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Influence of Amla Fruit (*Emblica officinalis)* Powder Supplementation on Some Reproduction Indicators of Adult Japanese Quail (*Coturnix Coturnix Japonica*) Reared Under Hot Climate

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ABSTRACT

This experiment was investigating the influence of different levels of Amla fruit powder supplementation on testis histology, Hatching and fertility characteristics of Japanese quail reared under hot climate(36±2.5c°).

162 males and females of the Japanese quail were used at the age of 9 weeks, divided randomly into six treatments by 18 females and 9 males each treatment of 3 replicates (six females and three males). Feed and water was *ad libitum* and the treatments of this study were: T1: Feed standard diet (control treatment). T2andT3 consumed standard diet supplemented with 0.25 gm/kg diet vit. C and 0.08 gm/kg diet Aspirin respectively, T4,T5 and T6 supplemented their ratio with 1, 1.5 and 2 gm/kg diet Amla fruit powder respectively.

The results showed that T6 increase significantly percentage of hatchability, area and thickness of germinal layer (GA) and (GT) . while no significantly differences (P≤0.05) observed between experimental treatments in percentage of fertility, area and diameter of seminiferous tubules lumen (LD) and (LA) compared with control treatment.

تأثير استعمال مستويات مختلفة من مسحوق ثمرة الاملا *(Emblica officinalis)* في بعض مؤشرات الأداء التناسلي لطيور السمان الياباني (*Coturnix coturnix japonica*) المربى تحت ظروف الاجهاد الحراري

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الخلاصة

اجريت هذه التجربة بهدف دراسة تأثير أضافة مستويات مختلفة من مسحوق ثمرة الاملا *(Emblica officinalis)* في بعض صفات الخصية النسيجية وصفات الخصوبة والفقس لطيور السمان المرباة تحت ظروف الاجهاد الحراري(36±2.5c°).تم استخدام 162 من ذكور واناث طيور السمان بعمر تسعة أسابيع، وزعت عشوائي على ست معاملات بواقع(18) انثى وتسعة ذكور لكل معاملة بواقع ثلاث مكررات (ستة اناث وثلاثة ذكور/ مكرر) غذيت الطيور بصورة حرة وكانت المعاملات التغذوية على النحو التالي: المعاملة الاولى، غذيت الطيور على عليقة قياسية (عليقة سيطرة). المعاملة الثانية والثالثة، غذيت الطيور على عليقة قياسية + فيتامين C بتركيز (0.25غم/كغم علف) و0.08 غم اسبرين /كغم علف على التوالي. والمعاملة الرابعة والخامسة والسادسة اضيف لها (1و1.5و2) غم مسحوق ثمرة الاملا / كغم علف على التوالي.

وجد ان المعاملة باستعمال مسحوق ثمرة الاملا بتركيز 2غم/كغم علف او فيتامين C أدت الى تحسن معنوي (P<0.05) في صفات الخصية النسيجية تمثل في زيادة معنوية في مساحة الطبقة الجرثومية وسمكها مما انعكس ايجابيا على حالة الخصوبة والفقس حيث ادت اضافة مسحوق ثمرة الاملا بتركيز 2غم الى ارتفاع معنوي في نسبة الفقس وتحسن معنوي في مقياس حجم غدة الرغوة لصالح المعاملة بفيتامين C والمعاملة السادسة مقارنة بمعاملة السيطرة.

الكلمات المفتاحية:
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INTRODUCTION

When the quantities of heat produced by an animal override the animal's capacity to get rid of heat to its surrounding environment heat stress happen. This may be caused by variations in a stress stimulates some of physiological modification (Akbarian *et al.*, 2016), including for example, troubles in metabolism, mineral balances, water, protein, energy and, enzymatic activity, blood metabolites and hormonal secretions and reduces production or reproduction performance in poultry(Lara & Rostagno, 2013).

