



## 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<sup>®</sup> 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 will be determine after exogenous crystalline progesterone on Lohman brown layers.*

Keywords: Progesterone, Mineral profile and 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 and Robert, 2000). Lohmann Brown hen is an egg-laying strain of chicken of hybrid origin and selectively bred from New Hampshire that commence 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, *et al.*, 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). There is a report by Fagari-Nobijari, *et al.*, (2012) that emphasized on involvement of zinc in the reorganization of ovarian follicles which are sites of progesterone production. Zinc is required for progesterone production and a deficiency can produce excessive prolactin secretion

(Brown and Pentland, 2007). Williams *et al.*, (2000) reported that Ca and P deficiencies in fast-growing broiler lines can cause skeletal abnormalities, such as rickets and tibial dyschondroplasia, which lead to lameness and increased morbidity as well as mortality. According to Sullivan(1994) the level of mortality as a result of the skeletal disorders has been estimated to be approximately 3% in the United States, of which is 60% of total mortality reported in the broiler industry by the NRC (2001).

## **MATERIALS AND METHOD**

### **Study Area**

The research was carried out at the Poultry Unit (GPS coordinate 11.97643<sup>0</sup>N, 008.42995<sup>0</sup>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<sup>®</sup>) which contains 16.0 % 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 (6) 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<sup>®</sup>, Techmel, USA). Sera were harvested and dispensed into labeled plain tubes using dropping pipettes. They were stored at -20 °C 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°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<sub>3</sub>) 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<sub>4</sub>) 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), 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

#### Serum magnesium

The effect of crystalline progesterone levels on serum magnesium concentrations in Lohmann Brown layers is presented in Table 2. There was no statistically significant ( $P > 0.05$ , Kruskal-Wallis Statistic = 3.688) difference in median serum magnesium concentrations (0.5, 0.6, 0.4, 0.7, 0.400 and 0.4 ppm) across respective progesterone treatments levels (0, 5, 10, 15, 20 and 25 mg). Minimum vs. maximum serum magnesium concentrations were 0.4 vs. 0.6, 0.4 vs. 0.6, 0.4 vs. 0.6, 0.6 vs. 0.8, 0.4 vs. 0.6 and 0.4 vs. 0.8 ppm across 0, 5, 10, 15, 20 and 25 mg crystalline progesterone treatments levels, respectively.

Table 1: 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	3.688 <sup>ns</sup>
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	
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 ( $P > 0.05$ )

ns = no of observation

#### Serum iron

The effect of crystalline progesterone levels on serum concentrations of iron in Lohmann Brown layers is presented in Table 2. There was no statistically significant ( $P > 0.05$ , Kruskal-Wallis Statistic = 9.115) difference in median serum iron concentrations of 0.13, 0.19, 0.11, 0.09, 0.07 and 0.07 ppm across

respective crystalline progesterone treatments levels (0, 5, 10, 15, 20 and 25 mg). Minimum serum iron concentrations recorded were 0.11, 0.11, 0.11, 0.07, 0.07 and 0.07 ppm across 0, 5, 10, 15, 20 and 25 mg crystalline progesterone levels, respectively. Crystalline progesterone treatments levels (0, 5, 10, 15, 20 and 25 mg) gave maximum serum iron concentrations of 0.15, 0.22, 0.15, 0.11, 0.11 and 0.11 ppm, respectively.

Table 2: Summary Statistics (including KW Statistic) of Serum Iron Concentrations 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	0.13	0.11	0.15	22.5	11.250	9.115 <sup>ns</sup>
5	3	0.19	0.11	0.22	40.0	13.333	
10	3	0.11	0.11	0.15	31.5	10.500	
15	2	0.09	0.07	0.11	12.0	6.000	
20	3	0.07	0.07	0.11	15.0	5.000	
25	3	0.07	0.07	0.11	15.0	5.000	

ns = not significant,  $P > 0.05$

ns = no of observation

### Serum sodium

The effect of crystalline progesterone levels on sodium serum concentrations in Lohmann Brown layers were presented in Table 3. 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 0 and 10 mg crystalline progesterone levels. All other comparison among crystalline progesterone levels gave statistically similar ( $P > 0.05$ ) serum sodium concentrations.

