Estradiol Benzoate Effects on Hormonal Level in Lohmann Brown Layers

[1] Ahmad Abubakar and [2]A.M. Abdussamad
[1,2] Department of Animal Science, Bayero University Kano, Nigeria.
Corresponding Author: Abubakar, A. 08039666036, drahsak@yahoo.com

ABSTRACT
An experiment was conducted to determine the effect of estradiol benzoate on serum hormonal levels in eighteen Lohmann Brown layers. The experiment was laid in a single factor completely randomized design and each treatment was replicated three times. Six different dose (0, 0.2, 0.4, 0.6, 0.8 and 1 mg/bird) of estradiol benzoate were administered intramuscularly via the keel muscle twice a week for a total period of six weeks. Blood samples were collected via the jugular vein or after slaughter or both as the case may be and serum was harvested thereafter. Serum was used for assay of estradiol-17ß, thyroxine and triiodothyronine. Data obtained were analyzed using Graphpad Instat statistical package. Results revealed that levels of estradiol benzoate did not affect serum levels of estradiol, thyroxine and triiodothyronine. In conclusion, estradiol benzoate injection had no significant effect on serum hormonal levels.

Keywords: Estradiol, Triiodothyronine, Thyroxine, Hormones, Poultry

1. INTRODUCTION

Hormones are essential for reproductive function in poultry (Samar, Hanaa & El-Sheikh, 2000). The term estrogen is mainly used when referring to estradiol (Cifti & Yildrim, 2013). Estradiol benzoate when administered to quails results in a significantly higher body weight at sexual maturity, increase in egg number, weight and mass and increase in shell gland size and serum calcium level (Samar & Ahmad, 2009). The same authors reported that estradiol enhances reproduction and production function in birds. According to Johnson (1986), estradiol stimulates vitellogenesis via its action on the liver and deposition of calcium in long bones. The fact that hormones have an intricate, complex and indispensable involvement with the reproductive process justifies investigation into the effect of exogenous administration of estradiol benzoate in Lohmann Brown layers acclimatized to our local environment.

The increase in usage of estradiol to increase egg production in poultry may deplete endogenous stores of some mineral elements (Houston, Donnan & Jones, 1995). The use of synthetic estradiol results in physiological changes within blood of poultry (Khan & Zafar, 2005). There is considerable evidence that steroid hormones are involved in the regulation of ovulation rate and oviductal development in poultry (Kang, Yuu, Seo, Hong & Ko, 2001). Estrogen dominance causes low thyroid function due to binding to the thyroid hormone receptor sites. This leads to the blocking of triiodothyronine hormone from being able to gain entry into the thyroid follicular cells, causing functional hypothyroidism which leads to low body temperature, depression and slows down metabolism (Alex, 2016).

2. MATERIALS AND METHODS

This experiment was conducted at the Poultry Unit of Faculty of Agriculture Teaching and Research Farm, Bayero University Kano, Nigeria (GPS coordinates: N11.97643°, E008.42995°). The experiment was carried out under the battery cage system. The cages are three-tiered comprising 18 compartments and coupled with aluminum feeder and a drinker made from half-opened plastic pipes. The pen is provided with foot-bath and water storage in two plastic containers with capacity of 100 litres each.

Eighteen layers were randomly selected based on Resource equation of Mead, Gilmour and Mead (2012). The experiment was laid out in a Single Factor Completely Randomized Design (CRD). The factor considered was estradiol benzoate which has six levels (0, 0.2, 0.4, 0.6, 0.8 and 1 mg). The control birds were given 1 ml normal saline. Each treatment was replicated three times. They were individually housed under battery-cage system, with one bird per cage and were allowed a stabilization period of two weeks.

During this period, they were fed *ad libitum* with Sovet's commercial layer ration (Super Layer®), water was also provided *ad libitum.* During the experimental period of six weeks, birds were fed at 8 am and water given *ad libitum*.

Estradiol benzoate injectable solution (Super estradiol®, Hebei New Century Pharmaceutical Company Limited, China) with a concentration of 2 mg/ml was used. A styrofoam box with ice pack was used to transport the estradiol benzoate from the site of refrigeration to the farm. A 2 ml syringe with 21-gauge needle was used to administer the injection deep into the breast muscle of the birds. The estradiol benzoate injection were given at a dosage of 0.2, 0.4, 0.6, 0.8 and 1 mg/bird, which translates into Treatments B, C, D, E and F, respectively, with 1 ml normal saline as Treatment A (Control). Injection was given at 10 am on Mondays and Thursdays each week throughout the experimental period.

