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Therapeutic Importance of Calcium Gluconate[r] in Cage Layer Fatigue (CLF), the Effects on Egg Production, Haematology and Serum Biochemistry of Commercial Layers

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Abstract: *The study was carried out to determine the therapeutic effects of different dosages of Calcium Gluconate (CG) on % egg production response, haematological and serum biochemical indices of three hundred Dominant Black CLF Layers in an intensive management system. Birds were allotted at 31wks of age into four treatments (T_0, T_1, T_2 and T_3) and each replicated thrice, 10% CG was administered intravenously at dosage of 0.00, 0.75, 1.50 and 2.25ml respectively. There was a positive response in % egg production from 65% to 80.50% (T_1), 85.00% (T_2) and 85.30% (T_3) within two weeks of treatment, all values are greater than T_0 (68%). The [Hb] for T_1 (16.00 ±0.00), T_2 (16.50 ±0.15) and T_3 (17.00 ±0.25) are statistically equal, but significantly ($P<0.05$) greater than the control T_0 (14.00±0.52), same trend was observed for wbc, MCH and MCHC. The Glucose level of T_2 (295.00±10.41) is equal to T_3 (298.00±25.79), but are significantly ($P<0.05$) greater than T_1 (268.00±2.29), which is in turn greater than the control (200.00±1.72) at $P<0.05$, the Calcium level also follow same trend. All other parameters measured show no significant difference between T_1, T_2, T_3 and the control T_0. Commercial layers with CLF can therefore be treated with 10% CG intravenously at a dosage of between 1.50 – 2.25ml per bird for five consecutive days without adverse effects on the haematology and serum biochemistry.*

Keywords: *Calcium gluconate, commercial layers, cage layer fatigue, % egg production, haematology, serum biochemistry*

1. INTRODUCTION

The skeleton of the commercial layer consists of structural bone (cortical and trabecular bone) which provides mechanical strength and supports the muscles, and a type of medullary (inner) bone special to birds, which has little mechanical function but acts as a reserve of calcium (especially the long bones, such as tibia, fibula, humerus and femur) and the vertebrae are the major store house for calcium, in form of carbonate and phosphate (Fasanmi and Olukole, 2010), these minerals are needed to form egg shells. The layer's medullary bone is formed just before the onset of sexual maturity and the start of egg laying and this coincides with a marked reduction in the volume of trabecular bone (the internal supporting framework of structural bone). 98% of body calcium and 80% of body phosphorus is present in the layer's skeleton, in the form of the mineral calcium hydroxyphosphate, which gives bones their strength and also acts as mineral reserve (Nipane and Mehare, 2011). During the formation of an egg the layer bird draws calcium from her bones to make the eggshell. In a healthy, well-fed bird the bones contain about three eggs worth of calcium. A number of problems (layers mash deficient in calcium, imbalance calcium-phosphorus ratio) can stop the bones from providing enough calcium to make the shell and when this happens the hen has three options. In reality most birds will select a combination of these three: they stop laying**,** produce thin or soft shelled eggs**,** and withdraw calcium from other organs other than the bones (Green, 2011) Egg-shell formation is most intense in the period of darkness when the hen does not eat. She therefore has to use calcium from the medullary bone, by a process of resorption. When this process leads to disease, it is because the medullary bone has become depleted of calcium and the hen starts to break down the structural bone to use the calcium for the formation of egg shell and to replenish the minerals in the medullary bone. The

result is a serious decrease in the amount of structural bone and the hen's skeleton becomes thin and brittle (Nipane and Mehare, 2011) The calcium deficiency in layer hens results in initial removal of calcium from bones, firstly from the medullary and then the bone walls. Although the severe calcium deficiency is often a triggering factor of cage layer fatigue (Dinev, 2012) Cage layer fatigue syndrome is a condition characterized by inability of hens to stand on their feet as a result of demineralization of the bone [osteoporosis] (Int. poultry Prod., vol.6, No.1, pg. 7&9), it is mainly found in young layer hens reared in battery cages in the period of peak egg production. The highly productive laying hen mat be on the threshold of its minimum endogenous calcium requirements, and may lack a mechanism to decrease egg production when calcium supply is insufficient (Bell and Siller, 1962). Affected birds are recumbent, go off feed and lay thin shelled or shelless eggs (Dinev, 2012), egg in the shell gland and active ova (Rosales, 2013). They also show paralysis around the time of peak production, this is as a result of the fractures of the fourth and fifth thoracic vertebrae causing compression of the spinal cord (Duncan, 2001). The pathological lesion of cage layer fatigue include muscular paralysis, sternal deformation, sigmoidal shaped ribs and in folding of the ribs caused by small fracture at the costochondrial junction, cortical bone is thin and medullary bone mass is decreased (Parkinson and Cransberg, 2003). The haematological parameters and serum biochemistry of chickens are essential for the diagnosis of various pathological and metabolic disorders, they also provide valuable information on the immune status of animals (Kral and Suchy, 2000), They can be used as a diagnostic tool in order to assess the health status of an individual and/or a flock; Haematological changes are commonly used to determine the health status and to assess the impact of environmental, nutritional and/or pathological stresses (Babatunde *et. al.*, 1992, Esonu *et. al.*, 2003, Elagib and Ahmed, 2011). Calcium is a vital intracellular and extracellular ion involved in neuronal activation, muscle contraction, enzymatic reactions, hormone secretion, and bone matrix (Schenck and Chew 2008).

