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Egg quality analysis and performance of laying hens fed different levels of calcium

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Abstract

This study was conducted to examine the quality of eggs and the performance of laying hens fed different levels of calcium. The study was carried out at the Poultry Unit of University of Port Harcourt Teaching and Research Farm, Choba, Port Harcourt, Rivers State. One hundred and twenty black Harco birds were used for the study using the completely randomized design. The laying birds were fed with diets consisting of different level of calcium (Ca), which comprised mainly of limestone and bone meal such that treatment 1 (T_1) had 2.5 % Ca, $T_{2\,fi}$ 3.5%, $T_{3\,fi}$ 4.5% and $T_{4\,fi}$ 5.5% Ca, respectively. All the birds were raised within four treatment groups which had three replicates of 10 birds each. At the termination of the study, the eggs and the feed records were used to compute the hen-day production (HDP), feed intake, number of eggs laid per hen, dozens of eggs laid per hen, feed consumed per dozen egg, cost of feed per dozen egg produced and mortality. Six eggs were collected from each replicate to study the eggs external quality (egg weight and width, the weight of the shell, the egg shape index, egg length, shell index and thickness) and internal quality (albumen weight, yolk diameter, yolk height and index, yolk pH and pH of the albumen, albumen height, yolk weight, yolk ratio and yolk albumen ratio). The study revealed that significant ($P<0.05$) differences existed in the performance parameters such as the hen-day production (HDP), feed intake, number of eggs laid per hen, dozens of eggs produced per hen, feed consumed per dozen egg produced and the cost of feed per dozen egg produced. The hens fed the T_2 and T_3 had the highest HDP, those fed the T_3 and T_4 had better feed conversion while the feed cost for T_3 feed was the best (the least). Significant differences were also observed in some of the external and internal parameters of the eggs, including, the egg weight, yolk weight, yolk height, yolk index and Haugh unit (HU) while others were not affected. Although the egg from T_4 had the highest HU, eggs from all the treatment were within the 'AA' group (HU of more than 72) termed high quality egg. The study showed that farmers can use Ca levels of 3.5 and 4.5% in the diet of the layers and obtain better HDP. However, the diet which had the 4.5% Ca inclusion, gave the best feed conversion and the least cost of the diet per dozen egg produced and was recommended for layers in the humid tropical zone.

Keywords: Calcium, Egg quality, Egg production, Laying hens, Performance, Haugh unit

Introduction

Calcium (Ca) is very necessary for layers. According to Hurwitz (1987), Ca regulates most of the body processes, including hormone biosynthesis, transference of cell information, cell replication and differentiation and it is useful during eggshell formation. The eggshell contains 90% mineral matter, 98% of it is made up of calcium carbonate (Mendonça-Junior, 1993). Losses resulting from egg shell defects ranges from 6 to 8% of the total eggs that are laid (Roland *et al*., 1977). But studies have shown that calcium and its inclusion level influences eggshell quality

(Faria, 2000). Butteler and Miles (2005) indicated that eggshell breakage is directly related to the quality of the eggshell and the capacity of the shell to withstand external forces. Estimates show that about 14.3% and 21.3% of all the eggs lay in the world either have cracks or are broken before they get to the final consumer (Chrystal, 2000).

The absorption of Ca is related to several factors, such as vitamin D, protein, phosphates, free fatty acids, and zinc levels. Eggshell works as a "package" However, several works have shown that the feed consumed of layers can be influenced by the dietary calcium level as the hypothalamus is capable of stimulating the release of norepinephrine (a neurotransmitter which acts in the central nervous system) to enhance the consumption of feed. Reduced feed consumption/intake of diets with higher calcium concentrations was reported by Clunies *et al.* (1992). They stated that when Ca was increased in diets, there was an increase in shell thickness, weight, and resistance to cracking but phosphorus had no effect on these parameters. Cheng and Coon (1990) evaluated different sources of limestone, levels of the diets, and the sizes of the particle, and concluded that the treatments that were applied did not affect the production of eggs or weight, but influenced eggshell quality.

