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Primary Audience: Nutritionists, Poultry Scientists

SUMMARY

A proper dietary electrolyte balance (DEB) is essential to ensure an optimum acid-base equilibrium and broiler performance. In low-CP diets, this balance can be affected by reduction of soybean meal and inclusion of high levels of synthetic amino acids. Although, some studies have related low-protein diets supplemented with amino acids and DEB, these relations are not well explained, because some research demonstrates confusion about the deficiency and balance of nutrients. The objective of these experiments was to evaluate the DEB effects of diets with low levels of protein supplemented with amino acids on broiler performance and bone development. Results indicated that DEB and CP content influenced broiler chick performance in the starter and growing periods. There was no significant effect due to the interaction between DEB and CP content for tibial dyschondroplasia incidence (TD) or in bone breaking resistance during the growing period of either experiment. The incidence of TD was reduced with 253 mEq/kg DEB in the starter period.

Key words: electrolyte balance, low protein, tibial dyschondroplasia

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DESCRIPTION OF PROBLEM

Broiler diets with CP content lower than the values suggested by the NRC [1], and supplemented with the limiting amino acids (lysine and methionine), can support performance results similar to those diets with higher levels of CP. Such diets can also reduce nitrogen losses, thereby decreasing environmental pollution. However, those results are not always obtained. The manipulation of the dietary electrolyte balance (DEB) has been proposed as one way to improve the performance of chickens fed low-CP diets. The DEB varies according to dietary CP [2], because the growth of chickens fed low-CP level diets decreases when DEB is altered by Na and K additions. However, the relationship between low-CP-amino-acid-supplemented diets and DEB in broiler chickens is poorly understood [3, 4].

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TABLE 1. Composition (%) and calculated nutrient content of starter diets in experiment 1

	Dietary electrolyte balance[A] (mEq/kg)					
	200		260		320	
	CP level (%)					
Ingredient	17	19	17	19	17	19
Corn grain	69.76	62.25	68.65	61.44	68.18	60.96
Soybean meal (45 % CP)	23.84	30.53	24.08	30.70	24.20	30.79
Dicalcium phosphate	1.86	1.83	1.86	1.84	1.87	1.84
Limestone	1.22	1.19	1.22	1.18	1.22	1.18
Vegetable oil	0.88	2.19	1.25	2.46	1.46	2.62
DL-Methionine, 99%	0.46	0.40	0.46	0.40	0.30	0.40
L-Lysine-HCl, 78%	0.57	0.36	0.56	0.35	0.56	0.35
Vitamin-mineral[B,C]	0.60	0.60	0.60	0.60	0.60	0.60
Antioxidant	0.01	0.01	0.01	0.01	0.01	0.01
Salt	0.05	0.33	0.04	0.21	0.05	0.10
$NaHCO_3$	0.26	0.01	0.75	0.51	1.27	1.02
KCl	0.49	0.30	0.52	0.30	0.28	0.13
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis						
ME (kcal/kg)	3,050	3,050	3,050	3,050	3,050	3,050
	(%)					
TSAA	1.00	1.00	1.00	1.00	1.00	1.00
Lysine	1.25	1.25	1.25	1.25	1.25	1.25
Calcium	1.00	1.00	1.00	1.00	1.00	1.00
Nonphytate P	0.45	0.45	0.45	0.45	0.45	0.45
Sodium	0.11	0.15	0.24	0.24	0.38	0.33
Chloride	0.30	0.38	0.31	0.31	0.20	0.16
Potassium	0.93	0.95	0.95	0.95	0.83	0.86

[A]DEB (mEq/kg) = %Na × 10,000/23* + %K × 10,000/39* − %Cl × 10,000/35.5* (* indicates molecular weight).
[A]Vitamin premix provided per kilogram of product 2,000,000 IU vitamin A, 400,000 IU vitamin D_3, 5,000 IU vitamin E, 600 mg vitamin K_3, 400 mg vitamin B_1, 1,200 mg vitamin B_2, 800 mg vitamin B_6, 200 mg folic acid, 6,000 mg nicotinic acid, 20 mg D-biotin; 2,400 mg pantothenic acid; 52,000 mg choline; 3,000 mg vitamin B_{12}; Se 80 mg; methionine 372,400 mg; antioxidant (BHT) 19,600 mg; coccidiostat (monensin) 100,000 mg; growth promoter (zinc bacitracin), 10,000 mg.
[C]Trace mineral premix provided per kilogram of product Fe ($FeSO_4 \cdot 7H_2O$), 100,000 mg; Mn ($MnSO_4 \cdot H_2O$), 16,000 mg; Zn ($ZnSO_4 \cdot 7H_2O$), 100,000 mg; Cu ($CuSO_4 \cdot 5H_2O$). 20,000 mg; Co, 2,000 mg; I ($Ca(IO_3) \cdot H_2O$), 22,000 mg. .

