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Effect of using different levels of dietary calcium and 1α hydroxycholecalciferol, on performance, from 70 to 82 weeks of age, in brown laying hens

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Abstract:	Brown laying hens, after the 70th wk of life, tend to increase the number of unsellable eggs produced due to a decrease in eggshell quality. Mortality due to prolapses and osteoporosis also increases after 65 to 70 wk of age making it difficult to reach the global industry goal of 500 eggs produced per hen in one laying cycle. Management of dietary calcium (Ca) and vitamin D3 (Vit D3) can influence both livability and eggshell quality. The industry´s nutritional data shows an increasing level of Ca in brown laying hens diets, because can increase productivity performance, nevertheless, negative impact of high Ca levels on digestibility and absorption of amino acids, fat, phosphorus (P) and microminerals potentially develop a long term problems for the bird. Additionally, levels of the active form of Vit D3: 1,25 dihydroxycholecalciferol (1,25(OH)2D3) decrease in layers (70th wk) and it has an important role in Ca metabolism because participated in: Ca transcellular absorption in the gut, Ca bone resorption and Ca in eggshell deposition. New strategies for Ca metabolic problems in older laying hens must be study and applied in the field, to decreasing the currently used of paracellular absorption in the gut (which needs more Ca) and increase the use Vit D3 metabolites like 1α hydroxycholecalciferol (1α(OH)D3) which has a similar bioefficiency as 1,25(OH)2D3 active form of Vit D3. The hypothesis of this research report is: that the concentrations of Ca in brown layer diets commercially used after 70 wk of age in Colombia are in excess and that the use of 1α(OH)D3 allows for lowering Ca concentrations in 10 even 20% in the diet without negatively impacting egg production, shell quality or mortality
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CALCIUM AND VITAMIN D_3 IN LAYING HENS

Effect of using different levels of dietary calcium and 1α hydroxycholecalciferol, on performance, from 70 to 82 weeks of age, in brown laying hens.

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SUMMARY

Brown laying hens, after the 70th wk of life, tend to increase the number of unsellable eggs produced due to a decrease in eggshell quality. Mortality due to prolapses and osteoporosis also increases after 65 to 70 wk of age making it difficult to reach the global industry goal of 500 eggs produced per hen in one laying cycle. Management of dietary calcium (**Ca**) and vitamin D_3 (**Vit D_3**) can influence both livability and eggshell quality. The industry´s nutritional data shows an increasing level of Ca in brown laying hens diets, because can increase productivity performance, nevertheless, negative impact of high Ca levels on digestibility and absorption of amino acids, fat, phosphorus (**P**) and microminerals potentially develop a long term problems for the bird. Additionally, levels of the active form of Vit D_3: 1,25 dihydroxycholecalciferol (**$1,25(OH)_2D_3$**) decrease in layers (70th wk) and it has an important role in Ca metabolism because participated in: Ca transcellular absorption in the gut, Ca bone resorption and Ca in eggshell deposition.

New strategies for Ca metabolic problems in older laying hens must be study and applied in the field, to decreasing the currently used of paracellular absorption in the gut (which needs more Ca) and increase the use Vit D3 metabolites like 1α hydroxycholecalciferol (**$1\alpha(OH)D_3$**) which has a similar bioefficiency as $1,25(OH)_2D3$ active form of Vit D_3.

The hypothesis of this research report is: that the concentrations of Ca in brown layer diets commercially used after 70 wk of age in Colombia are in excess and that the use of $1\alpha(OH)D_3$ allows for lowering Ca concentrations in 10 even 20% in the diet without negatively impacting egg production, shell quality or mortality.

Key Words: calcium, 1α-hydroxycholecalciferol, limestone, laying hens, egg production and egg shell quality.

DESCRIPTION OF PROBLEM.

Today, the goal in brown laying hens is to reach 100 wk of age with a production of 500 eggs per bird **(b)** and per cycle. In 2018, Asimetrix® (Asimetrix® Kra 50 # 2 sur 251, Medellin, Colombia) a company specialized in the systematization of production data, produced a diagnostic report of the results of different egg producing companies in Colombia. This analysis revealed that there were challenges to reaching the desired goals mainly due to problems that happens after the birds reach 70 wk of age: 1.) Increase in unsellable eggs between week 70 (2.5%) and 80 wk of age (6.5%); 2.) Higher mortality than breed guideline (Lohmann, 2016) the productive data evidence 7% vs 5 % in 70 wk and 21% vs 7% at 90 wk. 3.) Earlier average age of termination of laying hens in Colombia at 80 wk vs. 90 wk for Lohmann (2016).

Based on these identified issues, the question posed for this work was: how can the number of productive wk and sellable eggs be increased in laying hens in Colombia? To achieve these improvements both eggshell quality and livability must be improved.