According to Leeson and Summers (1997), when the medullar bones are used for eggshell formation, there is usually sudden loss of 2g of body calcium, therefore, a calcium bone reserve must be built up before the production period since eggshell works as a "package" for the egg contents and protects the embryo, and must be optimally strong to sustain egg laying, collection, grading, and transport, until it gets to the final consumer. Eggshell quality is therefore, very important, as consumers are always concerned with the safety of food since poor quality of shell could be a potential source of contamination (Kussakawa *et al.*, 1998).

It therefore, imply, that Ca and P are important dietary components needed for egg production, yet, the amounts required for the laying hen had always been debated. According to the NRC (1994), a Leghorn-type laying hen requires 2.71, 3.25 and 4.06% dietary calcium for feed intakes of 120, 100 or 80g/hen/day respectively. Holcombe *et al*. (1975) found that young hens exhibited the ability to differentiate between high and low levels of calcium in the diet. When two diets were offered, hens would increase consumption of the diet higher in calcium content. Holcombe *et al*. (1976) repeated the study using phosphorus and obtained similar results and stated that hens would regulate their phosphorus consumption when given the different diets that vary in the phosphorus levels. These studies suggest that P and Ca play a crucial role in the production of eggs by laying hens and can be detrimental if offered in excess or if deficient. Thus, the correct balance needs to be met to obtain optimal egg production in any given environment, since the established adequate P and Ca levels for layers seem to have been challenged because of the numerous advances in nutrition, genetic improvement, management and environment (Pelicia *et al*., 2009). Thus, to minimize broken eggshell percentage, production costs, and environmental impacts, Oliveira *et al*., (2002) stated that studies on optimal calcium levels in layer diets will be economically important. It is against this backdrop that this research was conducted to obtain the optimum level of Ca inclusion in layers' diet that is needed for good quality of egg and better performance.

Materials and methods

Location of study

The experiment was carried out at the Poultry Unit of the University of Port Harcourt Teaching and Research Farm, Choba, Port Harcourt, Rivers State.

Experimental materials, housing, and design

Black Harco birds numbering a hundred and twenty which were 42 weeks old were purchased for the study. The birds were properly housed in a deep litter system that was well demarcated into replicates. The birds were allocated to four treatments (T_1-T_4) which had 30 birds each (10 birds per replicate). The design of the experiment was the completely randomized design.

Management

All the birds had the same management condition during the period of the study. Routine management practices such as feeding, cleaning, washing of drinkers, washing of troughs, disinfection of foot dip and environmental sanitation were maintained out throughout the study period.

Experimental diet and duration of the study

Feed ingredients were bought and the layers' diets diet formulated as shown in (Table 1). The calcium content of the diet consisted of limestone and bone meal included as 2.5 % Ca in T_1, 3.5 % Ca in T_2 (control), 4.5 % Ca in T_3 and 5.5 % Ca in T_4. The experimental period was 12 weeks.

Table 1: Composition of experimental layers' diets

Ingredient (%)	T_1 (2.5% Ca)	T_2 (3.5% Ca)	T_3 (4.5% Ca)	T_4 (5.5% Ca)
Maize	37.25	37.25	38.25	38.25
Palm kernel cake	15.00	15.00	15.00	15.00
Soya bean meal	13.00	13.00	14.00	15.00
Groundnut cake	10.00	10.00	8.00	7.00
Fish meal	2.00	2.00	3.00	3.00
Wheat bran	14.00	11.00	7.50	5.00
Palm oil	2.00	2.00	2.00	2.00
Limestone	3.50	5.00	6.50	8.00
Bone meal	2.50	4.00	5.00	6.00
Dl meth	0.05	0.05	0.05	0.05
Lysine	0.05	0.05	0.05	0.05
[1]Vit/Min	0.25	0.25	0.25	0.25
Salt	0.40	0.40	0.40	0.40
Total	100.0	100.0	100.0	100.0
Calculated nutrient (%)				
Crude protein	19.40	19.33	19.05	19.02
ME (Kcal/kg)	2540.74	2508.06	2498.84	2496.81
Fat	5.16	5.04	4.99	4.98
Crude fibre	5.49	5.44	5.12	4.98
Lysine	0.88	0.87	0.89	0.88
Meth	0.33	0.33	0.34	0.34
Ca	2.50	3.54	4.54	5.51
Avail, P	0.99	1.17	1.31	1.42
Ca: P	2:1	3:1	3.5:1	4:1