Moreover, some research [5] suggests that diets supplemented with high levels of amino acids have harmful effects by interfering with the acid-base balance. The DEB alteration of these diets causes subclinical acidosis and reduces growth rate. Reduction of CP in diets results in less potassium, due to inclusion of less soybean meal [4]. The reduction of potassium in diets and consequent reduction of DEB can decrease performance and increase the incidence of tibial dyschondroplasia (TD).

It has been observed that by keeping Na + K + Cl balances between 150 and 300 mEq/kg [6, 7, 8, 9] during the starter period and 249 to 261 mEq/kg during the grower period [10] the incidence of TD is not increased, but the performance of broiler chickens is improved. Thus, the objective of these experiments was to evaluate the DEB effects in corn-soybean meal diets with low-CP content, supplemented with synthetic amino acids, on broiler performance, TD incidence, and bone breaking resistance during the growing period.

MATERIAL AND METHODS

Two experiments were conducted in a conventional open-sided house with side-wall curtains that was divided into pens of 8.1 m^2. Fresh wood shavings were used as litter. In the first experiment, 1-d-old Cobb male broiler chicks were randomly distributed in a three by two factorial arrangement of treatments with DEB and CP levels as main effects. Four replicates of 50 birds each were assigned to each

TABLE 2. Composition (%) and calculated nutrient content of grower diets in experiment 2

	Dietary electrolyte balance[A] (mEq/kg)								
	200			240			280		
	CP level (%)								
Ingredient	14	16	18	14	16	18	14	16	18
Corn grain	78.86	71.68	64.11	77.48	70.91	63.71	76.85	70.57	62.83
Soybean meal (45 % CP)	15.12	21.73	28.38	16.27	21.89	28.51	16.40	21.91	28.69
Dicalcium phosphate	1.52	1.49	1.47	1.36	1.50	1.47	1.36	1.50	1.47
Limestone	1.25	1.21	1.17	1.34	1.21	1.16	1.34	1.21	1.16
Vegetable oil	0.80	2.01	3.27	1.27	2.26	3.47	1.48	2.29	3.76
DL-Methionine, 99%	0.29	0.23	0.17	0.12	0.23	0.17	0.13	0.23	0.17
L-Lysine-HCl, 78%	0.65	0.44	0.23	0.35	0.44	0.23	0.34	0.44	0.23
Vitamin-mineral[B,C]	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55
Antioxidant	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Salt	0.02	0.19	0.34	0.02	0.17	0.29	0.02	0.16	0.31
$NaHCO_3$	0.57	0.33	0.08	0.87	0.66	0.41	1.20	1.00	0.80
KCl	0.36	0.14	0.20	0.36	0.17	0.02	0.32	0.13	0.02
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calculated analysis									
ME (kcal/kg)	3.150	3.150	3.150	3.150	3.150	3.150	3.150	3.150	3.150
	(%)								
TSAA	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Lysine	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
Calcium	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90
Nonphytate P	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38
Sodium	0.18	0.18	0.17	0.26	0.26	0.24	0.35	0.35	0.34
Chloride	0.22	0.22	0.25	0.22	0.22	0.22	0.20	0.20	0.26
Potassium	0.72	0.72	0.77	0.74	0.74	0.77	0.72	0.72	0.77

