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I am submitting herewith a dissertation written by Adesina Afisu Adekunmisi entitled "Broiler Chick Growth and Physiological and Metabolic Status as Affected by the Interaction Between Dietary Crude Protein and Dietary Electrolytes." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

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BROILER CHICK GROWTH AND PHYSIOLOGICAL AND METABOLIC STATUS AS AFFECTED BY THE INTERACTION BETWEEN DIETARY CRUDE PROTEIN AND DIETARY ELECTROLYTES

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Adesina Afisu Adekunmisi

December 1986

ACKNOWLEDGEMENTS

The author wishes to express his sincere gratitude and appreciation to the following:

Dr. K. R. Robbins, my major professor, whose excellent leadership, constructive criticism, enthusiasm and friendship were essential components for the successful completion of this program.

Dr. K. M. Barth, Dr. H. V. Shirley, Dr. R. Beauchene and Dr. J. P. Hitchcock, my graduate committee members, for their critique of the research plans and review of this manuscript.

Mr. George McGhee for his suggestions and assistance in data collection.

The staff of the University poultry farm for the care of the chickens during my experiments.

Dr. R. R. Shrode and his family, the sponsor of William Lester Brown Scholarship of the College of Agriculture which the author received during the course of his study.

Mrs. Regina Robbins and her children for their friendship and spiritual support.

Eddie Jarboe, Gena Gritzner, Linda Miller, Nancy Ramsey, Peter Osei and many others, who in one way or the other, contributed to the successful completion of this research work.

Finally, I gratefully acknowledge the sacrifice, patience and unflinching loving support of my wife, Aderonke, my son, Adedotun and the entire Adekunmisi family towards my quest for this education.

ABSTRACT

The research was designed to (1) assess the effects of dietary electrolyte balance (meq Na + K - Cl/kg diet), dietary crude protein (CP) level, and photoperiod on performance of broiler chicks; (2) evaluate the physiological and metabolic effects of dietary electrolyte balance in broiler chicks; and (3) determine the dietary electrolyte balance requirement of broiler chicks.

The results indicated a dietary electrolyte balance x crude protein interaction. Increasing the electrolyte balance by the addition of Na-and K-citrate improved feed consumption and gain of chicks fed high protein (28.6%) diets, but depressed feed consumption and gain of chicks fed low protein (14.3%) diets. Increasing Cl in high Na and K diets also depressed growth. The electrolyte balance x protein interaction on growth was less pronounced when feed intake was equalized. High Cl diets depressed blood pH, HCO_3, and base excess. Feeding high versus low electrolyte balance diets increased plasma uric acid concentration and decreased kidney asparaginase activity. The effect of dietary electrolyte balance on acid-base and asparaginase activity were independent of crude protein level. Amino acid supplementation of the low protein diet did not alleviate the growth depressing effect of increased electrolyte balance. Either the addition of a mixture of K and Na or addition of isomolar levels of K or Na alone depressed growth of chicks fed low protein diets. The results

indicated that the electrolyte balance which provides for optimum growth is dependent upon the dietary crude protein concentration. Acid-base status, kidney asparaginase activity and plasma uric acid concentrations were affected by alterations in dietary electrolytes, but these changes were not correlated with the growth response.

Dietary electrolyte balances of 250 meq, 325 meq and 400 meq/kg were found to be optimum for diets containing 14.3%, 21.4% and 28.6% CP, respectively. Increasing the electrolyte balance of a conventional 21% CP corn-soy broiler diet from 228 meq Na + K - Cl/kg to 325 meq by the addition of Na and K resulted in increased gain, feed intake and feed efficiency. It was concluded that within the range of 14% to 28% CP, the optimum electrolyte balance is increased by about 11 meq/kg per 1% unit increase in diet crude protein content.

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INTRODUCTION

Sodium, potassium and chlorine are the predominant electrolytes in the animal body. Sodium and chlorine are the major extracellular cation and anion respectively. Intracellularly, potassium predominates. As a result of the functional relationship among these monovalent electrolytes in maintaining normal osmolarity and acid-base balance, nutritional interactions among these minerals can be expected. In early nutritional studies, the requirement of domestic birds for these elements have most often been determined independently. During recent years, it has become abundantly clear that these interrelationships are not limited to these elements but involve other major nutrients as well--notably, amino acids and calcium.

The protein requirement for broilers is usually determined in relation to the calorie:protein ratio. In several studies, however, metabolism of basic amino acids has been shown to be influenced by dietary cations. For example, the severity of reduced feed intake and growth depression associated with lysine-arginine antagonism in diets containing excessive amount of lysine can be attenuated by mineral cations, especially potassium and sodium, while high dietary chloride tends to exacerbate the conditions. The production, excretion and plasma concentrations of the end products of protein metabolism in chickens can be altered by both dietary electrolyte balance and crude protein level. While several nutritional interrelationships,

as well as the extent to which their interrelationships alter nutritional requirements, have been established for certain nutrients (e.g. energy:protein; calcium:phosphorus; leucine, isoleucine and valine), no attempts have been made to evaluate the interrelationship between dietary electrolyte balance and crude protein content of the diet and the possible effect on their requirements. The fact that electrolyte balance is capable of influencing amino acid utilization, and vice versa, suggests that this nutritional interrelationship warrants further investigation in order to fully understand its nutritional and physiological implications and its practical application in poultry nutrition.

Another nutrient influenced by dietary electrolytes is calcium. Tibia dyschondroplasia, a cartilage abnormality, is one of the major problems confronting the broiler industry today. This abnormality has been attributed to imbalances in dietary monovalent electrolytes as a result of changes in acid base status. Alterations in calcium metabolism, for example, increases urinary calcium loss and negative calcium balance has been reported in a number studies with rats and humans. These separate observations further justify the need to evaluate the effect of dietary electrolyte balance and crude protein level on general performance of broilers. Such evaluation might provide methods of providing these essential nutrients for maximum growth and increased productivity.

With these considerations, therefore, the objectives of this study were: 1) to ascertain the relationship between dietary electrolyte balance and crude protein and to determine the effect on growth and

performance of broiler chicks, 2) To determine and measure the effects of the interrelationship between dietary electrolyte balance and crude protein on acid-base status, kidney asparaginase activity and plasma uric acid concentration, and 3) to quantify the dietary electrolyte balance requirement in relation to the crude protein content of the diet and to evaluate its practicability in today's broiler diet.

PART I

LITERATURE REVIEW

Interrelationships Between Dietary Electrolytes and Their Effects on Nitrogen Metabolism

The essentiality of the major dietary electrolytes (sodium, Na; potassium, K; and chlorine, Cl) for life has been recognized for decades. The National Research Council (1977; 1984) established the broiler chicken's requirements for these nutrients without reference to the possible interrelationships among them. This is not surprising since in most of the early requirement studies only the concentration of one of these elements was varied in a given experiment. In several studies in which both sodium and potassium or chlorine were studied, little or no sparing effect of one element on the requirement of the other was observed (Burns et al., 1953; McWard and Scott, 1961). Nonetheless, it has been demonstrated that the toxic effect of either Na or K could be reversed by increasing the dietary content of the other cation. Thacker (1959) found that an imbalance of cations and anions resulted in growth depression of rabbits. He indicated that physiological acidosis, a result of dietary cation-anion imbalance, could be corrected by addition of cations with metabolizable anions. Increasing the anion (SO_4) content of the diet while maintaining a constant cation content depressed growth (Leach et al., 1960). Nesheim et al. (1964) reported that the toxic effect of Cl could be reversed by increasing the dietary amounts of Na and/or K. Melliere and Forbes (1966) presented evidence indicating the importance of regulating the total cation-anion balance

[(Ca + Na + K + Mg) - (Cl + H_2PO_4)] in the diet of growing chicks. In their study in which only the levels of Na and K were varied, it was observed that the rate of growth of young chicks was depressed at both narrow and wide cation:anion ratios. Although all mineral cations and anions are probably involved, interest has been centered on the monovalent electrolytes (Na, K, Cl), largely because of their prominent effects on acid-base balance and because they are among the minerals most likely to be varied in diet formulation (Mongin, 1981). In fact, Mongin and Sauveur (1977) indicated that an electrolyte balance of 250 meq (Na + K - Cl)/kg diet was optimum for growth of young chicks. In a more recent study (Johnson and Karunajeewa, 1985), however, the optimum electrolyte balance was found to lie between 250 and 300 meq/kg diet. Moreover, the same authors demonstrated that electrolyte balance is preferable to the total cation-anion balance in describing mineral balance effects on growth of broiler chickens. It is evident from the available data that these three electrolytes are probably the key elements in the maintenance of metabolic acid-base balance.