[1]Vitamin and Trace mineral f or each kg contained: Mn 40g, Fe 20g, Zn 18g, Cu 0.8g, I 0.62g, Co 0.09g and Se 0.04g, pantothenic acid 1,000,000 I.U, cholecalciferol 1,100,000 I.U, quinones 0.8g, thiamin 0.6g and riboflavin 2.4g, folic acid 0.4g, biotin 0.02g, ascorbic acid 10.0g, Cholne chloride 120.0g, zinc bacitracin 80.0g, avatec 30.0g

Data collection

Weekly egg production record was kept during the experiment and used to obtain the hen day production, dozens of eggs produced and feed conversion (feed/dozen egg) while the feed intake record and the cost of ingredients were used to compute the feed intake and the cost of feed per dozen egg. At the end of the 12 weeks' study, six eggs were collected per replicate (18 eggs per treatment) for analysis of egg quality. The parameters studied were the external quality (egg weight, egg width, shell weight, egg shape index, shell index, egg length and shell thickness) and the internal quality (albumen weight, yolk diameter, yolk height, yolk index, yolk pH, albumen pH, albumen height, yolk weight, yolk ratio and yolk albumen: ratio).

The eggs were weighed with an electronic weighing scale and recorded in grammes. The length of the egg was measured with a Vernier caliper by taking the longitudinal distance between the narrow and broad ends of the egg while the egg width was obtained from the diameter of the widest cross-sectional region. These values were used to calculate the egg shape index (width of egg ÷ length of egg x100%). Individual eggs were carefully broken at the middle into a petri-dish and the yolk was separated from the albumen. The albumen and yolk weights were recorded. These values were used to calculate the yolk: albumen ratio (yolk weight ÷ albumen weight). The yolk height was measured and yolk diameter was obtained by measuring the maximum cross-sectional diameter using a pair of Vernier caliper. These were used to compute the yolk index (yolk height ÷ yolk diameter x100 %). The albumen height was also obtained by measuring the longest expanse of the albumen (between the yolk edge and the external edge of the thick albumen). The albumen and yolk pH were obtained using the universal pH indicator. The shells were washed with slight flowing water to properly clean them, they were left to dry at room temperature (along with the thin membranes) for 24 hours, after which they were weighed with a sensitive electronic weighing scale, while the shell thickness along with the membrane was recorded as the average value of the thickness taken from the broad end, narrow and the equatorial parts of each egg with the aid of a micrometer screw gauge. The shell weight and egg weight were used to calculate the shell index (shell weight ÷ egg weight x 100 %) while the Haugh unit was calculated using the expression: 100log $(H + 7.5 - 1.7W^{0.37})$, where: H = Albumin height in cm and W = egg weight in grams.

Data analysis

Data were analyzed using the one-way analysis of variance (ANOVA) of SAS (1990) while the differences in the mean that were significant were separated using Duncan Multiple Range Test.

Results

The influence of the different calcium levels in the diets on the performance of hens is shown in Table 2. The diets significantly ($P < 0.05$) affected the hen day production (HDP), feed intake, number of eggs lay per hen, dozen eggs/hen, feed/dozen egg (feed conversion) and feed cost/dozen egg while mortality was not affected. The highest HDP was obtained from birds fed the T_2 and T_3 diets, followed by the HDP of T_4, while the lowest was observed in T_1 (70.20%). The birds fed the T_1 diet had the highest feed intake followed by T_2, while T3 and T_4 had significantly lower feed intake. The number of eggs laid per hen and the dozen eggs/hen followed the same trend as the HDP with T_2 and T_3 being significantly higher followed by T_4 and lastly T_1. The feed conversion by the birds (feed/dozen egg) was significantly better for those fed the T_3 and T_4 diets follow T_2 and worst by those fed the T_1 diet. The cost of feed per dozen egg was however significantly higher for T_1 followed by T_2 and T_4 while the feed cost for T_3 feed was the best (the least). There was no mortality during the period of study.