[A]DEB (mEq/kg) = %Na × 10,000/23* + %K × 10,000/39* − %Cl × 10,000/35.5* (* indicates molecular weight).
[B]Vitamin premix provided per kilogram of product: 2,000,000 IU vitamin A; 400,000 IU vitamin D_3; 5,000 IU vitamin E; 600 mg vitamin K_3; 400 mg vitamin B_1; 1,200 mg vitamin B_2; 800 mg vitamin B_6; 200 mg folic acid; 6,000 mg nicotinic acid; 20 mg D-biotin; 2,400 mg pantothenic acid; 52,000 mg choline; 3,000 mg vitamin B_{12}; Se 80 mg; methionine 372,400 mg; antioxidant (BHT) 19,600 mg; coccidiostat (monensin) 100,000 mg; growth promoter (zinc bacitracin), 10,000 mg.
[C]Trace mineral premix provided per kilogram of product: Fe ($FeSO_4 \cdot 7H_2O$), 100,000 mg; Mn ($MnSO_4 \cdot H_2O$), 16,000 mg; Zn ($ZnSO_4 \cdot 7H_2O$), 100,000 mg; Cu ($CuSO_4 \cdot 5H_2O$), 20,000 mg; Co, 2,000 mg; I ($Ca(IO_3) \cdot H_2O$), 22,000 mg.

TABLE 3. Regression equations for performance parameters of broilers fed grower diets containing different levels of CP and dietary electrolyte balance (DEB) from 1 to 21 d of age (experiment 1)

Parameter	Regression equation	DEB × CP	R^2
Feed conversion$_{19\%CP}$ (g:g)	Y = 1.6448 − 0.0021939 DEB	CP = 19% DEB = negative linear effect	0.90
Feed conversion$_{17\%\ CP}$ (g:g)	Y = 1.94509 − 0.00311809 DEB + 0.000004755 DEB^2	CP = 17% DEB = 328 mEq/kg	0.89
Body weight (g)	Y = 773.80 + 0.604457 DEB	CP^{NS} DEB = positive linear effect	0.96
Feed intake (g)	Y = 1,113.31 + 0.461104 DEB	CP^{NS} DEB = positive linear effect	0.98

$^{NS}P > 0.05$.

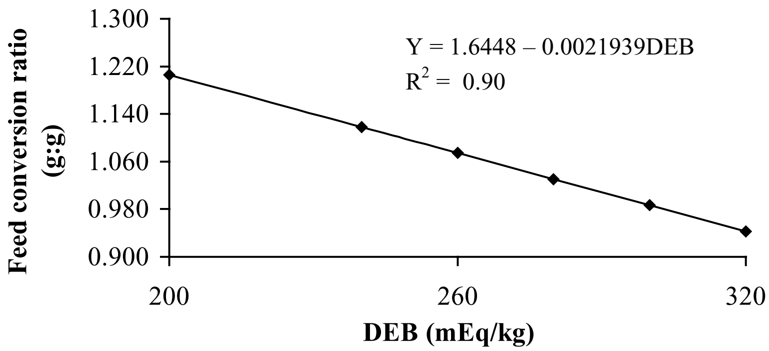

FIGURE 1. Effect of electrolyte balance (DEB) in diets with 19% CP on feed conversion ratio in broilers during the starting period (experiment 1, d 1 to 21).

treatment for a total of 1,200 birds. Treatments were corn-soybean meal diets (Table 1) formulated with three DEB levels (200, 260, and 320 mEq/kg) and two CP levels (17 and 19%). These diets were fed from 1 to 21 d of age. For each level of protein, the contents of methionine + cystine (1.0%) and lysine (1.25%) were maintained by supplementation with DL-methionine and lysine-HCl.

In the second experiment, 1,800 Cobb male broilers (21 d old) were divided into groups of 50 birds per pen. Four replicates were randomly distributed in a three by three factorial arrangement of treatments (DEB – 200, 240, and 280 mEq/kg × CP – 14, 16, and 18%). These diets were fed from 21 to 42 d of age. The birds received the same management and were fed a similar diet adequate in nutrient content [1] from 1 to 21 days of age. Corn-soybean meal experimental diets (Table 2) were formulated to contain 3,150 kcal ME/kg and 10% above recommended levels of lysine and methionine for the growing period [1]. These amino acid levels were maintained by supplementation with DL-methionine and lysine-HCl.

In both experiments, broilers were weighed at the start (1 or 21 d) and the end (21 or 42 d) of each experiment. Feed intake (FI), feed conversion ratio (FCR), and body weight gain (BWG) were evaluated at the end of each experimental period. Every bird that died or was removed was weighed, FI at that time was recorded, and these data were used for correction of FCR. Feed and tap water were provided ad libitum.