Our knowledge of electrolyte nutrition has been further enhanced by recent work related to amino acid metabolism and bone formation. The nutritional antagonism of lysine and arginine in diets containing excessive amounts of lysine has been shown in rats (Jones et al. 1966) and in chicks (Boorman and Fisher, 1966; Jones, 1964, Jones et al. 1966). Excess dietary lysine interferes with arginine utilization in chickens, in part by increasing renal arginase activity

and consequently increasing arginine degradation. The resulting effect is an increased requirement for arginine (Austic and Nesheim, 1970). High dietary levels of K decrease kidney arginase activity, increase muscle protein synthesis, and decrease intestinal bacterial activity in chicks (Stutz et al., 1972a). Increasing the dietary levels of arginine or mineral cations, especially K and Na, were reported to reduce the severity of the lysine-arginine antagonism in chicks if added in the form of metabolizable organic acids such as acetate or bicarbonate (O'Dell and Savage, 1966), while increasing dietary Cl exacerbates it (Austic and Calvert, 1981). The ameliorating effect of K might reside in the fact that its high dietary intake markedly increases hepatic catabolism of lysine to respiratory CO_2 (Austic et al., 1977; Scott and Austic, 1978).

In studies with rats, increased tissue concentrations of basic amino acids were noticed when diets containing deficient levels of potassium were fed (Iacobellis et al., 1956; Eckel et al., 1958; Arnauld and LaChance, 1980). Forsberg and Austic (1986) observed a similar response. They concluded from both in vitro and in vivo experiments that potassium deficiency appears to conserve lysine in the free form in the intracellular pool by enhancing cellular uptake and by decreasing the degradation of lysine and the utilization of lysine in protein synthesis. However, an earlier study of Robbins et al., (1982) indicated that there might be a species difference in tissue basic amino acid alterations in response to dietary potassium concentration. When chicks were fed crystalline

amino acid diets, concentrations of muscle free glutamine, lysine and arginine increased as the dietary K increased from 0.12% to 0.18% and decreased thereafter. Leach (1959) showed that increasing the protein content of the diet resulted in an increase in the K requirement of chicks. This response might be due to the role of K in protein biosynthesis (Schlessinger, 1964; Lubin and Ennis, 1964; Cohn and Lubin, 1978). This biosynthetic role of K was substantiated by the study of Reinhart et al. (1968) who indicated that chicks fed a semi-purified diet deficient in K incorporated significantly less L-leucine into skeletal muscle protein than did chicks which were pair-fed a diet containing adequate levels of potassium. A similar response was noted by Alexis et al., (1971). In another study (Stutz et al., 1972b), increased incorporation of L-lysine into muscle protein was noticed when a high-lysine, low arginine diet was supplemented with K-acetate. Thus, it could be concluded that the ameliorating action of dietary K on the lysine-arginine antagonism in chicks might be due to the increased lysine incorporation into muscle protein rather than increased lysine catabolism as indicated by Austic et al. (1977) and Scott and Austic (1978).

Uric acid is the main end product of nitrogen metabolism in chickens and there are indications that dietary electrolytes and protein may affect nitrogen metabolism. Okumura and Tasaki (1968) indicated that inclusion of HCl in the diets fed to adult male chickens with exteriorized ureters increased their NH_4 production and excretion while addition of $NaHCO_3$ had the opposite effect. Uric

acid production was unaffected by all the various dietary levels of $NaHCO_3$ or HCl employed. However, dietary deficiencies of Na and K caused a reduction in plasma uric acid concentration in chickens (Austic, 1980). The same author (Austic, 1978) previously reported that plasma uric acid concentration increased in chickens during metabolic alkalosis and decreased during acidosis. He also indicated that the amount of uric acid excreted was unaffected by dietary Na, K and Cl. Increased dietary protein increases the production and excretion of total nitrogen, uric acid, and ammonia (Featherston and Scholz, 1968; Hevia and Clifford, 1978; Tasaki and Okumura, 1964). In additon, increased dietary protein resulted in increased plasma uric acid levels (Okumura and Tasaki, 1968; Featherston, 1969). Dietary deficiencies of amino acids also increased plasma uric acid concentrations (Miles and Featherston, 1974).

Ammonia associated with protein metabolism in rats and birds alike is derived mainly from glutamine and/or asparagine. The increased ammonia production arises from increased activities of the mitochondrial enzyme glutaminase which exists in phosphate-dependent (PDG) and phosphate independent (PIG) forms and asparaginase in both liver and the kidney. However, Coon and Balling (1984) noted that asparaginase is more important than glutaminase in regulating acidosis in chickens as indicated by the large increase in kidney asparaginase activity compared to small increases in kidney PDG in chicks fed ammonium chloride containing diets, and the more normal urine pH in chicks fed additional dietary asparagine. Moreover, kidney

asparaginase activity was elevated by increased dietary protein in chicks but not in rats (Balling and Coon, 1981). Potassium deficiency or acidosis has been demonstrated to increase NH_4 excretion (Kamm and Strope, 1973; Tannen and McGill, 1976).

Some workers have reported improved growth when K and/or Na were added to low protein diets. Leibholz et al. (1966) showed that potassium acetate supplementation improved the performance of pigs fed protein deficient diets. Similar responses were observed when either sodium or potassium bicarbonate was added to lysine-deficient diets (Madubuike et al., 1980; Austic et al., 1983). Miyada and Cline (1983) also improved performance of pigs when they added potassium carbonate to a lysine-deficient diet.

The aforementioned studies have clearly indicated that the efficiency of protein utilization in chickens may be influenced greatly by both dietary electrolytes and protein. Evaluating the interrelationship between these two major nutrients may shed more light on factor(s) affecting their requirements and/or metabolism in chickens.

Effects of Dietary Electrolytes and Dietary Crude Protein on Calcium Metabolism

Another nutrient influenced by dietary electrolyte content is calcium. An imbalance in the monovalent cations and anions in the diet influence the incidence of leg problems in chicks (Mongin and Sauveur, 1977). A cartilage abnormality, tibial dyschondroplasia,

is characterized by the presence of uncalcified "plugs" of avascular hypertrophic cartilage in the proximal metaphysis of the tibiotarsus and less frequently in the tarsometatarsus (Siller, 1970). This leg abnormality is a major problem in the commercial broiler production and it occurs as early as one to two weeks in broiler chickens fed practical type rations (Riddell et al., 1971).

Sauveur and Mongin (1978) observed that the incidence of tibial dyschondroplasia was closely associated with metabolic acidosis in chickens fed a high chloride diet. In an earlier study (Sauveur et al., 1977) metabolic acidosis was shown to reduce the renal conversion of 25-hydroxycholecalciferol [25(OH)D_3] to 1,25-dihydroxycholecalciferol [1,25-$(OH)_2D_3$] by about 40% in rachitic chicks. They postulated that impaired bone mineralization might have resulted from an alteration in vitamin D metabolism. In support of this hypothesis, Lee et al. (1977) indicated that an acute acidosis induced by feeding ammonium chloride inhibited the conversion of 25(OH)D_3 to (25OHD_3) to 1,25$(OH)_2D_3$ in rats. In contrast to these findings, Edwards (1984) observed that supplementing chickens with 20 mg/day of either 1,25(OH_2) D_3 or 24,25 dihydroxycholecalciferol did not affect the incidence of dyschondroplasia. It is, therefore, difficult to explain the effect of a high chloride diet on tibial dyschondroplasia incidence by interference with vitamin D_3 metabolism.

In another study, however, significant urinary excretion of total acid, calcium and phosphorus has been associated with acid stress (Newell and Beauchene, 1975). Dietary electrolytes, particularly sodium, potassium and chlorine, affect acid-base balance.

Excess dietary chloride decreases blood pH, and HCO_3 and base excess unless Cl is balanced by equivalent concentration of Na and K. Also a negative correlation between dietary Cl and these blood variables was noticed in laying hens (Cohen and Hurwitz, 1974). Hamilton and Thompson (1980) observed decreased blood pH and bicarbonate levels and reduced egg shell strength in old hens by increasing the chloride content of the diet from 0.11 to 2.13%. Hunt and Aitken (1962) also reported that excessive levels of HCl depressed egg shell calcification. Although the preceding studies strongly pointed to the influence of dietary electrolytes on bone mineralization, other dietary factors, particularly protein in excess of the requirement produces a stress on an animal, in the classical sense, as evidenced by an increase in size of the adrenal glands and production of adrenocorticoids (Scott et al., 1982). High dietary protein can interfere with folic acid metabolism and consequently result in an increase in the incidence of leg problems (Creek and Vasaitis, 1963; Wong et al., 1977). The reason for this interaction was attributed to the role of folic acid in the prevention of perosis, and it is intimately associated with uric acid synthesis. Thus, high levels of dietary protein tend to increase the need for folic acid and aggravate the incidence of perosis in the chicken. Moreover, the workers also noted that uric acid output was maintained when folic acid deficient diets were fed, which indicated that folic acid was preferentially used for uric acid production at the expense of normal growth.

