











## Total electrolyte balance for laying hens

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**ABSTRACT:** *This study was done to determine the best value of the total electrolyte balance (BET) concerning the variables of performance and egg quality of the laying hens. We investigated 240 Hy-Line Brown laying hens, of 30 to 46 weeks of age, adopting the completely randomized experimental design, which included five treatments (1000, 1250, 1500, 1750 and 2000  $\mu\text{eq} / \text{kg}$ ), 8 repetitions and 6 birds per unit. The performance and quality of the birds' eggs were evaluated in the different treatments. The results were subjected to the analysis of variance and, wherever significant effect was noted, the regression equations were estimated, taking into account the electrolyte balance ( $\mu\text{eq} / \text{kg}$ ) as a concomitant variable, applying the SAS statistical program (2001). The consumption, production, and feed conversion variables by dozen eggs, final bird weight, egg density, yolk color, Haugh Unit, albumen weight, shell weight, and egg weight, were found to be unaffected by the experimental BET values. The values of the feed conversion per egg mass, yolk weight and uniformity were affected by the different BETs. The electrolyte balance values showing minimum feed conversion, higher yolk weight, and better uniformity were, respectively, BET = 1400, 1330, and 1250 in  $\mu\text{eq} / \text{kg}$  of loads at the different temperatures. From the regression equations, the value indicated was BET=1390 for the 30- to 46-week-old laying hens. The electrolyte balance of the diet was found to affect the laying hens in terms of performance and egg quality.*

**Key words:** electrolytes, avian physiology, egg formation, commercial feed.

## Balanço eletrolítico total para galinhas poedeiras

**RESUMO:** *Objetivou-se avaliar o melhor valor de balanço eletrolítico total (BET) para as variáveis de desempenho e qualidade dos ovos de galinhas poedeiras. Foram utilizadas 240 galinhas poedeiras da linhagem HyLine Brown, durante o período de 30 a 46 semanas de idade, distribuídas em um delineamento inteiramente casualizado, com cinco tratamentos (1000, 1250, 1500, 1750 e 2000  $\mu\text{eq}/\text{kg}$ ), oito repetições e seis aves por unidade experimental. Foram avaliados o desempenho e a qualidade dos ovos das aves com os diferentes tratamentos. Foi realizada análise de variância e, no caso de efeito significativo, foram estimadas equações de regressão considerando como variável concomitante o balanço eletrolítico ( $\mu\text{eq}/\text{kg}$ ), usando o programa estatístico SAS (2001). As variáveis de consumo, produção, conversão alimentar por dúzia de ovos, peso final das aves, densidade do ovo, coloração da gema, Unidade Haugh, peso do albúmen, peso da casca e peso do ovo, não foram afetados pelos BET experimentais. Os valores de conversão alimentar por massa de ovos, peso de gema e uniformidade foram influenciados pelos diferentes BET. Os valores de balanço eletrolítico que propiciaram conversão alimentar mínima, melhor peso de gema e melhor uniformidade foram, respectivamente: BET=1400, BET = 1330 e BET = 1250 em  $\mu\text{eq}/\text{kg}$  de cargas nas rações. Conforme as equações de regressão indica-se o valor de BET de 1390 para poedeiras de 30 a 46 semanas de idade. O balanço eletrolítico da dieta afeta o desempenho e a qualidade dos ovos de galinhas poedeiras.*

**Palavras-chave:** eletrólitos, fisiologia aviária, formação do ovo, rações comerciais.

## INTRODUCTION

In the hens, the maintenance of the acid-base balance was found to exert a strong influence on the physiological and biochemical aspects which, in turn, could affect the metabolic processes related

to growth, disease resistance, survival under stress and animal performance (BRETÂS et al., 2010). MORAES et al., (2019) reported that sodium, potassium, and chloride were fundamental to the maintenance of osmotic pressure and the acid-base balance of the body fluids.

The electrolytes present in the birdfeed were observed to alter the acid-base balance and, thus affect the metabolic processes concerned with growth, production, health, stress, and well-being (WANG et al., 2020).

This balance is linked to the electrolyte intake by the hens. The total electrolyte balance (BET) can affect growth, consumption, bone development, thermal stress, and cellular and osmotic metabolism (PATIENCE, 1990). Therefore, to avert acid-base imbalance, the rations needed to be supplemented with electrolytes like sodium bicarbonate and potassium chloride, which have been employed in the warm climate zones. The present diet contains many ingredients that can affect the electrolyte balance (BE); however, these are excluded in the model proposed by Mongin (1981), for example, the synthetic amino acids.