According to Arbe Ugalde (2019) and Lera, (2016) management and/or nutritional changes need to be made when mortality is high and egg shell quality low in older flocks. Correct feeding of Ca and P in long-cycle layers is a nutritional challenge, considering the bird's high Ca requirements for shell formation and maintenance of bone integrity (Bar et al., 1988 and 2002; Arbe Ugalde, 2019). In addition to the shell formation and bone maintenance functions of Ca, it also has numerous metabolic functions, among them, activation of enzymes, muscle contraction and hormonal activities (Zhang et al., 2017). About the Ca importance: even in a non-laying hen plasma ionizable Ca (iCa) is maintained at a constant concentration of 1.25 mM/L (Singh et al., 1986) while during egg formation iCa varies depending on timing of shell formation (Luck and Scanes, 1979; Singh et al., 1986). Ionizable Ca during shell formation may decrease to 1.15 m M/L but in non-shell formation times the concentration increases to 1.4 mM/L (Singh et al., 1986; Luck and Scanes 1979). To maintain these Ca levels in plasma, the hen must upregulate absorption during the shell formation phase of the egg laying cycle (Bar and Hurwitz, 1984) regulated by an increase in parathyroid hormone (Singh et al., 1986) that increases the conversion of 25 hydrocholecalciferol ($25OHD_3$) to 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) by the 1 α hydroxylaze primarily in the kidney (Abe et al., 1982) but also found in other tissues (Hewison et al., 2000) .

Acording to Abe et al., 1982) older layers of 91 with a laying rate of between 65.7% had greater cracked or soft shelled eggs (21.4%) as compared to 33 wk-old hens with a 92.1% production rate and 0% cracked or soft shell eggs. Young hens (33 wk) had more than double the concentration of plasma $1,25(OH)_2D_3$ (595 pg/ml) as compared to the 91 wk old hens (262 pg/ml\ml) and this was associated with an up to 3 times higher concentration of 1 α hydroxylase activity in the kidney.

Bar et al. (1988) reported that estrogenic activity in layers, decreases also after 70 wk of age and is associated with a decrease in the concentration of $1,25(OH)_2D_3$ in plasma. In addition, at that age there is a gradual deterioration of the kidney and the liver which reduces the enzymatic activity and with it, the hydroxylation of vitamin D_3 and $25(OH)D_3$, resulting in an inadequate production of $1,25(OH)_2D_3$ (Frost and Roland, 1990). The active form of vitamin D, $1,25(OH)_2D_{3,}$ is generated from dietary vitamin D_3 that is converted via two sequential hydroxylation. The first hydroxylation occurs in the liver by the 25, hydroxylase resulting in 25(OH)D_3, and the second in the proximal tubule of the kidney by 1α hydroxylase, resulting in the active form of vitamin D, 1,25$(OH)_2D_3$ (Soares, 1984; Biehl et al., 1998).

The 1α(OH)D_3 is an analog metabolite of 1,25$(OH)_2D_3$ (Holick et al., 1973) that can be hydrolyzed in the liver by 25 hydroxylaze to 1,25$(OH)_2D_3$ (Kaetzel and Soares, 1985; Ringe and Schacht, 2004). Work with this commercially available metabolite of vitamin D3 has been shown to improve tibial calcification and increase the bone breaking strength in Japanese quail (Soares et al., 1978). Moreover, 1α(OH)D_3 can improve absorption of Ca and P, and increase the thickness and quality of the shell, and decrease the loss of bone in laying hens (Abe et al., 1982).

Leeson and Summers (2009) suggested that to improve shell quality and increase viability in hens older than 70 wk, special attention must be paid to Ca and P and their optimal feeding concentrations. According to Bar (2009) and Rostagno et al. (2017) Ca concentration in brown layers should be 4% Ca. Given a consumption of 114 g feed/b/day suggested by the Lohmann® Brown Guide (2016) this would be a consumption of 4.56 g Ca/b/day. However, according to Asimetrix (2018), the typical diet in Colombia for 70 wk-old b consuming 110 g feed/day, contains 4.5% Ca resulting in a Ca consumption of 4.95 g Ca/b/day and by 80 wk Ca concentration is increased such that layers are consuming 5.5 to 6 g Ca b/day, at wk 80-85. To achieve these Ca consumptions the diet needs to contain 9 to 11% LS. These diets not only have excesses of Ca but the percent of the diet occupied by LS puts pressure on costs by requiring that other needed nutrients be supplied from more concentrated sources.

Excess amounts of Ca in the diet can affect digestibility of other nutrients. For example the excess soluble or reactive Ca can inhibit the action of phytase to hydrolyze phytic acid (Tamim et al., 2004; Li et al., 2015; Kim et al., 2018). Phytic acid is a strong chelator of Ca and other metal ions (Vohra et al., 1965; Koufman and Kleinberg, 1971) chelations that decrease the availability of P and the mineral cations chelated to phytic acid (Erdman, 1979) and phytate molecules that remain intact or not hydrolyzed by phytase will complex with proteins (O´Dell and De Boland, 1976) decreasing the availability of protein (Yu et al., 2012). Beyond the impact of excess Ca with phytate, Ca can also interact with other nutrients like fat resulting in lower Ca and fat digestibly (Atteh and Leeson, 1984) with formation of Ca soaps (Fuhrmann and Kamphues, 2016).

Separate feeding of part of the Ca in the form of LS has been reported to support better shell quality and decrease bone problems in older hens (Zarghi and Zakizadeh, 2016; Molnár et al., 2018). These authors suggested that 37.5% of the Ca be fed in the morning and the remaining 62.5% in the form of LS grit after 14:00 h.

Possible excesses in dietary Ca can cause hypercalcemia, which increases renal excretion of Ca and P (Hsu, 1997).