Hulan et al. (1980) reported that leg abnormalities were less pronounced in birds fed diets of low versus high protein content. Consumption of high protein diets resulted in the development of a mild osteoporotic condition in the Golden Syrian hamster (Lindsay et al., 1983), and increased excretion of calcium in both humans (Linkswiler et al., 1981; Marita, 1984) and in rats (Allen and Hall, 1978). Lutz (1984) noted similar protein-induced hypercalciuria in humans. This researcher demonstrated that raising protein intake in adult women from 44 g/day to 102 g/day increased urinary losses of calcium and resulted in a negative calcium balance. Supplementing the diet with 5.85 gm of sodium bicarbonate per day reduced urinary losses and re-established a positive calcium balance. Whitting and Draper (1980) suggested that oxidation of excess sulfur amino acids present in the high-protein diet resulted in increased production of sulfuric acid, and this might partly explain the phenomenon. Since both dietary electrolytes and protein seem to influence calcium metabolism, blood acid base-balance, and the general performance of birds, evaluation of their interrelationship might elucidate ways of providing these essential nutrients to enhance efficient broiler production.

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PART II

EFFECT OF DIET CRUDE PROTEIN LEVEL, ELECTROLYTE BALANCE AND PHOTOPERIOD ON GROWTH OF BROILER CHICKENS

Summary

Three experiments were conducted with broiler chicks to study the effects of dietary crude protein level, dietary electrolyte balance, and photoperiod on performance. Increasing the electrolyte balance (Na + K - Cl) from about 200 meq/kg to 350 meq/kg or more improved feed consumption and gain of chicks fed high protein (28.6%) diets, but depressed feed consumption and body gain of chicks fed low protein (14.3%) diets. Neither sex nor photoperiod affected the nature or magnitude of the crude protein x electrolyte balance interaction, although birds housed under 16L:8D were found to have significantly higher concentrations of plasma ammonia than did those housed under 23L:1D. Chicks fed diets containing a high vs. a low electrolyte balance had significantly higher concentrations of plasma uric acid, and significantly lower kidney asparaginase activities. Electrolyte effects on kidney asparaginase activity were not affected by diet crude protein content. Results indicate that the electrolyte balance which provides for optimum growth is dependent upon dietary crude protein concentration. Further, data suggest that diet electrolyte effects on metabolic acid-base homeostasis are not related to their effects on growth.

Introduction

It is well established that dietary mineral balance or, more specifically, electrolyte balance can affect growth and feed efficiency of poultry. Mongin and Sauveur (1977) reported that an electrolyte

balance (Na + K - Cl) of 250 meq/kg was optimum for growth of young chicks. Melliere and Forbes (1966) studied the effects of total cation-anion balance (Ca + Na + K + Mg - Cl - H_2PO_4 - HPO_4). However, recent work by Johnson and Karunajeewa (1985) demonstrated that electrolyte balance is preferable to total cation-anion balance in describing mineral balance effects on growth of broiler chickens. The mode of action of electrolyte balance on growth and feed consumption is not known, although it is clear that dietary electrolyte concentration affects metabolic acid-base balance (Johnson and Karunajeewa, 1985).

In the above studies, diets were formulated to contain crude protein levels at or near the chicken's estimated requirement and diet protein concentration was not included as an experimental variable. However, in other studies dietary electrolytes have been shown to affect efficiency of protein and amino acid utilization. For example, the lysine-arginine antagonism in chicks can be partially alleviated by high levels of dietary potassium and sodium, and exacerbated by high levels of dietary chloride (Austic and Calvert, 1981). Also, Leibholz et al., (1966) found that potassium supplementation of low-protein and/or low-lysine diets improved growth of swine.

The studies reported herein were conducted to determine if and to what degree dietary protein level affects the response of broiler chickens to changes in dietary electrolyte balance. Since photoperiod has also been shown to affect rate and efficiency of growth in broilers (Robbins et al., 1984), photoperiod was included as a third experimental variable.

Experimental Procedure

The composition of the semi-purified basal diets used in each experiment is presented in Table 1. All experimental diets were formulated to contain 3200 kcal metabolizable energy/kg diet. Although diet chloride content varied among experiments, diets within experiments were formulated to contain equal amounts of chloride. Experimental diets were prepared by the substitution of sodium citrate and potassium citrate for equal amounts of cellulose.

Hubbard crossbred broiler chicks were used. At one day of age, chicks were sexed with all morbid, underweight, and unthrifty chicks were discarded. The remaining chicks were then randomly distributed to 3 replicate groups of 6 chicks each per treatment. In Experiments 1 and 3, female chicks were used. Sex was an experimental variable in Experiment 2. Chicks were fed the experimental diets from day 1 to day 30 posthatching in Experiment 1; from day 1 to day 21 posthatching in Experiment 2; and from day 4 to day 30 posthatching in Experiment 3. Chicks in Experiment 3 were fed a corn-soy broiler starter diet for the first 3 days posthatching.

The chickens were housed in electrically heated battery-brooders in environmentally-controlled rooms which were kept at 28° C. Photoperiod was an experimental variable. During the light period, average light intensity at bird level was 14.5 lux. Feed and water were provided ad libitum.

TABLE 1. Percentage composition of the basal diets[a]

Ingredient	Protein level 14.3%	28.6%
Dextrose	25.00	25.00
Cornstarch	30.99	15.02
Isolated soy protein (85% CP[b])	16.65	33.30
Corn oil	7.50	7.50
Mineral premix[c]	4.65	4.65
Vitamin premix[d]	.20	.20
Choline chloride	.20	.20
DL-methionine	.25	.50
Cellulose	to 100.00	to 100.00

[a]Contained by calculation 3200 kcal ME/kg.

[b]As analyzed.

[c]Provided per kg diet: calcium chloride dihydrate, 4.14 g; calcium carbonate, 2.47 g; dibasic calcium phosphate, 25.2 g; monobasic calcium phosphate, 8.14 g; magnesium oxide, .568 g; manganous sulfate monohydrate, .651 g; ferric citrate, .50 g; zinc carbonate, .10 g; cupric sulfate pentahydrate, 20 mg; boric acid, 9 mg; sodium molybdate, 9 mg; ethylene diaminedihydroiodide, 40 mg; cobalt sulfate septahydrate, 1 mg; sodium selenite, .21 mg; silica, 4.65 g.

[d]Baker *et al.* (1979).

Experiment 1 consisted of a 2 x 2 x 2 factorial arrangement of treatments (Table 2), i.e., 2 levels of crude protein, 2 levels of dietary electrolytes, and 2 photoperiods. Feed intake, gain and feed efficiency were measured. The experimental diets were formulated based on ingredient compositions of NRC (1977). However, subsequent chemical analyses indicated that the levels of Na and K were significantly higher than calculated values, and this was due primarily to the difference in composition of isolated soy protein from that published (NRC, 1977). The experimental diets were calculated to contain either 150 or 350 meq Na + K - Cl/kg diet, but were analyzed to contain 185 and 467 meq/kg, respectively, in the low protein diets, and 274 and 564 meq/kg, respectively, in the high protein diets. Na and K were measured via atomic absorption spectrophotometry; Cl was measured using a Dionex ion chromatograph.

In Experiment 2, dietary protein was held constant at 14.3%. In this experiment the effects of electrolyte balance, photoperiod, and sex on feed consumption, growth and efficiency, and plasma ammonia and uric acid concentrations were evaluated. The treatments were arranged as a 2 x 2 x 2 factorial. Upon terminating the experiment, 1 ml of blood was obtained from each chick via heart puncture using heparinized syringes. Individual blood samples were pooled within replicate groups, thus yielding 3 samples per treatment. Plasma concentrations of ammonia and uric acid were determined enzymatically using commercial diagnostic kits (Sigma Chemical Co., St. Louis, MO).

TABLE 2. Effect of diet and photoperiod on performance of chicks (Experiment 1)[a]

Diet treatment			Photoperiod					
			23L:1D			16L:8D		
No.	Crude protein	Electrolyte balance[b]	Gain[c]	Feed cons.[c,d]	Gain/feed[d]	Gain[c]	Feed cons.[c,d]	Gain/feed[d]
	(%)	(meq/kg)	(g)	(g)	(g/kg)	(g)	(g)	(g/kg)
1	14.3	185	635	1136	559	625	1112	562
2	14.3	467	610	1146	532	509	969	525
3	28.6	274	598	932	620	544	897	606
4	28.6	564	650	1005	647	663	1010	654

[a]Data are means of triplicate groups of female chicks each. Chicks were fed the experimental diets from day 1 to day 30 posthatching. Average initial weight was 45 g. Pooled standard error of the mean for gain was 31.8, for feed consumption was 33.7, and for gain/feed was 21.5.

[b]Milliequivalents of Na + K - Cl per kg of diet (as analyzed). All diets contained 107 meq Cl/kg. Milliequivalents of Na and K per kg diet were: diet 1, 196 Na and 96 K; diet 2, 335 Na and 239 K; diet 3, 278 Na and 103 K; and diet 4, 422 Na and 249 K.

[c]The crude protein x electrolyte balance interaction was significant ($P<.01$).

[d]The main effect of crude protein was significant ($P<.001$).

