



Electrolyte balance in diets with reduced protein for semi-weighted laying hens in the second production cycle

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ABSTRACT - The objective was to evaluate the effect of electrolyte balance in diets with reduced protein for semi-weighted Bovans Goldline laying hens in the second production cycle. The design was completely randomized with five treatments and seven replicates of six birds per experimental unit. Treatment 1 consisted of a control diet containing 165.0 g/kg crude protein (CP), formulated with the addition of DL-methionine to meet birds requirements during the experimental period. To compose the treatments 2-5 (BE149, BE167, BE185 and BE203), a basal diet with reduced protein (135.0 g/kg CP) supplemented with synthetic amino acids DL-methionine, L-lysine, L- threonine, L-valine and L-tryptophan was formulated. This basal diet was supplemented with potassium carbonate, to replace the inert, so as to provide four levels of potassium (5.86, 6.56, 7.26 and 7.96 g/kg) corresponding to the electrolyte balance of 171, 149, 167, 185 and 203 meq/kg, respectively. There was increased linear effect for feed intake and decreasing linear effect for albumen weight and yolk percentage and quadratic effect for conversion per dozen and per egg mass. Crude protein is recommended at 135.0g/kg with 6.77 g/kg potassium and electrolyte balance of 172.51meq/kg in the diet of semi-weighted laying hens in the second production cycle.

Key Words: amino acids, crude protein, electrolytes

Introduction

Egg production, as an industrial activity, is always aimed at improving efficiency in production and economy by benefiting from the genetic potential presented by the current strains of commercial egg-producing hens. However, a constant concern lies on good nutrition. Given this aspect, one should be careful with the reduction of crude protein in diets for poultry due to a reduction in potassium levels also caused by the decrease in the soybean meal amount (Murakami et al., 2003). This is particularly important since the reduction of crude protein in diets is given by the decrease in soybean meal, ingredient responsible for supplying potassium to the formulation (Savaris et al., 2009), resulting in changes in the electrolyte balance of the diets.

The determination of optimal levels of sodium, chloride and potassium for each production cycle of the bird is of great importance, since these electrolytes play important roles in various physiological processes, such as osmotic pressure maintenance, synthesis of tissue proteins, intra and extracellular homeostasis maintenance, maintenance of the electrical potential of the cell membrane, acid-base

homeostasis, as well as the functioning of the enzyme and nerves. The diet should be extremely well balanced in order for the essential electrolytes to be used to perform biochemical and physiological functions of the body (Borges et al., 2003a).

Manipulation of dietary electrolyte balance has been proposed as a way to improve the performance of birds fed low crude protein diets. The dietary electrolyte balance varies with the protein diet, where the growth of animals fed low protein diets decreases when the dietary electrolyte balance is amended by adding Na and K (Murakami et al., 2003). Many studies have been directed to the development of simplified electrolyte balance expressions, in order to identify the critical ratios of electrolytes for use in feed formulations (Vieites et al., 2004).

According to Vieites et al. (2004), the acid-base balance is directly related to electrolytes ingested by birds. The electrolyte balance can influence growth, appetite, bone development, response to heat stress and metabolism of certain nutrients, such as amino acids, minerals and vitamins (Patience, 1990).

Given the aforementioned, this study aimed to evaluate the electrolyte balance in diets with reduced protein for semi-weighted laying hens in the second production cycle.

Material and Methods

The experiment was conducted at the poultry production module of the Center of Agricultural Sciences, Department of Animal Science, Universidade Federal da Paraíba. The experiment lasted 140 days, divided into five periods of 28 days. A total of 210 Bovans Goldline hens of 57 weeks of age were used. The design was completely randomized, consisting of five treatments and seven replications with six birds per experimental unit.

In the 15 days preceding the experiment, the egg production was recorded and the egg rate in the period was calculated to standardize experimental units. Birds were separated by category and egg production. Thus, the experiment was started by fitting the average egg production obtained with the experimental units formation. The average egg production per treatment was $77 \pm 0.02\%$. At the time of poultry distribution, the weighing per experimental unit performed for calculating the average weight that was 1.74 ± 0.01 kg/bird.

Treatment 1 consisted of a control diet containing 165.0 g/kg of crude protein (CP), formulated with the addition of DL-methionine with all amino acid levels reaching or slightly exceeding the recommendations of Rostagno et al. (2005) for semi-weighting laying hens. To compose treatments 2-5 (BE149, BE167, BE185 and BE203), a basal diet with reduced protein (135.0 g/kg CP) supplemented with the synthetic amino acids L-valine, L-tryptophan, L-lysine, DL-methionine and L-threonine was formulated. This basal diet was supplemented with potassium carbonate replacing the inert, so as to provide four levels of potassium in the diet (5.86, 6.56, 7.26 and 7.96 g/kg) and five electrolyte balance levels (171, 149, 167, 185 and 203 meq/kg - Table 1).