The hypothesis of this experiment is: that current concentrations of Ca in brown layer diets commercially used after 70 wk of age in Colombia are in excess and that the use of

$1\alpha(OH)D_3$ allows for lowering Ca concentrations in the diet without negatively impacting egg production, shell quality or mortality.

To test this hypothesis, The objectives of the trial were to determine, in 70 to 82 wk brown layers, the effect on egg production, shell quality and livability of: 1. Decreasing Ca in the diet by 10 and 20% (4.4 and 4 g/bird/day) relative to industry practices (4.9 g/bird/day); 2. Supplementing 1α-hydroxycholecalciferol ($1\alpha(OH)D_3$) and; 3. Interaction between Ca and $1\alpha(OH)D_3$. Feeding 4.4 g Ca/bird/day as compared to common industry practice for this age of 4.95 g/bird/day resulted in better egg production, better egg mass, and greater number of sellable eggs. The addition of $1\alpha(OH)D_3$ to the diets improved FCR per dozen eggs when the low Ca diet (4 g/bird/day) was fed as compared to the industry diet.

MATERIALS AND METHODS

Animals and Housing

The experimental protocol was approved by the animal ethics committee of the CES University (Cl. 10a #22 - 04, Medellín, Antioquia, Colombia).

A research barn with 68 wk old Lohmann® Brown laying hens (2250 b) belonging to a commercial company in El Carmen de Viboral, Antioquia (Colombia) situated at 2200 m above sea level, was made available for this work. The curtained barn contained 4 pyramidal battery cage lines. Each line had 2 sides (S) 12.35 m long with 3 levels and 26 cages per level per S. Each cage was 47.5 cm long by 41 cm high by 31 cm deep. The continuous feeder trough, placed outside the cages was 47.5 cm long with divider preventing feed movement between cages or hens being able to eat out of the neighbors feed. Each cage had 2 nipple drinkers.

This cage arrangement resulted in 8 S, with each S having 3 levels and 26 cages per level for a total of 78 cages per S. The number of b per pen varied due to mortalities up to 68 wk of age, with an average of 4 b per cage in the top and middle levels (368 cm^2/b) and 3 bs in the bottom level (491 cm^2/b).

Upon checks of mortality records up to 68 wk and measurements of light intensity (lux in lumens/m^2) at the level of the feeder in the 3 levels per S of the pyramid, the decision was made to discard 4 S that had low b numbers due to mortality and that had very low light intensities. Thus, for this experiment only 4 S were used (Figure 1) and only 1057 out of the 2250 b were included in the experiment. The last 2 cages per line (6 per S) were excluded and thus 24 cages per line, 72 per S were used. Due to this need to remove 4 S of cages and all birds contained in them from the study, the planned designed had to be cut back to maintained replicate and this resulted in an incomplete design. The new design allowed for some key contrasts but not for all original objectives to be tested.

From wk 68 to 70 the luminosity measurements, mortality and egg production data were analyzed, and replicates (composed of 6 cages, 2 on each level of the same S of the pyramid) were established and labeled.

Lohmann® Brown (1057 b) laying hens of 70 wk of age, were randomly distributed into 4 treatments (Trt). The block was the S, with each of the 4 S used having 3 replicates per Trt, resulting in a total of 12 replicates per Trt, with 21 to 23 b per replicate at the start of the trial. Birds were not moved between cages to maintain a behavior dynamics in the established groups, and avoid social instability that would lead to stress or induce aggressive behaviors (Estevez et al., 2007).

[Figure 1 about here]

Light intensity was measured (Lutron LM-8000 4 in 1 Environment Tester, Coopersburg, Pennsylvania, US) in the middle trough level of each S in two places selected to be equidistant between lights (HyLine, 2018), four times a day as follows: 07:00, 10:30, 14:30, 17:30 and an average per day reported.. The curtain barn had natural light and 750-lumen LED bulbs in the corridors between S. The average light intensity was 1,500 lux (range 400-2500) on the outer curtained S (S1 and S4) and 150 lux (range 120-170) in the internal corridors (S2 and S3) (Figure 1). Light was managed as it had been managed in this research site prior to 68 wk and as per company standard operating procedures. A 16 h light in 24 h photoperiod in all the production phase distributed as follows: lights were turned on at 05:30 and remained on until 19:30 Between 0:00 and 2:00, lights were turned on to allow consumption of any uneaten feed as specified in the 2016 Lohmann® Brown Management Guide (Lohmann, 2016). Those two hours of light were defined as midnight meal, although no additional food was supplied.

The selected b had been in the research barn starting on wk 16 of age, but had not been used for an experiment and thus had been fed the same diets until wk 68. The mash diet being consumed (110 g/b/day) by all hens from 46 to 68 wk, had a guaranteed nutrient content of: 17.6 g of crude protein (CP), 0.831 of digestible Lys (dLys), 4.18 g of crude fat (CF), 2830 Kcal ME/kg, 4.95 g of Ca and 0.4 g of available P (avP). All LS had been mixed in the feed with 60% the LS fed as grit [2.99 mm geometric mean diameter (GMD)] and 40% as fine LS (0.152 mm GMD) (Lohmann, 2016).

