EFFECT OF CRYSTALLINE PROGESTERONE LEVELS ON MINERAL PROFILE IN LOHMANN BROWN LAYERS

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ABSTRACT
The present experiment was conducted to determine the effect of crystalline progesterone (CP) on serum levels of minerals profile in eighteen Lohmann Brown layers. A single factor completely randomized design was employed with each treatment (0, 5, 10, 15, 20 and 25 mg/bird) administered intramuscularly via the keel muscle and replicated thrice. Data were analyzed using GraphPad InStat® statistical package. Statistically non-significant changes in median serum mineral levels across CP treatment groups were recorded, except for serum calcium, phosphorus and sodium.Median serum calcium (2.9 mmol/L) at CP dose of 20 mg decreased significantly by a magnitude of 0.7 mmol/L from the median calcium value (3.6 mmol/L) at 5 mg CP. Serum phosphorus decreased significantly from 2.8 mmol/L in control hens to 1.6 mmol/L in hens that received 20 mg CP. There was a significant increase in serum sodium median value (4.67 ppm) at treatment dose of 10 mg CP when compared to the corresponding sodium level (2.00 ppm) in the control group. It is recommended that serum biochemistry, hematological parameters and reproductive hormones were determined after exogenous crystalline progesterone on Lohman brown layers.
Keywords: Progesterone, Mineral profile, Lohmann Brown, layers.

INTRODUCTION
Poultry also refers to domesticated birds that are kept by man for the production of eggs and meat. Most poultry are classified as *Galliforms, Anseriforms* and *Columbiforms* (Oluyemi & Robert, 2000). Lohmann Brown hen is an egg-laying strain of chicken of hybrid origin and selectively bred from New Hampshire thatcommence laying at about 18 weeks of age; laying about 300 brown eggs a year (Feltwell, 2011). Progesterone in avian species is produced by granulosa cells of large mature hierarchical follicles (Zagahari, Taherkhani & Honarbakhsh, 2009). Essential minerals are those elements that are required to support adequate growth, reproduction and health throughout the lifecycle of an animal (Spears, 1999). The physiological role of mineral elements in animal body is very important, they are necessary to maintain body function, to optimize growth and reproduction and to stimulate immune response and also determine health status (Lopez-Alonso, 2012). Minerals such as calcium (Ca), phosphorus (P), magnesium (Mg), iodine (I), manganese (Mn), copper (Cu), selenium (Se) and zinc (Zn) play a vital role in reproductive performance of animals (Wilde, 2006). Trace elements such as copper and zinc play a vital role in progesterone production and regulation by luteal cells through involvement of the enzyme superoxide dismutase (Sales, Pereira, Bicalho & Baruselli, 2011).

MATERIALS AND METHODS
Study Area
The research was carried out at the Poultry Unit (GPS coordinate 11.97643^{0}N, 008.42995^{0}E) of the Department of Animal Science, Faculty of Agriculture, Bayero University Kano. The unit is equipped with battery cages that were used for the experiment. The sample size for this work comprised eighteen birds as determined by the resource equation of Mead, Gilmour and Mead (2012).
Experimental birds
The experiment was laid in a Single Factor Completely Randomized Design (CRD). Six treatments levels were imposed with each having three replications. The six treatments levels were 0, 5, 10, 15, 20 and 25 mg of exogenous progesterone/bird. Eighteen Lohmann Brown Strain of hens at age of twenty-four weeks were purchased from SOVET International Company Limited at Tarauni, Kano.

The birds were randomized and allocated individually to a battery cage. They were allowed to stay for two weeks before commencement of the experiment. The birds were provided with drinking water and fed *ad libitum* throughout the experimental period with SOVET layermash (Super layer®) which contains16.0 % crude protein, 5.0% fat, 6.0% fibre, 3.5 %calcium, 0.4 % phosphorus and 2600 kcal/kg metabolizable energy.