The experiment was terminated at the end of six weeks. Blood was collected from the right jugular vein, wing vein and after slaughter. Blood was immediately transferred into labeled plain sample bottles, which were transported to the laboratory. Blood was allowed to stay for two hours at room temperature to encourage clot formation. It was then subjected to centrifugation at 4000 revolutions per minute for 30 minutes in a centrifuge (Centrifuge 800D®, Techmel & Techmel, USA). Serum was transferred to a clean tube using transfer/dropping pipette and stored at -20 ^{o}C until further analysis.Serum estradiol, triidothyronine and thyroxine concentrations was determined using AccuBind® ELISA kits (Monobind Inc., USA) and result was read using microplate reader (Microplate Reader®, Model RT-2100C, Rayto Life and Analytical Science, Germany). .

DATA ANALYSIS

All statistical analyses were carried out using Graphpad Instat package (Graphpad Instat®, version 3.05, 32 bit for Win 95/NT, Graphpad Software, Inc, 2000).

3. RESULTS

Serum Estradiol

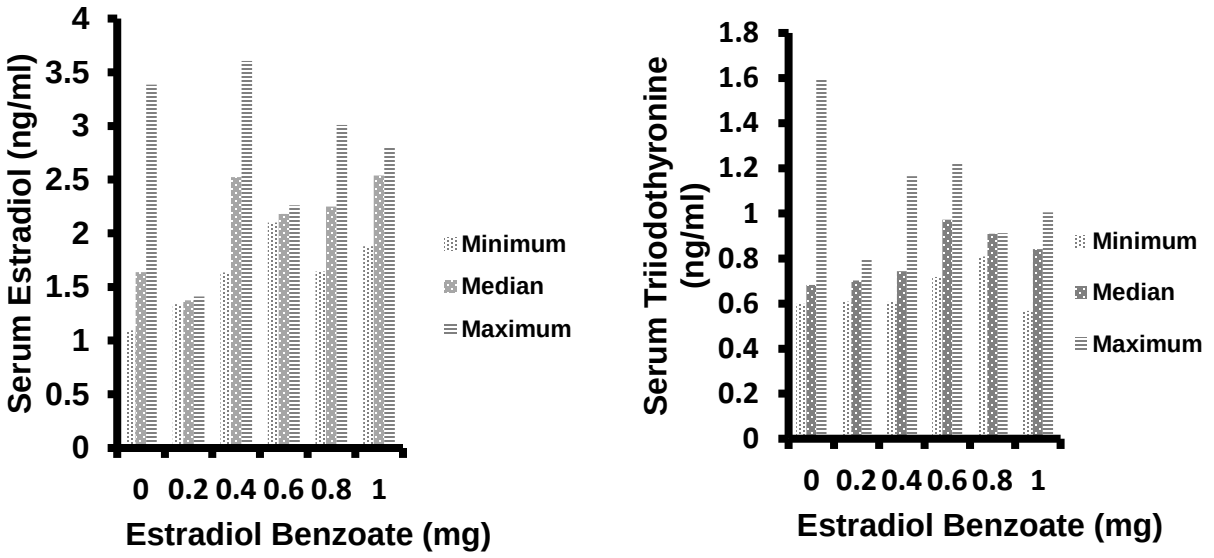
The effect of estradiol benzoate on median serum levels of estradiol in Lohmann Brown layers is presented in Figure 1. There was no statistically significant (P>0.05, Kruskal-Wallis statistic = 4.743) difference in median serum estradiol levels of 1.6, 1.4, 2.5, 2.2, 2.3 and 2.5 ng/ml across respective estradiol benzoate treatments (0, 0.2, 0.4, 0.6, 0.8 and 1 mg).

Serum Triiodothyronine

The effect of estradiol benzoate on median serum levels of triiiodothyronine in Lohmann Brown layers is presented in Figure 2. There was no statistically significant (P>0.05, Kruskal-Wallis statistic = 1.684) difference in median serum triiodothyronine levels (0.7, 0.7, 0.7, 1.0, 0.9 and 0.8 ng/ml) across respective estradiol benzoate treatments (0, 0.2, 0.4, 0.6, 0.8 and 1 mg).

Serum Thyroxine

The effect of estradiol benzoate on median serum levels of thyroxine in Lohmann Brown layers is presented in Figure 4. There was no statistically significant (P>0.05, Kruskal-Wallis statistic = 6.154) difference in median serum thyroxine levels of 26, 24, 9, 25, 22 and 12 ng/ml across respective estradiol benzoate treatments (0, 0.2, 0.4, 0.6, 0.8 and 1 mg).



