A^- is the conjugate base of the acid HA. The ease with which HA gives up its proton indicates the relative strengths of HA as an acid and A^- as a base. Conjugate pairs found in biological systems include H_2CO_3 / HCO_3^-, $H_2PO_4^-$ / HPO_4^{--}, NH_4^+ / NH_3 and various proteins including hemoglobin.

2-2. Regulation of blood acid-base balance

Efficient functioning of acid-base homeostatic mechanisms is an essential feature for optimal growth and production. The composition of blood is maintained within narrow limits to provide a stable environment for a complex inter play of biochemical and physiological events. For example, blood pH seldom moves outside the range 7.0-7.6 and, in fact, uncompensated shifts towards either extreme can be life threatening.

2-3. Acid-base homeostasis

Acid-base homeostasis refers to the tendency of an animal to maintain a constant intracellular and extracellular proton (H^+) concentration. When successful, the external balance of protons will be zero, because formation will be in equilibrium with excretion. Failure to maintain a relatively constant internal pH has a catastrophic effect on the animal. The size and charge of the hydronium ion (protons do not exist in free form, but are hydrated to form H_30^+, $H_5O_2^+$, etc.) allows it to interact intimately with other molecules, especially proteins, to alter their configuration. This results in structural changes which, among other things, alter the catalytic activity of enzymes, the nature of transport processes, the contractile properties of muscle and the oxygenation of tissues (Garrard *et al.*, 1985). Proton concentration generally is expressed in terms of pH; although widespread, the use of the pH scale requires caution due in part to the logarithmic scale employed (Stewart, 1978). This scale masks two critical features of proton concentration: the very small concentration of protons relative to other circulating ions and the magnitude of changes in H^+ concentration (e.g., a 1-unit change in pH represents a 10-fold change in H^+concentration). Consequently, H^+ concentration sometimes is expressed in nmoles per liter, rather than pH. Acid-base balance is not defined solely in terms of extracellular fluid pH, but includes evaluation of PCO_2, HCO_3^- and base excess. In combination, these parameters help to define not only the acid-base status of the animal, but also the nature of any deviation from normal. Both pH and $pC0_2$ can be measured directly, whereas $HC0_3^-$ and base excess are calculated according to the Henderson-Hasselbach equation and specific nomograms (Siggaard-Andersen, 1963), respectively. Typical arterial and venous blood acid-base parameters for swine are summarized in table 2-1.

Table 2-1: Typical acid-base balance parameters in porcine whole blood

parameter	Arterial	Venous
pH	7.48	7.41
H^+,nM	33	39
Pco_2,mmHg	44	54
HCO_3^-,mM	30	33

2-4. Evaluation of acid-base status

Although acid-base status usually is determined by analysis of the extracellular fluids, primarily arterial blood, this approach fails to describe the dynamic response of the animal to an acid-base challenge. Although pH, $pC0_2$, $HC0_3^-$ and base excess identify the relative contribution of the respiratory and metabolic components, it often is necessary to study urinary excretory patterns in order to learn how the animal is responding to a given dietary, environmental or pathological circumstance. Indeed, blood acid-base parameters will change only when respiratory and renal compensation are incapable of eliminating the total acid or alkaline load. For example, even though blood analysis revealed no acid-base disturbance, (Lutz, 1984) observed a typical renal response to an acid load. Blood assays identify acid-base status but provide no information on the physiological processes activated to achieve it. Because the kidney is involved primarily in the removal of fixed acid, monitoring urine composition provides useful insight into the animal's response to an acid or alkaline challenge. A valuable measurement is that of net acid excretion (NAE), which is defined as the sum of ammonium plus titratable acid (TA) minus HCO_3^- (Chan, 1981). Traditionally, NAE has been considered to be a useful estimate of endogenous acid production, equivalent to urinary sulfate plus organic acids plus 1.8 times dietary P (Relman *et al*., 1961). If an animal is in acid-base equilibrium, endogenous acid production should equal NAE. Lennon *et al*., (1966) demonstrated that NAE was correlated closely with the sum of urinary sulfate and salts of organic acids minus the dietary undetermined anion (dUA) plus fecal undetermined anion. In other words, the net acid load is generated primarily by the oxidation of sulfur amino acids (SAA), the formation of organic acids and the contribution of the diet in terms of acid or base.

Table 2-2: Examples of acid or base generating processes occurring in normal metabolism

1	Complete oxidation of carbohydrate or neutral lipids: Glucose + $6O_2 \rightarrow 6CO_2 + 6H_20$
2	Complete oxidation of sulfur amino acids: 2Methionine + $150_2 \rightarrow$Urea + $9Co_2 + 7H_20 + 4H^+ + SO_4^{--}$
3	Metabolism of salts of organic acids or bases: $2NH_4C1 + CO_2 \rightarrow$ Urea + $H_20 + 2H^+ + 2Cl^-$ $HCO_3^- \rightarrow OH^- + CO_2$
4	Oxidation of neutd lipids to organic acids (e.g.,ketosis): Triglyceride + $0_2 \rightarrow$ Acetoacetate+H^++ H_20
5	Oxidation of neutml carbohydrates to organic acids (e.g, lactic acidosis): Glucose$\rightarrow$ Lactate + $2H^+$
6	Bone formation: Ca^{2+}+ 4.8[HPO_4^{--}] + 1.2[$H_2pO_4^-$] + 2H20 $\rightarrow$Hydroxyapatite + 9.2 $^+$