The effects of dietary protein, dietary electrolyte balance, and photoperiod on growth, feed consumption and efficiency, and kidney asparaginase activity were assessed in Experiment 3. The treatments were arranged factorially as in Experiment 1. At the conclusion of the experiment, 3 randomly selected chicks from each replicate group (i.e., 9 chicks per treatment) were sacrificed and the kidneys were immediately excised. Approximately 3 g of kidney from each chick was homogenized in 4 volumes of ice cold .1 M tris buffer, pH 7.6. The crude homogenate was centrifuged at 1000 x g for 10 min at 5° C. Asparaginase activity in the supernatant was measured using methods described by Coon and Balling (1984). Activity was expressed as mg ammonia produced per g kidney per h.

All data were analyzed using analysis of variance procedures, with significance of each main treatment effect and their two- and three-way interactions tested using the general linear models procedure of SAS (1982).

Results

The results of Experiment 1 are presented in Table 2. Feed consumption and efficiency by birds fed the high protein diets were significantly ($P<.01$) higher. Moreover, there was a significant ($P<.01$) protein x electrolyte balance interaction for feed consumption and gain. Increasing the electrolyte balance (i.e., meq Na + K Cl/kg diet) in low protein diets resulted in an 5% reduction

in feed consumption and a 11% reduction in gain. Chicks fed the high protein diets responded oppositely to electrolyte balance, i.e., the higher electrolyte balance improved feed consumption by an average of 10% and gain by an average of 15%. The interaction between crude protein and electrolyte balance was more profound in chicks housed under 16L:8D photoperiod, and in fact, the three-way interaction of crude protein, electrolyte balance, and photoperiod approached significance for gain ($P<.10$) and feed consumption ($P<.06$).

The effect of electrolyte balance on performance of chicks fed low protein diets was further evaluated in Experiment 2 (Table 3). Feed consumption was reduced an average of 7% and gain by an average of 9% when dietary electrolyte balance was increased from 210 to 513 meq/kg. Gain and feed efficiency by birds housed under 16L:8D were significantly ($P<.05$) higher. Males gained more weight and consumed more feed than females. The response to changes in electrolyte balance was not affected by either sex or photoperiod. Plasma ammonia concentrations were significantly ($P<.05$) higher in birds housed under 16L:8D, but were unaffected by diet and sex. Associated with the reduced gain in birds fed 513 meq Na + K - Cl/kg diet, was a significant ($P<.05$) increase in plasma uric acid concentration.

In Experiment 3 (Table 4) the effect of dietary electrolyte balance, protein and photoperiod on growth and kidney asparaginase activity were evaluated. As in Experiments 1 and 2 there was a significant ($P<.05$) protein x electrolyte balance interaction. However,

TABLE 3. Effect of electrolyte balance, sex and photoperiod on performance and plasma concentrations of ammonia and uric acid of chicks (Experiment 2)[a]

Treatment							Plasma	
Diet No.	Electrolyte balance[b]	Sex	Photoperiod	Gain[c]	Feed cons.[d]	Gain/ feed[e]	Ammonia[e]	Uric acid[f]
	(meq/kg)			(g)	(g)	(g/kg)	(ug/ml)	(mg/dl)
1	210	M	23L:1D	453	1083	418	11.48	1.31
2	513	M	23L:1D	390	943	413	8.91	2.16
1	210	F	23L:1D	423	1034	410	9.24	1.59
2	513	F	23L:1D	393	948	414	7.47	2.04
1	210	M	16L:8D	490	1028	477	11.73	1.09
2	513	M	16L:8D	449	1008	446	10.85	1.90
1	210	F	16L:8D	447	1028	435	12.27	1.15
2	513	F	16L:8D	413	953	433	13.81	2.24
			pSEM[g]	15	25	12	.96	.15

[a]Data are means of triplicate groups of 6 chicks each. Chicks were fed the experimental diets from day 1 to day 21 posthatching. Average initial weight was 47 g.

[b]Milliequivalents of Na + K - Cl/kg diet (as analyzed). All diets contained 14.3% crude protein and 76 meq Cl/kg. Milliequivalents of Na and K were (by analysis): diet 1, 169 meq Na and 117 meq K/kg; and diet 2, 372 meq Na and 217 meq K/kg.

[c]The main effects of electrolyte balance, sex and photoperiod were signifcant ($P<.05$).

[d]The main effect of electrolyte balance was significant ($P<.05$).

[e]The main effect photoperiod was significant ($P<.05$).

[f]The main effect of electrolyte balance was significant ($P<.05$).

[g]Pooled standard error of the mean.

TABLE 4. Effect of diet and photoperiod on performance and kidney asparaginase activity of chicks (Experiment 3)[a]

Diet No.	Treatment: Crude protein	Treatment: Electrolyte balance[b]	Treatment: Photoperiod	Gain[c,d]	Feed cons.[c,d]	Gain/feed[c,d]	Asparaginase activity[e]
	%	(meq/kg)		(g)	(g)	(g/kg)	(mg NH_4/g kidney/h)
1	14.3	200	23L:1D	372	851	437	1.585
2	14.3	358	23L:1D	357	844	423	.909
3	28.6	190	23L:1D	542	984	551	1.262
4	28.6	350	23L:1D	639	1111	633	1.045
1	14.3	200	16L:8D	374	864	433	1.115
2	14.3	358	16L:8D	358	849	421	1.002
3	28.6	190	16L:8D	487	926	525	1.249
4	28.6	350	16L:8D	604	998	605	.987
			pSEM[f]	18	21	12	.168

[a]Data are means of triplicate groups of 6 female chicks each for gain, feed, and gain/feed; and means of 9 individual chicks for asparaginase activity. Chicks were fed the experimental diets from day 4 to day 30 posthatching. Average initial weight was 47 g.

[b]Milliequivalents of Na + K - Cl/kg diet (as analyzed). All diets contained 76 meq Cl/kg. Milliequivalents of Na and K were: diet 1, 196 meq Na and 80 meq K/kg; diet 2, 278 meq Na and 156 meq K/kg; diet 3, 196 meq Na and 70 meq K/kg; and diet 4, 283 Na and 142 meq K/kg.

[c]The crude protein x electrolyte balance interaction was significant ($P<.05$).

[d]The main effect of protein was significant ($P<.001$).

[e]The main effect of electrolyte balance was significant ($P<.01$).

[f]Pooled standard error of the mean.

in Experiment 3, the interaction was also significant ($P<.05$) for feed efficiency. Increasing the electrolyte balance from 200 to 350 meq/kg in low protein diets resulted in a 4% reduction in gain and a 2.5% reduction in gain:feed ratio. Conversely, a 20% improvement in gain and a 15% improvement in feed efficiency were observed when electrolyte balance was increased in high protein diets. Photoperiod was without effect, and gain, feed consumption and feed efficiency were significantly ($P<.001$) higher in birds fed the high protein diets. Kidney asparaginase activity was significantly ($P<.01$) higher in birds fed diets containing the lower electrolyte balance irrespective of protein level or photoperiod.

In none of the three experiments did mortality exceed 1%, nor was there any indication that mortality was treatment related.

Discussion

Electrolyte balance has been shown to affect growth and feed intake of chickens (Nesheim et al., 1964; Melliere and Forbes, 1966). Mongin and Sauveur (1977) indicated that 250 meq Na + K Cl/kg diet is the optimum electrolyte balance for growth of young chicks. More recently Johnson and Karunajeewa (1985) observed excellent growth in chicks fed diets containing between 250 and 300 meq/kg. Dietary nitrogen source and level varied considerably among these studies. Melliere and Forbes (1966) fed diets containing 19.5% of a mixture of crystalline amino acids; Nesheim et al. (1964) employed two basal

diets one containing casein, the other soy protein at levels equivalent to 25.9% and 24.8% crude protein, respectively; and Johnson and Karunajeewa (1985) employed practical broiler starter and finisher diets formulated to contain 22% and 18.5% crude protein, respectively. The diets used by Mongin and Sauveur (1977) were not described. However, in none of these studies was the effect of protein level evaluated despite evidence demonstrating an interrelationship between dietary electrolytes and amino acids (cf. Austic and Calvert, 1981).

Results of the studies reported herein indicate that optimum dietary electrolyte balance varies with dietary crude protein concentration. With low protein diets, growth of chicks was depressed diet electrolyte balance was changed by the addition of sodium and potassium. However, sodium and potassium addition to high-protein diets improved growth. In Experiments 1 and 2, the effect of diet electrolyte balance was primarily due to changes in feed consumption. But in Experiment 3, feed efficiency was also significantly affected by electrolyte balance. These results indicate that, within the levels of diet protein evaluated, the optimum electrolyte balance expressed as meq Na + K - Cl/kg is related to the level of diet crude protein.

Shirley (H. V. Shirley, Univ. TN, 1985, personal communication) found that atmospheric ammonia concentration was significantly higher when birds were housed under a 16L:8D vs. a 23L:1D photoperiod which is suggestive of photoperiod-induced changes in physiologic acid-base balance. Photoperiod has also been demonstrated to affect chicken

growth rate, body composition, and incidence of leg disorders (Robbins et al., 1984). Photoperiod was therefore included as an experimental variable. Although photoperiod was without significant effect in Experiment 1, it did tend to affect the magnitude of the protein x electrolyte balance interaction and was therefore retained as an experimental variable in Experiments 2-3. Birds fed the low-protein diets in Experiment 1 tended to gain more under 23L:1D, but in Experiment 2 the opposite was observed, i.e., gain was significantly lower in chicks under 23L:1D. There was no detectable photoperiod effect observed in Experiment 3. Given the lack of any repeatable photoperiod effect, it was concluded that photoperiod does not affect the magnitude or nature of the protein x electrolyte balance interaction.