The hens were housed in galvanized cages with dimensions $24 \times 37 \times 41$ cm and received water and feed *ad libitum*. The light program used was 17 hours a day.

At the end of each period, feed intake (g/bird/day), egg production (%), egg weight (g), egg mass (g/bird/day), feed conversion ratio per egg mass (kg/kg) and per egg dozen (kg/dozen), weights of albumen (g), yolk (g) and eggshell (g), percentages of albumen, yolk and shell and eggs specific gravity (g/cm^3) were evaluated.

At the end of each experimental period, the diet leftovers from each experimental unit were collected to calculate the feed intake. Egg collections were performed twice a day (at 9 and 16 h), recording the laying frequency, number of intact and defective eggs and the mortality rate. The egg production in percentage was calculated by dividing the total number of eggs per experimental unit by the

number of birds. The eggs of the last three days of each experimental period were weighed individually to obtain the average weight. The calculation of egg mass was conducted by the relationship between production and average egg weight per experimental unit. The feed conversion ratio per egg mass was calculated by the relationship between feed intake and egg mass produced. The conversion ratio per dozen eggs was calculated as the ratio of feed intake and production and the result multiplied by 12.

Of the total number of eggs produced per replicate, six units were selected for determination of weights and percentages of yolk, albumen and shell. After manual separation of these components, shells were kept in a forced-ventilation oven at 65°C for 4 hours. The percentages of yolk, albumen and shell were obtained by dividing the weight of each component by the egg weight and multiplying the result by 100.

The Haugh unit was determined after eggs had been weighed and then cracked and placed on a flat glass surface to measure the height of albumen thickness, with the aid of a clock micrometer. Haugh Unit values were calculated using the formula $\text{HU} = 100 \log (h - 1.7 \times w^{0.37} + 7.57)$, as follows: HU - Haugh unit; H - albumen height in millimeters, w: egg weight in grams.

The specific gravity was determined by the saline flotation method, according to the methodology described by Hamilton (1982). At the end of each experimental period, two eggs were selected representative sample per experimental unit, the eggs were subsequently immersed in salt solutions with densities ranging from 1.060 to 1.100 with an interval of 0.0025.

Eggs were placed in buckets with solutions from the lowest to the highest density and taken out when floating; density values corresponding to the solutions of containers were recorded. Before each evaluation, densities were checked with a petroleum densimeter.

Statistical analysis was performed using the software SAEG (Sistema de Análises Estatísticas e Genéticas, version 8.0.). Data were subjected to analysis of variance and the polynomial regression analysis was performed according to the electrolyte balance levels.

Results and Discussion

Increased linear effect of potassium levels ($P < 0.05$) was observed on feed intake, and results were dependent on the electrolytes used to vary the feed electrolyte ratio (Table 2).

Junqueira (2004) states that inadequate amounts of potassium can affect feed intake, which was observed in

this experiment, showing that even changing potassium levels in the diet, birds responded positively.

Imbalances in the electrolyte supplementation in the diet cause reduced appetite with weight gain and death when the compensatory mechanisms are not sufficient to maintain acid-base homeostasis (Borges, 2003b). When

imbalances are not compensated, they increase mortality (Mongin, 1981).

Egg production responded to increasing dietary potassium (K) levels in a quadratically way, increasing up to 7.03 g/kg K and 179.13 meq/kg electrolyte balance in the diet, decreasing at higher levels. Quadratic response was