2-5. Sources of acid and base

Acid is generated by normal metabolic processes; certain pathological states may increase its formation and (or) accumulation. The acid formed is of two types, volatile and fixed. Complete oxidation of carbohydrates and fat yields $C0_2$ and water; CO_2 sometimes is called an acid because upon reaction with water it forms carbonic acid (Table 2-2, Equation 1). The conversion of $C0_2$ and H_2O to H_2C0_3 is catalyzed by the widely distributed enzyme carbonic-anhydrase (Swenson, 1984). Provided that respiratory function is not impaired, CO_2 will not accumulate and acid will not accumulate. However, this is not always the case, even under well-managed conditions. For example, as in poultry, respiratory function is compromised during heat stress because thermal panting is required to assist in heat dissipation. Resulting hyperventilation induces a respiratory alkalosis as a consequence of depleted CO_2 reserves (Richards, 1970). This circumstance

provides an interesting example of the conflict between acid-base and other homeostatic mechanisms. It also demonstrates that discussion of acid-base balance must consider other aspects of the physical environment. Although the lungs function to remove H_2C0_3, they cannot remove fixed acids; this is the responsibility of the kidney. Fixed acids, such as sulfuric and phosphoric acid, are generated by a number of metabolic processes (Table 2), including SAA catabolism, incomplete oxidation of organic acids derived from neutral carbohydrate and fat, phospholipid metabolism (Lennon *et al*., 1966) and the deposition of hydroxyl-apatite in bone in the rapidly growing infant. Quantitatively, H_2C0_3 represents the largest source of acid in mammalian species.

2-6. Dietary electrolyte balance and its implications

The primary role of electrolytes lies in the maintenance of body ionic and water balance. Thus the requirements for strong ions that have characteristic effects on body fluids homeostasis cannot be considered individually because it is the overall balance that is important. It is well known that nutrition and environment influenced the bird's acid-base balance. Therefore, the maintenance of this balance can be an important measure to improve the performance of broilers raised under high temperatures and to overcome the harmful effects of respiratory alkalosis resulting from heat stress. Diets formulated with high anion contents (chloride: Cl^-) decrease blood pH and cause acidemia in broilers. Similarly high dietary cation contents (sodium: Na^+, potassium: K^+) increase blood pH and result in alkalemia. Both situations adversely affect the performance of broilers under thermoneutral environments. Cohen and Hurwitz (1974) indicated that the dietary addition of Na^+ (without Cl^-) increased plasma HCO_3^- and pH, while Cl^- addition (without Na^+) reduced plasma HCO_3^- and pH whereas the addition of both as NaCl (salt) caused a little change in plasma HCO_3^- and pH. Similarly, endogenous acid production by diets, particularly protein diets, has been known to influence the acid-base balance. In the view of these findings, it is clear that acid or base intakes, electrolyte balance, the environment, their interactions and implications on the performance of broilers still require further investigations. However, electrolyte availability may be influenced by intestinal and renal homeostatic regulation and by the greater absorption of monovalent ions (Na, K, and Cl) than divalent ions (Ca, Mg, P, S). The relationship between cations and anions and the acid-base balance has been explained by some researchers by developing equations. Melliere and Forbes (1966) described this interrelationship by the following equation: The cation-anion balance equation was also designed to relate performance to the concentration of selected minerals in the diet.

(Cation-Anion) = mEq ($Na^+ + K^+ + Ca^{2+} + Mg^{2+}$) - mEq ($Cl^- + SO_4^{2-} + H_2PO_4^- + HPO_4^{2-}$).

Mongin (1980) concluded that in order to keep the acid-base homeostasis as close as possible to normal, the bird has to regulate the input and/or the output of acidity. According to Mongin (1981) the net acidity intake can be measured by the difference between fixed anion and cations (Anion-Cation) intake. Likewise the net acidity output can be measured by the balance of ions excreted in the urine (Anion-Cation) outgo. The endogenous acid production (H^+ endo) by the metabolism of dietary components (particularly proteins) must also be considered in the following way;

$(Anion\text{-}Cation)_{intake} + H^+_{endo} - (Anion\text{-}Cation)_{outgo} = Zero$

The above equation describes the steady state situation where bird is in a constant acid-base balance, without either acid or base excess or deficiency. Under disturbed conditions (more acid intake or outgo) the blood base excess (alkali reserve; BEecf) will modify accordingly to achieve a steady state condition in the following way;

$(Anion\text{-}Cation)_{intake} + H^+_{endo} - (Anion\text{-}Cation)_{outgo} + BE_{ecf} = Zero$

This equation can be rewritten so as to better represent the extent of the modification of base excess;

$(Cation\text{-}Anion)_{intake} - (Cation\text{-}Anion)_{outgo} - H^+_{endo} = BE_{ecf}$

Under practical conditions the optimal electrolyte intake, in terms of acid-base balance can minimize the presence of base excess, tending to zero. The value of base excess closer to zero has been considered as optimal for better broiler performance. However, the above equation can be rewritten as in the following form to represent all electrolytes that can influence the acid-base homeostasis;

$(Cation\text{-}Anion)_{intake} = mEq(Na^+ + K^+ + Ca^{2+} + Mg^{2+}) - mEq(Cl^- + SO_4^{2-} + H_2PO_4^{4-} + HPO4^{2-})$

Usually phosphorus is added to poultry diets mostly as dicalcium phosphate, with the sole objective to meet the requirements rather than to balance cation and anions. Similarly calcium (Ca) is added as calcium carbonate ($CaCO_3$) to meet the growth and development requirements. Whereas magnesium (Mg) and sulphate (SO_4) are not normally supplemented in the diets, rather SO_4 is mostly present in the form of sulphate sources of supplemental minerals (like $FeSO_4$, $ZnSO_4$) or sulphur containing amino acids. Thus, the ions that are essential for the maintenance of the acid-base balance are Na^+, K^+ and Cl^-. Therefore, the above equation can be written as;