### Serum potassium

The effect of crystalline progesterone levels on serum concentrations of potassium in Lohmann Brown layers were presented in Table 4. There was no statistically significant ( $P > 0.05$ , Kruskal-Wallis Statistic = 5.180) difference in median serum potassium concentrations of 0.585, 0.830, 1.000, 0.830, 0.830 and 1.000 ppm across respective crystalline progesterone treatments levels (0, 5, 10, 15, 20 and 25 mg). Minimum serum potassium concentrations recorded were 0.50, 0.50, 0.83, 0.83, 0.67 and 0.83 ppm across 0, 5, 10, 15, 20 and 25 mg crystalline progesterone treatments levels, respectively. Crystalline progesterone treatments levels (0, 5, 15, 20 and 25 mg) gave respective maximum serum potassium concentrations of 0.67, 1.33, 1.00, 0.83, 1.170 and 1.17 ppm, respectively.

Table 5: Summary Statistics, KW Statistic and Selected Pairs Dunn's Multiple Comparisons Test for Serum Sodium 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	2.000	1.33	2.67	3.5	1.750	

5	3	4.000	4.00	4.00	22.5	7.500	11.720*
10	3	4.670	4.67	5.33	43.0	14.333	
15	2	4.335	4.00	4.67	21.0	10.500	
20	3	4.000	2.67	4.00	17.5	5.833	
25	3	4.000	4.00	4.67	28.5	9.500	

Comparison	Mean Rank Difference	Level of Significance
0 mg vs. 5 mg	-5.750	Ns
0 mg vs. 10 mg	-12.583	**
0 mg vs. 15 mg	-8.750	ns
0 mg vs. 20 mg	-4.083	ns
0 mg vs. 25 mg	-7.750	ns

ns = not significant; \*\*P<0.01, \*P<0.05

ns = no of observation

Table 6: Summary Statistics (including KW Statistic) of Serum Potassium Concentrations 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	0.585	0.50	0.67	5.0	2.500	5.180 <sup>ns</sup>
5	3	0.830	0.50	1.33	25.0	8.333	
10	3	1.000	0.83	1.00	31.5	10.500	
15	2	0.830	0.83	0.83	15.0	7.500	
20	3	0.830	0.67	1.17	25.5	8.500	
25	3	1.000	0.83	1.17	34.0	11.333	

ns = not significant, P>0.05

ns = no of observation

### Serum calcium

The effect of crystalline progesterone levels on serum calcium concentrations in Lohmann Brown Layers is presented in Table 7. There was statistically significant (P<0.05; mean rank difference = 11.000; Kruskal-

Wallis statistic = 14.837) difference in median serum calcium concentrations (3.6 mmol/L vs. 3.0 mmol/L) between birds administered crystalline progesterone at 5 and 10 mg. Also, a significant ( $P < 0.01$ ; mean rank difference = 13.667; Kruskal-Wallis statistic = 14.837) serum median calcium concentrations (3.6 mmol/L vs. 2.9 mmol/L) between birds administered crystalline progesterone levels at 5 and 20 mg was recorded.

### Serum phosphorus

The effect of crystalline progesterone levels on serum phosphorus concentrations in Lohmann Brown Layers is presented in Table 6. There was statistically significant ( $P < 0.05$ ; mean rank difference = 9.167; Kruskal-Wallis statistic = 13.245) difference in median serum phosphorus concentrations (2.8 mmol/L vs. 1.6 mmol/L) between the control and birds administered 5mg crystalline progesterone levels. Also, significant ( $P < 0.01$ ; mean rank difference = -10.667; Kruskal-Wallis statistic = 13.245) difference in median serum phosphorus concentrations (1.6 mmol/L vs. 2.8 mmol/L) was recorded between 20 and 25 mg progesterone treatment levels.