It was ARAÚJO et al., (2011) who proposed a new method of determining the electrolyte balance where these electrolytes were counted as the total electrolyte balance (BET). As the knowledge regarding these electrolytes and the influence of their  $\mu\text{eq} / \text{kg}$  on the metabolism of birds advanced, it was needful to account for them while calculating the electrolyte balance. Thus, the objective of this study was to assess the value of the best total electrolytic balance (BET) for the variables of performance and quality of the eggs in the laying hens.

## MATERIALS AND METHODS

The experiment was conducted in the poultry nutrition laboratory of the Federal Institute of Northern Minas Gerais, in Januária, Minas Gerais. The proposed objectives were achieved by adopting five diets, prepared to utilize the same electrolyte balance value ( $200 \mu\text{eq} / \text{kg}$ ) proposed by MONGIN (1981), according to Equation 1.

Equation 1: BE,  $\mu\text{eq} / \text{kg}$  of the feed =  $(\text{mg} / \text{kg}$  of Na + of the feed / 22.990) +  $(\text{mg} / \text{kg}$  of K + of the feed / 39.102) -  $(\text{mg} / \text{kg}$  of Cl- of the feed / 35.453).

Employing the equation proposed by ARAÚJO et al., (2011) the diets contained 1000, 1250, 1500, 1750, and 2000  $\mu\text{eq} / \text{kg}$ , respectively (Table 1).

Electrolyte balance equation according to ARAÚJO et al., (2011):

$$\text{BE, } \mu\text{eq/kg da ração} = [(\mu\text{eq/kg}^+/\text{kg } \text{Ca}^{2+} + \mu\text{eq/kg}^+/\text{kg } \text{K}^+ + \mu\text{eq/kg}^+/\text{kg } \text{Na}^+ + \mu\text{eq/kg}^+/\text{kg } \text{Mg}^{2+}) + (\mu\text{eq/kg}^+/\text{kg } \text{Lys} + \mu\text{eq/kg}^+/\text{kg } \text{Met} + \mu\text{eq/kg}^+/\text{kg } \text{Tre} + \mu\text{eq/kg}^+/\text{kg } \text{Trp} + \mu\text{eq/kg}^+/\text{kg } \text{Val} + \mu\text{eq/kg}^+/\text{kg } \text{Glu} + \mu\text{eq/kg}^+/\text{kg } \text{Chol})] - [(\mu\text{eq/kg}^-/\text{kg } \text{PO}_4^{3-} + \mu\text{eq/kg}^-/\text{kg } \text{SO}_4^{-2} + \mu\text{eq/kg}^-/\text{kg } \text{Cl} + \mu\text{eq/kg}^-/\text{kg } \text{HCO}_3^-) + (\mu\text{eq/kg}^-/\text{kg } \text{Lys} + \mu\text{eq/kg}^-/\text{kg } \text{Met} + \mu\text{eq/kg}^-/\text{kg } \text{Tre}$$

$$+ \mu\text{eq/kg}^-/\text{kg } \text{Trp} + \mu\text{eq/kg}^-/\text{kg } \text{Val} + \mu\text{eq/kg}^-/\text{kg } \text{Glu} + \mu\text{eq/kg}^-/\text{kg } \text{Chol}].$$

In this experiment, 240 laying hens of the Hy-Line Brown lineage in the 30- to 46-weeks-of-age class were used, with 4 periods of 28 days each, in completely randomized distribution design. Five treatments (1000, 1250, 1500, 1750, and 2000  $\mu\text{eq} / \text{kg}$ ), were done in total, with eight replicates, using six birds in each experimental unit.

The laying hens were standardized based on weight and production and accommodated in a screened masonry shed covered with clay tiles (5 m wide and 2 m in height), using galvanized wire cages with four  $25 \times 45 \times 40$  cm, and were laterally distributed on two floors, 0.80 m above the floor level. A galvanized channel type of feeder and drinker were used, covering the entire front extension of the cages. Diets were calculated based on the levels reported by ROSTAGNO et al. (2011) (Table 1).