Diets and Feeding

Diets were formulated to meet nutrient specifications for 70 wk old Lohman Brown layers (Lohmann, 2016) except for Ca (Tables 1 and 2). The same diets were fed throughout in the pretrial period (wk 68 and 69) and during the experimental period (70 to 82 wk of age). Four dietary Trt were tested as follows: Control **(C)** containing 4.5% Ca that when fed at 110 g/b/day in the am supplied 4.95 g Ca/b/day; Medium with (5 mM 1α(OH)D_3/kg feed) **(M+)** containing 2.01% Ca and fed at 104 g/day in the am with an additional supply (6 g/day) of grit LS (2.99 mm GMD) fed in the afternoon (14:00 h) for a total supply of Ca of 4.4 g Ca/b/day: Medium without 1α(OH)D_3 **(M-)**: Low with (5 mM 1α(OH)D_3/kg feed) **(L+)** containing 2.01% Ca and fed at 104 g/day in the am with an additional supply (5 g/day) of grit LS (2.99 mm GMD) fed in the afternoon (14:00 h) for a total supply of Ca of 4.01 g Ca/b/day. The split feeding of Ca used in Trt M+, M- and L+ followed recommendation made by (Zarghi and Zakizadeh, 2016; Molnár et al., 2018).

The original study was designed to contain 5 additional treatments: a control diet with split feeding of Ca; a control diet with no split feeding of Ca and with 1α(OH)D_3; a control diet with 1α(OH)D_3 and with split feeding of Ca; and a low diet without 1α(OH)D_3**.** As stated previously, having to discard half of the facility due to poor light intensity when measured before the study and high mortality in some S of the facility, only 4 Trt could be tested with enough replication. The 4 Trt tested were selected to try to answer specific questions in relation to their effect on mortality, productivity, and egg quality in older hen: 1. Does dietary Ca concentration impact mortality, productivity and egg quality in older hens? 1a. Impact of medium and low Ca diets (M+ vs L+) in diets containing 1α(OH)D_3 and 1b. Impact of high and medium Ca diets (C- vs M-); 2. Does supplementation with 1α (OH) D3 impact mortality, productivity and egg quality in older hen fed moderate Ca diet (M+ vs M-)?

[Table 1 about here]

The adaptation period to the new diets was 2 wk (68 and 69 wk of age), minimum adaptation time described by Kazue and Rostagno (2016), especially important for Trt M^+, M^- and L^+ b, which had to adapt to splitting the diet from 110 g/b/day fed at 6:30 h to laying hens getting 104 g of feed fed 6:30 h and 5 or 6 g of LS (2.99 mm GMD) supplemented at14:00 h.

The diet was formulated (Table 2) to contain 16% CP and based on the daily feed allowance an amino acids consumption of 0.831 dLys g/b/dayay and an amino acid ratio using dLys as 100% to the other digestible **(d)** amino acids as follows: 50.5% dMet; 91% total sulfur amino acids (dTSAA); 65% threonine (dThr), 19.25% tryptophan (dTrp) (Lemme, 2019). The diets supplied at based on the daily feed allowence, 308 kcal of ME/b/day, a Lohman, 2016). Diets were mixed 3 times with batch change occurring on wk 73 and 78. The LS particle size ratio used in the study was: 40% fine LS (0.152 mm GMD) and 60% grit (2.994 mm GMD) for treatment C; and 30% fine and 70% grit for Trt M+ and M-; and 33.4% fine and 66.6% grit for Trt L+. The reason for the differences in the proportion of fine to grit were driven by the amount of LS grit fed separate to the diet, at 14:00 h in Trt M+, M- (6 g LS/b/day) and L+ (5 g LS/b/day).

Laboratory Analysis

The geometric mean diameter (GMD) of the particles of feed and the LS were measured based on the dry sieving technique of American Society of Agricultural Engineers, (2003). Each batch of feed as well as the raw material were analyzed with for CP (Dumas: ISO 16634, 2018), CF (Intermediate filtration method: ISO 6865, 2017), EE (ISO 3596:2000) and minerals (Ca, P, Mg using ICP – ISO 27085:2009).

At 70 and 82 wk of age all b were weighed to determined BW gain or loss during the experiment. Feed intake (**FI**) was determined weekly when any residue left from the pre-weighed of feed given daily, was weighed. Mortality was check 2 times a d, BW recorded and feed in the trough removed and weighed to correct replicate feed intake based on hen d consumption. The following productive measurements were determined daily: egg production (**EP**) as well as cracked, broken and soft-shelled eggs and reported for each replicate as a percent per number of b alive daily. All eggs laid by each replicate on wk 72, 76, 80 and 82 were weighed and egg mass (**EM**) determined as follows: (EP percentage/100) x egg weight (**EW**) and FCR determined per mass or per dozen eggs. Six eggs per replicate were randomly selected on the last day of the week and shell weight (dry and without membranes), , shell thickness taken in the equatorial region using a Mitutoyo® micrometer with a precision of 0.0001 and reported in millimeters (Hamilton,1982) and shell breaking resistance (FUTURA® Egg-Shell-Tester, Gewerbering, Germany) done according to the procedure described by Hamilton, 1982.

Statistical Analyses

The statistical analyses were performed using JMP®, Version *15* SAS Institute Inc., Cary, NC, 1989-2019 through mixed model with repeat measurements over time; fixed effects: treatment and week, and block by S as a randomized effect. To assess if there was a difference between the Trt, as well as correlation between them and the replicate arranged in the batteries, Tukey's test (Tukey, 1949) was applied to each one of the variables that showed an statistical difference. Orthogonal contrasts were used to answer the research mean questions with the Trts: 1a. Impact of medium and low Ca diets (M+ vs L+) in diets containing $1\alpha(OH)D_3$ and 1b. Impact of high and medium Ca diets (C- vs M-); 2. Impact of $1\alpha(OH)D_3$ when used in moderate Ca diets (M+ vs. M-).

