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Research Article

Reproductive Hormones and Dimension of follicles of Hen fed Diets Supplemented with graded levels of Chromium Picolinate with or without Vitamin C at high Ambient Temperature.

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Abstract

The selection of follicles based on dimension determines those that will become atretic and those that will undergo further development to final ovulation. Two Hundred and Sixteen Point of Lay Noiler Pullets which was purchased from a reputable farm which were used for this study. The birds were assigned to 8 equal treatments in a completely randomized design (CRD) using 2 x 4 factorial arrangement (2 levels of vitamin C and 4 levels of CrPic). A basal diet was formulated, divided into eight equal portions and diet 1 to 4 were supplemented with 0.00 mg/kg CrPic, 0.40 mg/kg CrPic, 0.80 mg/kg CrPic and 1.20 mg/kg CrPic and diets 5 to 8 were supplemented with 200 mg/kg vitamin C; 0.40 mg/kg CrPic and 200 mg vitamin C; 0.80 mg/kg CrPic and 200 mg vitamin C and 1.20 mg/kg CrPic and 200 mg Vitamin C, respectively. The feeding trial lasted for 16 weeks for the studies. The reproductive hormones and dimension of follicles of laying birds are investigated . It was observed that there was no significant ($P > 0.05$) effect of CrPic and its interraction with vitamin C on the reproductive hormones of the chicken. However, at 1.2 mg/kg CrPic, progesterone, oestrogen, testosterone and FSH were higher than other levels for those without vitamin C, but decreased the luitenising hormone while progesterone, oestrogen, testosterone and FSH for those with vitamin C were also higher at 1.2 mg/kg CrPic than other levels of the diets. In addition, there was positive improvement in the values recorded on the follicle parameters measured in dimension by CrPic and Vitamin C supplement diet. This suggests that the dietary supplementation of CrPic with or without vitamin C could support the normal reproductive hormones and the rate of apoptosis

and atresia of the follicles of chicken raised at high ambient temperature. The study revealed that diets supplemented with Chromium Picolinate with or without Vitamin C does not have a negative effect on the reproductive hormones and dimension of follicles of laying birds.

Keywords:
Follicle, Graffian Follicle, Pre-ovulatory Follicle, Luteinising Hormone, Progesterone, Oestrogen, Testosterone and Vitamin C

Introduction:

Optimal reproductive efficacy in domesticated chickens is attained when follicular maturation occurs within a clearly defined hierarchical structure (Johnson, 2012). The development of small (pre-hierarchical) follicles is associated with follicle-stimulating hormone (FSH) and various other influences, including growth factors that increase the expression of FSH receptors in granulosa cells of pre-hierarchical follicles (Scanes, 2022). Typically, larger pre-ovulatory follicles are selected from the pool of smaller, developing follicles (Johnson, 2012). Wojtusik and Johnson (2012) indicated that vitamin D may directly influence the expression of Anti-Mullerian hormone (AMH), which, in turn, may affect follicle-stimulating hormone receptor (FSHR) expression, thereby playing a significant role in the follicular selection mechanism.

Research into the effects of chromium on the productivity of livestock has been carried out with considerable success (Lindemann et al., 1995; Amoikon et al., 1995), however, its utilization in poultry remains significantly limited. Nonetheless, Adebayo, (2024) documented the influence of chromium in its picolinate form on haematological parameters and growth hormone levels in birds subjected to elevated ambient temperatures.

In laying hens, the addition of Chromium has been associated with increased weight, enhanced production, greater mass, and improved quality of eggs (Uyanik et al., 2002). Garcia et al. (1997) investigated the impact of Cr-picolinate supplementation on tissue sensitivity to insulin, ovulation rates, and the secretion of progesterone and oxytocin. Although Chromium supplementation has shown a beneficial effect on tissue sensitivity to insulin (indicated by a decreased insulin-to-glucose ratio), the ovulation rate and progesterone levels remained stable. The subject of reproductive outcomes in relation to Chromium supplementation in cattle has garnered relatively minimal attention. Nonetheless, research has established a favorable influence of Chromium supplementation on the insemination index, duration of intervals, and service periods (Bonomi et al., 1997; Pechova et al., 2003), alongside a reduced occurrence of endometritis and placental retention (Chang et al., 1996; Villalobos et al., 1997). This study is therefore designed to explore the effect of CrPic with or without vitamin C on the number and dimension of follicles and reproductive hormones of hens under high ambient temperature.