Table 2: Influence of dietary treatments on performance of laying hens

Parameters	T_1	T_2	T_3	T_4	SEM
Hen day production (%)	70.20^c	79.30^a	78.20^a	73.90^b	1.50
Feed intake/g/hen/day	155.58^a	146.65^b	133.53^c	133.00^c	0.82
No of eggs laid/hen	59.00^c	66.60^a	65.70^a	62.07^b	1.01
Dozen egg/hen	4.92^c	5.55^a	5.48^a	5.17^b	0.12
Feed/dozen egg	2.66^a	2.22^b	2.05^c	2.16^c	0.15
Feed cost/dozen	358.35^a	299.15^b	276.24^c	291.13^b	8.57
Mortality	0.00	0.00	0.00	0.00	0.00

^{abc} = means within each row that bear superscripts different significant figure ($P<0.05$)

The effect of calcium on the external and external quality of the eggs is shown in Table 3. The diets significantly ($P<0.05$) affected egg weight, yolk weight, yolk height, yolk index and haugh unit (HU). The highest egg weight which was significantly different ($P < 0.05$) was obtained from T_4 while the other treatments had lower weight. There was significant ($P < 0.05$) difference in yolk weight with the highest observed in T4 which was not different from T_1 and T_2 and the least observed in T_3. The yolk height was highest in T4 although not significantly different from T_1 while T_2 was the lowest. The highest HU was obtained from eggs in T_4 and this was significantly ($P < 0.05$) different from the values obtained from the other treatments (T_1, T_2, T_3). There were no significant ($P > 0.05$) differences in egg width, egg length, shell thickness, shell weight, egg shape index, shell index, yolk diameter, albumen weight, yolk: albumen ratio, yolk pH, albumen pH and albumen height.

Table 3: Influence of dietary treatments on external and Internal quality of eggs

Parameters	*T_1*	*T_2*	*T_3*	*T_4*	*SEM*
External quality					
Egg weight (g)	63.67^b	62.00^b	62.67^b	69.00^a	1.59
Egg width (cm)	4.17	3.67	4.40	4.67	0.28
Egg length	5.60	5.67	6.17	6.00	0.35
Shell thickness (mm)	1.10	1.10	1.10	1.13	0.52
Shell weight (g)	7.00	6.33	7.00	6.67	0.31
Egg shape index	76.67	66.67	71.31	76.67	3.94
Shell index	11.00	7.67	11.67	9.67	1.43
Internal quality					
Yolk weight (g)	16.33^{ab}	16.00^{ab}	14.33^b	18.67^a	0.88
Yolk diameter (cm)	4.13	4.33	4.17	4.53	0.17
Yolk height(cm)	0.81^{ab}	0.57^c	0.73^{bc}	0.93^a	0.38
Albumen weight (g)	36.3	37.7	39.67	40.67	1.43
Yolk index	19.61^a	13.16^b	17.51^{ab}	20.53^a	1.78
Yolk albumen	0.45	0.42	0.36	0.46	1.98
Yolk P^H	5.00	5.00	5.00	5.00	0.00
Albumen P^H	9.00	9.00	9.00	9.00	0.00
Albumen height (cm)	4.00	3.70	4.00	4.70	0.08
Haugh unit	76.64^b	74.81^b	76.86^b	80.81^a	3.54

^{abc} = means within each column that bear superscripts different significant figure ($P<0.05$)

