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Original Article

Requirement of Sodium to Molted Laying Hens[1]

Author(s)

Melo RD[II] https://orcid.org/0000-0001-5982-9313
Cruz FGG[III] https://orcid.org/0000-0001-9076-9849
Melo LD[II] https://orcid.org/0000-0002-8243-670X
Feijó JC[II] https://orcid.org/0000-0001-8290-9741
Rufino JPF[IV] https://orcid.org/0000-0002-1605-5255
Brasil RJM[IV] https://orcid.org/0000-0002-8765-0780
Oliveira Fº PA[V] https://orcid.org/0000-0001-9754-2022
Silva FMF[VI] https://orcid.org/0000-0002-1692-0624

[I] Part of master's degree dissertation of the first author.
[II] Graduate Program in Animal Science, College of Agrarian Sciences, Federal University of Amazonas, Manaus, Amazonas, Brazil.
[III] Department of Animal and Vegetable Production, College of Agrarian Sciences, Federal University of Amazonas, Manaus, Amazonas, Brazil.
[IV] Graduate Program in Biodiversity and Biotechnology, College of Health Sciences, State University of Amazonas, Manaus, Amazonas, Brazil.
[V] Graduate Program in Fisheries Science in the Tropics, College of Agrarian Sciences, Federal University of Amazonas, Manaus, Amazonas, Brazil.
[VI] Animal Science Undergraduate, College of Agrarian Sciences, Federal University of Amazonas, Manaus, Amazonas, Brazil.

Mail Address

Corresponding author e-mail address
Ramon Duque Melo
College of Agrarian Sciences, Federal University of Amazonas, South Sector, University Campus, Av. General Rodrigo Octávio Jordão Ramos, 6200, Coroado I, Manaus, Amazonas, Brazil, 69077-000.
Phone: +55 xx 923305-1181 (R. 4082)
Email: ramon_duquemelo10@hotmail.com

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ABSTRACT

The objective of this study was to determine the ideal requirement of sodium to molted laying hens. The experimental period lasted 105 days, divided into five periods of 21 days. Hisex White laying hens 150 with 84 weeks-of-age were used. The experimental design was completely randomized in a factorial scheme (5x3) with treatments constituted by five levels of sodium (0.10, 0.15, 0.20, 0.25, and 0.30%) in the diets, and three postmolt stages (early = 21 days, medium = 63 days, and final = 105 days). Performance and egg quality results were evaluated by Tukey test at 5%. Eggshell resistance and weight gain results were evaluated by polynomial regression at 5%. Except the yolk height, all performance and egg quality variables were affected ($p<0.05$) by the sodium levels and the hens' age postmolt. The level of 0.15% of sodium in diets to postmolt laying hens provided better performance and egg quality, especially in the eggshell. Higher levels of sodium negatively affected the performance and egg quality. Hens in the early stage of postmolt period presented better performance and egg quality. At long-term, the postmolt hens presented worst results.

INTRODUCTION

Molting is a natural phenomenon in wild and domestic birds, causing the replacement of feathers and involving reproductive quiescence. This period is responsible for a significant decline in egg production and provides the equivalent of a 'reproductive rest' to the birds (Khan *et al.*, 2011). The low prices of eggs and spent flocks have resulted in the interest in replacing the natural molting process with the artificial one called induced molting (Berry, 2003).

Induced molting has become standard husbandry in many commercial egg operations. The main purpose of molting is to cease egg production in order for the hens to enter a nonreproductive state, which increases egg production and egg quality postmolt (Webster, 2003; Donaldson *et al.*, 2005). Although several successful procedures exist to induce molting in laying hens, most of these techniques differ in several aspects (Hussein, 1996).

The most effective moulting procedures create the least amount of stress, produce a rapid moult, and bring the flock back into egg production quickly (North & Bell, 1990). As a result, numerous studies have recognized the need for more information about molting procedures, timing and duration of the molting period, reproductive organ responses, bird behaviour, and nutritional requirements post molting (Wolford, 1984; Hussein, 1996). According to Teixeira & Cardoso (2011), the nutritional readjustment of all nutrients of the diet is essential to a successful molt program. Studies about nutritional requirements for laying hens are frequently updated in the literature (Domingues *et al.*, 2016). However, there is a great lack of information about requirements to molted laying hens.