The birds were provided free access to feed and water and 17 hours of light per day throughout the experimental period, by the management recommendations of the lineage manual (MANUAL ISA POULTRY, 2010). The birds and the rations were weighed at the commencement and completion of the experimental period to confirm the weight gain, feed intake, feed conversion per dozen eggs, and egg mass.

The following characteristics were assessed: feed consumption ( $\text{g} / \text{bird} / \text{day}$ ), egg production ( $\% / \text{bird} / \text{day}$ ), egg weight ( $\text{kg}$ ), feed conversion per egg mass ( $\text{kg} / \text{kg}$ ), feed conversion per dozen of eggs ( $\text{kg} / \text{dozen}$ ), final weight of birds ( $\text{g}$ ) and uniformity ( $\%$ ), Density ( $\text{kg} / \text{L}$ ), Haugh Unit, Yolk ( $\text{g}$ ), Albumin ( $\text{g}$ ), Shell ( $\text{g}$ ) and Total Egg Weight ( $\text{g}$ ).

The specific gravity was determined by immersing the eggs in saline solutions of density in the range of 1.070 to 1.095  $\text{g} / \text{cm}^3$ , with a 0.005  $\text{g} / \text{cm}^3$  interval, appropriately calibrated using a densimeter (OM-5565, Incoterm).

The weights of the yolk, albumen and shell were also determined, to accomplish which six eggs were randomly drawn from each repetition, from all the eggs collected over the last three days of each period. These were identified, weighed on a scale with 0.001 g accuracy. Then after breaking the egg, the shell was separated, washed, and dried in the open air for weighing at a later time.

The yolk was weighed immediately after breaking the egg, and the albumen weight was calculated as the difference between the total egg weight and yolk and shell weight. Statistical evaluation was then done using the analysis of variance and, when the significant effect was noted,

Table 1 - Percentage and chemical compositions of experimental diets formulated according to ROSTAGNO et al., (2011) and their respective electrolyte balances.

Ingredients (%)	-----Electrolytic balances (µeq/kg)-----				
	1000	1250	1500	1750	2000
Corn	56.502	56.486	56.461	56.428	57.028
Soybean meal	23.475	23.470	23.470	23.480	22.100
Soybean oil	4.455	4.460	4.470	4.480	4.335
Potassium carbonate	1.200	1.200	1.200	1.200	1.358
Calcium bicarbonate	1.945	1.495	1.040	0.588	0.127
Dicalcium phosphate	2.760	2.750	2.750	2.750	2.768
Limestone	8.430	8.905	9.370	9.840	10.310
Sodium chloride	0.477	0.477	0.477	0.477	0.477
DL-Methionine 99%	0.268	0.268	0.268	0.268	0.282
L-Lysine HCl 99%	0.080	0.080	0.080	0.080	0.123
L-Threonine 98%	0.074	0.075	0.075	0.075	0.750
L-Tryptophan	0.014	0.014	0.014	0.014	0.022
Vitamin supplement 1	0.100	0.100	0.100	0.100	0.100
Mineral supplement 2	0.100	0.100	0.100	0.100	0.100
Choline chloride 60%	0.100	0.100	0.100	0.100	0.100
Antioxidant3	0.020	0.020	0.020	0.020	0.020
-----Calculated composition-----					
Metabolizable energy, kcal/kg	2850	2850	2850	2850	2850
BE4, µeq/kg (Mongin, 1981)	250	250	250	250	250
BE4, µeq/kg (Araújo, 2011)	1000	1250	1500	1750	2000
Crude protein (%)	15.603	15.601	15.601	15.600	15.604
Digestible lysine (%)	0.780	0.780	0.780	0.780	0.780
Digestible methionine (%)	0.490	0.490	0.490	0.490	0.496
Methionine + digestible cystine (%)	0.710	0.710	0.710	0.710	0.710
Digestible threonine (%)	0.593	0.593	0.593	0.593	1.226
Digestible tryptophan (%)	0.179	0.179	0.179	0.179	0.179
Glycine+Total Serine (%)	1.384	1.383	1.384	1.384	1.330
Digestible valine (%)	0.651	0.651	0.651	0.651	0.626
Digestible isoleucine (%)	0.598	0.597	0.598	0.598	0.573
Arginine Dig (%)	0.955	0.954	0.954	0.955	0.913
Phenylalanine+ Digestible Tyrosine (%)	1.196	1.196	1.196	1.196	1.150
Digestible histidine (%)	0.396	0.396	0.396	0.396	0.382
Linoleic acid (%)	1.337	1.337	1.336	1.335	1.343
Calcium (%)	4.759	4.761	4.759	4.761	4.760
Match available (%)	0.601	0.599	0.599	0.599	0.600
Sodium (%)	0.206	0.206	0.206	0.206	0.206
Crude fiber (%)	2.647	2.643	2.643	2.642	2.630
Neutral detergent fibre (%)	10.584	10.576	10.574	10.571	10.541
Acid detergent fibre (%)	4.450	4.445	4.444	4.443	4.422