Calcium gluconate is an odourless, tasteless white powder or granules administered orally or intravenously to replenish the body's calcium stores (Free Dictionary.com, 2014). It is well established that the health and performance of birds are influenced by the nutrient and metabolites of blood that can be estimated by understanding the relationships between the haematological and serum biochemical parameters (Babatunde *et.al.*, 1987). The use of calcium gluconate has been well documented in various livestock (Kalaizatkis *et.al.*, 2011) and in dogs(Schenck and Chew 2008), but there is paucity of information vis-à-vis its usage in commercial poultry production in Nigeria, because in the Nigeria, recumbent commercial layers are culled rather than treated. The purpose of this study is, therefore, to evaluate if there are changes in the haematology and or serum biochemistry of cage layer fatigued hens following the administration of different dosages of calcium gluconate, and how the birds will respond thereafter. Also to disprove the belief of farmers that cage layer fatigue is irreversible.

2. MATERIALS AND METHOD

Three hundred (300) 28 weeks old commercial layers (dominant black breed) were selected from a flock of 3009 birds suffering from cage layer fatigue (80% hen day production, squatting on hock, paralysis of the limbs, thin shelled and shelless eggs), were allotted into four treatments, with each treatment replicated thrice and each replicate having twenty five recumbent commercial layer birds. Birds were kept in labeled cells of battery cages arranged in an open sided pen house in a Poultry Farm in Abeokuta, Ogun State, Nigeria from October -December, 2013. Birds were fed commercial (finished) layer diet; from point of lay (18wks) to 80% production (29wks) when the condition set in, and water was provided *ad libitum*. Light was provided 16 hours daily. Following the manifestation of the aforementioned clinical symptoms and pathological lesions of cage layer fatigue among the flock and the subsequent allotment of the 300 layers into the various treatments (T1, T2, T3 and T4) and each treatment

replicated thrice, with each replicate having 25 laying birds. Layers were injected with varying dosages of calcium gluconate (10%) using 5ml disposable syringe with 19 gauge needles through the wing vein (brachial), towards the direction of the heart, for 5 consecutive days with the exception of the control (T1). Treatment 2 (T2) was injected with 0.75ml), treatment 3 (T3) with 1.50ml and treatment 4 (T4) with 2.25ml

3. Blood collection and Analysis

Seven days post-administration of last dose of Calcium gluconate intravenously, blood samples were collected from four (4) birds per replicate, making twelve (12) birds per treatment. Bleeding was done from the punctured wing vein with a sterile gauge 19 needle; about 5ml of blood was collected from each bird into two sets of sterilized labeled sample bottles, one containing Ethylene Diamine Tetraacetic Acid (EDTA) and other bottles without anti-coagulant. Blood was collected, kept on ice in a cooler and transferred to the laboratory; the samples were immediately used for determination of haematological parameter values: Haemoglobin concentration was determined spectrophotometrically by cyanomethaemoglobin method (Schalm and Jain, 1975), RBC and WBC counts using Neubauer haemocytometer as described by Dacie and Lewis (1991), Packed Cell Volume (PCV) was determined by the microhaematocrit method, while the MCV, MCH and MCHC were calculated from RBC count, Hb concentration and PCV using the appropriate formulae as described by Mafuvadze and Erlwanger (2007).

Sera were collected from the second set of bottles without anti-coagulants through centrifugation with a macro centrifuge and used for analysis of the biochemical indices viz., Total protein was determined using the Kjedahl method as described by Kohn and Allen (1995), albumin was measured using dye binding technique as described by Doumas and Bigger (1972). Serum Calcium and Phosphorus were determined by the calorimetric method, while serum cholesterol was determined by the Roschlan methods, serum enzymes (ALT and AST were measured spectrophotometrically as described by Rej and Holder (1983) and Holder and Rej (1983) respectively.