Discussion

The result in Table 2 which showed that all the parameters were affected by the diets excluding mortality could be as a result of the different calcium (Ca) levels of the feed. The hen-day production (HDP), number of eggs laid per hen and dozen egg per hen which were highest in T_2 and T_3 tallied with that of Safaa *et al.* (2008) who found an increase in some performance parameters including egg production, egg mass and feed conversion when the level of Ca in diet was increased. Also, Roland and Bryant (1994) reported that when the Ca in the diet was increased from 3.3 to 4.1%, egg production, number of eggs laid per hen and dozen egg per hen were affected (increased) during 24th to 27th weeks of age. The result obtained was however contrary to those of Keshavarz and Nakajima (1993), Castillo *et al*. (2004) and Phalmast *et al.,* (2015) who did not find any effect of diet on egg production, number of eggs laid per hen and dozen egg per hen when Ca or Ca and vitamin D3 was increased to more than 3.5% in diets of laying hens. Cufader *et al.* (2011) also stated that significant effect of the diets was not noticed in egg production and egg weight when Ca levels of 3.0, 3.6 and 4.0% were included in the feed for old laying hens. Frost and Roland (1991) and Keshavarz and Nakajima (1993) observed that varying the levels of Ca in diet did not significantly affect the production of eggs, the number of eggs laid per hen and the dozens of eggs laid per hen. Similarly, Bar *et al.* (2002) stated that egg production, number of eggs laid per hen and dozen egg per hen when their age was 66 to 78 weeks were not affected when Ca intake increased from 3.6 to 4.9%, however, in a second trial, these authors observed an increase in egg production, number of eggs laid per hen and dozen egg per hen when dietary Ca of laying hens was increased from 3.5 to 4.8% from 57 to 65 weeks of age. The variations in the results obtained by researchers can be attributed to the rate of egg production, age of birds, nutrient content of diets and the number of cycles of lay. Also, improved metabolic functions which are normally associated with adequate Ca levels of diet may have resulted in the higher HDP, the number of eggs laid per hen and dozen egg per hen in T_2 and T_3 implying that T_1 and T_4 diets may not be best for hens at this age.

The decreasing feed intake level in this study as the Ca level increased tallied with the report by Araujo *et al.* (2011) who stated that there was lower feed intake by layers when the Ca in diet was increased from 3.5 to 4.5% in the post-moulting period. It was also in-line with the report of Clunies *et al.* (1992) who found that reduced feed intake was associated with higher Ca concentrations in the feed of the laying hens. Narvaez-Solarte *et al*. (2006) reported that there was decrease in the feed consumed daily as the Ca levels in the diet increased. Chandramoni and Sinha (1998) reported that when the dietary Ca levels increased, the feed intake tended to be increased but not significantly, attributing the discrepancies in results to the differences in age of bird, dietary energy density and feeding levels of Ca. However, Costa *et al*. (2008) and Nascimento *et al.* (2014) did not find any difference in feed intake when Ca levels of 3.5 to 5.0 % were tested. The effect of diets on feed intake and feed conversion in this study which was significant was contrary to the findings of Kussakawa *et al.* (1998) who stated that there was no effect on these parameters when Ca was increased from 3.0 to 4.0%. The different calcium ingredients used in their study which included calcite limestone and marine calcium may have contributed to this.

The better feed conversion ratio

(feed/dozen egg) obtained by birds fed the T_3 and T_4 diets may be associated with the standard Ca: P ratio of 4: 1 recommended by NRC (1994) for laying hens. This tallied with the report by Nascimento *et al*., (2014) who stated that feed conversion ratio (kg/kg and kg/dz) and egg production presented quadratic responses ($P < 0.05$). Pelicia *et al*., (2009) reported that the diet that contained 4.5% calcium had better feed conversion ratio (FCR) per dozen eggs and eggshell quality. Also, Safaa *et al*. (2008) stated that an increase in the Ca level of layers' diet from 3.5 to 4 % resulted in improved performance, including feed conversion ratio, egg production and egg mass in Lohmann Brown laying hens from 58 to 73 weeks of age. A better feed conversion ratio was also observed by Oliveira (2001) who reported improved FCR/kg eggs when feeding 3.74% of Ca in layers' diet. However, there was no effect of Ca level on feed conversion ratio (FCR) per kg eggs according to Araujo *et al*. (2005).

The absence of mortality of the layer during the period of this study confirmed that the available phosphorus (avP) in various diets were adequate since Sakomura *et al*. (1995) attributed increased mortality in laying flock to the deficiency of available phosphorus in the diet which causes osteoporosis of laying hens especially when they are kept in cages.

The significant effect of the diets on egg weight obtained in this study tallied with the report of Roland and Bryant (1994) who reported that as the Ca in diets increased from 3.3 to 4.1%, egg weight was affected in hens of 24 to 27 weeks of age. However, Keshavarz *et al.* (1993) recorded no difference in egg weight from 38 to 62 weeks of age of laying birds when the calcium content of the diet was increased from 3.5 to 4.0 %. Keshavarz and Nakajima (1993), Oliveira *et al*. (1997), and Oliveira (2001) who separately worked on increasing levels of Ca from 2.80 to 4.40 % did not observe any effect on egg weight. An initial study by Bar *et al.* (2002) stated that the weight of eggs obtained from hens which were 66 to 78 weeks of age were not affected when the Ca in the diets were increased from 3.6 to 4.9%. However, in a second trail, these authors found a trend toward decreased egg weight when dietary Ca was increased from 3.5 to 4.8% in the diet of hens which were 57 to 65 weeks of age.