$(Na + K - Cl) = (Cation\text{-}Anion)_{outgo} + H^+_{endo} + BE_{ecf}$

The total levels of Na^+, K^+ and Cl^- are used to calculate the dietary electrolyte balance (DEB) in poultry diets as follow; Dietary electrolyte balance

$(DEB) = mEq\ (Na^+ + K^+ + Cl^-)$

The ability of Na^+ or K^+ to neutralize hydroxyl groups (OH^-) and of Cl^- to neutralize hydrogen ions (H^+) is expressed in the term "milliequivalents" (mEq) which takes into account the atomic weight of each element or molecular weight of each molecule along with their respective valence or charge. In poultry nutrition, DEB is expressed as $Na^+ + K^+ - Cl^-$ in mEq/kg or 100g of diet. The optimal requirement of electrolyte balance was defined in terms of mEq $(Na^+ + K^+ - Cl^-)$/kg of feed around 250 mEq/ kg. Hooge (2003) reported that broiler and breeder diets usually have DEB indices ranging from about 100 to 250mEq/kg. It may be possible that the diets with different Na^+, K^+ and Cl^- contents may have the same DEB value. This is because of two cationic and one anionic mineral component in the equation used for determining DEB. The formula for determining mEq/kg is [(% in diet*10,000)*(valence)]/ [atomic or formula weight in g]. As an example the DEB of a diet containing Na^+ (0.30%), K^+ (0.68%) and Cl^- (0.30%) can be calculated by the following way;

Sodium (0.30%): 0.30%Na * 10,000/23.0 = 130 mEq Na^+/kg

Potassium (0.68): 0.68%K * 10,000/39.1 = 174 mEq K^+/kg

Chloride (0.30%): 0.30%Cl *10,000/35.5 = 84 mEq Cl^-/kg

DEB $mEq(Na^+ + K^+ - Cl^-) = 130 + 174 - 84 = 220$ mEq/kg of feed

The DEB based on dietary monovalent mineral contents could also be calculated by Using the factors reported by Hooge (1995) as;

Sodium (0.30%): 0.30%Na * 434.98 = 130 mEq Na^+/kg

Potassium (0.68): 0.68%K *255.74 = 174 mEq K^+/kg

Chloride (0.30%): 0.30%Cl *282.06 = 84 mEq Cl^-/kg

DEB $mEq(Na^+ + K^+ - Cl^-) = 130 + 174 - 84 = 220$ mEq/kg of feed

While adjusting the DEB for maximum bird performance, care must be taken that the total levels of Na^+, K^+ and Cl^- must be within acceptable ranges, neither deficient nor toxic (Mongin, 1981). The factors for calculating DEB are based on total contents of the nutrients in the chicken feeds, but in reality, bioavailabilities of nutrients affect the actual amounts of nutrients absorbed by the intestine into the blood stream (Hooge, 1995).

In the DEB equation it is assumed that only the minerals Na, K and Cl have an impact on the acid-base balance, without considering the source of electrolyte. The cations *(i.e.,* Na^+ and K^+) supplementation increases pH and blood HCO_3^-, while anion (Cl^-) addition decreases these parameters (Hurwitz *et al.*, 1973). Ruiz-Lopez and Austic (1993) compared the relative acidogenecities of several anions using chloride as a standard. In young birds, chloride significantly increased blood H^+ concentration at high levels (160-240 mEq/kg). They further reported that sodium sulphate was relatively more acidic than those of calcium sulphate and potassium sulphate, indicating the dependence of acidogenic properties of sulphate on the source (Ahmad *et al.*, 2005). Patience *et al.*, (1987), indicated that metabolisable anions like bicarbonate, carbonate and acetate have an influence on the acid-base balance by neutralizing acids and raising blood pH. Gorman and Balnave (1994) proved this in a study in which heat stressed broiler body weight gain associated with Na_2CO_3 and $NaHCO_3$ was significantly different in diets with identical electrolyte balance. They concluded that heat stress could lead to a metabolic requirement for the HCO_3 ion. Johnson and Karunajeewa (1985), and Gorman and Balnave (1994) indicated that DEB equation could not be used to predict the relative benefits of different mineral supplements or combination of minerals. The monovalent ions (Na^+, K^+ and Cl^-) have a greater electrolytic potential than divalent ions (Mg, S, P and Ca), with the electrolytic potential of the latter being greater than that of Fe, Mn, Zn, Cu, Se, Mo, Co and I. Mongin (1981) omitted these divalent ions from the DEB equation due to the followings; a) bivalent cations are not as rapidly absorbed as monovalent cations; b) Mg is commonly supplied in feeds; c) phosphate is hard to be quantified because it comes from various sources; d) calcium absorption rate is controlled by the endocrine system and is most commonly added as calcium carbonate for skeletal development; e) sulphate is included in small amounts as the anion for essential trace element, or to prevent methionine breakdown. However, different studies indicated that the divalent ions excluded from the DEB equation also exert significant effects on poultry performance. Hulan *et al.*, (1987) found little difference in the performance of chicks fed on diets in which DEB varied from 155 to 300mEq/kg. However, altering the Ca concentration of the diets altered both the absolute, and pattern of response to changes in DEB. Furthermore, increasing the P content of the diet tended to improve weight gain, although no primary P deficiency existed. Feeding $CaCO_3$ induced acidosis as compared to little effect by NaCl and KCl feeding. Similarly, significant effects were observed on the growth of chicks when the dietary cation and anion contents have been altered by varying the dietary Mg (Nelson *et al.*, 1981) or SO_4 (Ruiz-Lopez and Asutic, 1993; Ahmad *et al.*, 2005) contents. Patience (1990) also pointed out the effect of cations (Ca^{+2}, Mg^{+2}) and anions (HPO_4^{-2}, $H2PO_4^-$, SO_4^{-2}) on the acid-base and electrolyte balance in birds. Gorman and Balnave (1994) mentioned that these findings cast doubt on the validity of excluding all but Na^+, K^+ and Cl^- concentrations from the DEB equation. Though trace elements have also functioned as electrolytes, but due to their presence in small amounts in feed and in low concentrations in bird tissues, naturally reduces their impact on the acid-base balance and on the electrolyte balance in birds. Therefore, complete electrolyte equation would be:

$(Na^+ + K^+ + Ca^{+2} + Mg^{+2}) - (Cl^- + SO_4^{-2} + H_2PO_4^{-2} + HPO_4^-)$.