The proximate composition of the layer's mash was determined according to the official method of analysis (AOAC, 1990). All data collected were subjected to Analysis of Variance, and statistically different means separated using Duncan's Multiple Range Test (Duncan, 1955).

4. Results

Table1. *Ingredient Composition Of Commercial Layer Mash Fed To Cage Layer Fatigued Birds*

Ingredients	Quantity (Kg)
Maize	55.00
Soya Bean Meal	14.00
Groundnut Cake	10.00
Wheat offal	9.50
Bone	2.20
Limestone	8.50
Lysine	0.10
Methionine	0.10
Layers Premix (vitamins)	0.30
Salt	0.30
Total	**100.00**

Calculated Analysis

ME (Kcal/kg)	2694.21
CP	17.00
Ca(%)	3.78
P (%)	0.40

Table2. *Proximate composition of Layer's mash fed to cage layer fatigued birds*

Parameters	%
Moisture content	6.40
Crude protein	16.60
Crude fibre	3.20
Ash	4.20
Ether Extracts	3.40
Nitrogen free extract	66.20

Table3. *% Egg Production of Commercial layers before and after occurrence of Cage Layer Fatigue*

Month	Age/wks	% Production
September	20	15.80
October	21	19.00
	22	22.50
	23	41.50
	24	62.00
November	25	69.50
	26	74.00
	27	76.20
	28	78.00
December	29	82.30
	30	75.45
	31	65.00

Table4. *Response of % egg production to Calcium gluconate following Cage Layer Fatigue*

Age/weeks	T_0 Control	T_1 0.75ml CG/Bird	T_2 1.50ml CG/Bird	T_3 2.25ml CG/Bird
31	65.00	65.00	65.00	65.00
32	62.50	77.80	82.20	82.70
33	68.00	80.50	85.00	85.30
34	70.80	82.72	86.00	85.90
35	73.30	85.00	86.80	87.25

Table5. *Haematology Of Cage Layer Fatigued Comm. Layers Treated With Calcium Gluconate*

Parameter	T_0	T_1	T_2	T_3
	Control	0.75ml CG/Bird	1.5ml CG/Bird	2.25ml CG/Bird
PCV (%)	34.67 ± 0.72	34.50± 0.29	34.47 ± 0.15	35.10 ± 0.55
[Hb] (g/dl)	14.00 ± 052^{b}	16.00 ± 0.00^{a}	16.50 ± 0.15^{a}	17.00 ± 0.25^{a}
RBC (x10^{6} mm^{3})	2.80 ± 0.08	2.95. ±0.15	3.00 ± 0.10	3.00 ± 0.20
WBC (x10^{3} mm^{3})	23.00 ± 0.29^{b}	23.50 ± 0.76^{b}	28.00 ± 1.26^{a}	26.50 ± 0.58^{a}
Heterophil (%)	18.00 ± 038b^{c}	17.00 ± 0.50^{c}	19.00 ± 0.15^{b}	20.00 ± 0.58^{a}
Lymphocyte(%)	74.00 ± 0.96^{a}	73.00± 0.96^{a}	71.67 ± 0.29^{ab}	70.00 ± 0.76^{b}
Basophil (%)	2.00 ± 0.06^{b}	3.00 ± 029^{a}	2.00 ± 0.00^{b}	3.00 + 0.10^{a}
Monocyte (%)	4.50 ± 0.26	5.00 ± 0.12	5.00 ±0.00	5.00 ± 0.00
Eosinophil (%)	2.00 ± 0.05	2.00 ± 0.00	2.00 ±0.00	2.00 ± 0.00
MCV (fl)	124.12 ± 5.88	116.01 ± 4.19	115.17 ± 4.15	117.75 ± 5.64
MCH (pg)	44.27 ± 1.65^{b}	50.60 ± 0.00^{a}	52.18 ± 0.48^{a}	53.76 ± 0.80^{a}
MCHC (%)	40.41 ± 1.58^{b}	46.38 ± 0.39^{a}	48.36 ± 0.56^{a}	48.44 ± 0.13^{a}

abc – means with different superscript are significantly different P<0.05

Table6. *Serum Biochemistry of Cage Layer Fatigued Comm. Layers Treated With Calcium Gluconate*