Materials and Methods:

This research study was performed at the Poultry Unit of the Teaching and Research Farm of the Federal University of Technology, Akure (FUTA). The research took place during the height of the dry season in the region under study, specifically from January to February 2020. The wet and dry bulb temperatures of the experimental facility were recorded daily, both in the morning and in the evening. The mean daily temperature-humidity index (THI) for the experimental pen was found to be 34.08 ^{0}C±1.36. The THI, which serves as an indicator of the thermal comfort level for enclosed broiler chickens, was computed following the method established by Tao and Xin (2003) using the equation: THI= 0.85×Tdb+0.15×Twb where Tdb denotes the dry bulb temperature (^{0}C) and Twb represents the wet bulb temperature (^{0}C). A total of two hundred and sixteen (216) Noiler point-of-lay (POL) chickens, aged 18 weeks, were acquired from a reputable source. They were randomly allocated to eight distinct dietary treatments with three replicates per treatment, each containing nine birds. A basal diet was formulated, as depicted in Table 1. The proximate analysis of the standard diet was carried out in accordance with AOAC (1995). The basal diet was equally divided into eight portions and supplemented as follows: Diet 1 served as the control, diet 2 consisted of the basal diet plus 0.4 mg CrPi/kg diet, diet 3 comprised the basal diet plus 0.8 mg CrPi/kg, diet 4 included the basal diet plus 1.2 mg CrPic/kg, diet 5 was the basal diet plus 200 mg Vit C/kg, diet 6 was formulated as the basal diet plus (0.4 mg CrPic + 200 mg Vit C/kg), diet 7 was the basal diet plus (0.8 mg CrPic + 200 mg Vit C/kg), and diet 8 was the basal diet plus (1.2 mg CrPic + 200 mg Vit C/kg). The data acquired was subjected to an analysis of variance using General Linear Model approaches appropriate for a completely randomized design framed by a 2 × 4 factorial arrangement, incorporating 2 levels of Vitamin C and 4 levels of Chromium picolinate. The dimensions of the follicles in each bird slaughtered were measured using a divider and a measuring tape calibrated to the nearest 0.01mm and documented. The follicles were divided into matured/graafian follicles (follicle whose dimension is 2.5 cm or 25 mm and above); stage 3, pre-ovulatory or maturing follicles (20-24mm); stage 2, pre-ovulatory follicles (15-19mm); stage 1, pre-ovulatory follicles (10-14mm) and pre-vitellogenic follicles **(Myres et al., 2004).** Additionally, Serum growth and reproductive hormone concentrations were determined by double antibody RIA using commercial available RIA kits (IBL International GMBH, Flughafenstrasse 52a, D-22335 Hamburg, Germany) as described by **(Darras et al., 1992).** The data were analyzed for the effects of CrPic, Vitamin C, and the interaction between CrPic and Vitamin C, with significant effects leading to means being differentiated through Duncan's multiple range test using SPSS.

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Table 1: Composition of the Hens' Basal Experimental Diets

Ingredients	Quantity (kg)
Maize	55.00
Soya Bean Meal	15.00
Ground Nut Cake	7.00
Fish Meal	0.50
Palm Kernel Meal	5.67
Wheat Offal	5.48
Limestone	8.50
Dicalcium Phosphate	2.00
Lysine	0.15
Methionine	0.15
Salt	0.30
Layer Premix*	0.25
TOTAL	100

CALCULATED VALUES	
Crude protein (%)	16.85
ME (kcal/kg)	2687.36
Ca (%)	3.79
Crude fibre (%)	1.95
Lysine (%)	0.89
Methionine (%)	0.50
Phosphorus (%)	0.47