Progesterone Administration

Crystalline progesterone (25 mg in 1 ml ampoule) was used in this research. It was purchased in a box of 10 ampoules. Progesterone injection was administered twice weekly on Mondays and Thursdays in the morning (between 10:00 am and 11:00 am) throughout the experimental period of six weeks. Progesterone at 0, 5, 10, 15, 20 and 25mg/bird was injected via the breast muscle and designated as treatments A, B, C, D, E and F, respectively. The control (A) comprised normal saline administered at 1 ml/bird. At the end of the 6-week experimental period, blood sample was collected from wing vein using syringe and needle. Blood samples were collected in labeled plain containers, placed on ice pack in Styrofoam box and transported to the laboratory for serum harvesting. Blood samples were centrifuged at 4000 revolutions per minute for 30 minutes by using a centrifuge machine (Centrifuge 8000®, Techmel, USA). Sera were harvested and dispensed into labeled plain tubes using dropping pipettes. They were stored at -20 ^oC until needed for mineral profile determination.

Serum Digestion and Mineral Analysis

Nitric-perchloric acid method was used for wet-digestion according to the procedure of AOAC (1990) with little modification. Deionized water was used to wash the containers and each container was labeled. Serum sample which was stored at -20^{o}C was removed from the freezer and allowed to thaw for 2 hours at room temperature. One ml from each sample was collected using pipette and transferred into labeled 50ml beakers. Ten ml of nitric acid (HNO_3) was added to each of the samples. A hot plate was used to heat the content until all digestible matter is oxidized. Samples were allowed to cool and 5 ml of 70% perchloric acid ($HClO_4$) was added and the content boiled gently until white fumes appear. They were allowed to settle down and cool. Distilled water (20 ml) was added to each sample and boiled gently until white fumes appear. After cooling, Whatmann (No. 42) chromatographic paper was used to filter the sample. Thirty (30) ml of distilled water was then added and the content transferred into labeled sample plastic containers for storage until further analysis. Using Atomic Absorption Spectrophotometry (Atomic Absorption Spectrophotometer, Model AAS VGP210, Bulk Scientific, U.K), Magnesium (Mg), Zinc (Zn), and Iron (Fe) levels were determined in the digested sera. Flame Photometer (Model PF97, Jenway Ltd, UK) was used to determine serum Sodium (Na) and Potassium (K) levels. Colorimetric method (Ciba-Corning® Colorimeter, model 257, Sherwood Scientific Ltd., U.K) was used to determine serum Phosphorus (P) and Calcium (Ca) levels.

Data Analyses

Kruskal-Wallis Test was used to determine the effect of crystalline progesterone on levels of serum mineral concentration. Where significant differences exist, Dunn's Multiple Comparison Tests were used to compare the mean rank differences among the treatment groups. Statistical analyses of data were conducted using GraphPad InStat package (GraphPad InStat®,version 3.05, 32 bits for Win 95/NT, GraphPad Software Inc., 2000).

RESULTS AND DISCUSSION

Mineral Levels

Table 1: Summary Statistics (including KW Statistic) of Serum Zinc Concentration across Crystalline Progesterone Treatments Levels in Lohmann Brown Hens

Crystalline Progesterone Levels (mg)	n	Median	Minimum	Maximum	Sum of Ranks	Mean of Ranks	Kruskal-Wallis (KW) Statistic, corrected for ties
0	2	0.655	0.250	1.060	18.0	9.000	
5	3	0.670	0.170	0.720	23.5	7.833	
10	3	0.530	0.440	0.810	28.5	9.500	0.3097^{ns}
15	2	0.555	0.420	0.690	17.0	8.500	
20	3	0.530	0.170	1.220	26.0	8.667	
25	3	0.360	0.190	1.080	23.0	7.667	

ns = not significant, P>0.0

Serum zinc

The effect of crystalline progesterone levels on serum concentrations of zinc of Lohmann Brown layers is presented in Table 4. There were no statistically significant (P>0.05, Kruskal-Wallis Statistic = 0.3097) differences in median serum potassium concentrations of 0.655, 0.670, 0.530, 0.555, 0.530 and 0.360 ppm across respective crystalline progesterone treatment groups (0, 5, 10, 15, 20 and 25 mg). The minimum serum zinc concentrations recorded were 0.250, 0.170, 0.440, 0.420, 0.170 and 0.190 ppm across 0, 5, 10, 15, 20 and 25 mg crystalline progesterone treatments levels, respectively. Maximum serum zinc concentrations recorded were 1.060, 0.720, 0.810, 0.690, 1.220 and 1.080 ppm across 0, 5, 10, 15, 20 and 25 mg crystalline progesterone treatment levels, respectively.