Parameters	T_0	T_1	T_2	T_3
	Control	0.75ml CG/Bird	1.50ml CG/Bird	2.25ml CG/Bird
Total protein (g/dl)	4.40 ± 0.23	4.35 ± 0.08	4.60 ±0.23	4.75 ± 0.44
Albumin (g/dl)	3.28 ± 0.20	3.40 ± 0.06	3.37 ± 0.16	3.52 ± 0.56
Globulin (g/dl)	1.12 ± 0.36	0.95 ± 0.13	1.23 ± 0.24	1.23 ± 0.13
Glucose (mg/dl)	200.00 ± 1.72^{c}	268.00 ± 2.29^{b}	295.00 ± 10.41^{a}	298.00 ± 25.79^{a}
ALP (IU/L)	48.80 ± 5.62	52.30 ± 1.06	51.50 ± 1.32	54.00 ± 2.31
ALT (IU/L)	7.80 ± 057	8.40 ± 0.39	8.50 ± 0.41	7.90 ±0.28
AST (IU/L)	280 ± 11.55	275.00 ± 10.41	292 .00 ± 3.79	288.00 ± 7.21
Uric acid (mg/dl)	8.50 ± 0.35	7.80 ± 0.46	7.40 ± 0.41	8.20 ± 0.36
Cholesterol (mg/dl)	165.20 ± 3.61	168.00 ± 2.57	172.00 ± 12.07	162.00 ± 6.93
Calcium (mg/dl)	6.00 ± 0.06^{c}	27.00 ± 3.11^{b}	28.00 ± 1.17^{a}	30.00 ± 1.38^{a}
Phosphate (mg/dl)	6.50 ± 0.10	6.70 ± 0.21	6.70 ± 0.10	6.70 ± 0.10

abc – means with different superscript are significantly different P<0.05

ALP: Alkaline Phosphatase

ALT: Alkaline Aminotransaminase

AST: Aspartate Aminotransaminase

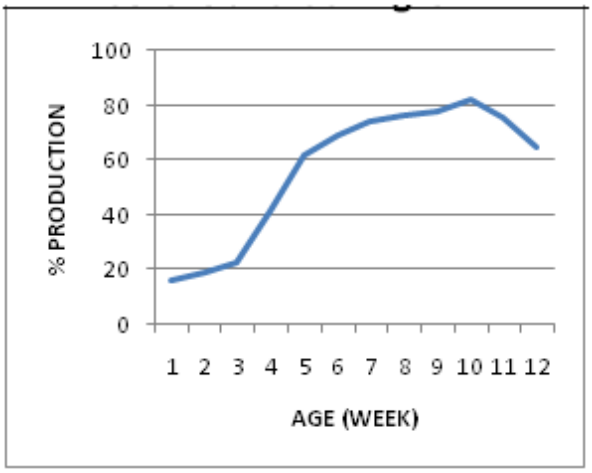

Fig1. *%production before and during CLF*

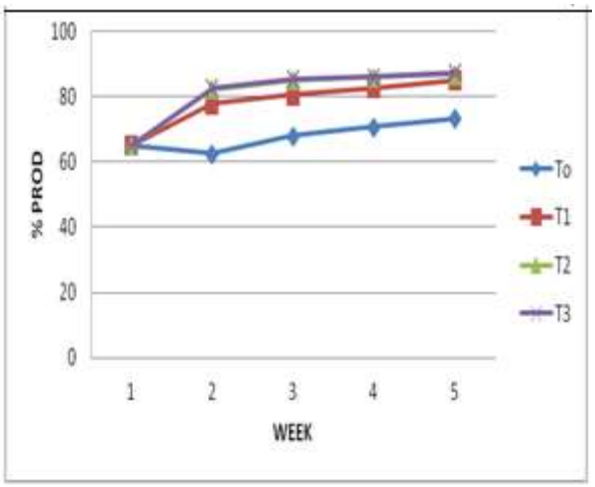

Fig2. *Response of EGG production to Calcium Glucanate Injection*

5. Results and Discussion

Table 3 shows the percentage production of the commercial layers prior to and immediately (2wks) after Cage Layer Fatigue (CLF). The percentage production has a positive progression from point of lay (18wk), at 20wk however, the percentage production rose to 35%, and got to 80% at 29wk of age. Thereafter due to high Calcium demand, this probably could be as a result of calcium and or vitamin D_3 deficient layer's mash; there is therefore a drastic drop in percentage production at 30wk (75.45%) and 31wk (65%), see fig 1. The high demand for calcium is characteristic of highly productive birds and birds at peak production, this is in consonance with Hamadani *et.al.*, (2013).

