Plant Archives Vol. 19, Supplement 1, 2019 pp. 160-164 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF HESPERIDIN ADDED TO THE DIET IN PHYSIOLOGICAL TRAITS AND SEX HORMONES OF MALES AND DOMESTIC CHICKENS

Saad Attallah Abdal Sada
Agriculture College, Al-Muthanna University, Iraq
atallah_saad@yahoo.com

Abstract

This study was conducted at the Poultry Farm Department of Animal production, College of Agriculture, University of Baghdad from 18 / 2 / 2017 to 18 / 8 / 2017 to investigate the effect of dietary supplementation with different levels of Hesperidin to ration in productive, physiological and reproductive performance in local Iraqi Fowl. A total 160 local Fowl were used in (120 females and 40 males) at 18 weeks of age. The chickens were distributed randomly at 20 weeks of age into four treatments, about one replicate for male and three replicates for females for each treatment. The Chicken were fed along experiment period which 12 week on the same diet conation 17.42% curd protein and 2751.7 kcal metabolic energy /kg feed and the Hesperidin added to the rations from the twenty weeks age until the end of experiment. The Chickens (male and female) were reared at separated cages from each other during the experiment period. The experiment treatments were : T1, control: 0 mg of Hesperidin T2 , 150 mg Hesperidin / kg of feed. T3, 300 mg Hesperidin / kg of feed and T4, 450mg Hesperidin / kg of feed. The Results showed no significant differences in blood serum traits for concentration of glucose, cholesterol and protein for males and females, while the protein concentration in males revealed the significant improvement in first period and overall mean of experiment and no significant improvement in blood serum concentration of testosterone hormone in males and estrogen and progesterone hormones in females.

Keywords : Physiological Traits, Hesperidin, Sex Hormones, Domestic chickens

Introduction

Hesperidin is one of the abundant flavonoids found in citrus peel and has an important role as an antioxidant by inhibiting fat oxidation and reducing oxidation (Jain *et al*., 2015). Hesperidin regulates cardiovascular function through its antioxidant properties, (Garc *et al*., 2001, and Liu *et al*., 2013). The molecular weight of Hesperidin is 610.57 Dalton, and its chemical form $C_{28}H_{34}O_{15}$ Hesperidin (Vesna Kuntic *et al*., 2014)Hesperidin is characterized by its fine needle-like shape or long bristles, with a pale yellow or dark color, odorless and odorless (Garg *et al*., 2001). Hesperidin promotes the activity of cellular antioxidant enzymes such as Super oxide dimutase (SOD) and Hemeoxygenase-1 (HO-1) and other catalase, increase the predominant cellular antioxidants and glutathione clotathione (Khedr, 2015; Roohbakhsh *et al*., 2015 and Parhiz *et al*., 2015).

Materials and Methods

This study was conducted in the poultry field of the Department of Animal Husbandry at the College of Agriculture/University of Baghdad. The field experiment lasted from 18/2/2017 to 18/5/2017 to study the effect of adding different levels of Hesperidin, In reproductive physiological performance. Used 160 local chickens (120 females and 40 males) at the age of 20 weeks, prepared by the Agricultural Research Department / Ministry of Agriculture in Baghdad. The birds were housed in one of the breeding halls in the cages located in the field of domestic birds, belonging to the Department of Animal Husbandry, both males and females, individually to get acquainted with the atmosphere of education in the hall. At the age of 20 weeks, birds were divided into four treatments, with 30 females + 10 males per treatment. Each five (5) cages were repeated to be three repeaters (each consisting of 10 females). In males, each male was fed on a uniform diet for 12 weeks, containing 17.42% crude protein and 2751.7 kcal. Representative energy / kg feed (Table 1). The male diet contains 13.50% raw protein, 2769.47 Kilocalories representative energy / kg feed (Table 2). The feed materials were prepared from the local market in Abu Ghraib, and the grains were crushed and mixed, in the poultry feed factory of the poultry field / Animal Production Department / College of Agriculture / University of Baghdad. Hesperidin was added to these diets with four concentrations (0, 150, 300, 450 mg / kg feed) for the four treatments T1, T2, T3 and T4, respectively, starting at 20 weeks until the end of the 12 week trial The 100% Pure Hesperidin Powder is used by Xian Lyphar Biotech Co, Ltd. The birds were housed in a single-layer mesh cage with a single layer, 45 x 40 x 45 cm in length, width and height, respectively. The cage contained two chickens. Male cages contained only one male. These cages were provided with a longitudinal mass divided by the coefficients and limited feeding. Cages were also provided with long

skirts made of galvanized zinc, so that the water would be available to the birds constantly. The room was equipped with air vents suitable for the size of the hall, the number of birds to ensure proper breeding conditions, a lighting program included 16 hours lighting and 8 hours of darkness / day throughout the duration of the breeding. Experimental data were recorded from the age of 20 weeks until the end of the actual 12-week trial (at the age of 32 weeks).