Crystalline Progesterone Levels (mg)	N	Median	Minimum	Maximum	Sum of Ranks	Mean of Ranks	Kruskal-Wallis (KW) Statistic, corrected for ties
0	3	3.1	3.1	3.1	33	11.000	14.837*
5	3	3.6	3.5	3.8	48	16.000	
10	3	3.0	2.9	3.0	15	5.000	
15	2	3.1	3.1	3.1	22	11.000	
20	3	2.9	2.7	2.9	7	2.333	
25	3	3.1	3.0	3.1	28	9.333	

Comparison	Mean Rank Difference	Level of Significance
5 mg vs. 10 mg	11.000	*
5 mg vs. 15 mg	5.000	ns
5 mg vs. 20 mg	13.667	**
5 mg vs. 25 mg	6.667	Ns

ns = not significant; \*\* $P < 0.01$ , \* $P < 0.05$

ns = no of observation

Table 7: Summary Statistics, KW Statistic and Selected Pairs Dunn's Multiple Comparisons Test for Serum Phosphorus 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	3	2.80	2.7	2.8	40.5	13.500	13.245*
5	3	1.60	1.6	2.0	13.0	4.333	
10	3	2.00	1.8	2.1	21.0	7.000	

15	2	2.65	2.5	2.8	24.5	12.250
20	3	1.60	1.4	2.0	11.0	3.667
25	3	2.80	2.7	3.0	43.0	14.333
		Mean				
		Rank				
Comparison		Difference	Level	of		
			Significance			
0 mg vs. 5 mg		9.167	*			
20 mg vs. 25 mg		-10.667	**			

ns = not significant; \*\*P<0.01, \*P<0.05

## DISCUSSION

Metabolic processes are coordinated by hormonal mechanisms, so the endocrine system of the animal is maximally required (Preda, *et al.*, 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). Luck and Scanes (1979) 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 phosphorus 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. Emura *et al.*, (1995) suggested that the secretory activity of golden hamster parathyroid glands may be stimulated in response to hypocalcaemia induced by intracutaneously administered progesterone in olive oil. In the female progesterone-treated group, mean serum calcium concentration was significantly low compared to the control group (Emura *et al.*, 1995). According to Oguike *et al.*, (2005), female hormones are complementary to parathyroid hormone and vitamin D in the control of calcium homeostasis in laying hens. It is, therefore, suggested that alteration of serum calcium homeostasis is significantly correlated with parathyroid hormone, vitamin D, estradiol and progesterone (Preda *et al.*, 2013). In the current study, serum levels of parathyroid hormone, vitamin D and estradiol were not determined.

Progesterone is an antagonist of aldosterone, presumably at the level of the renal tubules (Landau and Lugibihl, 1957). Progesterone-induced urinary sodium excretion (natriuresis) in rats results from inhibition of aldosterone binding to renal mineralocorticoid receptors (Wambach and Higgins, 1978). Because progesterone inhibits aldosterone binding to mineralocorticoid receptor (Quinkler *et al.*, 2002), increased progesterone production during the luteal phase leads to compensatory activation of the renin-angiotensin system and thus increased aldosterone production (Oelkers *et al.*, 1991). This compensation could be the likely explanation for the significant increase in serum sodium concentration in the present study following administration of 10 mg progesterone to laying birds; since progesterone, as reported by Szmiulowicz *et al.*, (2006), directly stimulates zona glomerulosa cell aldosterone production. It is also possible that beyond this dose further physiological changes in serum sodium level were nullified as observed in the current study. Likely confounders in the interpretation of this result include pre-treatment serum sodium level, dietary sodium intake and species differences.

## CONCLUSION

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

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