Result:

Reproductive Hormones of Hens Fed Diets Supplemented with Graded Levels of Chromium Picolinate (CrPic) with or without VitaminC

Reproductive hormones of hens fed diets supplemented with graded levels of chromium Picolinate (CrPic) with or without vitamin C is shown in Table 2. Selected hormones such as luteinising hormone, progesterone, oestrogen, testosterone and FSH were examined for any changes effected by the presence of CrPic with or without vitamin C. Generally, there were no significant difference (P > 0.05) in the observed parameters at the levels of vitamin C, the levels of the CrPic and the interaction of the two levels.

The interaction between levels of CrPic with the supplementationor non supplementation of vitamin C showed no significant differences (P > 0.05) at all the levels. At 1.2 mg/kg CrPic, progesterone (1.56ng/mL), oestrogen (460.13ng/dL), testosterone (22.13nmol/L) and FSH (4.59IU/L) were higher than other levels for those without vitamin C, but decreased the luitenising hormone(3.60 IU/L) while progesterone (1.54ng/mL), oestrogen (461.13ng/dL), testosterone (21.58nmol/L) and FSH (4.62IU/L) for those with vitamin C were also higher at 1.2 mg/kg CrPic than other levels of the additive.

The levels of CrPic administered did not significantly (P>0.05) affect luteinising hormone, oestrogen, testosterone and FSH. At 1.2 mg/kg CrPic, oestrogen (460.63ng/dL), testosterone (21.86nmol/L) and FSH (4.61IU/L) were higher numerically than other levels of CrPic. There were slight differences between those with vitamin C and those without vitamin C in hormonal production.

Discussion:

Reproductive Hormones of Hens Fed Diets Supplemented with Graded Levels of Chromium Picolinate (CrPic) with or without Vitamin C

Decrease in egg production traits of hens exposed to increasing levels of dietary Chromium picolinate was observed in the present study. As reported by **Farag et al., (2017)** the small white follicles produce more than 80% of the total ovarian oestrogen, which controls the reproductive tracts growth and development **(Devesa and Caicedo, 2019).** This shows that the ovary in poultry displays an essential role in the reproductive functions. Granulosa cells of large ovarian hierrachial follicles in laying hens secrete progesterone, the major steroid hormone **(Liu and Bacon, 2005).** Disorder of the ovarian function is responsible for reduced reproductive efficiency of hyperthemia hens **(Attia et al., 2015).**

Moreover, Oestradiol, a steroid hormone is the principal oestrogen in hens. It plays a key role in the control of reproductive behaviour and the regulation of the neuro-endocrine system. It is the hormones required for formation in the avian females. It stimulates the synthesis of egg albumen in the oviducts **(Ciftci, 2012).** Elevated CrPic supplementation as well as vitamin C supplementation increases the concentration of serum oestrogen and progesterone though not significant at 1.2mg/kg diet level of supplementation.

Since, it was earlier reported by **Adeyinka et al., (2007)** that egg production had a very high and positive correlation with progesterone level, the increase in serum progesterone as occasioned by the increased level of supplementation of CrPic did not agreed with this report as observed.

The decrease in the serum concentration of LH at 0.8mg and 1.2 mg/kg level of supplementation of CrPic in this present study did not also agreed with the earlier report by **Edens (2015)** that such reduction is responsible for reduced serum levels of progesterone and oestradiol-17β from granulosa and theca cells respectively leading to reduced ovulations and egg production. For this study, FSH, progesterone and oestrogen were found to increase despite the decrease in LH. Furthermore, the serum testosterone (TS) level steadily increased with increasing levels of CrPic on the diets up to 1.2 mg/kg CrPic having a non-significant increase when compared with the control group. Moreover, the present study also agreed with earlier report that documented positive effect of testosterone administration on egg production performance of laying hens **(Mohammadi et al., 2015)** as such positive effects were noticed at 1.2 mg/kg CrPic.