Chicks fed diets containing the low vs. high electrolyte balance had significantly lower concentrations of plasma uric acid and significantly higher kidney asparaginase activities. Okumura and Tasaki (1968) demonstrated that ammonia excretion was increased in chickens fed diets supplemented with hydrochloric acid. Coon and Balling (1984) reported that kidney asparaginase activity was increased in response to ammonium chloride-induced metabolic acidosis. It is therefore likely that the differences in kidney asparaginase activities observed in these experiments reflect changes in metabolic acid-base metabolism. And, since asparaginase activities were not affected by dietary protein level, the effects of diet electrolyte balance on growth were not related to the effects on acid-base phenomena.

If the changes in plasma uric acid concentration observed in Experiment 2 were the result of changes in rate of uric acid production, then the growth depressing effect of a high electrolyte balance in low protein diets may have been the result of less efficient protein utilization. However, Austic (1980) found that excess potassium increased, and excess chloride decreased, plasma uric acid concentrations as a result of alterations in renal clearance rather than synthesis of uric acid. This effect warrants further study.

The data from these experiments clearly demonstrate that the electrolyte balance requirement is dependent on the level of dietary crude protein. Although more research on the quantitative and qualitative aspects of this relationship is needed, it is likely that, for example, formulation of low-protein diets via crystalline amino acid supplementation, or the addition of excess protein to reduce abdominal fat content would affect the electrolyte balance requirement.

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PART III

EFFECT OF DIETARY ELECTROLYTE BALANCE ON GROWTH AND METABOLIC ACID-BASE STATUS OF CHICKS

Summary

Three experiments were conducted to evaluate the performance, and physiological and metabolic effects of dietary electrolyte balance in broiler chicks. Increasing the electrolyte balance (Na + K Cl) from 200 to 350 meq/kg in diets containing 76 meq of Cl/kg improved feed consumption and gain of chicks fed high protein (28.6%) diets, but depressed feed consumption and gain of chicks fed low protein (14.3%) diets. Increasing Cl to 226 meq/kg in high Na and K diets also depressed growth in both high and low protein diets. The growth responses were primarily the result of changes in feed consumption. The electrolyte balance x protein interaction on growth was less pronounced when feed intake was equalized. High Cl diets depressed blood pH, HCO_3, and base excess. Feeding high versus low electrolyte balance diets increased plasma uric acid concentration and decreased kidney asparaginase activity. The effect of dietary electrolytes on acid-base status and asparaginase activity were independent of crude protein level. Amino acid supplementation of the low protein diet did not alleviate the growth depressing effect of increased electrolyte balance. Further, either the addition of a mixture of K and Na or addition of isomolar levels of K or Na alone depressed growth of chicks fed low protein diets. The results confirmed the previous observation that the electrolyte balance which provides for optimum growth is dependent upon the dietary crude protein content. Moreover, acid-base status, kidney asparaginase activity and

plasma uric acid concentrations were greatly affected by alterations in dietary electrolytes, but these changes were not correlated with the growth response.

Introduction

The importance of a proper balance of dietary electrolytes (Na, K, and Cl) for optimal performance of chickens has been reported (Nesheim et al., 1964). Mongin and Sauveur (1977) concluded that an electrolyte balance (Na + K - Cl) of 250 meq/kg was optimum for growth. In a more recent study (Johnson and Karunajeewa, 1985) optimum electrolyte balance was found to lie between 250 and 300 meq (Na + K - Cl)/kg diet containing 73 meq of Cl/kg. Not only do dietary concentrations of each of the minerals affect utilization of the others, their ratios appear to affect utilization of other dietary nutrients as well. For example, amino acid metabolism is influenced by dietary electrolytes. The severity of the well-studied lysine-arginine antagonism on feed intake and growth (Jones, 1964; Jones et al., 1966; Boorman and Fisher, 1966) is reduced by increasing the concentrations dietary Na and K (O'Dell and Savage, 1966), while increasing dietary Cl exacerbates these detrimental effects (Austic and Calvert, 1981). Alterations in plasma and tissue amino acid concentrations have been associated with dietary K content (Eckel et al., 1958; Robbins et al., 1982), and urinary output of nitrogen in birds is influenced by acid-base balance (Okumura and Tasaki,

1968a) and dietary protein content (Okumura and Tasaki, 1968b; Featherston, 1969).

Results of the previous experiments (Part I) on these nutrients indicated that there is an interaction between dietary protein level and dietary electrolyte balance when dietary levels of Cl near the chick's requirement were fed. Namely, it appears that the electrolyte balance (i.e., meq Na + K - Cl/kg diet) required to maximize gain is related to the level of dietary protein. The objective of the studies reported herein was to determine if this protein x electrolyte balance interaction could be explained by dietary effects on metabolic acidbase balance.

Experimental Procedure

The compositions of the semi-purified basal diets are presented in Table 1. All experimental diets were formulated to contain 3200 kcal metabolizable energy/kg. Dietary sodium, potassium and chlorine concentrations were varied by the addition of sodium citrate, potassium citrate, sodium chloride and/or potassium chloride at the expense of cellulose. The Na and K concentrations of all feed ingredients were determined by atomic absorption spectrophotometry, and Cl was measured using a Dionex ion chromatograph.[1]

In each experiment female Peterson crossbred broiler chicks were used. At one day of age, chicks were sexed with all morbid

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[1]Chloride analyses were conducted by Galbraith Laboratories, Inc., Knoxville, TN.

TABLE 1. Percentage Composition of the Basal Diets[1]

Ingredients	Protein level, %[2] 14.3	28.6
Dextrose	25.00	25.00
Cornstarch	30.99	15.02
Isolated soy protein (85% CP)	16.65	33.30
Corn oil	7.50	7.50
Mineral premix[3]	4.65	4.65
Vitamin premix[4]	0.20	0.20
Choline chloride (50%)	0.40	0.40
DL-Methionine	0.25	0.50
Cellulose	to 100.00	to 100.00

[1]Contained by calculation 3200 Kcal ME/kg.

[2]As analyzed.

[3]Provided per kg diet: calcium chloride dihydrate, 414 g; calcium carbonate, 2.47 g; dibasic calcium phosphate, 25.2; monobasic calcium phosphate, 8.14 g; magnesium oxide, .568 g; manganous sulfate monohydrate, .651 g; ferric citrate, .50 g; zinc carbonate, .10; cupric sulfate pentahydrate, 20 mg; boric acid, 9 mg; sodium molybdate, 9 mg; ethylene diaminedihydroiodide, 40 mg; cobalt sulfate septahydrate, 1 mg; sodium selenite, .21 mg; silica, 4.65 g.

[4]Baker *et al*. (1979).

and underweight chicks discarded. The remaining chicks were then randomly distributed to three replicate groups of 6 chicks each per treatment. The chicks were housed in electrically-controlled battery-brooders in an environmentally-controlled room which was kept at 28° C.

A 23 h light and 1 h dark photoperiod was maintained. The average light intensity at bird level was 14.5 lux. Chicks were fed the experimental diets from day 4 to day 28 posthatching in Experiment 1; from day 5 to day 28 posthatching in Experiment 2; and from day 2 to day 32 posthatching in Experiment 3. Chicks were fed a corn-soy broiler starter diet for the days preceding initiation of the experiments. Feed and water were provided ad libitum in Experiments 1 and 3. In Experiment 2 feed was restricted, but chicks had unlimited access to water.

In Experiment 1, the effects of dietary electrolytes, electrolyte balance and crude protein on performance, kidney asparaginase activity, plasma uric acid, and blood acid-base balance were evaluated. The experimental treatments consisted of 2 levels of dietary crude protein and 2 levels of dietary electrolyte balance (meq Na + K Cl/kg diet). Diets 1 and 4 were formulated to contain 199 meq of Na, 77 meq of K and 76 meq of Cl with an electrolyte balance of 200 meq/kg. The high electrolyte balance diets (Diets 2 and 5) were achieved by addition of Na- and K-citrate while maintaining the same Na:K meq ratio as in the low electrolyte balance diets. The high Cl levels in diets 3 and 6 were attained by addition of KCl and NaCl,

thus maintaining the same Na and K levels as in the high electrolyte balance diets, and the same electrolyte balance of 200 meq/kg as in the low electrolyte balance diets. Following the 24-day feeding period, blood samples were collected in lithium-heparinized syringes via cardiac puncture from 3 randomly chosen chicks from each replicate group. Blood samples were pooled within replicate groups, thus providing 3 samples per treatment. The blood samples were immediately analyzed for pH, pCO_2, HCO_3 and base excess (Dobson, 1980) using an automated blood pH-gas analyzer (Model 158, Corning Ltd. Galstead, Essex, England) at a temperature of 38°C and standardized at a hemoglobin concentration of 15.0 g/dl. The same birds were then sacrificed by cervical dislocation and the kidneys were immediately excised. Approximately 3 grams of kidney from each chick was homogenized in 4 volumes of ice-cold 0.1M tris buffer, pH 7.6. The crude homogenate was centrifuged at 1000 x g for 10 minutes at 5° C. Asparaginase activity in the supernatant was measured using methods described by Coon and Balling (1984). Activity was expressed as mg ammonia produced per g kidney per h. From the 3 remaining birds in each replicate group, 4 ml of blood was obtained via heart puncture using lithium-heparinized syringes. Plasma was collected and frozen for later analysis. Plasma concentrations of uric acid were determined enzymatically using a commercial diagnostic kit (Sigma Chemical Co., St. Louis, MO).