Table 1 : Percentages of the ingredients used of female in the study and their chemical composition

Feeding materials	Usage percentage%
Yellow corn	30
Wheat	35.4
Soybeans (48% protein)	20
CP	5
Sun Flower Oil	1
Clay stone	7.2
DCP	1
Vitamins and minerals premix	0.2
Salt(NaCl)	0.2
Total	100
Calculated Chemical Analysis (2)	
Crude protein (%)	17.42
Energy represented (kg / kg feed)	2751.7
Lysine (%)	0.92
Methionine (%)	0.37
Methionine + Cysteine (%)	0.7
Calcium (%)	3.30
Available phosphorus (%)	0.45

(1) Proteins Center LAYCON - 5 SPECIAL W: Dutch origin. Each containing 40% raw protein, 5.00% fat, 2.10% fiber, 5% calcium, 2% phosphorus available, 3.80% lysine, 2.85% methionine, 3.29% methionine + Kg Vitamin E, 40 mg Vitamin K, 40 mg B1, 90 Vitamin B2, 150 mg B3, 60 mg B6, 500 mg B12, 15 mg Folic acid, 100 µg biotin, 1 mg iron, 200 mg copper, 1.600 mg manganese, 1.200 mg zinc, 20 mg iodine, 5 mg selenium, 100 mg anti-oxidant (BHT) (2) according to the chemical analysis of the mixture according to NRC (1994).

Table 2: Percentages of the ingredients used of male in the study and their chemical composition

Feeding materials	Usage percentage%
Yellow corn	17
Wheat	46.1
Soybeans (48% protein)	4
CP	5
Barley	18
Wheat bran	7
Limestone	2
DCP	0.5
Vitamins and minerals premix	0.2
Salt (NaCl)	0.2
Total	100
Calculated Chemical Analysis (2)	
Crude protein (%)	13.5
Energy represented (kg / kg feed)	2769.47
Lysine (%)	0.599
Methionine (%)	0.315
Methionine + Cysteine (%)	0.7
Calcium (%)	1.17
Available phosphorus (%)	0.35

(1) Proteins Center LAYCON - 5 SPECIAL W: Dutch origin. Each containing 40% raw protein, 5.00% fat, 2.10% fiber, 5% calcium, 2% phosphorus available, 3.80% lysine, 2.85% methionine, 3.29% methionine + Kg Vitamin E, 40 mg Vitamin K, 40 mg B1, 90 Vitamin B2, 150 mg B3, 60 mg B6, 500 mg B12, 15 mg Folic acid, 100 µg biotin, 1 mg iron, 200 mg copper, 1.600 mg manganese, 1.200 mg zinc, 20 mg iodine, 5 mg selenium, 100 mg anti-oxidant (BHT) (2) according to the chemical analysis of the mixture according to NRC (1994).

Chemical Properties of Blood

Blood Collection : Blood collection through the venipuncture of the cutaneous ulnar vein using a 5 ml syringe equipped with a 25 gauge Needle, according to Al-Daraji *et al*. (2008). The bird is placed lying on its back and spreading one of the wings, and remove the feathers covered to the region of the skin ulnar vein quickly to avoid causing the pain to the bird, and then draw blood from the vein with the syringe with a needle measuring 25, after the vein hole upward, and draw blood from the way of causing pressure to enter the blood After removal of the needle from the plastic syringe in plastic tubes of 10 ml, these tubes are placed in the centrifuge at 4000 cycles / minute and for 30 minutes, to separate the serum from the cell segment. After separation the serum samples are transferred to other plastic tubes, 20º C until the tests. The tests were performed three times throughout the experiment (once / for each period). Blood was collected from 10 females of each treatment. After the serum was separated from the cell, the following tests were performed: glucose concentration, cholesterol and total protein.

Glucose concentration : Determine the concentration of glucose in the sperm plasma depending on the implementation steps listed in the kit manufacturer's manual. Biomaghreb's instructions, which produced the kit, were followed using a Spectrophotometer. As described by Asatoor and King (1954).

Cholesterol concentration : The accompanying leaflet followed the standard kit produced by Biomaghreb, which is based on the enzymatic analysis of cholesterol level, according to the Allain (1974) method, reading absorption at 505 nanometers, using a spectrophotometer.

Total protein concentration : The equipment produced by Biomaghreb, based on the Biuret method, was used by Wotton and Freeman (1982). This method is based on the interaction of copper ions in the basal medium with peptide bonds to produce a complex color.