Heat stress causes release of mineralocorticoid and glucocorticoid from adrenal cortex, and stimulate lipid peroxidation in cell membrane (Feenster, 1985; Pardue *et al*., 1985; Burley *et al.*,1993). Most cell energy is produced by oxidative phosphorylation inside cells mitochondria .heat stress leads to overproduction for mitochondria reactive oxygen species (ROS) that can harm proteins, lipids, and DNA, further reducing energy generation efficiency and increasing ROS production in mitochondria. Vitamin C is used in the poultry nutrition because of their anti-stress effects and decline its levels during the heat stress (Renaudeau *et al*.,2012). Many researches explained benefit of dietary vitamin C supplementation to laying hens during heat stress it can protect the birds from the heat and it can also improve deficiency (Sahin *et al.* , 2003; Lohakare *et al.* , 2005). Vitamin C is most important water soluble antioxidants in biological systems and break the chain of lipid peroxidation (Verstraeten *et al*., 2015).

Acetylsalicylic acid, known as an antipyretic drug, the active ingredient of aspirin (Weissmann, 1991). Its act to inhibits prostaglandins synthesis; hence, it may reset the hypothalamic thermostat. Supplemented stressed bird's diet with (ASA) lead to improvement production and physiological performance (Hassan *et al*., 2003; Abou El-Soud *et al.*, 2006 & Abdel-Fattah, 2006). Fed laying hens with diets containing ASA at levels more than 0.4% at first 13 months of egg production no significant effects on fertility percentage and hatchability percentages from total eggs set and from fertile eggs, as compared with un- supplemented group

Amla tree can be considered as medium sized deciduous trees, spread in throughout India, Pakistan, Uzbekistan, Sri Lanka, South East Asia, China and Malaysia. Amla tree grows about 8-18m height, its leaves simple light green, sub-sessile, closely set along the branchlets looks like pinnate leaves; flowers are yellowish green ; fruits are globose, fleshy, pale yellow with six obscure vertical furrows enclosing six seeds in two seeded three crustaceous cocci (Patel & Goyal,2012). Amla is highly nutritious and considered as most important sources of vitamin-C, amino acids and minerals (Walia & Boolchandani,2015). It contains several chemical constituents like tannins, alkaloids and phenols. Antioxidant properties in this plant due to three among all hydrolysable tannins, Emblicanin A and B; gallic acid, ellagic acid (Gaikwad *et al*., 2016). The fruit also contains phyllemblin activity directed fractionation revealed the presence of several phytochemicals like gallic acid, corilagin, furosin and geraniin. (Verstraeten *et al*., 2015).Flavonoids like quercetin, alkaloids like phyllantine and phyllantidine found. Amla fruit having phytoesrogenes that help to balance sexual hormones levels in stressed a animals (Liu *et al*.,2014).

The objective of this study was to investigate the influence of supplemented different levels Amla fruit powder on some reproductive characteristics of stressed quail bird by rearing under heat stress conditions compared with that supplemented with artificial Vitamin C and Acetylsalicylic acid.

MATERIALS AND METHODS

This study was conducted at the poultry farm of Animal Production Department / College of Agriculture / University of Tikrit during the period from 13/7/2016 to 8/9/2016 for 56 days to investigate the influence of different levels of Amla fruit powder supplementation on some testis histology, hatching and fertility parameters of Japanese quail reared under heat stress conditions.

Animals: 162 adult males and females of the Japanese quail age of 9 weeks living in a properly ventilated animal house that had the typical conditions of temperature, humidity, and a consistent schedule of lighting with 16 hours of lighting and 8 hours of darkness. These birds were given a

standard diet which containing 2878 Kcal metabolisable energy and 19.9% crude protein, while water was available to them all the time.

