

EFFECT OF *Hibiscus sabdariffa* CALYX ON THE REPRODUCTIVE HORMONES OF LAYING HENS

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Abstract

This study evaluated the effects of *Hibiscus sabdariffa* calyx supplementation on reproductive hormones of laying hens for 56 days. The treatments consisted of a basal diet with varying levels of roselle calyx supplemented at 0%, 1.5%, 3%, and 4.5% respectively. Sixty Isa Brown layers were divided into 4 groups of 15 birds each and randomly assigned to the four experimental diets in a completely randomized design (CRD). Each group was sub-divided into 3 replicates of five birds each and each replicate housed in a pen. Feed and water were provided *ad libitum* four 8 weeks. Blood samples collected before and after 6 weeks of feeding the experimental diets were analyzed for LH, FSH, cortisol and estradiol using the standard protocols of (ELISA) kit. The results showed that dietary supplementation with *Hibiscus sabdariffa* calyx had no significant effect ($p > 0.05$) on FSH and cortisol but significantly impacted LH and estradiol concentration ($p < 0.05$). The highest LH level was observed in birds fed 1.5% supplementation, which was significantly higher ($p < 0.05$) than in those given 3%. The control and 4.5% groups were similar ($p > 0.05$) to both 1.5% and 3% levels of supplementation. For FSH, the peak concentration was obtained in birds fed 3% *Hibiscus sabdariffa*, while the 1.5% and 4.5% groups were comparable ($p > 0.05$) to the control. Cortisol concentration was highest ($p > 0.05$) in the control group, whereas birds fed 1.5%, 3%, and 4.5% *Hibiscus sabdariffa* had similar ($p > 0.05$) values. Estradiol levels were significantly higher ($p < 0.05$) in the control and 3% groups, while the 1.5% and 4.5% treatments resulted in similar lower values ($p > 0.05$). In conclusion, supplementing the diets of laying hens with *Hibiscus sabdariffa* calyx had no deleterious effect on FSH, LH and cortisol but slightly decreased the concentration of the estradiol.

Key words: cortisol, Estradiol, FSH, *Hibiscus sabdariffa*, LH

Description of problem

Reproductive efficiency in poultry, particularly in laying hens, is largely influenced by hormonal balance, with luteinizing hormone [1], follicle-stimulating hormone (FSH), estradiol [2] and cortisol playing critical roles in follicular development, ovulation, egg production, and stress regulation [3]. However, the high cost of conventional feed additives and synthetic hormone regulators has led to growing interest in natural plant-based alternatives that could modulate these physiological processes [4]. *Hibiscus sabdariffa* calyx is widely consumed as a beverage and used in traditional medicine due to its rich bioactive compounds, including flavonoids, polyphenols, and anthocyanins which have been associated with antioxidant, anti-inflammatory, and phytoestrogenic activities

[5]. Despite its common use, there is limited scientific evidence on how *Hibiscus sabdariffa* supplementation may influence reproductive endocrinology in poultry. Therefore, understanding its effect on LH, FSH, estradiol, and cortisol concentrations in laying hens is necessary to determine its potential as a natural feed additive for improving reproductive performance and stress management.

Materials and Method

Experimental Site

The experiment was carried out at the University of Ilorin Teaching and Research Farm while the laboratory analysis was done at Department of Animal Production laboratory, University of Ilorin, Ilorin, Kwara State, Nigeria.

Preparation of Test Material

Fresh *Hibiscus sabdariffa* calyx also known as Zobo leaves were purchased from the market. The calyx were air-dried and crushed and thereafter stored in airtight containers until it was added to the feed.

Experimental Birds and Management

Sixty point-of-lay (16 weeks old) Isa brown hens purchased from Yamfy farms in Ilorin, Kwara State were used for the experiment. Before the arrival of the hens, the pen and cages were cleaned, washed, thoroughly disinfected. On arrival, the birds were given vitamins and antistress.