In certain situations, it may also be necessary to take into account either the specific effects of each ion or the individual requirements for these ions/electrolytes (Na^+, K^+, Cl^-). These factors have not been considered in the DEB equation and may limit its use. Individual nutritional recommendations for these electrolytes for broilers also vary, NRC (1994) recommends 0.20, 0.30 and 0.20; 0.15, 0.30 and 0.15% for Na^+, K^+ and Cl^- from 0 to 3 and 3 to 6 weeks of age, respectively. The nutritional requirements for broilers also vary with age. Therefore, recommendations for K may vary from 0.21 to 0.73% (Robbins *et al.*,

1982; NRC, 1994) and for Na and Cl from 0.41 to 0.12% and from 0.53 to 0.12%, respectively (Edwards, 1984; NRC, 1994). Birds have been shown to tolerate an excess of K^+ to a greater degree than an excess of Na^+ (Sauveur and Mongin, 1974). Hurwitz *et al*., (1973) noted an improvement in the growth of birds when Na:Cl of 1:1 and 200 mEq/kg DEB ($Na^+ + K^+ - Cl^-$) was achieved by varying Na and Cl levels in the diet. Ahmad *et al.*, (2005) reported higher weight gain and feed: gain in heat stressed broilers with DEB 50 mEq/kg, maintained by supplementing basal diet with $NaHCO_3$ and NH_4Cl to have Na (0.26 and 0.20%), K (0.71 and 0.65%) and Cl (0.88 and 0.71%), in the starter and finisher diets, respectively. They were of the view that dietary NH_4Cl supplementation reduced the Na:Cl ratio and beneficial effects may be due to both H^+ and Cl^- dissociation. Contrarily, Borges *et al*., (2004b) increased the contents of Na and K, and noticed lower feed intake and weight gain in broilers for DEB 40 and 340 mEq/kg and worse feed conversion for DEB 340 mEq/kg of feed. They attributed these responses to Na:Cl imbalance due to low Na contents (0.15%) and/or high Cl contents (0.70%) in DEB 40 and high K^+ contents (1.06%) associated with Na^+ (0.30%) in DEB 340 mEq/kg diet. These differential responses to dietary concentrations of Na^+, K^+ and Cl^- emphasized the importance of rectifying any deficiency or excess of an individual mineral ion before achieving the desired DEB. The interrelationship between mineral ions, environment (thermoneutral, cold and heat stress) and other nutrients, particularly amino acids, must be considered while computing the preferred DEB. Under heat stress situations lowered retention and greater excretion of K^+ increased the demand for the ion Whereas, Borges *et al*., (2004b) noted poor performance in broilers with 1.06% K, 0.30% Na and DEB 340 mEq/kg. These findings suggest that response to supplemental K is linked to ambient temperature and that once the nutritional requirements are met; the ratio among Na, K and Cl is a determining factor for performance.

2-7. Inter-relationships between egg shell quality, blood acid-base balance and dietary electrolytes

2-7-1. Metabolism of ingested food: Food is a large contributor of excess H^+ in laying hens. Production of CO_2 by various oxidative pathways is the main cause. Carbohydrates, lipids and glucogenic amino acids are broken down to CO_2 via pyruvate. Fatty acids and ketogenic amino acids are converted to acetoacetate and 8 hydroxybutyrate and then to CO_2. Sulphuric acid is a major oxidation end product of breakdown of excess cysteine and methionine (Lemann and Relman., 1959). Phosphoric acid is formed from catabolism of phospholipids, phosphoproteins and phosphoric esters.

2-7-2. Bone metabolism: Under normal circumstances, bone formation (H^+ production) and reabsorption (H^+ withdrawal from plasma) have relatively minor effects on acid-base balance in mammals. However, in laying birds, particularly high producing domestic fowl, demands for calcium lead to considerable reabsorption of medullary bone, the unique calciferous tissue deposited in the medulla of the long bones e.g., tibia. While the effects of bone reabsorption on acid-base balance (and vice versa) have received little attention, it seems likely that supply of up to l g of calcium from medullary bone of a domestic hen over a period of just a few hours could have a significant effect. Likewise, daily replenishment of medullary bone from dietary calcium would have a similar effect, but in the opposite direction.

2-7-3. Shell formation: Secretion of $CaCO_3$ (about 5-6g over 18-20 hours in the domestic hen) induces a severe metabolic acidosis. Mongin and Sauveur (1979) presented a model which describes the secretion of HCO_3^- and Ca^{++} from uterine mucosal cells to the lumen

and the movement of H^+ into the plasma. Production of H^+ during shell formation is sufficient to maintain a pH differential across the uterus of at least 0.03 for 20 hours and to produce an average maximum differential of 0.08 about 14-15 hours after the egg enters the uterus (Hodges, 1969).

2-7-4. Respiratory compensation

The primary function of the respiratory system in acid-base regulation is to control the total quantity of CO_2 in blood. The respiratory system regulates CO_2 transport by erythrocytes, CO_2 excretion from the lungs, and restoration of the carbonic acid/bicarbonate buffer system in plasma and erythrocytes. The respiratory system is, therefore, ultimately responsible for total CO_2 in all fluid compartments of the body due to exchange of CO_2 or HCO_3^- with blood. In a steady state of exchange, arterial and alveolar pC02 are similar and likely identical (Scheid and Piiper, 1980).