1 Vitamin mixture (kg of product): vit. A - 10,000,000 I.U.; Vit. D3 - 2,000,000 I.U.; Vit. E - 30,000 U.I. A; vit. B1 - 2.0 g; Vit. B2 - 6.0 g; Vit. B6 - 4.0 g; Vit. B12 - 0.015 g; ace. pantothenic - 12.0 g; biotin - 0.1 g; Vit. K3 - 3.0 g;ác. Folic - 1.0 g; ace. Nicotinic - 50.0 g; If - 250.0 mg. 2 Mineral mixture (kg of the product): Fe - 80 g; Cu - 10 g; Co - 2 g; Mn - 80 g; Zn - 50 g; I - 1 g. 3 Antioxidant: BHT (Butylhydroxy toluene). 4 Electrolytic Balance.

the regression equations were performed taking into account the electrolyte balance ( $\mu\text{eq} / \text{kg}$  as a concomitant variable), through the PROC MIXED in the statistical program SAS (2001). Later, the

BET effects were tested using Dunnett's test at 5% probability, by comparison of the control treatment with the others. The  $R^2$  value was achieved from the data noted in each of the experimental repetitions.

## RESULTS AND DISCUSSION

In this experiment, we observed the maximum and minimum temperature to be 32.7 °C and 19.4 °C, on average, respectively, and the relative humidity on average, as 47.75%. The variables of consumption, egg production, feed conversion per dozen eggs, the final weight of the birds remained unaffected ( $P>0.05$ ) according to the experimental BET (Table 2).

CALDERON & JENSEN (1990) reported no significant differences for the egg production of the laying hens fed on different quantities of protein in the feed. These results concur with the findings of MANJU et al., (2015) who, in their study on crude protein levels (12, 14, 16, 18%) and lysine content (0.85 and 1.0%), also discovered that the egg production, consumption of ration and egg quality remained unaffected.

The findings from this experiment correspond with those of some authors such as SILVA et al., (2006) who reported no difference in feed conversion per dozen eggs. In their studies, LAURENTIZ et al., (2005) recorded that the birds fed with 16.4% of CP showed improvement in the variables of feed conversion by dozen birds, egg production and weight of the eggs when the AAST levels were boosted from 0.45% to 0.60% in the diets for the Lohmann Brown hens in the second production cycle.

For the variable, the final weight of the birds, the BET treatment 1250  $\mu\text{eq} / \text{kg}$ , showed an improved result of 1678 g ( $P>0.05$ ), a value very close to that of the treatment using 1000  $\mu\text{eq} / \text{kg}$ . A BE value below 180  $\mu\text{eq} / \text{kg}$  and above 300  $\mu\text{eq} / \text{kg}$  lowers the weight of the birds (JOHNSON & KARUNAJEEWA, 1985). HULAN et al., (1987) in their research on the effect of diets containing Na + + K + - Cl- in different

doses, revealed that the lowest and the highest weight gains were achieved with 174 and 215  $\mu\text{eq} / \text{kg}$ . Also, BORGES et al., (1999) observed that the best electrolyte balance was noted in concentrations of 199  $\mu\text{eq} / \text{kg}$  and the Na + and Cl- ions.

The feed conversion per egg mass and uniformity values were influenced by the different BET values (Table 2,  $P<0.05$ ). For uniformity, birds given the BET treatment 1250  $\mu\text{eq} / \text{kg}$ , showed the best result. Therefore, for this parameter, the birds revealed an excellent electrolyte balance with 1250 $\mu\text{eq} / \text{kg}$ , reiterating that they were more adapted to this relationship in the diet than with the other treatments.