The significant difference observed in the yolk weight was due to the difference in the weight of the eggs while the difference in the yolk index which was significant may be attributed to the difference in the yolk height. But Oliveira (2001) found no effects of dietary Ca and avP levels on yolk percentage in his study.

The haugh unit (HU) of all the eggs across the treatment groups which were high (above 70), with T_4 being significantly higher tallied with the report by Albano-Junior *et al*. (2000) who stated that 2 and 4% Ca levels in diet supported higher HU when compared to 5% Ca, while 3 and 6% Ca levels in diet were not significantly different from the other levels. The high HU obtained in this study implied that the eggs were good for human consumption. Eggs with HU less than 40 had been classified as inferior eggs that were not good for consumption (Garba *et al.*, 2010). The classification of eggs according to haugh unit as AA for HU of 72 and above, A (60-71 HU), B (31-59 HU) and C (30 HU) with the AA and A, as eggs of high quality B and C as those with low quality and a general consideration of egg with HU of 70, as been acceptable (USDA 2000), imply that all the eggs obtained from this study will be acceptable to consumers.

The non-significant effect of the diets on shell weight and shell thickness weight in this study agreed with the report of Keshavarz (1998) who observed that shell weight and shell percentage were not changed when there was an increase in the dietary Ca from 3.1 to 3.8% however, there was improvement in the shell thickness from 0.372 to 0.86mm. The result obtained was also in agreement with the report of Rao and Roland (1990) who observed that the shell weight and shell thickness of eggs were not significantly affected when the Ca in the diets was increased from 3.2 to 4.5 %. Accordingly, Rodrigues (1995) also did not find any significant effect of varying the Ca levels on eggshell thickness. But Rodrigues *et al*., (2005) reported that 3.5% Ca in the diet improved the thickness of the eggshell when compared to 2.0% Ca.

The internal egg qualities such as, yolk diameter, albumen weight, yolk: albumen ratio, yolk P^H, albumen P^H and albumen height which did not give any significant differences in this study agreed with several researchers (Keshavarz and Nakajima, 1993, Roland and Bryant, 1994, Castillo *et al*., 2004) who recorded no additional improvement in the internal egg quality when there was an elevation in the Ca level of the diet to more than 3.5%.

Conclusion

The performance of the birds showed that the T_2 and T_3 diets were better in terms of egg production. Thus, farmers can use Ca levels of 3.5 and 4.5% in diet for laying hens and obtain better result. However, the T_3 diets (4.5% Ca) which had the best conversion of feed to eggs, as well as the least cost of production is termed the best diet. Based on the egg quality, all diets were good with T_4 being the best with the higher egg weight and HU. However, since the goal of every producer is to make more profit using cheapest diet, the T_3 diet which had the highest HDP (although with T_2), best feed conversion lowest feed cost and good weight of eggs and HU is recommended for layer production in the humid tropical zone.

References

Albano-Junior M., Albuquerque, R. and Lima, C.G. 2000. Desempenho e qualidade dos ovos de diferentes linhagens de poedeiras comerciais pós-muda forçada recebendo rações com níveis variáveis de cálcio. *Brazilian Journal Veterinary Research Animal Science*. 37(4):334 – 338

Araújo, J.A., *Silva, J.H.V., Costa, F.G.P., Givisiez, P.E.N. and Sakomura, N.K.* 2005. Levels of calcium, available phosphorus and phytase in the diet of laying hens after forced molting. *Annals of the 42nd Annual Meeting of the Brazilian Zootechnical Society. Goiânia, Goiás. Brazil*.