Two birds from each experimental unit were used for tibial evaluations at the end of each experiment. In the second experiment, during the growing period, the right legs of chickens were taken to measure bone breaking resistance with a manual press of simple com-

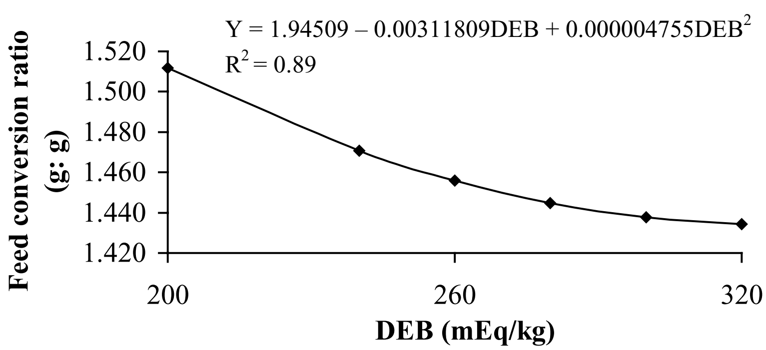

FIGURE 2. Effect of electrolyte balance (DEB) in diets with 17% CP on feed conversion ratio in broilers during the starting period (experiment 1, d 1 to 21). [2]Quadratic effect (regression equation).

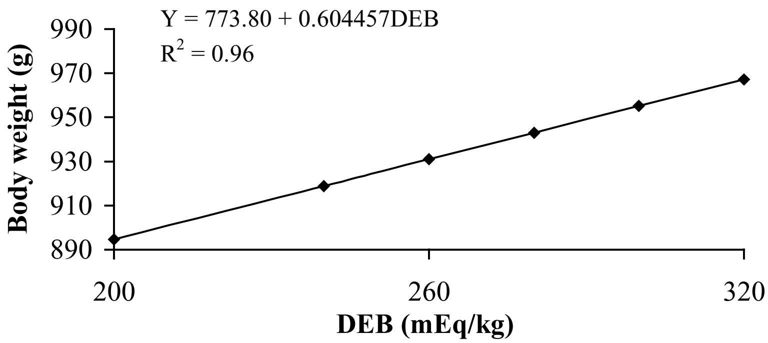

FIGURE 3. Effect of electrolyte balance (DEB) in low-protein diets on body weight in broilers at 21 d of age (experiment 1, d 1 to 24).

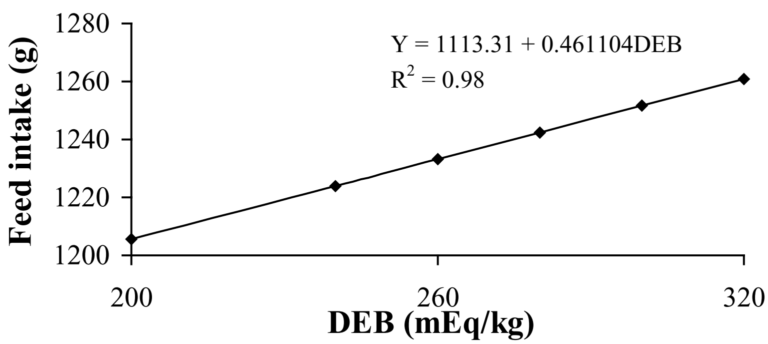

FIGURE 4. Effect of electrolyte balance (DEB) in low-protein diets on feed intake in broilers during the starting period (experiment 1, d 1 to 21).

pression at 50 kg. Left tibias were fixed in Bouin solution for histological analysis using an image analysis procedure in both experiments [8, 11]. Data were analyzed by response surface regression analysis.

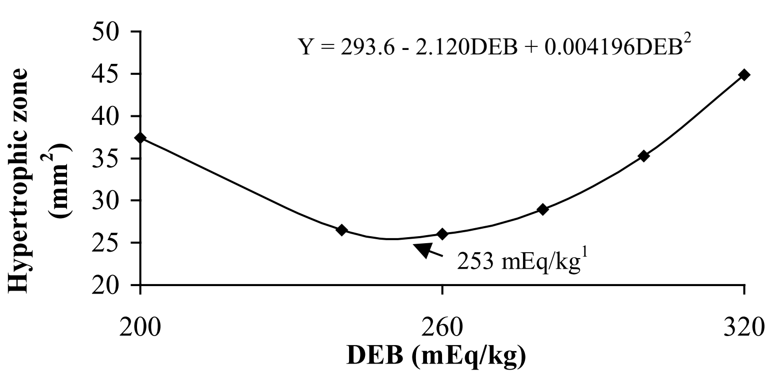

FIGURE 5. Effect of electrolyte balance (DEB) in low-protein diets on hypertrophic zone (A2) of broilers tibial cartilage during the starting period (experiment 1, d 1 to 21). [1]Optimum DEB level (253 mEq/kg). [2]Quadratic effect (regression equation).