The weekly mortality data did not meet the assumptions of normality, specifically due to the data volume at zero, which is why they were reported as a **summation** of the daily mortality rates reported from 70^{th} to 82^{th} wk. An arcsine transformation was conducted and the data were analyzed using the Wilcoxon test based on nonparametric variables; no statistical differences were found.

RESULTS AND DISCUSSION

Upon statistical analysis of the data with S as a block, no S effect was seen on any of the parameters measured. Replicate hen BW was not different ($P>0.05$) at the start of the experiment (70 wk, data not shown) with average hen BW at the start of 1.967 kg. At the end of the experiment, there was an effect ($P<0.05$) of Trt: on BW, FI, EP, EW, EM, sellable eggs, and FCR both per egg mass and per dozen eggs (Table 3). Overall BW was lower at 70 and 82 wk for all Trt compared with that expected based on the breed guide

(Lohman, 2016). Mortality over the 12 wk experiment was similar ($P>0.05$) for all Trt (Table 3) and was between 2.60 and 4.06% which was lower than Colombian mortality averages (6.4%) for Lohmann Brown hens between 70 and 82 wk (Asimetrix, 2018). Egg weight was lower (average 63.7 g) than the breed guide (Lohman, 2016) expectation (69.0 g), and lower than average EW for Lohmann Brown hens in Colombian in 2018 (Asimetrix, 2018) of 66.2 g. Egg breaking resistance measured in Newtons were similar ($P>0.05$) for all Trt. Based on Hamilton (1982) the egg breaking resistance seen in this study fell within the range (31 to 45 Newtons) defined as belonging to medium breaking resistance eggs. No differences between Trt were detected in shell weight (average 6.32 g), shell thickness (average 0.474 mm), or percent cracked or broken eggs (data not shown).

To better interpret data, specific contrast were done, only when the overall effect of Trt on the measured parameter was significant ($P<0.05$), to answer the following questions: 1a. Impact of medium and low Ca diets (M+ vs L+) in diets containing $1\alpha(OH)D_3$ and 1b. Impact of high and medium Ca diets (C- vs M-); 2. Impact of $1\alpha(OH)D_3$ when used in moderate Ca diets (M+ vs. M-).

1. Does dietary Ca concentration impact mortality, productivity and egg quality in older hens?

To answer this question, two contrasts were done: 1a. Impact of medium and low Ca Trt (M+ vs L+) where both contained $1\alpha(OH)D_3$ and 1b. Impact of high and medium Ca Trt (C- vs M-) where neither Trt contained $1\alpha(OH)D_3$.

Contrast 1a. No differences (P>0.05) were detected in BWG, egg weight or mortality between laying hens fed the L+ and M+ Trt (Table 3). Average BW at 70 and 82 wk was 1967 and 1988 g, respectively (data not shown). While BWG was similar, hens fed the L+ Trt consumed less feed (P<0.05) than those fed the M+ Trt.

Even though hens on the L+ Trt consumed less (P<0.05) Ca (4.14 g Ca/b/day) than those on the M+ Trt (4.55 g Ca/b/day) (Table 3), they had greater (<0.05) EP and sellable eggs and better (P<0.05) FCR per dozen eggs. Other measures (EW, EM, FCR per EM, and egg breaking resistance were not affected (P>0.05) by consumed Ca.

Contrast 1b. Laying hens fed the M- Trt had greater EP, egg mass, sellable eggs (P<0.05) than the layers fed the C- Trt (Table 3) even though hens fed the M- Trt consumed less (P<0.05) Ca than those fed the C- Trt (4.66 and 4.98 G Ca/b/day, respectively). This was similar to what was seen in contrast 1a where FI also decreased as Ca intake decreased. It is important to note that this is confounded by timing of Ca feeding. In the C- Trt, all Ca (4.95%) was fed in the am as part of the mash diet while in M- Trt Ca was split between the 6:30 h (2.01% Ca) feeding and a feeding (2.39% Ca from limestone grit) at 14:00 h.

The effect of Ca concentration on FI varies greatly in the literature. In contrast to what was found in this research, Bar et al. (2002) did not find difference FI in 66 wk old Lohmann layers consuming different concentrations of Ca (2.8, 4.2 or 5.8 g Ca/b/day) for 12 wk. Similarly, Leeson et al. (1993) reported no effect on FI or on most productive parameters in Isa Brown layers fed diets containing between 2.8 and 4.2% Ca for 52 wk (from 19 to 71 wk of age) with a consumption between 67 and 71 wk of between 3.4 to 5 g Ca/b/day.

Opposite to what was found in this research, and differing to what was reported by Bar et al (2002) and Leeson et al (1993), Hurwitz et al. (1969), reported a reduction in FI as Ca concentration increased, an opposite impact to that found in the current trial. Recently (Attia et al., 2020) reported no effect of feeding different diet Ca (3.5, 4.0 and 4.5% Ca in the diet) on FI in H&N Brown Nick layers under heat stress. In this last paper, no analytical values for diet Ca were given and it is not clear if FI was curtailed by amount of feed fed daily.