Table 2: Reproductive Hormones of Hens Fed Diets Supplemented with Graded Levels of Chromium Picolinate (CrPic) with or without Vitamin C

Level of CrPic(mg)	Vitamin C (mg)	LHormo(IU/L)	Progest(ng/mL)	Oestrogen(ng/dL)	Testosterone(nmol/L)	FSH(IU/L)
Level of CrPic						
0		3.65±0.02	1.52±0.00	458.30±1.40	20.83±0.43	4.54±0.02
0.4		3.66±0.03	1.53±0.01	458.68±2.02	21.04±0.42	4.59±0.01
0.8		3.63±0.01	1.52±0.00	457.04±1.29	21.08±0.36	4.56±0.02
1.2		3.62±0.01	1.55±0.01	460.63±0.79	21.86±0.26	4.61±0.02
Vitamin C						
	0	3.63±0.02	1.53±0.00	458.98±1.05	21.17±0.28	4.56±0.02
	200	3.64±0.02	1.53±0.00	458.98±1.05	21.24±0.28	4.59±0.01
CrPic xVit c						
0	0	3.64±0.02	1.52±0.01	457.49±2.62	20.58±0.41	4.52±0.04
0.4	0	3.64±0.04	1.52±0.01	459.38±3.05	21.35±0.68	4.59±0.01
0.8	0	3.65±0.02	1.52±0.01	456.39±1.64	20.59±0.40	4.54±0.05
1.2	0	3.60±0.01	1.56±0.03	460.13±0.58	22.13±0.32	4.59±0.03
0	200	3.66±0.05	1.52±0.00	459.10±1.50	21.08±0.84	4.57±0.01
0.4	200	3.67±0.04	1.53±0.01	457.98±3.26	20.73±0.57	4.59±0.02
0.8	200	3.60±0.01	1.52±0.01	457.69±2.27	21.57±0.50	4.58±0.01
1.2	200	3.64±0.01	1.54±0.01	461.13±1.60	21.58±0.41	4.62±0.01
Statistical significance						
CrPic level		0.6036	0.0801	0.4673	0.2838	0.1597
Vitamin C		0.5933	0.7014	0.6959	0.8504	0.1161
CrPic level*Vitamin C		0.4852	0.7331	0.9017	0.3922	0.7760

Means within the same column with different superscripts (a, b, c) are significantly different (P≤0.05). LHormo- luteinising hormone; Progest-Progesterone; FS follicle Stimulating Hormone

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Result and Discussion:

Dimensions of Follicles of Hens Fed Diets Supplemented with Graded Levels of Chromium Picolinate (CrPic) with or without Vitamin C

Shown in table 3 is the dimensions of follicles from hens fed diets supplemented with graded levels of chromium picolinate (CrPic) with or without vitamin C. The matured or Graafian follicles were those with diameter of 25 mm and above. The three stages of the Pre-ovulatory follicles had diameters of 20-24 mm (stage 3), 15-19 mm (stage 2) and 10-14 mm (stage 1) respectively. Follicles at the various levels of developments were measured and compared among the diets with or without vitamin C, in which there were slight variation in the dimensions of these follicles at several stages of developments. It was observed that there were no significant treatments effect on gfollicle, preovutwo to preovuone, on the other hand, Preovuthree was significantly ($p < 0.05$) influenced by the levels of CrPic in the diets. Dimension follicles of preovuthree were significantly higher at 0.4 mg/kg CrPic (22.67cm) than other levels of CrPic. At gfollicle (21.33cm) and preovutwo (16.83cm), 0.4 mg/kg CrPic had reduced size than other levels of CrPic and the control. The diets without vitamin C supplementation showed gfollicle dimension of 22.92cm, the preovuthree showed a dimension of 21.71cm and preovuone showed a dimension of 10.93cm but were higher at the supplementation of 200 mg of vitamin C except preovutwo, which had higher dimension of 17.04cm in the absence of vitamin C supplementation. The selection of follicles based on dimension determines those that will become atretic and those that will undergo further development to final ovulation **(Myres et al., 2004).** The dimensions of follicles of the hens, used in this study, were unaffected by neither the level of supplementation of chromium picolinate nor the interaction between the CrPic and vitamin C which affirms that the rate of apoptosis and atresia of the follicles could be said to be normal.