TABLE 4. Regression equations for histological analysis of tibial epiphysis of broilers fed grower diets containing different levels of CP and dietary electrolyte balance (DEB) from 1 to 21 d of age (experiment 1)

Parameter	Regression equation	Levels of DEB × CP
Hypertrophic zone (A2)	$Y = 293.6 - 2.120\ DEB + 0.004196\ DEB^2$	CP^{NS} DEB = 253 mEq/kg
Total area of epiphysis (A3)	$Y = 307.7 - 1.756\ DEB + 0.003539\ DEB^2$	CP^{NS} DEB = 248 mEq/kg

$^{NS}P > 0.05$.

RESULTS AND DISCUSSION

First Experiment— Starter Period (1 to 21 d)

A significant interaction ($P < 0.05$) was observed between CP and DEB levels for FCR but not for other variables (Table 3). A positive linear effect of DEB for FCR at 19% CP after unfolding the interaction (Figure 1) and a quadratic effect at 17% CP (Figure 2) were observed. At this level, the best FCR was estimated to occur at 328 mEq/kg DEB. This value was out of the study range, showing that perhaps FCR could continue to improve with increasing DEB. The DEB had a linear effect ($P < 0.05$) on body weight at 21 d (Figure 3) and FI (Figure 4). There were significant differences ($P < 0.05$) among all variables under evaluation between the two CP levels (17 or 19%). However, the broiler performance observed in this experiment was not different from the genetic line standards [12]. Results of this study confirmed earlier findings [2] that indicate optimum DEB varies with CP concentration. Previous research at this laboratory [8] has concluded that broiler chickens in the starter period, fed with diets containing around 22% of CP, need DEB between 246 and 315 mEq/kg for maximum performance. Other authors [7, 9] have suggested that poultry perform adequately when fed approximately 240 mEq/kg DEB.

The histological evaluation of the tibia epiphysis areas demonstrated that the growing cartilage zone had normal distribution and did not show variations ($P < 0.05$) in function of DEB (Table 4). The data from the epiphysis hypertrophic area, however, did not represent normal distribution. The generalized linear model (GLIM) was used for analysis [13]. It was determined that these data plotted according to γ-distribution. A model with link function identity provided the best adjustment to data distribution in the function of DEB. In this model (Figure 5), a minimum tibia hypertrophic area was estimated at 253 mEq/kg DEB. The total area of epiphysis decreased (Figure 6) as DEB increased. The smallest area was estimated at 248 mEq/kg.

A minimum level around 250 mEq/kg has been suggested in previous research [8] to

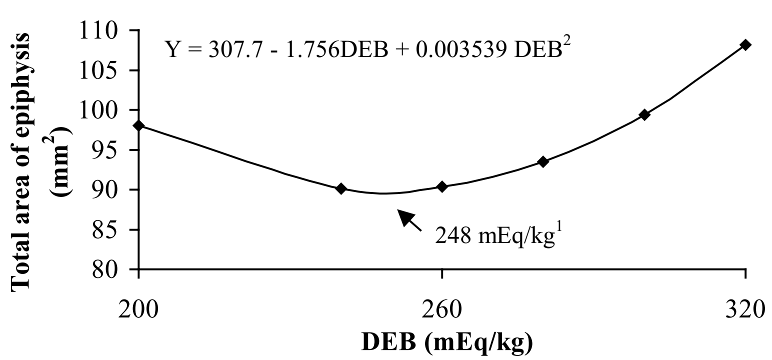

FIGURE 6. Effect of electrolyte balance (DEB) in low protein diets on the total area of epiphysis (A3) of broiler tibial cartilage during the starting period (experiment 1, d 1 to 21). [1]Optimum DEB level (248 mEq/kg). [2]Quadratic effect (regression equation).