In the present study as Ca intake decreased, egg production increased (P<0.01) but egg weight and egg mass were not affected (P>0.05). As with the impact of diet Ca content and differences in Ca consumption per hen per day on FI, it is hard to come to a consensus as to the effects when published literature is reviewed. This differs from the observations reported by Bar et al. (2002), who did not find significant difference when feeding 4.5 or 5 g Ca/b/day, and from the results reported by Leeson et al. (1993), who found a reduction in egg size when Ca in the diet increased. Hurwitz et al. (1969), on the other hand, reported no productive differences were observed between hens fed either 3 or 3.69 and 5.35 g/b/day.

The reason for the contrasting effects are not evident. Differences in design, age and strain of hens and overall hen management may explain part of these differences. Levels of analyzed Ca and P in the diets fed are often not reported (Hurwitz et al., 1969; Attia et al., 2020) and in some cases only the basal diet Ca and P analysis are reported (Leeson et al., (1993).

The actual amounts of the nutrients being tested in the diet is an essential component of a report and allows for interpretation. This is especially important for Ca where in general analyzed Ca levels in the diets vary to those formulated. The source, particle size and quality of the limestone used in the diets also becomes important as they affect how the hens digests Ca (Zang and Coon, 1997; Lichovnikova, 2007; Sanders-Blades et al., 2009; Sinclair-Black et al 2019

2. Does supplementation with 1α (OH) D3 impact mortality, productivity and egg quality in older hen fed moderate Ca diet (M+ vs M-)?.

Contrast 2. Laying hens fed the M- has greater final BW, FI, EP, egg weight, egg mass, egg mass, FCR by dozen, sellable eggs, which differs from the results reported by Bar et al. (1988), who did not obtain a significant difference in production with the same concentration of 5μg/kg of $1\alpha(OH)D_3$, but did observe a significant decrease ($p>0.01$) in the number of unsellable eggs of 31.5%, in birds of 72^{th} to 84^{th} wk when using $1\alpha(OH)D_3$.

On another note, Frost and Roland (1990) reported no significant difference in terms of production, feed conversion ratio and egg weight when using 4.5 μm/kg of $1\alpha(OH)D_3$ with respect to a product without $1\alpha(OH)D_3$. The intake of Ca was significantly higher ($p> 0.01$) for the Trt M-, and we have a confusing result because we do not know if it because FI intake was higher ($p> 0.01$) or because Ca level was higher than expected in the diets (2.01%, formulated vs 2.14 analyzed).

CONCLUSION AND APPLICATIONS

When the Ca levels of 4.95 g/bird/day reported in Colombia we reduced by 10% at a 4.4% level in birds older than 70 wks, significant differences are observed in these regards: higher BW, EP, egg weight shell quality, sellable eggs and lower FCR.

When using 1α(OH) D_3 in diets with 4.4% Ca, the production response is different to that of 4.4% Ca without the metabolite, as a higher production performance was found for the latter in terms of: number of sellable eggs, EP, egg weight, egg mass and FCR. When 1α(OH)D_3 is used at a level of 4% Ca g/bird/day, the results include a better production response than when using 4.4% with the metabolite, which consists in: higher BW, EP, egg mass, sellable eggs and lower FCR, those impact positively the price of production of eggs and generate new nutritional alternatives in poultry industry.

By reducing the Ca level by 20% using 1α(OH)D_3 compared to the levels reported for Colombia in layers of 70 wks, from 4.95 to 4.0% Ca g/bird/day, FI was lower and similar results are obtained, maintaining the BW by using 1α(OH)D_3 in birds older than 70 wk.

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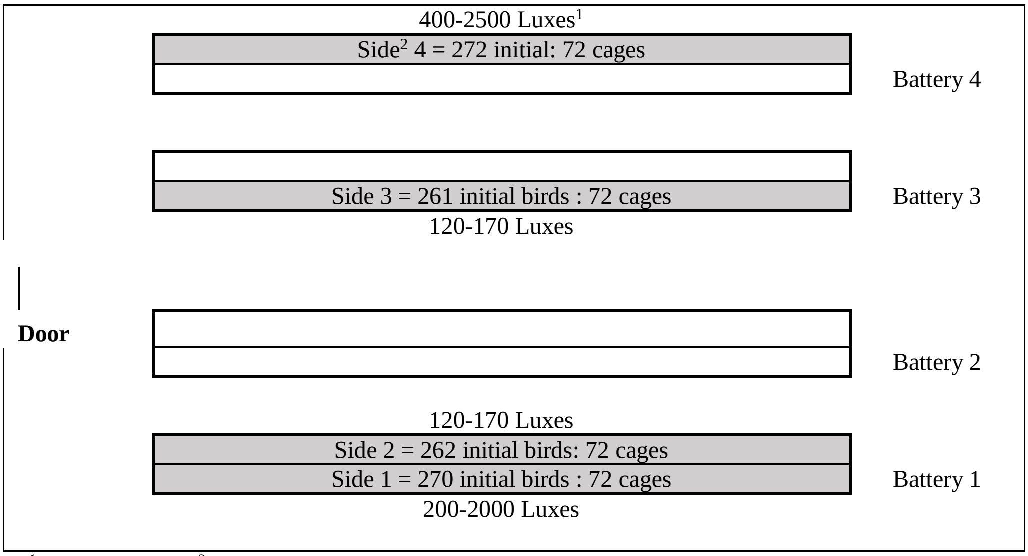
FIGURES AND TABLES

Figure 1: Drawing of the experimental shed with shaded sides representing sides used in the experiment and results of light measurements (lux)



[1] Luxes in lumens/m^2, measured at at feeder height at each of the three midpoint in each corridor (Technical Bulletin, Hy-Line (2017))

[2] SIDE(S): Shaded S selected to be used (Block) in the experiment based on prior lower mortality and greater uniformity in light.