Studies investigating the sodium requirement are less found in the literature than other nutrients to laying hens in the first and second cycle. According to Borges (2001) and Borges *et al.* (2002), sodium, together with chlorine and potassium, present high metabolic activity in the acid-base balance, maintenance of the cellular osmotic pressure and metabolism of water in the tissues. These elements must be in constant balance so as not to compromise the good functioning of the enzymes responsible for several metabolic reactions. Both deficiency and excess of sodium in the poultry diets cause problems to the birds, mainly affecting the performance (Ribeiro, 2007; Lima *et al.*, 2015).

Considering the above, the purpose of this study was to determine the ideal requirement of sodium to molted laying hens.

MATERIAL AND METHODS

This study was conducted in the facilities of Poultry Sector, College of Agrarian Sciences, Federal University of Amazonas, Manaus, Amazonas State, Brazil. The experimental procedures were approved by the Ethics Committee in Use of Animals (protocol number 041/2018) of the Federal University of Amazonas, Manaus, Amazonas, Brazil.

The experimental period lasted 105 days divided into five periods of 21 days. Birds were subjected to an adaptation period of seven days to feed and facilities. The aviary had galvanized wire cages (1.00 x 0.45 x 0.50 m), trough feeders, and nipple drinkers. A stocking density of 13.3 birds/m^2 was used, with six birds per cage. Throughout the experimental period, 16 hours of light/day were provided to the birds (12 hours of natural + 4 hours of artificial), with water and feed *ad libitum*. The temperature and air relative humidity were recorded twice a day (9 and 15 hours) from a digital hygrometer positioned at the height of the cages, obtaining averages results of 29.35 ºC and 63.57% respectively. Egg collection was also performed twice a day (9 and 15 hours).

From 80 to 83 wks of age, hens were submitted to a molt program using nonfeed removal method (Biggs *et al.*, 2003; Mazzuco & Hester, 2005) for 28 days. 150 Hisex White laying hens with 84 weeks of age were used. The birds were weighed at the beginning and at the end of the experimental period, presenting an initial average weight of 1.38±0.0019 kg, and a final average weight of 1.43±0.013 kg. The experimental design was completely randomized in a factorial scheme (5x3) with treatments constituted by five levels of sodium (0.10, 0.15, 0.20, 0.25, and 0.30%) in the diets, and three postmolt stages (early = 21 days, medium = 63 days, and final = 105 days). Experimental diets (Table 1) were calculated according to the requirements provided by Rostagno *et al.* (2017) using the software SUPERCRAC (2008).

At the end of each period of 21 days, we calculated the feed intake (g/bird/day), egg production (%), feed efficiency (kg of feed used / kg of egg), feed efficiency (kg of feed used / dozen eggs), and egg mass (g). At the end of each 21 days period, four eggs to each plot were randomly selected to evaluate egg weight (g), specific gravity (g/cm^3), yolk (%), albumen (%), eggshell (%), yolk height (mm), albumen height (mm), yolk color, eggshell thickness (μm), Haugh unit, and eggshell resistance (N).

The eggs were stored for one hour in room temperature and weighed using an electronic balance (0.01 g). The eggs were placed in wire baskets and immersed in buckets containing different levels of sodium chloride (NaCl) with density variations from 1.075 to 1.100 g/cm^3 (interval of 0.005) to evaluate the specific gravity.

Then, the eggs were placed on a flat glass plate to determine albumen and yolk height, and yolk diameter using an electronic caliper. To separate albumen and yolk a manual separator was used. Each one was placed in a plastic cup and weighed in an analytical balance.

Eggshells were washed, dried in an oven (50 ºC) for 48 hours, and weighed. Dry eggshells were used to determine the eggshell thickness using a digital micrometer. Average eggshell thickness was analyzed considering three regions: basal, meridional, and apical.

The yolk color was evaluated using a ROCHE© colorimetric fan with a scale of 1 to 15. Haugh unit was calculated using the egg weight and albumen height values in the formula H_{unit} = 100 x log (H + 7.57 – 1.7 x W0.37), where H = albumen height (mm), and W = egg weight (g).