TABLE 5. Regression equations for performance parameters of broilers fed grower diets containing different levels of CP and dietary electrolyte balance (DEB) from 21 to 42 d of age (experiment 2)

Parameter	Regression equation	DEB × CP	R^2
Feed intake* (g)	$Y = -7.1454 + 0.03466\ DEB - 0.000039276\ DEB^2 + 0.846151\ CP - 0.0202151\ CP^2 - 0.000898022\ (DEB \times CP)$	CP = 15.9% DEB = 270.9 mEq/kg	0.64
Body weight gain* (g)	$Y = -8.1757 + 0.02348\ DEB - 0.0000233368\ DEB^2 + 0.829412\ CP - 0.0185565\ CP^2 - 0.000710246\ (DEB \times CP)$	CP = 17.94% DEB = 230.03 mEq/kg	0.89
Feed conversion ratio* (g:g)	$Y = +8.15666 - 0.0066783\ DEB - 0.579113\ CP + 0.0125163\ CP^2 + 0.000395395\ (DEB \times CP)$	CP = 16.89% DEB = Negative linear effect	0.95

*Significant $P < 0.05$.

guarantee a good acid-base balance and adequate mineralization to avoid TD by using the same histological analysis reported herein. The metabolic acidosis observed in diets with lower DEB could increase the incidence of this leg problem, if other concomitant conditions are present. The same level of DEB seems to be necessary when the diets have low CP content to reduce TD incidence. However, low CP levels had a detrimental effect on broiler performance but did not affect the incidence of TD.

Second Experiment—Growing Period (21 to 42 d)

In this period, an interaction ($P < 0.05$) between CP and DEB for all broiler performance variables was noted (Table 5). The DEB and CP levels affected FI in a quadratic pattern and the BWG of broilers. Maximum FI was estimated at 270.9 mEq/kg and 14.9% CP (Figure 7), and BWG at 230.03 mEq/kg and 17.94% CP (Figure 8). The DEB had a linear effect on FCR, whereas CP had a quadratic effect. The best FCR was estimated at 16.89% CP (Figure 9), and for optimum FCR, the DEB was calculated at 246.33 mEq/kg.

The DEB levels suggested to optimize BWG and FCR were lower than estimated previously for this period [10] but agreed with other researchers [2, 4, 6, 7, 9] that have evaluated DEB in grower broiler diets with higher contents of CP and synthetic amino acid supplementation.

Because the levels were not lower than those reported to be the minimum CP necessary for good performance of broilers during this period [14], the benefits of DEB must be considered cautiously, and further research needs to evaluate the benefits of DEB in low-CP diets supplemented with synthetic amino acids.

No effects ($P > 0.05$) of DEB and CP on the epiphysis areas analyzed (hypertrophic cartilage zones that characterize TD (A_2)] or on bone resistance were observed (Table 6). Chickens in the growing period could already have a good bone formation that is not significantly affected by DEB manipulation or at least within the range evaluated in this experiment. Although, the levels of CP studied were lower than those assayed in other research [15], bone development was not affected by dietary pro-

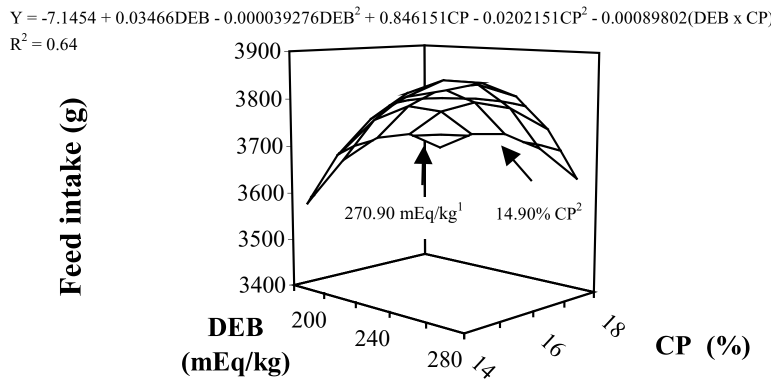

FIGURE 7. Effect of dietary electrolyte balance (DEB) in low-protein diets on feed intake of broilers during the growing period (experiment 2, d 21 to 42). [1]Optimum DEB level (270 mEq/kg). [2]Optimum CP percentage (14.90%).