Table 1. Description of treatments

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Treatments[1]	Feed and/or limestone offered, bird/day				Ca intake (g/bird/day)
	Time of day birds were fed				
	6:30 h		14:00 h		
	Mash feed (g)	**% Ca (FML) [2]**	**Limestone added/b/day**	**Total feed offered g**	
Control (C) [1]	110	4.5%	0	110	4.95
Medium+(1α $(OH)D_3$)[1]	104	2.01%	6	110	4.40
Medium-[1]	104	2.01%	6	110	4.40
Low +(1α $(OH)D_3$)[1]	104	2.01%	5	109	4.00

[1]Control (C) contained 4.95% Ca as part of the mash diet fed at 6:30 h;[1]Medium with 1α$(OH)D_3$ (M+) contained 2.01% Ca in the mash diet fed at 6:30 h and 6 g of limestone (2.39% Ca) grit fed at 14:00 h; [1]Medium without 1α$(OH)D_3$ (M-) with the same Ca management as M+; [1]Low with 1α$(OH)D_3$ (L+) contained 2.01% Ca in the mash diet fed at 6:30 h and 5 g of limestone (1.99% Ca) grit fed at 14:00 h;

[2] FML: % Ca formulated into the mash feed.

Table 2. Ingredients, formulated and analyzed nutrients in the diets.

	Inclusion percentage		
Ingredient	**C[1]**	**M+-L+[1]**	**M-[1]**
Corn	61.570	66.240	66.240
Soybean meal (47.14 CP)	19.960	19.930	19.930
Fine Limestone [2]	4.000	2.800	2.800
Wheat bran	0.990	2.000	2.000
Corn DDGS	1.000	2.000	2.000
Meat & bone meal	2.050	1.780	1.780
Others[3]	0.440	0.439	0.440
$1\alpha(OH)D_3$[4]	0.000	0.001	0.000
Palm oil kernel	1.000	1.000	1.000
Corn gluten	0.500	1.000	1.000
Gift Limestone [5]	6.100	0.780	0.780
Soybean oil	1.000	0.500	0.500
Sea salt	0.400	0.400	0.400
Dicalcium phosphate (18.27%)	0.050	0.220	0.220
Fungi Inhibitor	0.100	0.100	0.100
Methionine DL (99%)	0.260	0.230	0.230
Sodium bicarbonate	0.010	0.010	0.010
Choline cholride (56%)	0.400	0.400	0.400
Lysine HCl (78%)	0.150	0.150	0.150
L-Thereonine (98%)	0.020	0.020	0.020
TOTAL	**100.000**	**100.000**	**100.000**
Formulated (analyzed ±SD) %			
ME, kcal/kg	280	296.15	296.15
Crude protein [6]	16(±0.16)	16.92.(16.7±0.15)	16.92. (17.3±10.39)
Ether Extract[6]	4.4 (3.82 ±0.58)	4.65 (4.15±0.50)	4.65 (4.88 ±0.23)
Crude fiber [6]	2.5 (2.85± 0.35)	2.64 (2.85 ± 0.21)	2.64 (2.76 ± 0.12)
Ca Feed[7] (%)	4.5% (4.61± 0.11)	2.01% (2.05±0.04)	2.01% (2.14 ± 0.13)
Ca, fed 14:00 hr (GMD *2,994 mm*) (g)	0	6	5
Total Ca g/bird/d	4.95	4.4	4
Available P (calculated)	0.36	0.39	0.39
Total phosphorus[8]	0.51 (0.50 ± 0.01)	0.54 (0.54 ± 0.0)	0.54 (0.55 ± 0.01)
dLys [9]	0.76	0.8	0.8
dMet [9]	0.39	0.41	0.41
dMet+Cys[9]	0.69	0.73	0.73
dTre [9]	0.53	0.56	0.56
dTrp [9]	0.16	0.17	0.17

[1] Control (C) contained 4.95% Ca as part of the mash diet fed at 6:30 h; [1] Medium with $1\alpha(OH)D_3$ (M+) contained 2.01% Ca in the mash diet fed at 6:30 h and 2.39% Ca from limestone grit fed at 14:00 h; [1]Medium without $1\alpha(OH)D_3$ (M-) but the same Ca management as M+; [1]Low with $1\alpha(OH)D_3$ (L+) contained 2.01% Ca in the mash diet fed at 6:30 h and 1.99% Ca from limestone grit fed at 14:00 h.

[2] Fine Limestone: Analyzed Ca: 39.72% Mg: 0.22% (ICP-ISO 27085:2009) geometric mean diameter in microns (GMD) 0.152 mm.