Experimental design: there was random assortment of 162 adult male and female into 6 groups having 18 animals per each by 18 female and 9 males each treatments of 3 replicates (six females and three males) placed in a ground cages with dimensions (80 × 40 × 50 cm) . Treatments of this study were :

T1: standard diet (control treatment).
T2: diet supplemented with 0.25 gm./kg diet vit. C .
T3: diet supplemented with 0.08 gm./kg diet Aspirin .
T4: diet supplemented with 1 gm./kg diet Amla powder .
T5: diet supplemented with 1.5 gm./kg diet Amla powder .
T6: diet supplemented with 2 gm./kg diet Amla powder .

To study characteristics of reproductive performance , three males per treatment were weighed and slaughtered then testis were excised to extraction the relative weight of them and study the histological traits of testis .

Histological Examinations: The testis of the birds were removed following the sacrifice. Formalin (10%) kept the left testis in place for 24 hours. After it had been dehydrated with ethyl alcohol in rising concentration 70-100 and moved in two content of xylol, the samples were surrounded in paraffin, partitioned by the rotary microtome at 5µm. After this, the slide samples moved through falling levels 100-70% of ethylic alcohol in xylol. Hematoxylin and Eosin stain (Luna, 1968) was used to stain the histological slides. The seminiferous tubules diameter (D.S), seminiferous tubules lumen diameter (D. L), germinal layer area (GA) and seminiferous tubules lumen area (LA) were measured with the help of an ocular micrometre. The formulas for this calculation were suggested by Taha (2008):

$$GA = \frac{\pi}{4} * \left(DS^2 - DL^2 \right)$$

$$LA = \frac{\pi}{4} * DL^2$$

Cloacal gland size measurements: A digital calliper was used to obtain the measurements of length (mm) and width (mm). Using these measurements, by using the formulas put forward by Chaturvedi et al. (1993)

(4/3 × 3.5414 × a × b2, where a = 0.5 × long axis and b = 0.5 × short axis).

Cloacal gland measurement was obtained for each of the birds at intervals of 1-8 week, following the commencement of treatment.

Hatching and fertility characteristics: To study hatching and fertility characteristics selected 90 eggs per treatment by 30 eggs per replicate and Incubated in automatic hatch for 17 days then used for the measurement of the fertility , hatchability and total of embryo mortalities by the method of AL-Zubaidy (1986) .

Statistical analysis: The experiment was carried out as a complete randomized design (CRD). The data was analysed with the help of SAS (2005). The variation between the treatment methods was contrasted with the help of Duncan's multiples range tests (Duncan, 1955).

RESULTS & DISCUSSION

Testis tissue characteristics:

The results in table 1 showed non-significantly different between the treatments in testis percentage and seminiferous tubules diameter (SD) comparison with the control group , while significant different (P<0.05) were found between treatments in seminiferous tubules lumen diameter (LD) and seminiferous tubules lumen area (LA) . T6 (addition of 2 gm Amla powder per kg of diet) recorded decreased significant (P<0.05) in LD and LA compared with control group and T3 . As for the germinal layer area (GA) and germinal layer thickness (GT) The results was opposite as noted previously .

Table 1 : Influence of Amla powder supplementation on some testis histological characteristics of Japanese quail reared under hot climate conditions (means ± SE)

Characteristics / treatments	testis relative weight %	SD(µm)	LD(µm)	GA(µm²)	LA(µm²)	GT(µm)
T1	0.38 ± 3.20	4.61 ± 260.58	2.08 ± 93.17 A	1.98 ± 131.53 B	1.63 ± 73.20 A	2.52 ± 167.41 B
T2	0.38 ± 2.86	2.66 ± 280.36	2.38 ± 88.15 Ab	3.21 ± 151.02 Ab	1.87 ± 69.26 Ab	4.08 ± 192.21 Ab
T3	0.72 ± 2.38	8.57 ± 263.11	2.41 ± 91.95 A	8.63 ± 134.48 B	1.89 ± 72.25 A	10.98 ± 171.16 B
T4	0.30 ± 2.93	± 261.33 12.22	0.76 ± 89.51 Ab	9.00 ± 134.99 B	0.60 ± 70.33 Ab	11.45 ± 171.81 B
T5	0.22 ± 3.00	7.34 ± 265.31	2.66 ± 87.14 Ab	4.73 ± 139.99 Ab	2.09 ± 68.47 Ab	6.02 ± 178.17 Ab
T6	0.15 ± 2.42	5.96 ± 286.05	1.33 ± 84.24 B	5.73 ± 158.55 A	1.05 ± 66.19 B	7.30 ± 201.80 A
Significance level	**N.S**	**N.S**	*	*	*	*