In Experiment 2 the diets and treatment design were the same as in Experiment 1. However, feed intake was equalized across all

treatments. Experiments 1 and 2 were conducted at the same time. The daily feed offered to the birds in Experiment 2 was based on the lowest feed consumed by any group in Experiment 1 during the previous day.

Experiment 3 was designed to specifically determine the effect of dietary cation source and electrolyte balance on performance of chicks fed the low protein (14.3%) diets. The low (150 meq) electrolyte balance diet (Diet 1) contained 95 meq Na, 131 meq K, and 76 meq Cl. In Diet 2 a mixture of Na- and K-citrate (1:2.33 molar ratio) was added to the basal diet to achieve an electrolyte balance of 350 meq/kg. To Diet 3 was added 140 meq K/kg (isomolar to that added to Diet 2); and to Diet 4 was added 200 meq K/kg (isomolar to the total added cation level in Diet 2). Similarly, to Diets 5 and 6 were added 60 and 200 meq Na/kg, respectively, which are levels isomolar to the level of added Na and total cation, respectively, in Diet 2. The calculated amino acid composition of the low protein diet was deficient in methionine, theronine, and lysine when compared to National Research Council (1984) requirements. For this reason, diets identical to Diets 1 and 2 were supplemented with these limiting amino acids to evaluate their possible involvement in the growth depressing effect of a high electrolyte balance in the low protein diet.

All data were analyzed using analysis of variance procedures, with the significance of treatment effects tested using the GLM procedure of the Statistical Analysis System (1982).

Results and Discussion

The results of Experiment 1 are shown in Tables 2 and 3. Feed consumption, gain and efficiency of gain were significantly ($P<.001$) higher in birds fed the high protein diets (Table 2). In addition, there was a significant ($P<.05$) electrolyte balance x protein interaction for gain and feed consumption. An increase in the electrolyte balance from 200 to 350 meq/kg by addition of Na and K to low protein diets depressed feed consumption by an average of 4% and growth by an average of 5%. The reverse occurred in chicks fed the high protein diets, i.e., the higher electrolyte balance improved feed consumption and gain by an average of 10% and 8% respectively. Increasing the Cl content (Diets 3 and 6) while maintaining the same electrolyte balance as in Diets 1 and 4 depressed ($P<.05$) feed intake and gain but this effect was independent of protein level. The lack of a significant electrolyte balance effect on feed efficiency suggests that the growth response to changes in electrolyte balance were primarily the result of changes in feed consumption.

It has been reported (Leach et al., 1960) that increasing the anion (SO_4) content of the diet while maintaining constant cation content depressed growth of chicks. Sauveur and Mongin (1978) also indicated that metabolic acidosis due to ingestion of excess Cl resulted in a growth depression which could be overcome by supplementing the diet with Na and K. It might therefore be expected that the deleterious effect of excess dietary cation (Na and K) might be ameliorated by increasing the dietary anion (Cl) content. However, in

TABLE 2. Effect of Dietary Electrolyte Balance and Crude Protein on Performance of Chicks (Experiment 1)[a]

Diet no.	Electrolyte Na	K	Cl	Electrolyte balance	Crude protein	Gain[b-f]	Feed Intake[c,d]	Gain/Feed[b,e,f]
	--(meq/Kg)--			(meq/kg)	(%)	(g)	(g)	(g/kg)
1	199	77	76	200	14.3	622	1088	571
2	308	118	76	350	14.3	591	1040	568
3	308	118	226	200	14.3	573	1038	552
4	199	77	76	200	28.6	650	968	671
5	308	118	76	350	28.6	703	1042	675
6	308	118	226	200	28.6	631	960	658
				pSEM[g]		13	26	7

[a]Data are means of triplicate groups of 6 chicks each. Average initial weight was 69 g. Chicks were fed the experimental diets from day 4 to day 28 posthatching.

[b]The interaction of electrolyte balance x crude protein (Trt 1 + 4 vs. 2 + 5) was significant ($P<.03$).

[c]The main effect of protein was significant ($P<.001$).

[d]The interaction of electrolyte balance x crude protein (Trt 1 + 4 vs. Trt 2 + 5) was significant ($P<.05$).

[e]The effect of Cl in diets containing equal electrolyte balance (Trt 1 + 4 vs. Trt 3 + 6) was significant ($P<.05$).

[f]The effect of Cl in diets containing the high levels of Na and K (Trt 2 + 5 vs. Trt 3 + 6) was significant ($P<.05$).

[g]Pooled standard error of the mean.

TABLE 3. Effect of Dietary Electrolyte Balance and Crude Protein on Blood Acid-Base Status, Kidney Asparaginase Activity and Plasma Uric Acid Concentration of Chicks (Experiment 1)[a]

Diet no.[b]	Plasma uric acid[c,d,e]	pH[c,e,f]	pCO_2	HCO_3[e]	Base excess[e,f]	Asparaginase activity[c]
	(mg/dl)		(mmHg)	(mmole/L)	(mmole/L)	(mg NH_4/g kidney/h)
1	3.62	7.462	33.733	23.600	2.200	1.021
2	4.36	7.399	40.000	24.333	1.166	0.899
3	4.01	7.309	40.833	20.067	-4.567	1.119
4	7.13	7.451	34.967	23.600	2.000	1.004
5	7.40	7.403	38.167	23.367	0.533	0.916
6	7.63	7.336	38.566	20.267	-3.667	1.104
pSEM[g]	0.14	0.20	3.10	1.70	1.70	0.04

[a]Data are means of nine individual observations for plasma uric acid and asparaginase activity. All other data are means of three observations.

[b]See Table 2 for diet description.

[c]The interaction of electrolyte balance x crude protein (Trt 1 + 4 *vs*. 2 + 5) was significant ($P<.05$).

[d]The main effect of protein was significant ($P<.001$).

[e]The effect of Cl in diets containing the high levels of Na and K (Trt 2 + 5 *vs*. Trt 3 + 6) was significant ($P<.05$).

[f]The effect of Cl in diets containing equal electrolyte balance (trt 1 + 4 *vs*. Trt 3 + 6) was significant ($P<.05$).

[g]Pooled standard error of the mean.

this experiment the detrimental effect of excess cation in Diet 2 (Table 2) was further exaggerated by Cl supplementation (Diet 3). Thus, the concept of an optimum "electrolyte balance" as described by Sauveur and Mongin (1978) may only be valid within a narrow range of dietary chloride levels.

The effects of dietary electrolyte balance and protein concentration on acid-base balance, plasma uric acid concentration, and kidney asparaginase activity are shown in Table 3. Birds fed the high Cl diets (Diets 3 and 6) were indeed acidotic as indicated by the depressed ($P<.05$) blood pH, HCO_3, and base excess (BE). The addition of Na and K to low Cl diets (Diets 2 and 5) resulted in a slight depression in pH and a significant ($P<.05$) decrease in BE. The response of chicks to diets containing high levels of Cl was similar to that previously reported (Cohen et al., 1972; Sauveur and Mongin, 1978). However, it is difficult to explain why BE was depressed when Na and K were added to the diets. Perhaps this was an indirect effect of the observed reduction in asparaginase activity coupled with increased plasma uric acid concentration, since both of these changes would tend to depress BE. The blood acid-base responses to alterations in dietary electrolyte balance at low Cl levels (76 meq) were independent of protein level and hence not correlated with the effects of electrolyte balance on growth. These findings are similar to those of Johnson and Karunajeewa (1985) in that they too were unable to correlate the effects of electrolyte balance (at low chloride levels) on growth with blood acid-base status.

Ammonia production as measured by kidney asparaginase activity was unaffected by protein level, but was depressed ($P<.05$) by an average of 10% in chicks fed diets containing high electrolyte balance (350 meq/kg). Unlike the effect on growth, diets formulated to contain 200 meq/kg with either a low or high level of Cl elicited the same effect. Other researchers have reported changes in ammonia production as a result of dietary electrolyte modifications. Metabolic acidosis induced by feeding diets containing ammonium chloride increased kidney asparaginase activity (Coon and Balling, 1984). Okumura and Tasaki (1968) demonstrated that both ammonia production and excretion were increased in chickens fed diets supplemented with hydrochloric acid, whereas the addition of sodium bicarbonate had the opposite effect.