To determine the eggshell resistance, an electronic machine of mechanical (model Instron 5984) located in the Materials Laboratory of the Superior College of Technology of the State of Amazonas University, was used. This machine was connected to a computer, generating the power levels (represented in Newton) used to break the eggshell.

All data collected in this study were analyzed using the GLM procedure of SAS (Statistical Analysis System, v. 9.2) and estimates of treatments were subjected to ANOVA. Performance and egg quality results were evaluated by Tukey test. Eggshell resistance and weight gain results were evaluated by polynomial regression. Results were considered significant at $p \leq 0.05$.

Table 1 – Experimental diets composition.

	Levels of sodium, %				
Ingredients	0.10	0.15	0.20	0.25	0.30
Corn 7.88%	69.2669	69.1117	68.9566	68.8016	68.6464
Soybean meal 46%	18.5293	18.5584	18.5875	18.6166	18.6457
Limestone	9.6489	9.6486	9.6482	9.6478	9.6475
Dicalcium phosphate	1.6723	1.6727	1.6731	1.6734	1.6738
Salt	0.1686	0.2946	0.4206	0.5466	0.6726
PREMIX vitaminic/mineral	0.5000	0.5000	0.5000	0.5000	0.5000
DL-Methionine 99%	0.2140	0.2140	0.2140	0.2140	0.2140
L-Lysine	0.0372	0.0397	0.0421	0.0446	0.0471
L-Tryptophan	0.0271	0.0276	0.0281	0.0285	0.0290
L-Threonine	0.0073	0.0085	0.0098	0.0110	0.0124
Total	100.00	100.00	100.00	100.00	100.00
Nutrients					
Metabolizable energy, kcal.kg[-1]	2,799.92	2,795.28	2,790.65	2,786.02	2,781.38
Crude Protein, %	14.500	14.500	14.500	14.500	14.500
Calcium, %	4.200	4.200	4.200	4.200	4.200
Available phosphorus, %	0.400	0.400	0.400	0.400	0.400
Sodium, %	0.100	0.150	0.200	0.250	0.300
Total methionine, %	0.422	0.422	0.422	0.422	0.422
Digestible methionine, %	0.403	0.403	0.403	0.403	0.403
Digestible met. + cys., %	0.450	0.500	0.550	0.600	0.650
Digestible lysine, %	0.653	0.653	0.653	0.653	0.653
Digestible threonine, %	0.498	0.498	0.498	0.498	0.498
Digestible tryptophan, %	0.172	0.172	0.172	0.172	0.172

[1] Guaranteed levels per kilogram of the product: Vitamin A 2,000,000 IU, Vitamin D3 400,000 IU, Vitamin E 2,400 mg, Vitamin K3 400 mg, Vitamin B1 100 mg, Vitamin B2 760 mg, Vitamin B6 100 mg, Vitamin B12 2,400 mcg, Niacin 5,000 mg, Calcium Pantothenate 2,000 mg, Folic acid 50 mg, Coccidiostat 12,000 mg, Choline 50,000 mg, Copper 1,200 mg, Iron 6,000 mg, Manganese 14,000 mg, Zinc 10,000 mg, Iodine 100 mg. Selenium 40 mg. Vehicle q.s.p. 1,000 g.

RESULTS

All performance variables were affected ($p<0.05$) by the sodium levels and the hens' age postmolt. Birds fed diets with 0.10% and 0.15% of sodium presented better egg production, feed efficiency, and egg mass. High levels of sodium significantly reduced the performance. In the final stage of postmolt period, the hens presented larger feed intake and lower egg production, which provide worst feed efficiency, and egg mass (Table 2).

Table 2 – Feed intake (FI), egg production (EP), feed efficiency (FC, kg.kg^{-1}), feed efficiency (FC, kg.dz^{-1}), and egg mass (EM) of postmolt laying hens fed diets with different levels of sodium.