Sex hormones in the serum : Blood concentrations of males and females were measured at the end of the experiment. Blood samples were taken three times every 30 minutes of 6 males of each treatment to measure the concentration of testosterone and three consecutive samples every 30 minutes of 6 females of each treatment. Concentration of estrogen and progesterone. After separating the serum from the cell, the plastic tubes containing the serum were transferred by a cool box to the Bashayr Al-Harthiya laboratory in Baghdad / Al-Harthiya to perform the tests. The following tests were performed: Measuring the concentration of the testosterone hormone in the male serum.

The data of this study were analyzed according to the Complete Randomize Design (CRD), to study the effect of different coefficients in the studied traits, using the available statistical program SAS (2004). The differences between the averages were compared with the test Duncan (1955).

Results and Discussion

Table 3, 4 and 5 show no significant differences in concentrations of glucose, cholesterol and protein in local female chicken serum, and tables 6 and 7 in the concentration of glucose and cholesterol in domestic chicken male serum among the four experimental treatments during the three study periods (first, second and third) as well as the general rates of the above-mentioned qualities. Table 8 showed a significant improvement in serum protein concentration in favor of T2, T3, and T4 compared to T1 control during the first period of study. No significant differences were observed in the second and third periods in protein concentration Between transactions. The overall rate of protein concentration in male serum, From Table 8, T3 and T4 were significantly higher) P≤0.05 (on T1 and T2 respectively, with no significant differences between T1 and T2. The serum protein concentration was 3.58, 4.00, 4.15, 4.14 (g / 100) Ml) for T1, T2, T3 and T4, respectively.

Table 3 : Effect of addition of Hesperidin to local chicken diets in the concentration of glucose (mg / ml100) in female blood (mean ± standard error)

Glucose concentration (mg /100 ml) in female blood				General Mean
Treatment	**First Period**	**Second Period**	**Third Period**	
T1	253.33 ±2.72	305.6 ±20.30	287.33 ±11.55	282.08 ±11.52
T2	245.33 ±27.57	267.66 ±3.92	268.00 ±14.43	260.33 ±15.30
T3	234.00 ±54.15	264.66 ±16.89	266.33 ±6.17	254.99 ±25.73
T4	249.66 ±1.76	261.33 ±1.85	279.33 ±8.45	263.44 ±4.02
Significant	**NS**	**NS**	**NS**	**NS**

T1: 0 mg Hesperidin / kg feed,T2:150 mg Hesperidin/kg feed, T3: 300 mg Hesperidin / kg feed, T4: 450 mg Hesperidin / kg feed**.** Duration: Each period is 28 days. First (20-24) week, second (24-28) week and third (28-32) week**.** NS: Not significant.

Table 4 : Effect of addition of Hesperidin to local chicken diets in the concentration of cholesterol (mg / 100ml) in female blood (mean ± standard error)

Cholesterol concentration (mg /100 ml) in female blood				General Mean
Treatment	**First Period**	**Second Period**	**Third Period**	
T1	167.53 ±47.78	448.98 ±192.36	167.53 ±47.78	289.94 ±82.39
T2	273.83 ±52.59	369.93 ±31.04	273.83 ±52.59	273.47 ±65.93
T3	294.33 ± 68.43	408.16 ±177.60	294.33 ± 68.43	330.27 ±135.28
T4	137.43 ±1.36	279.26 ±51.04	137.43 ±1.36	206.45 ±27.77
Significant	**NS**	**NS**	**NS**	**NS**

T1: 0 mg Hesperidin / kg feed, T2:150 mg Hesperidin/kg feed, T3: 300 mg Hesperidin / kg feed, T4: 450 mg Hesperidin / kg feed. Duration: Each period is 28 days. First (20-24) week, second (24-28) week and third (28-32) week. NS: Not significant.

Table 5 : Effect of addition of Hesperidin to local chicken diets in the concentration of Total protein (g / 100ml) in female blood (mean ± standard error)

Total protein concentration (mg /100 ml) in female blood				General Mean
Treatment	**First Period**	**Second Period**	**Third Period**	
T1	4.80 ±0.59	6.21 ±1.36	5.43 ±0.06	5.48 ±0.67
T2	5.66 ±0.38	5.79 ±0.16	5.94 ±0.44	5.79 ±0.32
T3	6.23 ±0.87	4.82 ±1.29	5.44 ±0.64	5.49 ±0.93
T4	4.99 ±0.02	5.88 ±0.31	0.22 ±5.68	5.51 ±0.18
Significant	**NS**	**NS**	**NS**	**NS**

T1: 0 mg Hesperidin / kg feed,T2:150 mg Hesperidin/kg feed, T3: 300 mg Hesperidin / kg feed, T4: 450 mg Hesperidin / kg feed**.** Duration: Each period is 28 days. First (20-24) week, second (24-28) week and third (28-32) week. NS: Not significant.