[3] Contained a vitamin and mineral premix with a guaranteed per kg of diet: vitamin A, 10,000 IU; vitamin D_3, 3,000 IU; vitamin E, 20,0 IU; vitamin B12, 0.015 mg; riboflavin, 5mg; niacin, 25 mg; pantothenic acid, 8 mg; vitamin K3, 3 mg; folic acid, 0.75 mg; biotin, 0.5 mg; thiamine, 2 mg; pyridoxine, 2.5 mg; zinc from zinc oxide, 100 mg; manganese from manganese sulfate, 80 mg; iron from iron sulfate 80 mg; selenium from Prokel® Se from Se glycinate complex 0.1%), 0.3 mg; copper from copper sulfate, 1.5 mg; iodine from calcium iodate, 0.9 mg. Also contained of diet: red pigment, 3.5 gr; yellow pigment, 1.5 gr; bacitracin zinc, 60 gr; halquinol, 40 gr; phytase Natuphos E, 300,000 FTU; antioxidant, 125 gr.

[4] $1\alpha(OH)D_3$ 12.5 g Alpha D3 green/TM guaranteed 5μg/kg of $1\alpha(OH)D_3$. Produced by Adiquim kilometer 1.5 Autopist Medellin-Bogota. Guarne-Colombia

[5] Gift Limestone: Analyzed Ca: (39.07%) Magnesium: 0.26% (ICP-ISO 27085:2009). GMD 2.994 mm.

[6] Crude protein: Dumas (ISO 16634, 2018). Ether Extract (ISO 3596:2000). Crude fiber: Intermediate filtration method (ISO 6865, 2017)

[7] Total calcium and phosphorus in feeding stuffs ((ICP-ISO 27085:2009))

[8.] Total phosphorus analysis: UV-Vis spectrophotometry. Colombian Technical Standard (NTC) 4891

[9] Digestible amino acids were calculated based on the analysis reported in raw materials by means of: NIRS Aminodat 4.0® (Evonik Industries, 2010) from raw materials used in the diet.

Table 3. Effect of diet treatments fed from 70 to 82 wk of age on productive and eggshell quality measures

Treatments[1]	**BWG**[2] **70th to 82th wk (g)**	**Feed Intake (g)**	**Ca Intake (g/b/day)**	**Egg production (%)**	**Egg weight (g)**	**Egg mass**[3] **(g)**	**FCR per Egg mass**[4]	**FCR per dozen**[5]	**Mortality %**[6]	**Sellable ,eggs, %**[7]
C^{-}	3.08	108.06^{b}[9]	4.98^{a}	79.41^{b}	63.96^{a}	50.79^{ab}	2.14^{ab}	1.64^{b}	4.06	77.83^{ab}
M^{+}	14.95	107.65^{b}	4.34^{c}	77.40^{c}	63.42^{ab}	49.08^{c}	2.19^{a}	1.67^{a}	3.11	75.70^{c}
M^{-}	11.12	108.56^{a}	4.46^{b}	80.95^{a}	64.07^{a}	51.84^{a}	2.11^{b}	1.62^{c}	2.60	79.28^{a}
L^{+}	8.98	106.79^{c}	3.93^{d}	79.08^{b}	63.26^{b}	49.96^{bc}	2.15^{ab}	1.62^{c}	3.42	77.33^{bc}
SEM[10]	4.0007	0.2303	0.0147	0.006	0.2335	0.4587	0.0243	0.0159	0.1256	0.0061
P Value	NS	<0.001	<0.001	<0.001	0.039	<0.001	0.01	0.05	0.8662	<0.01
C- vs. M-	NS	0.005	<0.001	0.009	NS	0.017	NS	NS	NS	0.0264
M+ vs M-	NS	<0.001	<0.001	<0.001	0.046	<0.001	0.0080	0.0004	NS	<0.001
M+ vs L+	NS	<0001	<0.001	0.005	NS	0.0469	0.1075	0.0015	NS	0.0107
Lohmann, 2016[12]	27.00	109	4.50	66.85%	69	47.7		1.65		

[1] Control (C) contained 4.95% Ca as part of the mash diet fed at 6:30 h; [1] Medium with $1\alpha(OH)D_3$ (M+) contained 2.01% Ca in the mash diet fed at 6:30 h and 2.39% Ca from limestone grit fed at 14:00 h; [1]Medium without $1\alpha(OH)D_3$ (M-) but the same Ca management as M+; [1]Low with $1\alpha(OH)D_3$ (L+) contained 2.01% Ca in the mash diet fed at 6:30 h and 1.99% Ca from limestone grit fed at 14:00 h.

[2]BWG= body weight

[3] Egg mass calculated as (egg production (%)/100)* egg weight (g)

[4] FCR per egg mass calculated as feed intake (g) / egg mass

[5]FCR per dozen eggs calculated as feed intake (Kg) / (eggs laid/12)

[6] Cumulative mortality over the 12 wk of the experiment

[7]Sellable eggs calculated as egg production (%) – [broken eggs (%) + cracked eggs (%) + soft-shelled eggs (%)]

[8] Lohmann guide suggests that breaking resistance should be greater than 40 Newtons for the whole production cycle (18-90 wk)

[9] Values in a column with different superscript letter are different (P<0.05) based on Tukey multiple comparison test.

[10]SEM: standard error of the mean

[11]NS=Not significant (>0.05)

[12] Lohman 2016 standard.