Factors	Variables				
	FI (g/bird/day)	EP (%)	FC (kg.kg^{-1})	FC (kg.dz^{-1})	EM (g)
Sodium, %					
0.10	106.29^{c}	79.62^{ab}	2.07^{b}	1.61^{b}	51.53^{ab}
0.15	111.06^{a}	83.86^{a}	2.07^{b}	1.59^{b}	53.89^{a}
0.20	107.49^{b}	72.80^{b}	2.39^{ab}	1.84^{ab}	46.69^{b}
0.25	107.24^{b}	72.91^{b}	2.47^{a}	1.83^{ab}	46.55^{b}
0.30	107.81^{b}	72.64^{b}	2.48^{a}	1.89^{a}	45.61^{b}
Postmolt stage					
Early	100.59^{b}	83.33^{a}	2.06^{b}	1.60^{b}	53.89^{a}
Medium	110.79^{a}	82.82^{a}	2.13^{ab}	1.63^{b}	52.96^{a}
Final	112.54^{a}	62.95^{b}	2.69^{a}	2.02^{a}	39.72^{b}
Effect			*p*-value		
Sodium, %	0.01*	0.01*	0.01*	0.01*	0.01*
Postmolt stage	0.01*	0.01*	0.01*	0.01*	0.01*
Interaction	0.41^{ns}	0.24^{ns}	0.16^{ns}	0.15^{ns}	0.16^{ns}
CV (%)	6.40	12.87	6.54	5.84	13.26

CV - Coefficient of variation; * Significant effect ($p<0.01$); ns – non-significant.

Egg weight and its main structures (yolk, albumen and shell) were affected (*p*<0.05) by the sodium levels and hens' age. Birds fed diets with 0.10% and 0.15% of sodium produced heavier eggs, which reflected in larger percentages of its main structures. High levels of sodium significantly reduced its parameters (Table 3).

Hens in the early stage of postmolt period produced heavier eggs, with better percentages of its main structures. Medium stage of postmolt period also provided great results to egg weight and yolk percentage. From the increase of the hens' postmolt age, there was a reduction in its main structures, especially in the yolk and eggshell.

Table 3 – Egg weight, (EW), percentage of yolk (PY), percentage of albumen (PA), and percentage of eggshell (PS) of eggs from postmolt laying hens fed diets with different levels of sodium.

Factors	Variables			
	EW (g)	Yolk, % (%)	Albumen (%)	Eggshell (%)
Sodium, %				
0.10	64.26ab	29.04a	60.63a	10.00a
0.15	64.74a	29.11a	60.74a	10.11a
0.20	64.14ab	28.12b	59.93b	9.89b
0.25	63.26b	28.03b	59.19b	9.84b
0.30	62.28c	27.99c	59.22b	9.65b
Postmolt stage				
Early	64.69a	60.51a	29.07a	10.32a
Medium	63.98a	60.37a	28.33b	9.90b
Final	62.91b	58.73b	27.92c	9.23c
Effect	*p*-value			
Sodium, %	0.01*	0.04**	0.01*	0.01*
Postmolt stage	0.05**	0.01*	0.01*	0.04**
Interaction	0.10ns	0.06ns	0.07ns	0.12ns
CV (%)	4.08	3.70	4.75	5.43

CV - Coefficient of variation; * Significant effect (*p*<0,01); ** Significant effect (*p*<0,05); ns – non-significant.

Except for the yolk height, all performance variables were affected (*p*<0.05) by the sodium levels. Birds fed diets with 0.15% of sodium presented better egg quality. High levels of sodium significantly reduced the internal and external quality of the eggs (Table 4).

All egg quality variables were affected (*p*<0.05) by the hens' postmolt age. Eggs from hens in the early stage of postmolt period presented better internal and external quality. From the increase of the hens' postmolt age, there was a significant reduction in the egg quality, especially the external quality.

Table 4 – Albumen height (AH), yolk height (YH), eggshell thickness (ST), specific gravity (SG), Haugh unit (HU), and yolk colour (YC) of eggs from postmolt laying hens fed diets with different levels of sodium.