Table 6 : Effect of addition of Hesperidin to local chicken diets in the concentration of glucose (mg /100 ml) in male blood (mean ± standard error)

Glucose concentration (mg / 100ml) in female blood				General Mean
Treatment	First Period	Second Period	Third Period	
T1	300.00 ±10.00	223.00 ±30.00	296.00 ±12.00	273.00 ±17.33
T2	303.50 ±21.50	289.50 ±10.50	297.50 ±3.50	296.83 ±11.83
T3	317.00 ±5.00	310.50 ±30.50	316.50 ±12.50	314.66 ±16.00
T4	291.00 ±19.00	284.00 ±5.00	278.00 ±12.00	284.33 ±12.00
Significant	NS	NS	NS	NS

T1: 0 mg Hesperidin / kg feed, T2:150 mg Hesperidin/kg feed, T3: 300 mg Hesperidin / kg feed, T4: 450 mg Hesperidin / kg feed. Duration: Each period is 28 days. First (20-24) week, second (24-28) week and third (28-32) week. NS: Not significant.

Table 7: Effect of addition of Hesperidin to local chicken diets in the concentration of cholesterol (mg /100 ml) in male blood (mean ± standard error)

Cholesterol concentration (mg /100 ml) in female blood				General Mean
Treatment	First Period	Second Period	Third Period	
T1	112.35 ±10.15	95.45 ±12.55	90.00 ±7.00	99.26 ±9.90
T2	125.90 ±22.50	103.90 ±20.20	104.00 ±8.00	111.26 ±16.90
T3	124.15 ±14.55	108.40 ±12.40	97.00 ±4.00	109.85 ±10.31
T4	126.50 ±1.40	113.90 ±0.90	103.50 ±10.50	114.63 ±4.26
Significant	NS	NS	NS	NS

T1: 0 mg Hesperidin / kg feed, T2:150 mg Hesperidin/kg feed , T3: 300 mg Hesperidin / kg feed, T4: 450 mg Hesperidin / kg feed. Duration: Each period is 28 days. First (20-24) week, second (24-28) week and third (28-32) week. NS: Not significant.

Table 8: Effect of addition of Hesperidin to local chicken diets in the concentration of Total protein (g /100 ml) in male blood (mean ± standard error)

Total protein concentration (g /100 ml) in female blood				General Mean
Treatment	First Period	Second Period	Third Period	
T1	3.49 ±0.03b	3.63 ±0.42	3.64 ±0.19	3.58 ±0.21 b
T2	4.09 ±0.18a	3.68 ±0.60	4.25 ±0.32	4.00 ±0.36ab
T3	4.32 ±0.11a	4.12 ±0.23	4.03 ±0.22	4.15 ±0.18a
T4	4.12 ±0.06a	4.32 ±0.01	3.99 ±0.15	4.14 ±0.07a
Significant	*	NS	NS	*

T1: 0 mg Hesperidin / kg feed, T2:150 mg Hesperidin/kg feed, T3: 300 mg Hesperidin / kg feed, T4: 450 mg Hesperidin / kg feed. Duration: Each period is 28 days. First (20-24) week, second (24-28) week and third (28-32) week. NS: Not significant.*significantP<0.05.

Table 9 : The addition of Hesperidin to local chicken diets in the serum concentrations of (female and male reproductive hormones (mean ± standard error)

Sex hormones in the serum			
Treatment	Estrogen (Pgm / ml serum)	Progesterone (Ng / ml serum)	Testosterone (Ng / ml serum)
T1	5.63 ±0.51	0.02±0.13	0.48±0.93
T2	9.31±0.71	0.01±0.17	0.13±1.06
T3	3.90±0.80	0.02±0.14	0.11±0.76
T4	11.95±0.62	0.02±0.12	0.32±0.87
Significant	NS	NS	NS

T1: 0 mg Hesperidin / kg feed,T2:150 mg Hesperidin/kg feed , T3: 300 mg Hesperidin / kg feed, T4: 450 mg Hesperidin / kg feed. NS: Not significant.

The increased concentration of protein in the blood of domestic chicken males may be due to the role of the antioxidant Hesperidin, which maintains the pathways of the biological reactions within the body, including the maintenance of amino acids and peptides from oxidizing agents that cause damage (Rao and Shen, 2002). Or perhaps due to the needs of the male body to a protein (amino acids) more to build muscle structure of the body for reasons related to sex, or sex hormones did not affect the secretions of secreted glands secrete these hormones.

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