Alveolar air CO_2, (gas phase) $\rightarrow$ Blood $C0_2 + H_2O \rightarrow H^+ + HC0_3^-$

Small perturbations to $pC0_2$ in either micro-environment will cause an immediate respiratory response. For example, an increase in inspired CO_2 will result in greater minute volume due mainly to an increase in tidal volume with little or no change in breathing frequency (Anderson *et al*., 1986). On the other hand, a large decrease in arterial $pC0_2$ (with associated decrease in HCO_3^- and increase in pH) can occur during heat stress in response to increased breathing frequency and minute volume but decreased tidal volume (Brackenbury *et al*., 1982).

2-7-5. Renal compensation

The kidneys are ultimately responsible for excretion of excess H^+ and anions such as $H_2PO_4^-$, SO_4^{--} and HCO_3^- which are formed or released by metabolic processes associated with food, bone and shell. Also in regard to acid-base balance, the kidneys contribute to the restoration of the H_2CO_3 /HCO_3^- buffer system by reabsorption of HCO_3^-. These functions are integrated with osmoregulation and nitrogen excretion. A disturbance in one of the functions of the kidney will affect the other functions (Hodges, 1969). Non-volatile anions from metabolism of food and other sources are filtered from the blood by glomeruli to become urinary buffers. These include $H_2PO_4^-/HPO_4^{--}$ and uric acid hate buffer systems. The physiological significance of NH_4^+/NH_3 as a buffer system lies in the ability of NH_3 to combine readily with H^+ without an increase in titratable acidity i.e., considerably more H^+ can be secreted beyond that amount needed to acidify urine to pH 4.5. Secretion of H^+ and reabsorption of Na^+ and HCO_3^- can continue after saturation of other urinary buffers provided NH_3 production continues.

2-8. Metabolic change during shell formation

2-8-1. Acid-base balance

Mongin's (1978) model describing shell calcification shows secretion of HCO_3^- into the lumen of the shell gland. The HCO_3^- is derived from hydration of CO_2, under the influence of the enzyme carbonicanhydrase. For each mole of $HCO3^-$ secreted one mole of H^+ passes into the plasma. Another mole of H^+ is released when HCO_3^- changes to CO_3^{--} (Sturkie and Mueller, 1976), although Mongin (1978) has not included this in his model.

Hodges (1969) demonstrated an increase in H^+ concentration in blood perfusing the uterus during shell formation. The pH differential between the sciatic artery and the anterior uterine vein rose from about 0.03 after the egg entered the shell gland, reached a maximum of about 0.08 after 15 hours of calcification, and then fell to about 0.04 at oviposition. The non-constant pH differential suggests that the secretion rate of HCO_3^- is also not constant.

The results of Prashad and Edwards (1973) support the latter finding and they showed that plasma phosphate reached a peak at about the same time.

2-8-2. Calcium homeostasis

The physiological significance of blood ionic calcium (Ca^{++}) concentration in calcium metabolism is discussed in detail by (Schraer *et al*., 1973). He has pointed out that three hormonal systems are involved in the control of plasma Ca^{++}. These are parathyroid hormone (PTH) secreted by the parathyroid gland, calcitonin (CT) secreted by the ultimobranchial gland and 1, 25 dihydroxy-cholecalciferol (1, 25-$(OH)_2 D_3$) secreted by the kidney. There is evidence that the functions of at least two of these hormonal systems are influenced by blood acid-base balance, i.e., calcium metabolism is inter-related with carbonate metabolism. Examples of metabolic pathways or reactions which are sensitive to acid-base disturbance are:

(i) Impairment of hydroxylation of 25-(OH) D_3 in the kidney to 1, 25-$(OH)_2 D_3$ Due to reduced activity of 25-(0H) D_3-I-hydroxylase.

(ii) Equilibrium between plasma Ca^{++} and bound calcium (Schraer *et al*., 1973).

(iii) Uptake of calcium by shell gland mitochondria (Schraer *et al*., 1973).

(iv) Regulatory interaction between PTH and Ca^{++}.

(v) Anomalies in excreted levels of urinary cyclic AMP (an indicator of PTH activity) in rats made alkalotic by feeding $NaHCO_3$with and without supplements of 1,25-$(OH)_2D_3$.

Further evidence of the possible involvement of blood-acid-base balance in calcium metabolism was presented by Nys and deLaage (1984). They found that active transport of Ca^{++} across intestinal and uterine tissue requires a highly active form of $Mg^{++}HCO_3^-$ ATPase which in turn requires high activity carbonic-anhydrase. The results of Odom and Harrison (1985) support this idea since they found that the calcium flux in uterine tissue was influenced by $pC0_2$ of the medium bathing the serosa. They suggested that low blood $pC0_2$ produced by hyperventilation during heat stress might restrict calcium transport, or as a late study showed, by affecting the ionic calcium pool (Odom *et al*., 1986). Another possibility is that hormonal control of ovulation (and hence rate of lay) is influenced by the calcium balance of the hen, mediated through plasma Ca^{++}.

2-8-3. Blood and urine

The previous section deals mainly with theoretical aspects of calcium homeostasis. In these section actual changes in calcium and inorganic phosphorus concentrations in blood plasma and urine during shell formation are considered.