The following equations were adjusted for the feed conversion by mass of the eggs (CAM), respectively:  $\text{CAM} = (-4.59 \times 10^{-7} \text{BE}^2) + (0.0014 \text{BE}) + 3.0815$ , ( $R^2 = 0.47$ ), achieving a BET value = 1525 $\mu\text{eq} / \text{kg}$ . However, these results differ from those recorded by LOPES et al., (2011) who found no significant effect for this parameter, in their study. By contrast, MORAES et al., (2019) reported a significant effect ( $P<0.05$ ) concerning the electrolyte balance, indicating that the more quantity of  $\text{NaHCO}_3$  included in the diet the greater the feed conversion. These studies have considered only a small portion of the dietary electrolytes applying the classic BE calculation (MONGIN, 1981).

Regarding the variables of quality as shown in table 3: Colorimetric fan, density, Haugh unit, albumen weight, shell weight, and total egg weight, remained unaffected by the experimental BET.

The yolk weight alone was influenced by the different BET ( $P<0.05$ ). The birds that were given the BET treatment 1250  $\mu\text{eq} / \text{kg}$ , revealed better yolk weights, which indicates that this electrolytic balance with 1250 $\mu\text{eq} / \text{kg}$  was more effective in this situation, with the objective being to raise the yolk

Table 2 - Performance of laying hens under different BET in diets.

Variables	BET levels ( $\mu\text{eq}/\text{kg}$ )					SEM	CV	P-value
	1000	1250	1500	1750	2000			
Consumption (g/bird/day)	116	111	111	114	111	2.55	0.045	0.172
Egg production (%)	84.7	84.9	82.3	85.1	81.66	21.72	0.051	0.369
Feed conversion per egg mass (kg/kg)	2.19	2.06	2.10	2.09	2.19	0.026	0.016	0.022
Feed conversion per dozen eggs (kg/dozen)	1.65	1.57	1.61	1.60	1.64	0.071	0.044	0.267
The final weight of birds (g)	1676	1678	1625	1657	1640	0.11	0.066	0.074
Uniformity (%)	89.6	93.8	85.4	89.6	89.6	-	-	-

SEM= Standard error mean; CV= Coefficient of Variation; P-value= Probability Value; BET in  $\mu\text{eq}/\text{kg}$ = Total Electrolyte Balance in  $\mu\text{eq}/\text{kg}$ ; \* Significant effect ( $P<0.05$ ). CA egg mass =  $(-4.59 \times 10^{-7} \text{BE}^2) + (0.0014 \text{BE}) + 3.0815$ , ( $R^2=0.47$ ).

Table 3 - Quality of eggs of laying hens under different BET in diets.

	BET levels ( $\mu\text{eq/kg}$ )							
Variables	1000	1250	1500	1750	2000	SEM	CV	P-value
Colorimetric range (Roche)	4.2	4.2	4.2	4.3	4.4	0.878	0.206	0.855
Density (kg/L)	1.108	1.107	1.104	1.107	1.102	0.004	0.004	0.058
Haugh Unit	129.7	128.8	130.4	129.8	130.1	1.19	0.009	0.071
Yolk (g)	15.2	16.2	15.3	15.1	15.1	0.809	0.053	0.022
Albumin (g)	36.8	38.2	38.5	38.8	38.2	1.74	0.046	0.211
Egg shell (g)	5.7	5.9	5.7	5.7	5.5	0.338	0.059	0.265
Total egg weight (g)	62.1	65.8	64.0	63.8	62.9	4.32	0.068	0.589

SEM= Standard error mean; CV= Coefficient of Variation; P-value= Probability Value; BET in  $\mu\text{eq/kg}$ = Total Electrolyte Balance in  $\mu\text{eq/kg}$ . \*Significant effect ( $P < 0.05$ ). Yolk =  $(1.10 \times 10^{-8} \text{ BE}^3) - (5.24 \times 10^{-5} \text{ BE}^2) + (0.078 \text{ BE}) - 21.73$  ( $R^2 = 0.59$ ).

weight, which ranks among the foremost features desired by the consumers.

Strikingly different from this study, LIMA et al., (2015) in their work using the sodium levels in quail diets to stabilize the electrolyte balance, concluded that the 0.10; 0.15, 0.20, 0.25 and levels did not significantly affect ( $p > 0.05$ ) egg performance and quality. JUNQUEIRA et al., while (2000) assessing the effects of the sources and levels of sodium, chlorine, and potassium, as well as the  $(\text{Na} + \text{K}) / \text{Cl}$  ratio, on the performance and characteristics of the hen laying blood plasma also reported no change ( $p > 0.005$ ) in the variables of the quality of eggs.