Araujo, J.A., Silva, J.H.V., Costa, F.G.P., Souza, J.M.B., Givisiez, P.E.N. and Sakomura, N.K. 2011. Effect of the levels of calcium and particle size of limestone of limestone on laying hens. *Revista Brasileira de Zootecnia*. 40(5): 997 - 1005

Bar, A., Razaphkovsky, A.V. and Vax E.2002. Re-evaluation of calcium and phosphorus requirements in aged laying hens. *British Poultry Science*. 43:261-169

Butteler, G. D. and Miles, R. D. 2005. Concept of egg shell quality http://www.ofn.org/povitry/flxman4.htm

Castillo, C., Cuca, M., Pro, A., Gonzalez, M. and Morales, E. 2004. Biological and economic optimum level of calcium in White Leghorn laying hens. *Poultry*

Science. 83(6): 868-872

Chandramoni. J.S.B. and Sinha R.P. 1998. Effect of dietary calcium and phosphorus concentrations on retention of these nutrients by caged layers. *British Poultry Science*. 39: 544-548

Cheng, T.K. and Coon, C.N. 1990. Effect of calcium source, particle size, solubility in vitro limestone, and calcium intake level layer on bone status and performance. *Poultry Science*. 69 (12): 2214-2219.

Chrystal, P. 2000. South African Limestone: The cheap ingredient. http://www.spesfeed.co.za/avtvmn%2.2000.htm.

Clunies, M., Parks D., and Leeson S. 1992. Calcium and phosphorus metabolism and eggshell formation of hens fed different amounts of calcium. *Journal of Applied Poultry Research*.71:482-489.

Costa, F.G.P., Oliveira, C.F.S., Dourado, L.R.B., Neto, R.C.L., Campos, M.A.S.F. and Lima, A.G.V.O. 2008. Níveis de cálcio em dietas para poedeiras semipesadas após o pico de postura. *Revista Brasileira de Zootecnia*. 37(4):624-628.

Cufador, Y., Olgun, O. and Yildiz A.O. 2011. The effect of dietary calcium concentration and particle size on performance, egg shell quality, bone mechanical properties and tibia mineral contents in moulted laying hens. *British Poultry Science*. 70: 963-969

Faria, J.O.M., Sakomura N.K. and Santana, A.E. 2000. Power systems and supplemental flour shell oysters on the performance and quality of shell eggs from hens commercial. *Brazilian Journal of Animal Science*. 29 (5): 1394-1401

Garba, S., Jabir, M. and Omojola, A.B. 2010. Egg Quality of Commercial hens fed diet fed with increasing substitution level of metabolizable energy of pearl millet for corn. *Proceeding of 35th Annual Conference of Nigerian Society for Animal Production, University of Ibadan, Nigeria*. pp. 308-310

Holcombe, D. J., Roland, D. A. and Harms, R. H. 1975. The ability of hens to adjust calcium intake when given a choice of diets containing two levels of calcium. *Poultry Science*. 54:552-561.

Holcombe, D. J., Roland, D.A. and Harms. R. H. 1976. The ability of hens to regulate phosphorus intake when offered diets containing different levels of phosphorus. *Poultry Science*. 55:308-317.

Hurwitz, S. 1987. Effect of nutrition on egg quality. In: Wells RG, Belyavin, C.G. Egg quality current problems and recent advances. *London: Butterworths*. pp. 1522-1527.

Kesavarz, K. and Nakajma S. 1993. Re-evaluation of calcium and phosphorus requirements of laying hens for optimum performance and eggshell quality. *Poultry Science*. 72:144-153

Keshavarz, K. 1998. Further investigations on the effect of dietary manipulation of protein, phosphorus, and calcium for reducing their daily requirement for laying hens. *Poultry Science*. 77: 1333-1346.

Kussakawa, K.C.K., Murakami, A.E. and Furlan, A.C. 1998. Combinations of calcium sources in diets of laying the final stage of production and after molting.

Brazilian Journal of Animal Science. 27 (3): 572-578.