- *: means significant difference at (P≤0.05)
- N.S: means non-significant difference at (P≤0.05)

T1 : control treatment . **T2:** control diet supplemented with 0.25 gm/kg diet vit. C . **T3:** control diet supplemented with 0.83 gm/kg diet Aspirin . T4: control diet supplemented with 1 gm/kg diet Amla powder . **T5:** control diet supplemented with 1.5 gm/kg diet Amla powder . **T6:** control diet supplemented with 2 gm/kg diet Amla powder

Hatching and fertility characteristics:

Table 2 showed non-significantly different (P<0.05) between all treatments in fertility , hatchability of fertile eggs and embryo mortalities when addition Vitamin C , Aspirin and different levels of Amla powder comparison with control treatment , while increase significant (P<0.05) were found in hatchability when addition of 2 gm Amla powder per kg of diet (T6) compared with control and other treatments but not significant with T2 .

Table 2 : Influence of Amla powder supplementation on characteristics of hatching and fertility of Japanese quail reared under hot climate conditions (means ± SE)

Characteristics / treatments	Fertility %	Hatching %	hatchability of fertile eggs %	embryo mortalities %
T1	1.92 ± 80.00	Bc1.92 ± 70.00	0.30 ± 87.48	0.30 ± 12.51
T2	2.93 ± 87.77	Ab2.93 ± 77.77	0.37 ± 88.58	0.37 ± 11.41
T3	4.84 ± 82.22	Bc1.92 ± 70.00	2.81 ± 85.46	2.81 ± 14.53
T4	3.85 ± 80.00	C 2.93 ± 67.77	4.31 ± 84.98	4.31 ± 15.01
T5	1.11 ± 84.44	Bc2.22 ± 74.44	2.31 ± 88.15	2.31 ± 11.84
T6	1.92 ± 90.00	A1.92 ± 83.33	0.15 ± 92.58	0.15 ± 7.41
Significance level	**N.S**	*	**N.S**	**N.S**

- *: means significant difference at (P≤0.05)
- N.S: means non-significant difference at (P≤0.05)

T1 : control treatment . **T2:** control diet supplemented with 0.25 gm/kg diet vit. C . **T3:** control diet supplemented with 0.83 gm/kg diet Aspirin . T4: control diet supplemented with 1 gm/kg diet Amla powder . **T5:** control diet supplemented with 1.5 gm/kg diet Amla powder . **T6:** control diet supplemented with 2 gm/kg diet Amla powder

Cloacal gland volume

The effect of adding Vitamin C , Aspirin and different levels of Amla powder to Japanese quail diet reared under heat stress conditions on cloacal gland size (mm^3) are given in **Figure1** .We noted significantly($P<0.05$) increasing in cloacal gland size with adding 2 gm/kg diet Amla powder(T6) as compared with control group ,T3,T4and T5 . While non-significantly different were showed in cloacal gland size between T6 and T2