Plasma uric acid concentration was also influenced by the various alterations in dietary electrolytes and protein. An increase in electrolyte balance from 200 to 350 meq/kg by addition of Na and K (Diets 1 and 4 versus 2 and 5) resulted in an increase in plasma uric acid, with the magnitude of this response being greater in chicks fed the low protein diets. Increasing Cl content while maintaining the same cation concentrations did lower plasma uric acid content in chicks fed the low protein diets (Diet 3 versus 2), but unexpectedly increased the plasma uric acid concentration in chicks fed the high protein diets (Diet 6 versus 5). In general, these results support the conclusion of Austic (1980) that diets favoring acidosis (a low cation:Cl ratio) lowered plasma uric acid concentrations, whereas

those favoring alkalosis (a high cation:Cl ratio) increased plasma uric acid concentrations. The increase in plasma uric acid in response to high protein diets is consistent with earlier studies (Okumura and Tasaki, 1968; Featherston, 1969).

Because the nature and the magnitude of the electrolyte balance x crude protein interaction was apparently related to a specific effect on feed intake, the same treatments were repeated in Experiment 2 under conditions of equalized feeding. In this experiment (Table 4) only dietary crude protein significantly ($P<.0001$) affected growth rate and feed efficiency. However, even under these conditions there was a numerical tendency for reduced gain and efficiency when Na and K were increased in low protein diets (Diets 1 versus 2), and improved gain and efficiency when added to high protein diets (Diet 4 versus 5), but the effect was not significant.

Some workers have reported improved performance when K and/or Na were added to low protein diets. Leibholz et al. (1966) showed that potassium acetate improved the performance of pigs fed protein-deficient diets. Similar responses were observed when either sodium or potassium bicarbonate was added to lysine-deficient diets (Madubuike et al., 1980; Austic et al., 1980). However, results of the preceding experiments (Part I) indicated that increasing the electrolyte balance by addition of Na and K consistently depressed growth on low-protein diets. In light of these observations the specific effects of dietary cations, electrolyte balance and amino acid supplementation on growth of chicks fed the low protein diet were further assessed.

TABLE 4. Effect of Dietary Electrolyte Balance and Crude Protein on Performance of Chicks when Feed Intake was Equalized (Experiment 2)[a]

Diet no.	Electrolyte Na	Electrolyte K	Electrolyte CL	Electrolyte balance	Crude protein	Gain[b]	Gain/Feed[b]
	-----(meq/KG)-----			(meq/kg)	(%)	(g)	(g)
1	199	77	76	200	14.3	521	547
2	308	118	76	350	14.3	507	532
3	308	118	226	200	14.3	520	545
4	199	77	76	200	28.6	582	577
5	308	118	76	350	28.6	591	619
6	308	118	226	200	28.6	587	615
					pSEM[c]	9	9

[a]Data are means of triplicate groups of 6 chicks each. Average initial weight was 79 g. Average feed intake was 954 g per replicate. Chicks were fed the experimental diets from day 5 to day 28 posthatching.

[b]The main effect of protein was significant ($P<.0001$).

[c]Pooled standard error of the mean.

The results of this experiment are presented in Table 5. As in Experiment 1, the high electrolyte balance (350 meq/kg) depressed ($P < .005$) feed consumption and gain. The independent addition of K (Diet 3) or Na (Diet 5) at levels equivalent to those in Diet 2 also depressed ($P < .025$) feed consumption growth. The addition of K (Diet 4) or Na (Diet 6) at levels equivalent to the total amount of added cation (200 meq/kg) in Diet 2 further depressed ($P < .005$) fed consumption and gain. Although both Na and K were growth depressing, Na was more toxic on a molar basis. Similar observations were made by Sauveur and Mongin (1974). Talbot (1978) found that the toxic effect of excess Na could be overcome by the dietary addition of either K or Cl. Amino acid supplementation (Diets 7 and 8 versus 1 and 2) did significantly ($P < .005$) improve feed consumption and gain, but the effect of electrolyte balance still manifested itself.

The results of these studies clearly demonstrate that the dietary electrolyte balance which provides for optimal growth is dependent on diet protein concentration. Although alterations in dietary electrolyte balance did not affect kidney asparaginase activity, plasma uric acid concentration, and acid-base status, the changes observed were not correlated with the growth response. The nature and the magnitude of the electrolyte balance x protein interaction observed in these experiments were primarily the result of a specific effect on feed intake.

TABLE 5. Effect of Dietary Cation Level, Electrolyte Balance and Supplemental Amino Acids on Performance of Chicks (Experiment 3)[a]

Diet		Electrolyte balance	Gain[b]	Feed intake[b]	Gain/ Feed
		(meq/kg)	(g)	(g)	(g/kg)
1	Basal diet[c]	150	571	1147	498
2	Basal + 60 meq Na^+ + 140 meq K^+	350	513	1047	490
3	Basal + 140 meq K^+	290	534	1064	502
4	Basal + 200 meq K^+	350	545	1091	500
5	Basal + 60 meq Na^+	210	550	1066	517
6	Basal + 200 meq Na^+	350	515	1064	483
7	Diet 1 + 0.10% DL-Met. + 0.10% L-Thr + 0.05% L-Lys HCl	150	621	1189	522
8	Diet 2 + 0.10% DL-Met. + 0.10% L-Thr + 0.05% L-Lys HCl	350	566	1103	513
		pSEM[d]	11	16	7

[a]Data are means of triplicate groups of 6 chicks each. Average initial weight was 46 g. Chicks were fed the experimental diets from day 2 to day 32 posthatching.

[b]Effect of supplemental Na and K (Trt 1 vs. Trt 2) was significant ($P<.005$); effect of K and Na in isomolar concentration to that added to diet 2 (Trt 1 vs. Trt 3 + 5) was significant ($P<.025$); effect of K and Na in isomolar concentration to the total cation added to diet 2 (Trt 1 vs. Trt 4 + 6) was significant ($P<.005$); effect of supplemental amino acids (Trt 1 + 7 vs Trt 2 + 8) was significant ($P<.005$).

[c]Contained 95 meq Na^+, 131 meq K^+ and 76 meq Cl^- (Table 1, 14.3% CP).

[d]Pooled standard error of the mean.

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PART IV

DIETARY ELECTROLYTE REQUIREMENT OF BROILER CHICKS AS AFFECTED BY DIETARY PROTEIN CONTENT

Summary

A soy protein based semi-purified diet was used to estimate the optimum electrolyte balance (meq Na + K - Cl/kg diet) at 3 levels of dietary crude protein. Results indicated that in diets containing 14.3% crude protein (CP) the optimum electrolyte balance was approximately 250 meq/kg; at 21.4% CP the optimum was about 325 meq/kg; and at 28.6% CP it was about 400 meq/kg. A conventional 21% CP corn-soy broiler diet which, by calculation, contained 228 meq Na + K - Cl/kg was used in two subsequent experiments. In both experiments, it was found that feed intake, gain and feed efficiency were significantly improved ($P<.05$) by the addition of 100 meq Na + K/kg diet in diets containing 56.4 meq Cl/kg, thus confirming an optimum electrolyte balance of about 325 meq/kg in diets containing 21% CP. It was concluded that the optimum dietary electrolyte balance of broiler chicks is dependent on diet crude protein content. Further, within the range of 14% to 28% CP, the optimum electrolyte balance is increased by about 11 meq/kg per 1 percentage unit increase in diet crude protein content.

Introduction

The results of the preceding experiments (parts 2 and 3) have shown that there is a marked interaction between dietary crude protein level and dietary electrolyte balance (meq Na + K - Cl/kg diet).

Namely, increasing the electrolyte balance from 200 meq/kg to 350 meq/kg or more improved gain and feed consumption of birds fed high protein diets, but depressed growth of birds fed low protein diets. It was found that although dietary electrolyte balance did affect kidney asparaginase activity, plasma uric acid concentration and metabolic acid-base status, these observed effects were not statistically correlated with the growth response. The nature and the magnitude of the electrolyte balance x protein interaction observed in these experiments were apparently the result of a specific effect on feed consumption.

Interest has been centered on these monovalent electrolytes (Na, K, Cl) largely because of their prominent effect on metabolic acid-base balance and because they are among the minerals most likely to be varied in diet formulation (Mongin and Sauveur, 1977; Leach, 1979). Mongin and Sauveur (1977), indicated that an electrolyte balance of 250 meq (Na + K - Cl/kg diet) was optimum for growth of young chicks. Johnson and Karunajeewa (1985) observed that the optimum electrolyte balance lies between 250 and 300 meq/kg diet. However, in neither of these reports was the effect of protein level evaluated. The electrolyte balance x crude protein interaction observed in preceding studies (parts 2 and 3) strongly suggests that the electrolyte balance required to maximize growth is proportional to the level of dietary protein.

The present studies were undertaken to quantify the optimum dietary electrolyte balance at three protein levels utilizing semi-purified diets, and to evaluate the applicability of such estimates to a conventional corn-soy diet.