A **B**

Figure 1. Effect of Estradiol Benzoate on (A) Median Serum Estradiol Levels and (B)Median Serum Triiodothyronine Levels in Lohmann Brown Layers.

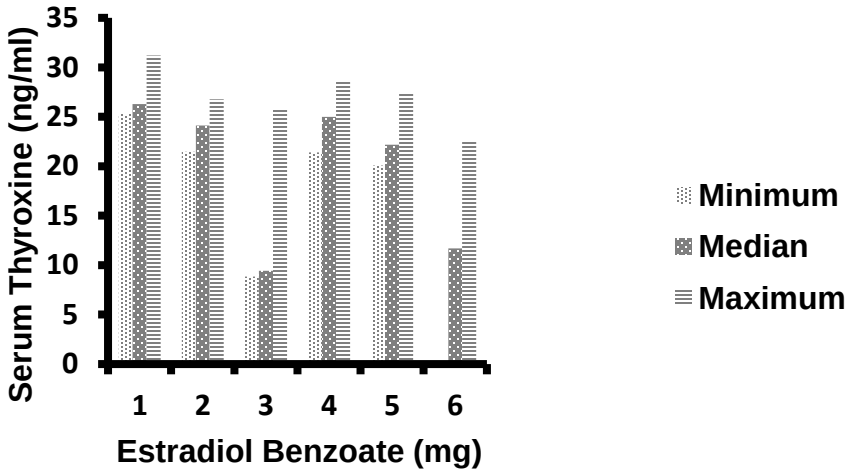


Figure 2. Effect of Estradiol Benzoate on Median Serum Thyroxine Levels in Lohmann Brown Layers.

4. DISCUSSION

Estradiol benzoate did not confer any changes in endogenous levels of the three serum hormones (estradiol, triiodothyronine and thyroxine) monitored. Several reasons could be proffered in order to explain this outcome. Blood sampling was done terminally for convenience sake not serially per time point as reported by other authors (Guémené & Williams, 1994; Souza, Cunha, Caraviello & Wiltbank, 2005; Zaghari, Taherkhani & Honarbakhsh, 2009; Preda, Balaceanu & Dojana, 2013). Since endogenous hormones exhibit peak concentrations at certain time points, these peak levels could be missed with terminal bleeding as hormonal concentrations might have returned to basal levels at the time of bleeding. In the current study, terminal bleeding was a more practical option because of the difficulty of maintaining an intravascular line for serial sampling per time point and the fact that the study was a composite one where other groups

monitored egg production which could be affected by the stress associated with frequent handling due to serial blood sampling. Another question is whether the pharmacodynamics and pharmacokinetics of estradiol esters in laying hens differ from other species. Since no attempt was made in the present study to curtail the endogenous production of estradiol-17ß, could the absence of effect of estradiol benzoate on serum estradiol-17ß level be a result of the masking effect of endogenously produced estradiol-17ß?

Although some studies demonstrated the effects of estrogens on the development, physiology and histology of the thyroid gland, questions about the effects of estrogen replacement therapy on the thyroid gland remained unanswered (Banu, Arosh, Govindarajulu & Aruldhas, 2001). Estrogen and androgen administration modulate the pituitary-thyroid axis through alterations in thyroid hormone-binding globulin metabolism, but the effect of sex steroids on extrathyroidal thyroxine (T4) to triiodothyronine (T3) conversion in rats are unknown (Kordi & Khazali, 2015). Regarding the effect of estradiol on thyroid activity, numerous studies have demonstrated contradictory results such as stimulatory effect through activation of pituitary-thyroid axis following estradiol treatment (Banu, Govindarajulu & Aruldhas, 2002; De Araujo *et al*., 2006) or no alterations of thyroid stimulating hormone (TSH) although decreased T3 in ovariectomized rats which became normal after estradiol treatment (Lima *et al*., 2006). In women treated with conjugated estrogen 0.625-2.5 mg/kg/day, there was no change in serum TSH concentrations (Abdalla *et al*., 1987). In other studies, perimenopausal and postmenopausal women had "elevated" serum TSH concentrations after 1 month (Contreras & Parra, 2000) or 6 weeks (Marqusee, Braverman, Lawrence, Carroll & Seely, 2000) of estrogen therapy. Having in mind the afore-mentioned discrepancies in literature, the present study looked at the response of the thyroid following administration of estradiol benzoate by determining serum levels of T4 and T3 in terminal blood samples. The "no effect" outcome may be connected to the Fact that the current study determined serum concentrations of thyroid hormones (T4 and T3) at a time when there was no peak in serum estradiol-17ß.