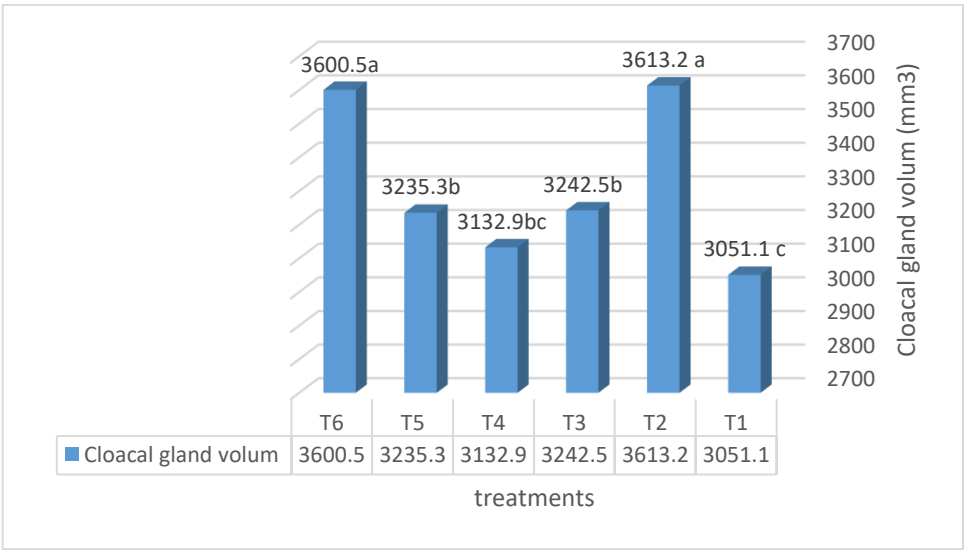

Figure1: Influence of Amla powder supplementation on the cloacal gland volume (mm^3) of Japanese quail reared under hot climate conditions (means ± SE)

- The different liters refers to significant difference at (P≤0.05)

T1 : control treatment . **T2:** control diet supplemented with 0.25 gm/kg diet vit. C . **T3:** control diet supplemented with 0.83 gm/kg diet Aspirin . **T4:** control diet supplemented with 1 gm/kg diet Amla powder . **T5:** control diet supplemented with 1.5 gm/kg diet Amla powder . **T6:** control diet supplemented with 2 gm/kg diet Amla powder

To find out the positive effect of Amla powder to Hatching and fertility characteristics we must explain two important points :

The first is pertain of male Japanese quail , noticed used Amla fruit lead to significant increase ($p \leq 0.05$) in germinal layer area (GA) and germinal layer thickness (GT) of semenifeurs tubules in testis of male Japanese quail (table 1) . This improvement of testis tissue characteristics reflection the improvement of testis tissue antioxidant (Taha & Al-Douri, 2013) . The improvement of testis tissue characteristics have positive correlation with hatchability (Taha , 2008) . Through the results in this study we can say that use Amla powder lead to production sperms able to fertilizing through maintaining sperm cell and spermatogonium cells and maintained of antioxidant damage Shaban &Taha, (2017) indicated reduced malondialdehyde (MDA) level and rising glutathione (GSH) level when used Amla powder in broiler nutrition. Taha (2016) observed that improving of testosterone and testis tissue depends to improvement of antioxidant statues, size of the cloacal gland correlated with testosterone hormone. Massa *et al.* (1980) reported that development of cloacal gland depends to secretion of this hormone, also Oishi & Konishi (1983) found positive correlation between testosterone hormone concentration with testis weight and cloacal gland size in Japanese quail male.

The second important point pertain of female Japanese quail , egg components are the main source of embryonic development (Sturkie , 1986) therefore , any different in its components is closely related to embryonic development , so (Surai ,1999) reported that feeding parent stock broiler to diet high content of tocoferol led to increase antioxidant percentage in embryo brains . Of this was concluded that Amla powder enhance antioxidant in egg components of female Japanese quail which leads to raise growth and embryonic development until hatching. This improvement may be due to Amla powder effect to lipoprotein by lower LDL and raise HDL through Inhibit HMG Co-A reductase enzyme and increase activity of Lecithin-Cholesterol Acyl transferees (LCAT) enzyme which lead to reducing Cholesterol concentration (Anila & Vijayalakshmi 2002).

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