Table 3:Dimensions of Follicles of Hens Fed Diets Supplemented with Graded Levels of Chromium Picolinate (CrPic) with or without Vitamin C

	Level of CrPic (mg)	Vitamin C (mg)	GFOLLICLE (cm)	PREOVUTHREE (cm)	PREOVUTWO (cm)	PREOVUONE(cm)
Level of CrPic						
	0		25.33 ± 0.33	21.42 ± 0.33	16.92 ± 0.52	11.43 ± 0.55
	0.4		21.33 ± 4.28	22.67 ± 0.60	16.83 ± 0.42	11.33 ± 0.49
	0.8		25.00 ± 0.00	21.17 ± 0.38	16.92 ± 0.58	11.58 ± 0.52
	1.2		25.83 ± 0.83	22.58 ± 0.27	17.42 ± 0.69	10.92 ± 0.49
Vitamin C						
		0	22.92 ± 2.08	21.71 ± 0.39	17.04 ± 0.34	10.93 ± 0.25
		200	25.83 ± 0.44	22.21 ± 0.28	17.00 ± 0.43	11.71 ± 0.40
CrPic xVit c						
	0	0	25.00 ± 0.00	20.83 ± 0.44	16.67 ± 0.33	10.87 ± 0.47
	0.4	0	16.67 ± 8.33	22.33 ± 1.20	16.67 ± 0.88	11.00 ± 0.58
	0.8	0	25.00 ± 0.00	21.17 ± 0.60	17.00 ± 0.58	11.33 ± 0.67
	1.2	0	25.00 ± 0.00	22.50 ± 0.50	17.83 ± 0.93	10.50 ± 0.50
	0	200	25.67 ± 0.67	22.00 ± 0.00	17.17 ± 1.09	12.00 ± 1.00
	0.4	200	26.00 ± 0.58	23.00 ± 0.50	17.00 ± 0.29	11.67 ± 0.88
	0.8	200	25.00 ± 0.00	21.17 ± 0.60	16.83 ± 1.17	11.83 ± 0.93
	1.2	200	26.67 ± 1.67	22.67 ± 0.33	17.00 ± 1.15	11.33 ± 0.88
Statistical significance						
CrPic level			0.4503	0.0494	0.903	0.8388
Vitamin C			0.1910	0.2630	0.9468	0.1661
CrPic level*Vitamin C			0.4064	0.773	0.8693	0.9788

Means within the same column with different superscripts (a, b, c) are significantly different (P≤0.05)

Conclusion

The study revealed no significant effect of CrPic and its interraction with vitamin C on the reproductive hormones of the chicken. However, at 1.2 mg/kg CrPic, progesterone, oestrogen, testosterone and FSH were higher than other levels for those without vitamin C, but decreased the luitenising hormone while progesterone, oestrogen, testosterone and FSH for those with vitamin C were also higher at 1.2 mg/kg CrPic than other levels of the diets. In addition, there was positive improvement in the values recorded on the follicle parameters measured in dimension by CrPic and Vitamin C supplement diet. This suggests that the dietary supplementation of CrPic with or without vitamin C could support the normal reproductive hormones and the rate of apoptosis and atresia of the follicles of chicken raised at high ambient temperature.

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Credit Author Statement

The authors declared that they were all participated and designate roles from the conceptualisation of the research, data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; software; supervision; validation; visualization; writing and reviewing of the original draft.

Authorship Contribution

Dr Francis Bosede Adebayo: Research conception, materials and method development and selection, and data preparation.

Stephen Oluwaseun Ibikunle: Literature review, data and laboratory analysis

Oluwafolaranmi Segun Omoleye: Data preparation and materials and methods

Nathaniel Olu Alamuoye: Laboratory analysis and data preparation

J.C Olowoyeye : Literature review and supervision

Femi Awotokun: Data preparation and supervision

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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