5. CONCLUSION

In conclusion, the administration of estradiol benzoate at 0.2, 0.4, 0.6, 0.8 and 1 mg/bird had no effect on serum estradiol, triiodothyronine and thyroxine levels. Serum mineral levels did not significantly change with estradiol benzoate administration.

REFERENCES

Abdalla, H.I., Beastall, G., Fletcher, D., Hawthorn, J.S., Smith, J., and McHart, K.D. (1987).Sex Steroid Replacement in Post-Menopausal Women: Effects on Thyroid Hormone Status. *Maturitas*, 9: 49-54.

Alex, T. (2016). Estrogen Dominance Symptoms, Golden Wellness Centre, Retrieved from www.holistic-back-relief.com/estrogen-dominance-symptoms.html

Banu, S.K., Arosh, J.A, Govindarajulu, P. and Aruldhas, M.M. (2001). Testosterone and Estradiol Differentially Regulate Thyroid Growth in Wistar Rats from Immature to Adult Age. *Endocrinology Research*, 27 (4): 447-463.

Banu, S.K., Govindarajulu, P. and Aruldhas, M.M. (2002). Developmental Profiles of TSH, Sex Steroids and their Receptors in the Thyroid and their Relevance to Thyroid Growth in Immature Rats. *Steroids*, 67: 137-144.

Cifti, H.B. and Yildrim, I. (2013). Effects of Estradiol 17β on Serum Mineral Concentrations of Female Japanese Quails. *QScience Connect*.doi: http://dx.doi.org/10.5339/connect.2013.37

Contreras, I. and Parra, D. (2000). Estrogen Replacement Theraphy and the Prevention of Coronary Heart Disease in Post-Menopausal Women.*American Journal of Health –System Pharmacy*, 57: 1963-1968.

Guemene, D. and Williams, J.B. (1994). Relationships Between Broodiness Expression Laying Persistency and Concentrations of Hormones During the First Productive Period in Turkey Hens (*Meleagris galloparo*), *Reproduction Nutrition Development* 34 (4): 371-381.

Houston, D.C., Donnan, D. and Jones, P.J. (1995). The Source of the Nutrients Required for Egg Production in Zebra Finches (*Poephilia guttata*). *Journal of Zoology*, 235 (3): 469-483.

Johnson, A.L. (1986).*Reproduction in the Female* in: Avian Physiology, P.D Sturkie, Newyork, Springer Press, USA.

Kang, W.J., Yuu, J.S., Seo, D.S., Hong, K.C. and Ko, Y. (2001). Relationship among Egg Productivity, Steroid Hormones (Progesterone and Estradiol) and Ovary in Korean Native Ogol Chicken.*Asian-Australasian Journal of Animal Sciences*,14 (7): 922-928.

Khan, T.A. and Zafar, F. (2005). Haematological Study in Response to Varying Doses of Estrogen in Broiler Chickens. *International Journal of Poultry Science*, 4 (10): 748-751.

Kordi, F., and Khazali,H. (2015) The Effects of Ghrelin and Estradiol on Mean Concentration of Thyroid Hormones. *International Journal of Endocrinology and Metabolism.* 13 (1): 1-5.

Marqusee, E., Braverman, L.E., Lawrence, J.E., Carrol, J.S. and Seely, E.W. (2000). The Effect of Droloxifen and Estrogen on Thyroid Function in Postmenopausal Women. *Journal of Clinical Endocrinology and Metabolism*, 85: 4407-4410.

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

Preda, C., Balaceanu, R. and Dojana, N. (2013). Evolution of Hormonal Control of Calcium and Phosphorus Metabolism in Different Hen Breeds During the Laying Cycle. *Veterinary Medicinel*, 70(2): 286-291.

Samar, A.E., Hanaa, M.K. and El-Sheikh, A.M. (2000). Estradiol and Progesterone Levels During Ovulatory Cycle of Two Local Strains of Laying Hens. *Egyptian Poultry Science*, 22 (III):869-878.

Souza, A.H., Cunha, A.P., Caraviello, D.Z. and Wiltbank, M.C. (2005). Profile of Circulating Estradiol-17β after Different Estrogen Treatments in Lactating Dairy Cows. *Animal Reproduction*, 2(4): 224-232.

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