Table 2: Summary Statistics (including KW Statistic) of Serum Magnesium Concentration across Crystalline Progesterone Treatments in Lohmann Brown Hens

Crystalline Progesterone Levels (mg)	N	Median	Minimum	Maximum	Sum of Ranks	Mean of Ranks	Kruskal-Wallis (KW) Statistic, corrected for ties
0	3	0.5	0.4	0.6	16.0	8.000	
5	3	0.6	0.4	0.6	27.5	9.167	
10	3	0.4	0.4	0.6	20.5	6.833	
15	2	0.7	0.6	0.8	27.0	13.500	3.688^{ns}
20	3	0.4	0.4	0.6	20.5	6.833	
25	3	0.4	0.4	0.8	24.5	8.167	

ns = not significant, P>0.05

Serum sodium

The effect of crystalline progesterone levels on sodium serum concentrations in Lohmann Brown layers is presented in Table 11. A statistically significant (P<0.01; mean rank difference = -12.583; Kruskal-Wallis Statistics = 11.720) difference was recorded in serum sodium concentrations (2.000 ppm *vs*. 4.335 ppm) between 0and 10 mg crystalline progesterone levels. All other comparison among crystalline progesterone levels gave statistically similar (P>0.05) serum sodium concentrations.

Discussion

Metabolic processes are coordinated by hormonal mechanisms, so the endocrine system of the animal is maximally required (Preda, Bălăceanu & Dojană, 2013). This statement is applicable to the actively laying hen which has become a true metabolic bomb due to effects of high pressure selection for egg production (Preda *et al*., 2013). (Sales, Pereira, Bicalho & Baruselli, 2011) identified daily evolutions of blood calcium level in hens which were probably related to the evolution of the level of gonadotropin-releasing hormone (GnRH). In the present study, the decrease in serum calcium as well as serum sodium in the 20 mg progesterone-treated group could be linked to female sex hormones which were reported by Emura, Shoumura and Isono (1982) to have marked effects on calcium metabolism. Moreover, sex hormones are under the influence of GnRH and its associated tropic hormones. It is also possible that beyond this dose further physiological changes in serum sodium level were nullified as observed in the current study.

CONCLUSION

In conclusion, serum calcium, and serum sodium decreased significantly affected negatively by crystalline progesterone (CP) injection in Lohmann Brown laying hens.

REFERENCE

Emura, S., Shoumura, S. and Isono, H. (1982). Ultrastructural Changes of the Parathyroid Gland of the Oestrogen-Treated Golden Hamsters. *Journal of Clinical Electron Microscopy*, 15: 113-126.

Feltwell, R. (2011). *Small Scale Poultry Keeping:A Guide to Free Range Poultry Production*. London, U.K.: Faber & Faber.

Mead, R.,Gilmour, S.G. and Mead, A. (2012). *Statistical Principles for the Design of Experiments.* New York, U.S.A.: Cambridge University Press.

Oluyemi, Y.A. and Robert, F.A. (2000). *Poultry Production in Warm Wet Climate*.London, U.K.:

Preda, C., Bălăceanu, R. and Dojană, N. (2013). Evaluation of Hormonal Control of Calcium and Phosphorus Metabolism in Different Hen Breeds During the Laying Cycle. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca: Veterinary Medicine*, 70 (2): 286-292.

Sales, J.N., Pereira, R.V., Bicalho, R.C. and Baruselli, P.S. (2011). Effect of Injectable Copper, Selenium, Zinc and Manganese on the Pregnancy Rate of Crossbred Heifers (*Bos indicus* × *Bos taurus*) Synchronized for Timed Embryo Transfer. *Livestock Science*, 142 (1-3): 59-62.

Spears, J.W. (1999). Re-evaluation of the Metabolic Essentiality of Minerals. *Asian-Australasian Journal of Animal Science*, 2 (6):1002-1008.

Wilde, D. (2006). Influence of Macro- and Micro-minerals in the Peri-parturient Period on Fertility in Dairy Cattle. *Animal Reproduction Science*, 96 (3-4): 240-249.

Zagahari, M., Taherkhani, R. and Honarbakhsh, S. (2009). Effects of Progesterone Injection on Performance, Plasma Hormones and Ovarian Morphology of *Ad Libitum* and Restricted Fed Broiler Breeder Hens. *African Journal of Biotechnology*, 8 (22): 6481-6489.