Leeson, S. and Summers, J.D. 1997. Commercial Poultry Nutrition. 2nd edn. *Guelph University Books*. 355pp

Mendonca-Junior C.X. 1993. Factors involved in the nutritional quality of the egg. Proceedings of the 3rd Symposium Technical Production of eggs São Paulo, SP. Brazil. São Paulo: APA; p.29-51. Metabolism and production in laying hens. *British Poultry Science*. 18:633-640

Narvaez-Solarte, W., Rostagno, H.S., Soures, P.R., Uribe-Velasquez, L.F. and Silva M.A. 2006. Nutritional requirement of calcium in white laying hens from 46 to 62 weeks of age *International Journal of Poultry Science*. 5:181-184

Nascimento, G.R., Murakami, A.B., Guerra, A.F.Q.M., Ospinas-Rojas, I.C., Ferreira, M.F.Z., and Fanhani J.C. 2014. Effect of different vitamin D sources and calcium levels in the diet of layers in the second laying cycle. ***Revista Brasileira de Ciencia Avicola***. 16(2): 37-42

National Research Council. 1994. Nutrient Requirements of Poultry. 9th revised edition. *National Academy Press, Washington D C. U.S.A*. ISBN-13: 9780309048927, Pages: 155.

Oliveira, J.E.F., Oliveira, B.L. and Bertechini, A.G. 1997. Níveis de cálcio granulometria e horário de fornecimento de calcário no desempenho e na qualidade do ovo de poedeiras leves no segundo ciclo de postura. *Ciência e Agrotecnologia*. 21:502-510.

Oliveira, J.R. 2001. Níveis de cálcio e de fósforo em dietas de poedeiras leves e semipesadas no segundo ciclo de produção [dissertação]. *Lavras: Universidade Federal de Lavras*.

Oliveira, J.R., Bertechini, A.G. and Fassani, E.J. 2002. Calcium levels in diets for laying light and semi-heavy in the second production cycle. *Science and Agrotecnologia*. 26: 1060-1067.

Pelicia, K., Garcia, E.A., Faitarone, A.B.G., Silva, A.P., Berto, D.A., Molino, A.B. and Vercese, F. 2009. Calcium and available phosphorus levels for laying hens in second production cycle. *Brazilian Journal of Poultry Science*. 11(1): 39 - 49

Phalmast, H., Kijparkorn, S. and Ittitanawong, P. 2015. Effects of vitamin D-3 and calcium on productive performance, egg quality and vitamin D-3 content in egg of second production cycle. *The Thai veterinary medicine*. 45(2) :189-19

Safaa, H.M., Serrano, M.P., Valencia, D.G., Frikha, M., Jimenez-Moreno, E. and Mateos, G.G. 2008. Productive performance and egg quality of brown egg-laying hens in the late phase of production as influenced by the level and source of calcium in the diet. *Poultry Science*. 87: 2043-2051.

Sakomura, N.K., Silva, G.S., Pinheiro, J.W., Resende, K.T., and Junqueira, O.M. 1995. Exigências nutricionais de fósforo para galinhas poedeiras. *Revista Brasileira de Zootecnia*. 24(6):936-951.

SAS Institute 1990. SAS/STAT User's Guild. *SAS Institute Inc., Cary, NC, USA*.

Rao, K.S. and Roland S.R. 1990. In-vivo solubilization of limestone in commercial Leghorns: Role of dietary calcium level, limestone particle size, in vitro solubility rates limestone, calcium and the status of the hen. *Poultry Science*. 69: 2170-2176.

Rodrigues, P.B. 1995. Fatores nutricionais que afetam a qualidade do ovo de poedeiras de 2º ciclo [dissertação]. *Lavras Universidade Federal de Lavras*.

Rodrigues, E.A., Janqueira, O.M., Velerio, M., Andreotti, M.O., Cancherini, L.C. Faria, D.E.and Fillardi, R.S. 2005. Níveis de cálcio em rações de poedeiras comerciais no segundo ciclo de postura. *Acta Scientiarum Animal Sciences*. 27(1): 49-54.

Roland, D.A., Putman E.C. and Hilburn R.L. 1977. The relationship of the ability of hens to maintain eggshell calcification when stressed with adequate dietary calcium. *Poultry Science*. 57 (6): 1616-1621.

Roland, D.A. and Bryant, M.M. 1994. Influence of calcium on energy consumption and egg weight of commercial leghorns. *Journal of Applied Poultry Research*. 3: 184-189.

USDA 2000. United States Department of Agriculture, United States Grades and Classes for Eggs. *AMS 56210. ASM, USDA, Washington, DC.*

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