Experimental Procedure

In each experiment female Peterson crossbred broiler chicks were used. Chicks were then randomly distributed to four replicate groups of 6 chicks each per treatment in Experiment 1 and to three replicate groups of 6 chicks each per treatment in both experiments 2 and 3. The chickens were housed in electrically-heated battery brooders in an environmentally-controlled room which was kept at 25°C. Chicks were fed the experimental diets from day 4 to day 32 posthatching in Experiment 1; from day 5 to day 26 posthatching in Experiment 2; and from day 1 to day 29 posthatching in Experiment 3. A corn-soy broiler starter diet was fed to the chicks for the day preceding the initiation of the experiments. Feed and water were provided ad libitum throughout the experimental periods.

The composition of the semi-purified basal diets and the elemental composition of all experimental diets utilized in Experiment 1 are presented in Tables 1 and 2, respectively. All diets were formulated to meet or exceed the nutrient requirements for growing chicks (NRC, 1984). The experimental treatments consisted of 3 levels of dietary electrolyte balance (meq Na + K - Cl/kg diet) at each of the three protein levels. The choice of the various electrolyte balances used was based on preceding experiments (parts 2 and 3) and governed by the Na and K content of the basal diets. The concentrations of these elements increase as the protein level increases. These electrolyte balances were achieved by the addition of Na- and K-citrate

TABLE 1. Percentage Composition of the Basal Diets (Experiment 1)[a]

Ingredient	Protein level[b] 14.3%	21.4%	28.6%
Dextrose	25.00	25.00	25.00
Cornstarch	30.99	23.00	15.02
Isolated soy protein (85% CP[b])	16.65	24.97	33.30
Corn oil	7.50	7.50	7.50
Mineral premix[c]	4.65	4.65	4.65
Vitamin premix[d]	.20	.20	.20
Choline chloride	.40	.40	.40
DL-methionine	.25	.375	.50
Cellulose	to 100.00	100.00	100.00

[a]Contained by calculation 3200 kcal ME/kg.

[b]As analyzed.

[c]Provided per kg diet: calcium chloride dihydrate, 4.14 g; calcium carbonate, 2.47 g; dibasic calcium phosphate, 25.2 g; monobasic calcium phosphate, 8.14 g; magnesium oxide, .568 g; manganous sulfate monohydrate, cupric sulfate pentahydrate, 20 mg; boric acid, 9 mg; sodium molybdate, 9 mg; ethylene diaminedihydroiodide, 40 mg; cobalt sulfate septahydrate, 1 mg; sodium selenite, .21 mg; silica, 4.65 g.

[d]Baker *et al.* (1979).

TABLE 2. Elemental Composition of the Experimental Diets (Experiment 1)[a]

	Diets								
	1	2	3	4	5	6	7	8	9
Sodium, meq/kg	94	174	254	139	221	304	183	268	352
Potassium, meq/kg	82	152	222	112	180	247	153	208	274
Chloride, meq/kg	76	76	76	76	76	76	76	76	76
Electrolyte balance, meq/kg[b]	100	250	400	175	325	475	250	400	550

[a]Calculations based on mineral analyses of all raw ingredients of the diet.

[b]Calculated as meq (Na + K - Cl)/kg diet.

at the expense of cellulose. The Na and K concentrations of all dietary ingredients were determined by atomic absorption spectrophotometry, and Cl was measured using a Dionex ion chromatograph.

Experiments 2 and 3 were conducted to evaluate the applicability of the determined optimum electrolyte balance (325 meq/kg at 21.4% crude protein) estimated in Experiment 1 to a conventional corn-soy based diet containing 21% crude protein. The experimental diets were formulated based on ingredient compositions of NRC (1984). The electrolyte balance of the basal (control) diet was calculated to be 228 meq/kg (Table 3). The experimental diet was prepared by the additon of 100 meq Na + K (1:2.27 molar ratio) to the basal diet at the expense of corn, thus achieving an electrolyte balance of 328 meq/kg.

Results and Discussion

The results of Experiment 1 are presented in Table 4. The quadratic effect of electrolyte balance on gain was significant ($P<.05$) at each level of crude protein. There were no significant effect of electrolyte balance on feed intake of chicks fed diets containing either 21.4% or 28.6% dietary crude protein, however, there was a significant ($P<.006$) quadratic effect in chicks fed diets containing 14.3% crude protein. The quadratic effect of electrolyte balance on feed efficiency was significant ($P<.005$) when diets containing either 14.3% or 21.4% crude protein were fed. The linear effect of electrolyte balance on feed efficiency was significant ($P<.005$) for chicks fed 28.6% CP diets.

TABLE 3. Composition of the Basal Diet (Experiments 2 and 3)

Ingredient	%	Calculated Analysis	
Corn	61.36	CP, %	21.00
Fish meal	3.55	ME, kcal/kg	3200.00
Rock phosphate	1.49	Ca, %	1.00
Limestone	.63	P (avail.), %	.50
Salt	.23	Na, %	.20
Soybean meal	27.71	K, %	.773
Soy oil	4.18	Cl, %	.20
Vitamin premix[a]	.60	Met + Cys, %	.94
DL-methionine	.25	Lys, %	1.20
Total	100.00	Na + K - Cl, meq/kg	228

[a]Provided per kg diet: vitamin D_3, 750 ICU; vitamin A, 4,175 IU; riboflavin, 4.74 mg; vitamin B_{12}, .011 mg; choline, 468 mg; niacin, 41.7 mg; D-Ca-pantothenate, 7.34 mg.

TABLE 4. Effect of Dietary Electrolyte Balance on Performance of Chicks at Different Protein Levels (Experiment 1)[a]

Diet No.	Treatment: Electrolyte balance[b]	Treatment: Crude protein	Gain[c]	Feed cons.[d]	Gain/feed[e]
	(meq/kg)	(%)	(g)	(g)	(g/kg)
1	100	14.3	620	1256	494
2	250	14.3	667	1316	507
3	400	14.3	602	1230	489
4	175	21.4	725	1253	579
5	325	21.4	755	1274	593
6	475	21.4	720	1229	586
7	250	28.6	702	1179	596
8	400	28.6	733	1200	611
9	550	28.6	714	1171	609
		pSEM[f]	13	25	5

[a]Data are means of quadruplicate groups of 6 female chicks each. Chicks were fed the experimental diets from day 4 to day 32 posthatching. Average initial weight was 71 g.

[b]See Table 2 for complete description.

[c]Quadratic effect of electrolyte balance was significant ($P<.005$) at both 14.3% and 21.4% CP levels and was significant ($P<.05$) at 28.6% CP.

[d]Quadratic effect of electrolyte balance was significant ($P<.005$) at 14.3% CP.

[e]Quadratic effect of electrolyte balance was significant ($P<.005$) at both 14.3% and 21.4% CP levels. Linear effect of electrolyte balance was significant ($P<.005$) at 28.6% CP diets.

[f]Pooled standard error of the mean.

Within each level of crude protein, feed efficiency and gain were highest when diets contained the middle level of electrolyte balance, thus indicating optimum electrolyte balances of near 250 meq/kg in diets containing 14.3% CP, near 325 meq/kg in diets containing 21.4% CP, and near 400 meq/kg in diets containing 28.6% CP. The optimal electrolyte balance of 325 meq/kg for diets containing 21% CP is close to the range (250-300 meq/kg) reported by Johnson and Karunjeewa (1985). Mongin and Sauveur (1977) reported an optimum electrolyte balance of 250 meq/kg, but it is not clear what levels of dietary crude protein were used in their experiments. The results of Experiments 2 and 3 are presented in Table 5. Addition of Na- and K-citrate to the conventional corn-soy diet to achieve an electrolyte balance of 328 meq/kg resulted in a significant ($P<.05$) increase in feed intake, body weight gain, and feed efficiency in both Experiments 1 and 2.

The present studies demonstrated that an electrolyte balance of about 325 meq/kg is required to support maximum gain and feed efficiency of broilers fed typical corn-soy-based commercial broiler diets containing 21% CP. Moreover, the data indicated that, within the range of 14 to 28% dietary crude protein, the optimum electrolyte balance is increased by approximately 11 meq (Na + K - Cl)/kg per 1 percentage unit increase in diet protein content.

TABLE 5. Effect of Electrolyte Balance on Growth of Chicks Fed a Practical-Type Diet (Experiment 2 and 3)

Variable	Electrolyte balance, meq Na + K - Cl/kg diet					
	Experiment 2[a]			Experiment 3[b]		
	228	328	(pSEM)[c]	228	328	(pSEM)[c]
Gain, g	532	580	(9)	695	761	(13)
Feed consumed, g	870	929	(14)	1104	1188	(20)
Gain/feed, g/kg	611	629	(2.9)	629	641	(3.5)

[a]Data are means of 3 groups of 6 chicks each fed the diets from day 5 to day 26 posthatching. Average initial weight was 59 g. The effect of electrolyte balance was significant ($P<.04$) for each variable.

[b]Data are means of 6 groups of 6 chicks each fed the diets from day 1 to day 29 posthatching. Average initial weight was 40 g. The effect of electrolyte balance was significant ($P<.01$) for each variable.

[c]Pooled standard error of the mean.

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