Experimental Diets

Treatment 1 (control): 100% basal diet
 Treatment 2: 1.5% of roselle calyx + 98.5% basal diet
 Treatment 3: 3% of roselle calyx + 97% basal diet
 Treatment 4: 4.5% of roselle calyx + 95.5% basal diet

Each experimental diet was in form of mash and hens were fed *ad libitum* and had access to clean water throughout the period of the experiment

Table 1: Proximate composition of basal diet

Nutrient	Proportion
Crude protein	16.5%
Crude fat	4.5%
Crude fibre	5.0%
Calcium	3.6%
Available phosphorus	0.45%
Lysine	0.80%
Methionine	0.4%
Metabolizable energy	2650 kcal/kg

Collection of Blood Samples

Blood samples were collected from four birds per treatment after six weeks of feeding them with the experimental diets. 3ml of blood was drawn from of each bird at

eighteen (18) weeks and twenty-four(24) weeks of age using a sterilized disposable syringe and needle. Blood was collected through the brachial wing vein into Ethylene-Diamine-Tetra-Acetic Acid (EDTA) coated bottles and then stored in a vacuum flask containing ice to maintain a cold chain during transportation to the laboratory for hormonal analysis.

The blood samples were subsequently assayed for luteinizing hormone (LH), follicle-stimulating hormone (FSH), cortisol and estradiol levels using the standard protocols of (ELISA) kit.

Statistical Analysis

One-way analysis of variance (ANOVA) was used to analyze the data collected with significance level of 5%. The difference between means were separated using Duncan's multiple range test.

Results and Discussion

The effects of graded levels of *Hibiscus sabdariffa* (HS) calyx supplementation on reproductive hormones of laying hen are shown in Table 2. *Hibiscus sabdariffa* did not significantly affect FSH and cortisol ($p>0.05$), but significantly impacted LH and estradiol concentrations. The highest LH concentration was obtained from birds fed 1.5% HS which was significantly higher ($p<0.05$) than birds fed 3% HS. However, the control and 4.5% HS groups were similar ($p>0.05$) to both 1.5% and 3% HS fed hens. Estradiol was significantly influenced ($p<0.05$) with control and 3% HS fed group showing higher concentrations than 1.5% and 4.5% HS supplemented groups.

The LH concentration obtained from this study ranges between 0.17mIU/ml in birds fed 3% *Hibiscus sabdiffara* to 0.50mIU/ml in birds fed 1.5% *Hibiscus sabdiffara*. The FSH concentration obtained from this study ranges between 1.42mIU/ml in birds fed 4.5% *Hibiscus sabdiffara* and 2.51mIU/ml in birds fed 3% *Hibiscus sabdiffara*. The complementary action of LH and FSH is crucial for follicular growth during the

reproductive processes [1]. The Cortisol concentration ranged between 36.63pg/ml in birds fed 4.5% *Hibiscus sabdariffa* and 43.27pg/ml in birds fed control. Reduced cortisol levels indicate lower stress levels, which can improve overall health and productivity [6]. Cortisol levels, an indicator of stress, were unaffected, suggesting that *Hibiscus sabdariffa* supplementation at the doses used did not significantly influence stress response in the birds [7]. The estradiol concentration ranged between 50.86pg/ml in birds fed 4.5% *Hibiscus sabdariffa* to 67.03pg/ml in birds fed control. The increased Estradiol levels in birds fed 3% HS and control diets, compared to those fed 1.5% and 4.5% HS, suggests a potential

estrogenic effect of HS [8]. Similar findings have been reported when [9] investigated the effect of Roselle calyx in two preparation forms in layer diets on egg production performance, egg quality and TBARS value in plasma and yolk. This finding is also supported by a study that reported modulated hormone levels in rats administered *Hibiscus sabdariffa* extract [10]. The selective effect of *Hibiscus sabdariffa* on Estradiol levels, without impacting LH, FSH and Cortisol, may be attributed to the phytoestrogenic properties of HS which can bind to estrogen receptors and influence estrogen-regulated pathways [11].

Table 2: Effects of *Hibiscus sabdariffa* calyx on the reproductive hormone of laying hens

Time of collection (T)	LH (mIU/ml)	FSH (mIU/ml)	ESTRADIOL (pg/ml)	CORTISOL (ng/ml)
Week 20 (Base line)	0.45 ^a	3.18 ^a	73.20 ^a	55.14 ^a
Week 26 (HS)	0.15 ^b	0.42 ^b	44.90 ^b	23.45 ^b
P-value	0.0086	<.0001	<.0001	<.0001
Inclusion (I)				
0%	0.33 ^{ab}	1.71	67.03 ^a	43.27
1.5%	0.50 ^a	1.56	52.39 ^b	37.61
3%	0.17 ^b	2.51	65.92 ^a	39.66
4.5%	0.20 ^{ab}	1.42	50.86 ^b	36.63
P-value	0.1247	0.3009	0.0010	0.1989
T*I	0.1544	0.2696	0.1280	0.6774
SEM±	0.2940	1.2110	8.8881	6.4168

Conclusion and application

This study demonstrates that *Hibiscus sabdariffa* calyx can modulate reproductive hormones in laying hens, particularly by enhancing LH and estradiol at moderate inclusion levels. The hormonal response is likely associated with its rich phytochemicals, including flavonoids, anthocyanins, tannins, and saponins, which are known for their antioxidant and phytoestrogenic properties. Overall, moderate supplementation (1.5–3%) appears beneficial for supporting reproductive function without adverse effects on stress hormones, suggesting its potential use as a natural feed additive to improve reproductive performance in poultry.