Factors	Variables					
	AH (mm)	YH (mm)	ET (µm)	SG (g/mL^3)	HU	YC
Sodium, %						
0.10	7.76ab	16.49	0.39a	1,085.58ab	86.95ab	5.68b
0.15	7.92a	16.56	0.39a	1,086.08a	88.33a	5.78a
0.20	7.59b	16.72	0.38b	1,084.91b	85.58b	5.66b
0.25	7.45b	16.65	0.37c	1,083.66b	84.90c	5.65b
0.30	7.31c	16.55	0.37c	1,065.91c	84.13c	5.23c
Postmolt stage						
Early	7.73a	17.85a	0.40a	1,088.15a	86.96a	6.12a
Medium	7.54b	16.20b	0.40a	1,085.50b	85.62b	5.82b
Final	7.54b	15.72c	0.34b	1,070.05c	85.36b	4.87c
Effect	*p*-value					
Sodium, %	0.03**	0.86ns	0.02**	0.01*	0.01*	0.01*
Postmolt stage	0.04**	0.01*	0.01*	0.01*	0.02**	0.01*
Interaction	0.08ns	0.13ns	0.18ns	0.12ns	0.17ns	0.14ns
CV (%)	7.37	3.71	5.84	2.89	3.89	7.24

CV - Coefficient of variation; * Significant effect (*p*<0,01); ** Significant effect (*p*<0,05); ns – non-significant.

Sodium levels significantly affected (*p*<0.05) the eggshell resistance (Table 5) both in the vertical and horizontal positions. Birds fed diets with 0.15% of sodium produced the most resistant eggshells in the vertical position ($y = - 0.8479x^2 + 0.25381x + 49.338$ $R^2 = 0.73$), however most fragile in the horizontal position ($y = - 0,8121x^2 + 0,25119x + 39,004$ $R^2 = 0,85$).

Table 5 – Resistance of eggshells in vertical (REV) and horizontal (REH) positions of eggs from postmolt laying hens fed diets with different levels of sodium.

Sodium, %	REV, N	REH, N
0.10	35.33	39.83
0.15	47.97	27.74
0.20	31.18	33.64
0.25	30.98	33.46
0.30	32.67	36.86
p-value	0.05	0.02
Effect	Q	Q
CV (%)	2.09	8.83

CV - Coefficient of variation. *p*-value - Coefficient of Probability. Q - Quadratic effect.

Sodium levels significantly affected (*p*<0.05) the weight gain (Table 6) of postmolt laying hens. Birds fed diets with 0.10% of sodium present a lower weight gain ($y = -1,3936x^2 + 0,70316x + 11,7$ $R^2 = 0,89$). All postmolt hens presented weight gain, regardless of the sodium level used in the diets.

Table 6 – Weight gain of postmolt laying hens fed diets with different levels of sodium.

Sodium, %	Weight gain, %
0.10	1.90
0.15	11.13
0.20	11.63
0.25	11.70
0.30	11.39
p-value	0.01
Effect	Q
CV (%)	7.56

CV - Coefficient of variation. *p*-value - Coefficient of Probability. Q - Quadratic effect.

DISCUSSION

The increase levels of sodium caused a significant effect on the birds' performance. Sodium levels above 0.20% caused a significant lower in feed intake, consequently affecting the feed efficiency, egg production and egg mass. These results corroborate Murakami *et al.* (2003) and Ribeiro *et al.* (2008), where the authors reported that sodium levels between 0.15 to 0.20% were sufficient to provide a better requirement to postmolt hens, presenting a better performance and egg quality, especially the eggshell. The same authors also commented that both deficit and higher sodium levels are adverse to hens, providing worst results.

High levels of sodium in the poultry diets tend to stimulate an excessive water intake, restricts the feed intake, negatively interferes on respiratory alkalosis, electrolyte balance and nutrient absorption by the sodium and potassium pump, generating significant economic losses to the hens (Ribeiro, 2007; Lima *et al.*, 2015). The literature presents a great variance in the requirement of sodium to molted laying hens, where Fassani *et al.* (2002) suggested values between 0.19 to 0.22%, Murakami *et al.* (2003) proposed 0.13%, and Rodrigues *et al.* (2004) recommended 0.25%. Murakami *et al.* (2003), and Lima *et al.* (2015) attributed this significant variation to the use of birds of different strains, ages, environmental conditions among other factors that may influence the physiological response of the hens. However, all authors reported that the performance and the quality of the eggs are negatively affected by high levels of sodium.