Table4 shows the percentage production response of layers to treatment with different dosages of Calcium Gluconate (CG) for five consecutive days following the incidence of CLF, and placed on a basal diet of layers mash (Table 1). Following the administration of CG, at 32wk T_0 which is the control suffered a regression in percentage egg production to 62.50% because CG was not administered to the birds thereby causing further drop in serum calcium and hence shelless eggs, drop in number of eggs and percentage production, which is in agreement with the findings of Fard *et. al.*,(2010) and Rosales (2013). The drop in production was for a week, but at 33wk of age the percentage production of the control started to rise (68.0%) and continued until the termination of this work. T_0, with only the layer's mash and no CG injection did not give an immediate response , rather there was a further drop in percentage egg production, this is because of the route and time of attainment of the threshold level of calcium before it can be fully functional and useful for shell formation and muscular activities. But for the other treatments T_1, T_2 and T_3 with different dosages of CG (0.75ml, 1.50ml and 2.25ml respectively) and same dietary calcium in layer's mash, there was almost instantaneous response of percentage egg production to the CG injection; this is due to the intravenous route used (Short and Van Poznak, 1992), for the administration of the source of calcium, but this response varies as the dosage increases, there are corresponding increase in the response of percentage production, at week 33, T_1(80.50%), T_2(85.00%) and T_3(85.30%), there were progressive increment in the percentage egg production throughout, until the termination of this work (see fig. 2). It is however noteworthy, that irrespective of the dosage of CG administered, the response of percentage egg production is still better than the control, at week 35, T_0 (73.30%), T_1 (85.00%), T_2 (86.80) and T_3 (87.25%), T_3 has the best percentage production, followed by T_2.

Table 5 shows the haematology (1wk after treatment) of CLF commercial layers with CG. As the dosage of CG increases there are corresponding increases in some of the haematological parameters ([Hb], wbc, MCH and MCHC). The [Hb] for T_1, T_2 and T_3 are statistically equal, but significantly greater ($P<0.05$) than the control (14.00±0.52), whose birds were not treated with CG. The values of [Hb] recorded for all the treatments however fall within the normal range (Aiello and Mays, 1998, Okeudo *et,al.*, 2003). The wbc count and the differential counts did not follow a particular order, just that the WBC of T_0 is not different from T_1, possibly because of the dosage of CG (0.75ml)

which is low, but both of them (T_0 and T_1)are less ($P>0.05$) than T_2 and T_3, which are not significantly different from one another. Out of the calculated haematological parameters, only MCH and MCHC show significant difference ($P<0.05$) across the treatments, they displayed same trend for all the treatments, T_0 is less than T_1, T_2 and T3, which are all statistically equal ($P>0.05$). Just like the [Hb], all the values fall within the normal range (Okeudo *et.al.*, 2003). All other haematological parameters measured (PCV, RBC and MCV) are the same for all the treatments, no significant differences, and all values fall within the normal range (Mitruka and Rawnsley, 1977).

Table 6 shows the serum biochemistry of CLF commercial layer, 1wk after treatment with CG. Most of the parameters measured (TP, albumin, globulin, ALP, ALT, AST, Uric acid, cholesterol and phosphate) did not differ across the treatment irrespective of the treatment dosage with CG. Calcium gluconate tends to elevate the Blood Sugar Level (BSL) as the dosage of CG increases [T0 (200±4.72), T1 (268±2.29), T2 (295.001±0.41) and T3 (298.002±5.79)], this increase in BSL is somehow strange because avians are known to maintain a high and relatively constant BSL even in low nutritional state (Liukkonen-Antilla, 2001). Also, CG significantly ($P<0.05$) affect calcium level in the blood, the level increases correspondingly as the dosage of CG increases, the control T_0 has a value of 6.00±0.06 which is lower than T1 (0.75ml) with 27.00±0.76, T2 (1.50ml) with 28.001±.17 and T3 (2.25ml) with calcium level of 30.00±1.38, but both T2 and T3 are significantly greater ($P<0.05$) than T1.

6. CONCLUSION

Calcium gluconate is a good source of calcium for commercial layers, and it is indicated when there is deficiency of calcium (dry season, nutritional deficiency of calcium and osteoporosis), it has tendency to increase blood calcium level and hence instantaneous response in percentage egg production. Irrespective of the dosage of calcium gluconate administered (0.75-2.25ml/layer), it has a positive effect on some haematological ([Hb], wbc, MCH and MCHC) and serum biochemical (glucose and calcium) indices, and does not have adverse or negative effects on every other parameters measured.

It is therefore recommended that 10% Calcium gluconate can be used in laying birds with CLF at a dosage of 1.50-2.25ml per layer intravenously for five days.

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