Regarding the yolk weight the equations mentioned were adjusted,  $\text{Yolk} = (1.10 \times 10^{-8} \text{ BE}^3) - (5.24 \times 10^{-5} \text{ BE}^2) + (0.078 \text{ BE}) - 21.73$  ( $R^2 = 0.59$ ). Achieving the values of  $\text{BET} = 1330 \mu\text{eq} / \text{kg}$  and  $\text{BET} = 2000 \mu\text{eq} / \text{kg}$  to attain maximum values of yolk weight are described by the cubic equation. However, the weight values of the buds estimated under  $\text{BET} = 1330 \mu\text{eq} / \text{kg}$  were even greater than those below  $\text{BET} = 2000 \mu\text{eq} / \text{kg}$ .

With respect to the colorimetric range, the yolk pigmentation in the treatments revealed color intensities that were very close. In fact, OLIVEIRA et al., (2007) reported that the higher yolk pigmentation may have some connection to the inclusion of more corn in the diet because corn possesses natural xanthophylls that intensify the pigmentation. In terms of density, all the eggs were assessed in different NaCl solutions, and the treatment that attained the highest value was  $\text{BET} 1000 \mu\text{eq} / \text{kg}$ , with a solution of  $1.108 \text{ kg} / \text{L}$  ( $P > 0.05$ ). In their studies, SILVA et al., (2006) reported that these data did not fall within the normal values for laying hens, which are in the range of  $1.080$  to  $1.090 \text{ g} / \text{cm}^3$ .

Regarding the Haugh unit, the  $\text{BET} 1500 \mu\text{eq} / \text{kg}$  treatment produced numerically improved results ( $P > 0.05$ ). The result drawn from the Haugh Unit revealed excellent egg quality, as it exceeded the minimum value of 72 recommended by the EGG - GRADING MANUAL (2000).

Considering albumen weight,  $\text{BET} 1750 \mu\text{eq} / \text{kg}$  demonstrated a better numerical result ( $P > 0.05$ ) compared to the other treatments. These data corroborate those found by

No significant effect ( $P > 0.05$ ) was observed for the relative weight of the eggshell. By altering the prosthetic and amino acid levels of the diet, egg size can be changed to possibly minimize the problems of shell quality observed by the completion of the first and second production cycles. This occurred, most likely, because the quantity of shell deposited gets reduced at a level equal to or higher than the degree to which the albumen or yolk is reduced (PAVAN et al., 2005).

According to PINTO et al., (2002), egg weight is dependent upon the quality and quantity of protein consumed by the laying hens from their diet and can be a useful reference to guarantee their amino acid requirements. The high protein level and amino acid content of the diet remarkably influenced the egg size (ATTIA et al., 2020). In this study, the  $\text{BET}$  treatment  $1250 \mu\text{eq} / \text{kg}$  enabled a good total egg weight ( $P > 0.05$ ) to be attained. A comparison was made of these results with those of MAKLED and CHARLES (1987) who reported that egg production remains unaffected when  $\text{NaHCO}_3$  is added to the diet.

These findings are exceptional from those found in the literature for the method of BE calculation used, according to ARAÚJO et al. (2011). In fact, MURAKAMI et al. (2006) in their evaluation using the classic BE method for the first and second

cycles of the hen layers, reported ideal values of 205  $\mu\text{eq} / \text{kg}$ , for the first production cycle, and 174  $\mu\text{eq} / \text{kg}$ , for the second one. However, in this methodology, a much lower electrolyte volume than the one used in this study was evaluated.

## CONCLUSION

The different BET values used were observed to affect the performance of the hens. The electrolyte balance values which gave the lowest possible feed conversion, improved yolk weight, and induced better uniformity were, respectively,  $\text{BET} = 1525$ ,  $\text{BET} = 1330$  and  $\text{BET} = 1250$  in  $\mu\text{eq} / \text{kg}$  of the feed loads. From the regression equations, the BET value of 1390 was identified as ideal, for laying hens 30 to 46 weeks of age.

## BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The project was submitted and approval was granted by the ethics committee for the use of animals (CEUA-IFNMG) under protocol 00124-2018.

## DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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## AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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