Table 1 - Ingredient and chemical composition of the diets

Ingredient	Treatment				
	1	2	3	4	5
Corn	60.153	68.222	68.222	68.222	68.222
Soybean meal	27.260	17.373	17.373	17.373	17.373
Soybean oil	0.621	0.026	0.026	0.026	0.026
Dicalcium phosphate	1.487	1.554	1.554	1.554	1.554
Limestone	9.703	9.736	9.736	9.736	9.736
Salt	0.487	0.499	0.499	0.499	0.499
L-lysine HCl	-	0.128	0.128	0.128	0.128
DL-methionine	0.139	0.219	0.219	0.219	0.219
L-threonine	-	0.018	0.018	0.018	0.018
L-tryptophan	-	0.017	0.017	0.017	0.017
L-valine	-	0.058	0.058	0.058	0.058
Choline chloride 60g/kg	0.060	0.060	0.060	0.060	0.060
Mineral premix ¹	0.050	0.050	0.050	0.050	0.050
Vitaminic premix ²	0.025	0.025	0.025	0.025	0.025
Stafac	0.005	0.005	0.005	0.005	0.005
Antioxidant ³	0.010	0.010	0.010	0.010	0.010
Inert ⁴	-	1.877	1.753	1.629	1.505
Potassium carbonate	-	0.123	0.247	0.371	0.495
Chemical composition					
Metabolizable energy (kcal/kg)	2.700	2.700	2.700	2.700	2.700
Crude protein (g/kg)	165.0	135.0	135.0	135.0	135.0
Calcium (g/kg)	42.0	42.0	42.0	42.0	42.0
Available phosphorus (g/kg)	3.75	3.75	3.75	3.75	3.75
Digestible lysine (g/kg)	8.11	6.79	6.79	6.79	6.79
Digestible met.+cys. (g/kg)	6.18	6.18	6.18	6.18	6.18
Digestible methionine (g/kg)	3.74	4.13	4.13	4.13	4.13
Digestible threonine (g/kg)	5.64	4.48	4.48	4.48	4.48
Digestible arginine (g/kg)	11.02	8.13	8.13	8.13	8.13
Digestible tryptophan (g/kg)	1.93	1.56	1.56	1.56	1.56
Digestible valine (g/kg)	7.14	6.11	6.11	6.11	6.11
Chlorine (g/kg)	3.46	3.56	3.56	3.56	3.56
Sodium (g/kg)	2.30	2.30	2.30	2.30	2.30
Potassium (g/kg)	6.60	5.86	6.56	7.26	7.96
Eletolyte balance (meq/kg)	171	149	167	185	203

¹ Mineral premix per kg feed: Mn - 60 g; Fe - 80 g; Zn - 50 g; Cu - 10 g; Co - 2 g; I - 1 g; excipient q.s. - 500 g.

² Vitamin premix (Concentration/kg): vit. A - 15,000,000 UI; vit. D₃ - 1,500,000 UI; vit. E - 15,000 UI; vit.B₁ - 2.0 g; vit.B₂ - 4.0 g; vit.B₆ - 3.0 g; vit.B₁₂ - 0.015 g; nicotinic acid - 25 g; pantothenic acid - 10 g; vit. K₃ - 3.0 g; folic acid - 1.0 g; selenium - 250 mg; excipient q.s. - 1,000 g.

³ Ethoxyquin. - 10 g, and excipient q.s. - 1,000 g.

⁴ Sand.

Table 2 - Means of feed intake, egg production, egg weight, egg mass, and mass conversion of laying hens

Electrolyte balance	Feed intake (g/bird/day)	Egg production (%)	Egg weight (g)	Egg mass (g/bird/day)	Mass conversion (kg/kg)	Feed conversion (kg/dozen)
171	124.45	81.48	68.78	56.29	2.36	1.96
149	113.18	71.32	66.42	47.54	2.48	1.99
167	116.04	78.23	66.49	51.96	2.36	1.88
185	114.60	74.56	66.91	49.91	2.41	1.94
203	123.25	74.52	67.37	50.21	2.52	2.04
Means	118.30	76.02	67.19	51.18	2.43	1.96
Effect	L*	Q*	NS	Q*	Q*	Q*
CV (%)	2.12	1.98	2.17	2.43	3.10	2.51

L and Q* - linear and quadratic effects, respectively (P<0.05); NS - not significant; CV - coefficient of variation.

observed also for the variables egg mass, feed conversion ratio per egg mass and feed conversion ratio per dozen eggs, in which bird response improved with increasing levels of K to 7.12, 6.82 and 6.77 g/kg and electrolyte balance of 181.43, 173.79 and 172.51, respectively (Table 3).

Potassium is considered the most abundant intracellular cation and is involved in many metabolic processes, including nerve conduction, muscle excitation-contraction and cell volume regulation. Consequently, changes in K+ homeostasis profoundly affect cellular functions. According to Vieites et al. (2005), feed with electrolytes influences the acid-base balance and consequently affects metabolic processes, resistance to diseases and stress, and performance parameters. Possibly, the acid-base balance considerably affected the productive response of birds whose diets were supplemented with high levels of potassium carbonate.

The electrolyte balance of the diet has a direct connection with the acid-base balance of the animal. Therefore, when changing the protein content of the diet, it is necessary to adjust the dietary electrolyte balance. Maintaining this balance in the animal is of great physiological and biochemical importance (Sousa et al., 2002), whereas cellular activities, electrolyte exchanges and maintenance of the structural condition of body proteins are profoundly influenced by small changes in blood pH (Macari et al., 1994; Murakami, 2000). The electrolyte balance may also interfere with the hepatic, renal and blood metabolisms.