The eggshell is the most affected parameter by the sodium levels variation in the diets due to acid-base balance to be an important factor in the eggshell formation (Mongin, 1968). Hall & Helbacka (1959) suggested that the deposition of calcium carbonate in the eggshell is dependent upon blood pH, where the ion sodium is the most important regulator. In this sense, the metabolic acidosis and alkalosis could be produced by altering the dietary sodium to chloride ratio, regardless of the total concentration of these two ions in the diet (Cohen *et al.*, 1972). Frank & Burger (1965) reported that an ideal sodium supplementation result in thicker eggshells. Miles & Harms (1982) indicated that a correct supplementation of sodium associated with the calcium content in the diet result in an increase on egg specific gravity.

According to Gal-Gaber *et al.* (2003) and Costa *et al.* (2012) excessive sodium consumption may affect the kinetic behavior of the small intestine of birds, possibly reducing the absorption, because the affinity of the sodium pump is reduced with excess of sodium in the organism. Thus, less calcium will be absorbed and could be available to eggshell formation, producing eggs with a more fragile shell, where the internal contents will be more susceptible to the action of microorganisms.

On the other hand, our results pointed that the postmolt stage also affected the performance and egg quality. After the molting process, the birds tend to presented great performance and egg quality due to ovarian and oviducal regression (Gongruttananun *et al.*, 2013; Gongruttananun & Saengkudrua, 2016). However, approaching 100 weeks, there was a significant reduction on performance and egg quality. Normally, the molt procedure cause a significant increase on performance and egg quality at short-term and these indexes tend to decrease according to the age of the bird due to the natural wear caused by constant egg production in industrial scale (Wu *et al.*, 2007). Physiologically, there are great wear on oviduct tissues, and difficulty to absorb the nutrients and its transfer to the egg's composition (Newman and Lesson, 1999; Bar *et al.*, 2001; Heflin *et al.*, 2018; Elhamouly *et al.*, 2019).

Different strains may ~~to~~ present different responses on performance after molt, especially on laying hens and semi-heavy hens (Said *et al.*, 1984; Gongruttananun *et al.*, 2017; Hu *et al.*, 2019). There is very limited literature about the performance, egg composition, egg solids, egg quality, and profits of postmolt hens (Wu *et al.*, 2007), especially at long-term, a period near or exceeding ~~to~~ 100 weeks. An induced molting not only may improve performance and eggshell quality, but also increase profits by optimizing the use of replacement pullets on commercial layer farms (Lee, 1982; Barker *et al.*, 1981; Bell, 2003; Mazzuco *et al.*, 2011; Gongruttananun, 2018) proving the importance to understand its effect at long-term.

Wilson *et al.* (1967) studying several methods of inducing a molt and subsequent rest period, observed that molted hens present a better performance and shell thickness at short-term without affect the egg weight and albumen quality, regardless to the molt process used. However, this effect significantly reduces at long-term, where the hens present clear signals of fatigue due to the old age (Chanaksorn *et al.*, 2019).

At the end of the experimental period, when the hens presented nearly 100 weeks, all birds presented a gain in the weight. However, the level of sodium in the diet directly influenced this parameter, where laying hens fed diets with the lower sodium level present a significant lower weight gain. Normally, a reduction in body weight of 15 to 30% during the molt is necessary to maximize the performance of laying hens at the second cycle (Barker *et al.*, 1981). The birds lose this weight during the molt period and gain (recover) at a faster rate after the molt (Wilson *et al.*, 1967). McDaniel (1985) reported that the lightest birds before molt have a tendency to gain the greatest percent weight from the end of the molt to the onset of egg production. In this sense, Buhr and Cunningham (1994) also commented that during the postmolt period the body weight gain increased, with the 15% weight loss groups maintaining a slightly heavier body weight throughout the postmolt period. The same authors affirmed that the molt method directly affect the body weight, performance, and egg quality.

CONCLUSIONS

It was concluded that the level of 0.15% of sodium in diets to postmolt laying hens provided better performance and egg quality, especially in the eggshell. Higher levels of sodium negatively affected the performance and egg quality. Hens in the early stage of postmolt period presented better performance and egg quality. In the long-term, the postmolt hens presented worst results.

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