2-8-3-1 Calcium: Hodges (1969) observed an increase in withdrawal of calcium from blood perfusing the uterus in the period 0-4 hours after the egg entered the shell gland. From 4-16 hours rate of withdrawal was steady, although the overall concentration of calcium fluctuated. After 16 hours the rate of withdrawal diminished. Hodges (1969) observed a steady decline in concentration of Ca^{++} in blood taken from the brachial vein once the egg entered the shell gland. A low concentration was maintained betweeen 12 and 15 hours, and then it rose. Parsons and Combs (1981) also reported a decline in concentration of Ca^{++} coinciding with an egg entering the shell gland, a low level being maintained for several hours, then a rise to the pre-calcification level commencing about three hours before oviposition. Buss *et al*., (1980) reported that urinary calcium concentration remained constant except when the hen was forming a shell (about 7-9 times lower). There were no significant differences between lines of hens selected for thick or thin egg shells.

2-8-3-2. Phosphorus: In addition to Ca^{++}, concentrations of inorganic phosphorus in blood plasma and urine also change significantly during shell formation (Parsons and Combs, 1981). This is thought to arise from PTH stimulation of bone reabsorption to counter a fall in plasma Ca^{++} due to shell formation. Mongin and Sauveur (1979) observed that the peak in plasma phosphate concentration was reduced if hens were given a separate meal of crushed sea shells, i.e., the diet supplied a greater proportion of the calcium needed for shell formation and bone reabsorption was reduced accordingly.

2-8-3-3. Sodium, potassium and chloride:

(i) Composite effect: Mongin (1981) has proposed a theoretical model of acid base homeostasis which, in its simplest form, states that:

$(Na + K - Cl)_{intake} = (cation\text{-}anion)_{output} + H^{+}_{endogenous} + base_{excess}$

Where base excess is a measure of the capacity of blood to resist changes in pH. Attempts by various workers to utilise this concept to improve egg shell quality and laying performance have produced contradictory results. Comparisons between experiments are made difficult by the absence of data on blood acid-base changes in some studies.

The data of Sauveur and Mongin (1974) indicate no effect of (Na + K - C1) over the range 160-360 mEq/kg on shell weight/surface area. Hamilton and Thompson (1980) claimed there was a lack of effect on shell quality, but did observe depressed rate of lay and feed intake at low (330) and high (620) mEq/kg levels. Their diet low in (Na + K - C1) depressed pH, HCO_3^- and shell quality, however. Vogt and Harnisch (1983) observed production of thinner shells at low (68) and high (296) levels, but no effect on rate of lay or feed intake.

Austic and Keshavarz (1984) observed a weak tendency (r = 0.2) for thicker shells as (Na + K - Cl) increased. They concluded that reduced feed intake on high C1 diets (0.86 or 0.94%) was responsible for shell thinning. The diets depressed blood HCO_3^- but it is more likely that low dietary calcium (2.0%) was the cause. It has been observed depression of feed intake and rate of lay at low (8 and 33) and high (319 and 418 mEq/kg) levels of (Na + K - C1) in two separate experiments (Hughes 1985). In one experiment using old hens (86 weeks of age) following a molt (at 70 weeks), no effect of (Na + K - C1) on shell thickness but did observe linear increases in pH and HCO_3^- with (Na + K - C1) similar to those results reported by Cohen and Hurwitz (1974). In the other experiment using young hens (32 weeks of age), is observed a curvilinear increase in shell thickness as (Na + K - C1) increased from about 150 meq/kg. There are several possible reasons for these discrepancies. Firstly, some diets used by Sauveur and Mongin (1974) and by Hamilton and Thompson (1980) were probably deficient in sodium and chloride and, therefore, any effects of (Na + K - C1) might have been 'masked (Mongin, 1981). Secondly, extreme levels of (Na + K - C1) used in several of the studies were obtained using individual dietary levels of Na, K or C1 well beyond practical recommendations, and therefore not in accord with assumptions implicit in Mongin's (1981) model. Other possible reasons include differences in breed, age and health of the hens, and environmental conditions, particularly degree of heat stress.

Models to account for the composite effects of Na, K and C1, such as (Na + K - C 1) proposed by Mongin (198l), or (Na + K)/C1 used by Cohen and Hurwitz (1974) do not adequately describe the effects of Na, K and C1 on laying performance or shell formation. The value of such models for commercial formulation is uncertain. After dietary deficiencies and excesses have been considered, the supporting evidence is contradictory. Further work in this area should emphasise the respective metabolic roles of Na, K and C1.

2-9. Dietary electrolyte balance (Na^++K^+-Cl^-) and broiler

2-9-1. Dietary electrolyte balance (Na^++K^+-Cl^-) and broiler performance

Mongin (1981) reported that optimal chick growth performance, when fed purified diets, was achieved using DEB (Na^++K^+-Cl^-) of around 250 mEq/kg with a relation (K+Cl)/Na > 1. Weight of birds, when assessed at 42 d, decreased when DEB was lower than 180 mEq/kg and higher than 300 mEq/kg (Johnson and Karunajeewa, 1985). An optimal DEB was found for feeds containing from 250 to 300mEq/kg. Hulan *et al*., (1987) determined the effect of feeds containing Na^++K^+- Cl^- in different ratios with varying Ca level and noted that the worst and the best weight gains were achieved when the DEB was 174 and 215 mEq/kg, with 1.38 and 0.95% Ca, respectively. Some researchers are of the view that manipulated electrolyte influenced the best DEB (Rondon *et al.*, 2001) for broilers. Borges *et al*., (2003a), noted best weight gain in broilers with DEB 199 mEq/kg, when Na^+ and Cl^- were manipulated. They recommended that extreme Cl^- (0.15 and 0.71%), K^+ (0.98 and 1.21%) and Na^+ levels (0.15 and 0.60%) should be avoided in prestarter diets as these extremities would lead to mineral toxicity. Rondon *et al*., (2001) observed the best DEB as 250mEq/kg when Na^+ levels varied and 319 mEq/ kg when K^+ was manipulated. Similarly, in growing (21-42 d) broilers maximum feed intake was noted by Borges *et al.*, (2004b), in DEB 264 mEq/kg treatment, when Na^+ level was increased in the diet, and 213 mEq/kg, when K^+ and Na^+ levels were concurrently increased in the diet. This indicates that there is a limit over which feed intake is depressed as a function of excessive Na^+ and/or K^+. Rondon *et al.*, (2001), and Murakami *et al.*, (2001) established with modern broiler strains and practical diets, an optimal DEB for the starter phase between 246 and 315 mEq/kg and for the grower one between 249 and 257 mEq/kg. Fixter *et al*., (1987) mentioned that optimal DEB for growing broilers varied with ambient temperature, being 250mEq/kg for moderate temperatures (18 to 26°C) and 350 mEq/ kg for high temperatures (25 to 35°C). Borges *et al*., (2003a) noticed best broiler performance with DEB varying from 186 to 250mEq/kg. However, a high DEB (340 and 360 mEq/kg) resulted in metabolic alkalosis.