It is verified that the interrelation between electrolytes affects bird performance both when providing mineral levels that are out of the nutritional requirement or changing the optimum relation between them (Vieites et al., 2004).

Hamilton & Thompson (1980) found negative effects of (Na + K)/Cl ratios below 1.91 or above 2.83 on feed efficiency, egg production and eggshell weight. In this study, the (Na + K)/Cl ratio that resulted in better performance was around 2.6.

The electrolyte balance corresponding to the K levels which optimized production performance were between 172-181 mEq/kg in a range of study from 171 to 203 mEq/kg. Few studies have been conducted to determine the electrolyte balance in diets for semi-weighted laying hens in the second production cycle.

The albumen weight was significantly influenced by treatments, showing a linear increase as potassium levels increased (Tables 4 and 5).

The potassium involvement in the processes of amino acids absorption may also contribute to the increase in albumen weight, due to a greater availability of nutrients for the synthesis of egg components such as albumen and eggshell (Nelson & Cox, 2000). In the mechanism of active transport of sodium and potassium pump, when sodium is pumped against its concentration gradient; potassium, amino acids and glucose enter the cell both by active transport and passive diffusion, favoring the albumen development by the deposition of solids which consist primarily of protein.

Table 3 - Equations estimated for the productive parameters of laying hens

Productive parameters	Equation	Set point	R ²
Feed intake	$y = 88.367 + 4.110x$	-	0.69
Egg production	$y = -98.335 + 49.852x - 3.546x^2$	7.03	0.58
Egg mass	$y = -55.059 + 29.902x - 2.102x^2$	7.12	0.62
Feed conversion ratio per egg mass	$y = 7.806 - 1.597x + 0.117x^2$	6.82	0.96
Feed conversion ratio per egg dozen	$y = 6.805 - 1.451x + 0.107x^2$	6.77	0.94

Table 4 - Means of weight of albumen, yolk and shell, percentages of albumen, yolk and eggshell, yolk pigmentation specific gravity and Haugh unit of eggs from hens

Electrolyte balance	Albumen weight (g)	Yolk weight (g)	Shell weight (g)	Albumen (%)	Yolk (%)	Egg shell (%)	Yolk pigmentation	Specific gravity	Haugh unit
171	43.26	18.32	6.51	61.96	26.23	9.34	6.91	1.080	83.52
149	39.70	17.99	6.40	60.49	27.43	9.62	7.60	1.081	84.65
167	40.55	17.58	6.40	60.49	26.69	9.54	7.53	1.081	85.35
185	40.58	17.65	6.49	60.50	26.15	9.57	7.68	1.081	86.66
203	42.43	17.59	6.47	61.65	25.76	9.43	7.56	1.079	86.92
Means	41.30	17.83	6.45	61.02	26.45	9.50	7.46	1.0810	85.42
Effect	L*	NS	NS	NS	L*	NS	NS	NS	NS
CV (%)	1.87	2.29	2.53	1.44	2.75	1.90	2.53	0.23	4.11

L and Q* - linear and quadratic effects, respectively (P<0.05); NS - not significant; CV - coefficient of variation.

Table 5 - Equations estimated for the egg quality parameters of laying hens

Productive parameters	Equation	Set point	R ²
Weight of albumen	$y = 32.701 + 1.174x$	-	0.85
Yolk	$y = 31.986 - 0.793x$	-	0.98

Significant effect was observed ($P < 0.05$) for yolk percentage, which behaved in decreasing linear way to increasing potassium levels in the diet. The variations observed for egg constituents have indicated that the yolk proportion is inversely related to variations in proportions of albumen and shell. This is because when obtaining the proportion of egg constituents (%), each portion is related to the egg weight and variation in one of the constituents implies change in proportion of the other, and this variation cannot be directly proportional. Thus, potassium addition at the level of 7.96 g/kg increased the albumen percentage, making the yolk proportion decrease in relation to the egg weight.

According to Souza et al. (2002), the K concentration stimulates the release of growth hormone which inhibits lipogenesis and increases lipolysis, where this impact can explain the yolk weight decrease, since much of its content is composed of lipids.

Conclusions

It is possible to reduce the crude protein content from 165.0 g/kg to 135.0 g/kg with 6.77 g/kg potassium supplementation, which corresponds to 173 meq/kg electrolyte balances in the diet of laying hens.

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