References

1. Prastiya, R. A., Madyawati, S. P., Sari, S. Y., & Nugroho, A. P. (2022). Effect of follicle-stimulating hormone and luteinizing hormone levels on egg-laying frequency in hens. *Veterinary world*, 15(12), 2890–2895. <https://doi.org/10.14202/vetworld.2022.2890-2895>
2. Yanfen Ma, Jinwei Yao, Shuo Zhou, Yuling Mi, Xun Tan, Caiqiao Zhang (2020). Enhancing effect of FSH on follicular development through yolk formation and deposition in the low-yield laying chickens, *Theriogenology*. 157. (418-430), <https://doi.org/10.1016/j.theriogenology.2020.07.012>.
3. Wang, X. J., Liu, L., Zhao, J. P., Jiao, H. C., & Lin, H. (2017). Stress impairs the reproduction of laying hens: an involvement of energy. *World's Poultry Science Journal*, 73(4), 845–856. doi:10.1017/S0043933917000794
4. Wondimagegn Tadesse Alem (2024). Effect of herbal extracts in animal nutrition as feed additives. *Heliyon*, 10(3). <https://doi.org/10.1016/j.heliyon.2024.e24973>. <https://www.sciencedirect.com/science/article/pii/S2405844024010041>.
5. Okereke, C. N., Chukwuma, M.O., & Iroka F.C. (2015). Phytochemical analysis and medicinal uses of *Hibiscus sabdariffa*. *International Journal of Herbal Medicine*. 16. 16-19.
6. Rogerson, O., Wilding, S., Prudenzi, A., & O'Connor, D. B. (2024). Effectiveness of stress management interventions to change cortisol levels: a systematic review and meta-analysis. *Psychoneuroendocrinology*, 159, 106415. <https://doi.org/10.1016/j.psyneuen.2023.106415>
7. Caulfield, M. P., & Padula, M. P. (2020). HPLC MS-MS Analysis Shows Measurement of Corticosterone in Egg Albumen Is Not a Valid Indicator of Chicken Welfare. *Animals : an open access journal from MDPI*, 10(5), 821. <https://doi.org/10.3390/ani10050821>
8. Ndlovu, N., Erlwanger, K. H., & Chivandi, E. (2025). Effect of Dietary Fortification with *Hibiscus sabdariffa* Calyces Meal on Egg Production and Egg Quality of Japanese Quail (*Coturnix coturnix japonica*). *Veterinary medicine and science*, 11(1), e70141. <https://doi.org/10.1002/vms3.70141>
9. Sukkhavanit, P., Angkanaporn, K., & Kijparkorn, S. (2013). Effect of Roselle (*Hibiscus sabdariffa* Linn.) Calyx in Laying Hen Diet on Egg Production Performance, Egg Quality and TBARS Value in Plasma and Yolk. *The Thai Journal of Veterinary Medicine*, 41(3), 337–344. retrieved from <https://he01.tci-thaijo.org/index.php/tjvm/article/view/9592>
10. Lorenzana-Martínez, G., San Juan-García, C. A., Santerre, A., Andrade-González, I., & Bañuelos-Pineda, J. (2023). The Phytoestrogenic Effect of *Hibiscus sabdariffa* Involves Estrogen Receptor α in Ovariectomized Wistar Rats. *Plant foods for human nutrition (Dordrecht, Netherlands)*, 78(2), 426–431. <https://doi.org/10.1007/s11130-023-01061-x>
11. Saleh, A. A., Ahmed, E. A. M., & Ebeid, T. A. (2019). The impact of phytoestrogen source supplementation on reproductive performance, plasma profile, yolk fatty acids and antioxidative status in aged laying hens. *Reproduction in domestic animals, Zuchthygiene*, 54(6), 846–854. <https://doi.org/10.1111/rda>