Flemming *et al.*, (2001) compared three different DEB (Na^++K^+-Cl^-) levels *i.e.*, high, medium and low, in male broilers during the summer season and noticed that weight gain, feed conversion, viability and productive efficiency index (PEI) were not affected by the DEB, and the low DEB showed the lowest PEI. On the other hand, Borges *et al.*, (2004b) reported that increase in DEB (40 to 340) caused a quadratic effect on weight gain and feed: gain, and a linear increase in feed intake. However, feed intake was at maximum for DEB of 202 mEq/ kg. The ideal DEB observed was between 246 and 277 mEq/kg in overall results. Borges *et al.*, (2003a) noticed that DEB 240 mEq/kg gave the best body weight gain and feed conversion ratio versus DEB 0, 120, and 360 mEq/kg, in broiler raised during summer season (max. 31°C, min. 23°C; RH 75.5%). These four dietary treatments contain NaCl, $NaHCO_3$, and NH4Cl at the highest DEB level $KHCO_3$, with K^+ levels of 0.52% in starter and 0.47% K in grower diets. Optimal DEB levels predicted from curvilinear regression were 236 mEq/kg for body weight gain and 207 mEq/ kg for feed conversion ratio (average 221.5 mEq/kg) from 0-42 d of age. These DEB corresponded to estimated (interpolated) values in predicted optimal 207 to 236 mEq/kg starter, Na 0.409 to 0.445% and Cl 0.326 to 0.372% (K=0.52%), and grower Na 0.410 to 0.445%, Cl 0.315 to 0.267% (K=0.47%). Lower Na^+ and higher Cl^- contents in DEB 207 might have reduced the feed intake that resulted in optimal feed conversion ratio. However, there was a limit to electrolyte addition, because high DEB (360 mEq/kg; containing 0.11-0.12% K^+ from $KHCO_3$; increased Na^+ from $NaHCO_3$ and 1.53-1.61% HCO_3), resulted in poor live performance, as it intensified the problem of alkalosis. In conclusion, a practical DEB range of 220 to 240 mEq/ kg minimum should normally be adequate for broiler chickens raised during summer season (max. 31°C, min. 23°C; RH 75.5%). Borges *et al.*, (2003b),

reported that under thermoneutral environment (max. 32 to 25°C, min. 28 to 19°C; RH 49 to 58%) DEB 240 mEq/kg (0.35 and 0.35% Na^+; 0.37 and 0.29% Cl^- in starter (0.75% K) and grower (0.67% K), respectively, increased 42 d weight gain in Ross broiler chickens than that of DEB 40 mEq/kg. The DEB treatment had no effect on performance of broilers exposed to cyclic daily heat stress (wk 1 and 2, thermoneutral; wk 2 to 6, max. 35, 35, 33, 33°C, min. 23, 20, 19, 19°C; RH 51 to 54%). However, regardless of ambient temperature, increasing DEB stimulated feed intake, which may possibly be due to increasing Na^+ levels in diets (0.15 to 0.45%) used colostomies male broiler chickens and reported no significant effect of the DEB treatments (140, 240, or 340 mEq/kg) on feed intake, BW gain and N balance in birds exposed daily to cyclic heat stress (22.5±3.5°C for 14 h and 33±2.0°C for 10 h). On the basis of water, electrolyte and nitrogen metabolism results, it was concluded that DEB of 240 mEq/ kg was most favorable for broilers reared in either thermoneutral or daily cyclic heat stress environments. Borges *et al.*, (2004b), noted that in growing (21-42 d) broilers the DEB 40 mEq/kg reduced feed intake and weight gain. They attributed this response to Na:Cl imbalance due to excess Cl^- (0.70%) and low Na^+ levels (0.15%). On the other hand, the DEB 340 mEq/kg resulted in worse feed conversion that may be due to excess Na^+ (0.45%) in the diet. A quadratic effect of DEB on weight gain and feed: gain was noted when the electrolyte ratio was increased only by the supplementation of Na^+. The ideal DEB, obtained by the manipulation of Na^+ and Cl levels, was between 202 and 235 mEq/ kg.