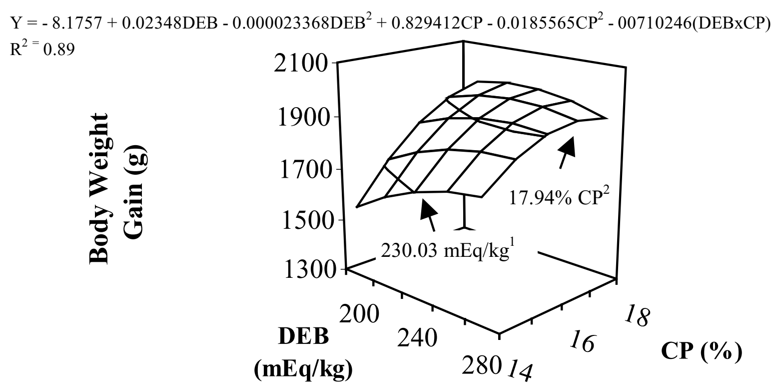

FIGURE 8. Effect of dietary electrolyte balance (DEB) in low-protein diets on body weight gain of broilers during the growing period (experiment 2, d 21 to 42). [1]Optimum DEB level (230.03 mEq/kg). [2]Optimum CP percentage (17.94%).

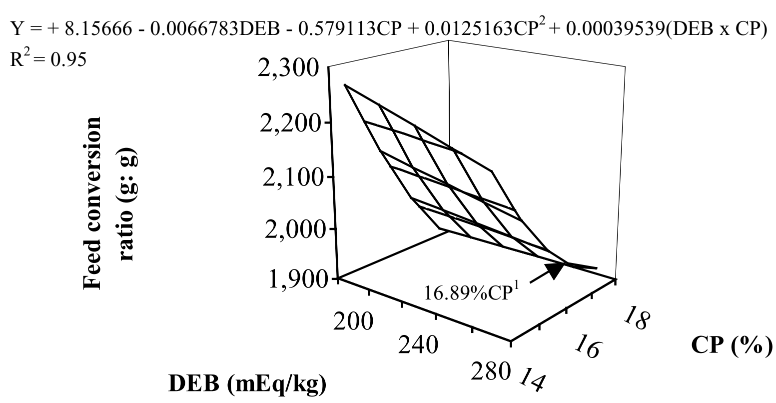

FIGURE 9. Effect of dietary electrolyte balance (DEB) in low-protein diets on feed conversion ratio of broilers during the growing period (experiment 2, d 21 to 42). [1]Optimum CP percentage (16.89%).

TABLE 6. Femur and tibia bone breaking resistance and tibia epiphysial areas at 42 d of age of broilers fed grower diets with different levels of dietary electrolyte balance and crude protein (experiment 1)

Treatments		Bone resistance (kg/mm^2)		Area[B] (mm^2)		
DEB[A] (mEq/kg)	CP (%)	Femur	Tibia	A_1	A_2	A_3
200	14	30.16	35.23	20.35	33.17	118.90
	16	30.03	35.72	24.01	42.93	146.71
	18	30.50	38.77	31.81	37.96	142.59
240	14	28.35	32.53	24.21	31.18	122.05
	16	31.16	39.34	23.91	36.12	138.92
	18	26.46	33.93	25.24	30.11	125.07
280	14	29.18	37.19	22.79	31.18	116.58
	16	28.51	34.33	19.08	33.15	122.20
	18	32.88	43.98	17.18	26.21	104.39
Standard error		5.12	7.50	2.22	2.44	6.42

[A]Dietary electrolyte balance (mEq/kg) = %Na × 10,000/23* + %K × 10,000/39* − %Cl × 10,000/35.5* (* indicates molecular weight).
[B]A_1 = growing cartilage zone; A_2 = hypertrophic zone; A_3 = total area of epiphysis.

tein level. Probably under other conditions, such as at high environmental temperatures and higher growth rates, the low-CP diets could cause different broiler responses as it has been previously reported [16].

CONCLUSIONS AND APPLICATIONS

1. Crude protein and DEB affect the performance of growing broiler chickens from 1 to 42 d of age.
2. Tibial dyschondroplasia incidence could be reduced in the starter period, if the DEB is maintained at 253 mEq/kg regardless of the dietary CP content. The DEB and CP seemed to have no effect on TD incidence or bone breaking resistance in the growing period.
3. The best performance was obtained with starter diets containing 328 mEq/kg and 19% CP and with grower diets varying from 230 to 246 mEq/kg and CP from 16.9 to 17.9% with supplementation of synthetic lysine and methionine.

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