2-9-2. Mortality and carcass characteristic

Borges *et al*., (2003a) observed non-significant effects of DEB treatments (0, 120, 240, 360 mEq/kg) on mortality in broilers reared under moderately high ambient temperature and relative humidities, in both starter (0-21 d) and finisher (21-42 d) phases. They also noted non-significant effects of DEB on carcass yield, breast, thigh plus leg, back, wing, not noticed any significant effect of DEB (40, 140, 240 and 340 mEq/kg) on the live ability of growing (21-42 d) broilers reared in mild environment. While evaluating the different sources of dietary electrolytes Ahmad *et al.*, (2005), indicated significantly high carcass weight and dressing percentage in heat stressed broilers fed diets supplemented with $NaHCO_3$ Significantly higher breast meat yield in birds fed $NaHCO_3$, Na_2CO_3 and NH_4Cl supplements and lowest abdominal fat in $NaHCO_3$ and NH_4Cl supplements was noticed along with significant effect on mortality at fixed DEB values of 250 and 50mEq/kg. Musthaq *et al.*, (2005), noticed non-significant effect of increasing levels of dietary Na (0.20, 0.25, and 0.30%) and Cl (0.30, 0.40, and 0.50%) on mortality (0 to 2.78%) in heat stressed (32-39؛C) 28-days-old broilers. In their experiment the DEB was fixed at 250mEq/kg, that is why the negative effects of increasing levels of Na and Cl may be corrected. The use of $NaHCO_3$ in addition to NaCl as a source of Na may also attributed towards broiler survivability.

2-9-3. Water consumption, rectal temperature and litter moisture

Dissipation of more than 80% of the heat produced via evaporative cooling highlighted the importance of enhancing water consumption in heat stressed broilers. Among several factors, water consumption depends upon the bird's age, physiological state, ambient temperature, water temperature and pH, dietary protein levels, and amount and types of salts added in feed and water. The increase in water consumption benefits the bird by acting as a heat receptor as well as increasing the amount of heat dissipated per breath (Belay and Teeter, 1993). The increase in water consumption by 20% over basal levels can increase heat loss per breath by as much as 30% (Belay and Teeter, 1993). According to Borges *et al*., (2002), water consumption depends directly on bird age and on the Na^++K^+-

Cl^- ratio in the feed. Increased water consumption caused by the greater Na^++K^+Cl^- ratio had a direct impact on litter moisture and it also reduced the rectal temperature in birds. Similarly, Borges *et al.*, (2003a), observed linear increase in water intake of heat stressed broilers with increasing DEB levels (increased Na^+ and K^+ intake) in the diet and with bird's age. However, water turnover increased with increasing DEB, but decreased with increasing bird age. This increase in water intake occurred to maintain the osmotic balance and to quench the thirst caused by increased Na^+ and K^+ intake as blood osmotic pressure in birds is a thirst-regulating factor. On the other hand, low DEB (diets with high Cl contents) did not stimulate water intake. The internal body temperature of broilers decreased linearly as the DEB increased, and birds that were fed diets with 240 and 360 mEq/kg had the lowest temperature and smallest body heat variation from morning to afternoon during tropical summer conditions (max. 31°C, min. 23°C; RH 75.5%). This was attributed to increased water consumption by these birds and presumably heat dissipation and the efficiency in evaporative heat loss also increased with increased water intake. This supported the hypothesis that stimulus to increase water intake is a crucial factor for enhancing the survivability of heat stress birds. Similarly, in broilers grown (21- 42 d) in mild temperature conditions (av. min. and max. temp. 17-27؛C and 21-28؛C, respectively, RH 69.0-74.8%), water consumption and litter moisture showed a linear trend as the DEB increased (40, 140, 240 and 340 mEq/kg; Borges *et al.*, 2004b). The authors were of the view that increase in water consumption was particularly important at high temperatures, where it could contribute to bird survivability. However, as confirmed by rectal temperatures (40.4 to 40.6°C) no significant effect of DEB treatments was noted on body temperature (Borges *et al.*, 2004b). In contrast to the above findings, Borges *et al.*, (2004a), reported increased water consumption in colostomized male broiler chickens by 22.4% from 254 to 300 ml/kg0.75 in heat stress (22.5±3.5°C for 14 h and 33±2.0°C for 10 h) compared to thermoneutral environment. However, in either environment no effect of DEB levels (140, 240, or 340 mEq/kg) was observed on water consumption, excretion and other water related parameters. Various factors can affect the excreta and litter moisture content, some of them are related to management and housing (amount and type of litter, temperature, ventilation, heating, drinking system, density), other factors are related to diseases caused by various infections (*coccidiosis, E. coli, Campylobacter, Spirochaetes*) (Francesch and Brufau, 2004). The dietary factors that may affect water consumption and excretion may also affect the moisture contents of the excreta and litter. Moisture content of litter may also be altered by the excreta composition, which can affect their water retention capacity and limit the evaporative water losses (Francesch and Brufau, 2004). As mentioned earlier, the excess of Na^+ and K^+ promoted increase in litter moisture and water intake (Mongin, 1981), whereas the increase of Cl anions seemed unrelated to excreta moisture (Rondon *et al.*, 2001). The effect of dietary Na^+ levels on water intake and excreta moisture has been well documented and a wide agreement existed between researchers that excess of Na^+ in chicken diets increases excreta moisture. In majority of reports, the increase of excreta moisture is linearly dependent on the increase of added Na^+ (Fleet and Saylor, 1983; Murakami *et al.*, 1997; Rondon *et al.*, 2001). Smith *et al.*, (2000b) reported that increasing dietary concentration of Na^+, K^+ or P gave linear increase in the water intake of birds and linear increases in the moisture content of their excreta. Each 0.1% increase in dietary mineral increased the moisture content of the excreta by 0.904 (±0.157), (±0.202) and 0.559 (±0.031) % (standard error) for Na^+, K^+ and P, respectively. Likewise, the increase in dietary K^+ level has also been associated with an increase in water consumption and excreta moisture of layer (Smith *et al.*, 2000b) and broiler (Borges *et al.*, 2003a) birds. Borges *et al.*, (2003a), reported that water intake increased linearly as the DEB increased and the increase of water intake was also reflected in a progressive