



Impact of Heat Stress on Reproductive Behavior, Performance and Biochemical Parameters of Pigeon: A Trial to Alleviate Heat Stress by Propolis or Wheat Diets

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ABSTRACT

A limited research on effect of heat stress on pigeon. This study evaluates the effect of heat stress on reproductive behavior, performance, net return, economic efficiency, total aerobic microbial count, carcass traits and biochemical parameters, in addition to investigating the role of propolis or wheat diets to alleviate its effect. A total of 24 pairs of pigeons were allocated into 24 house pens (4 treatment x3 replicate x 2pair for each replicate) which were reared under heat stress condition (summer season in Egypt for 3 months). Reproductive behavior and performance of pigeons were observed daily. While, carcass traits, total aerobic microbial count and biochemical parameters were investigated at the end of an experiment.

The obtained data revealed that, heat stress condition lead to increasing the aggressive part of courtship behavior, decreased egg production, egg weight, fertility% and hatchability %.

In conclusion, the addition of propolis to basal diet helped in relieving the effect of heat stress, however, the wheat diet had a negative impact on heat stress condition in pigeon

1. INTRODUCTION

Egypt characterized by hot summer from May to October as temperature reaches 45° C, while the suitable temperature for poultry breeding is between 16-25° C (Sahin et al., 2006) so the performance of birds can be reduced drastically (Abdel-Mohsein et al., 2014).

The reproductive hormones play a vital role as they regulate various stages of development and function in the female reproductive system. The high ambient temperature as a result of climate change may affect the reproductive rhythm via the hypothalamic-hypophyseal-ovarian axis (Reddy and Rao, 2014). Various studies revealed a significant negative correlation between environmental temperature and the reproductive hormone concentration, which in turn cause compromised reproductive efficiency in farm animals such as reduced plasma luteinizing hormone

(LH) and reduce ovarian steroids (progesterone, testosterone, estradiol) levels, and increased plasma prolactin (PRL) levels which lead to incidence of brooding behavior and may terminated egg laying. (Rozenboim et al., 2004) in addition to, prolactin cause suppression of gonadal activity, leading gonads regression, inhibition of FSH and LH which in turn shut off the secretion of sex hormones which reflected by inhibition of the sexual behavior thus preventing further reproduction effort until the young had hedged (Manning, 1972).

Reproductive failure and decrease fertility associated with environmental heat stress in birds may be due to 1st, the interrelationship between elevated temperature and cessation of egg and semen production in birds. 2nd, compromising the functions of the reproductive tract and disrupting the hormonal balance as a reduction in ovarian blood supply

because of peripheral vasodilatation.

3rd, hyperprolactinemia which decreases reproductive activity by acting on the hypothalamus and inhibiting gonadotropin-releasing hormone release thus reduced reproductive efficiency causing termination of egg production and elongated expression of brooding behavior (El Halawani et al., 1984). Also, Prolactin hormone increased the incidence of crouching or sitting in the nest and enhance the incidence of parental feeding invitations (squab-oriented bill openings) (Buntin et al., 1991)

The productive and reproductive performance of poultry were negatively affected by heat stress as there was decrease in egg production (Star et al., 2009 ; Deng et al., 2012), decrease in egg weight (Star et al., 2009 ; Mack et al., 2013), reduced fertility (Christopher et al., 1995), reduced hatchability (Rozenboim et al., 2007), however, there was an increase in prolactin hormone level with increased expression of brooding behavior (Rozenboim et al., 2004). Moreover, a reduction in T₃ level (Mack et al., 2013), total protein, albumin, globulin (Faisal et al., 2008) and calcium level (Mahmoud et al., 1996), in which the former and latte are required for egg formation.

The whole wheat grain diet could have several advantages as its local availability, no grinding processing cost, and a positive effect on gizzard weight (Nir et al., 1990) besides that, Feeding of whole grain improve growth performance, gut health and gut microbiota of poultry (Engberg et al., 2004) and reduce feed costs with no adverse effect on broiler performance (Amerah and Ravindran, 2008). In addition to that, Propolis has antioxidant, antibacterial, immunomodulatory and or anti-inflammatory functions (Bankova et al., 2014) which helped in facing the negative impact of heat stress.

2. MATERIAL and METHODS

2.1. Pigeons and management:-

A total 24 pairs (18:24 months old) of Local Egyptian Baladi pigeons (Columbia Livia) reared in an indoor housing system under natural daylight (12 L\, 12 D) and exposed to surrounding environmental temperature and humidity which were recorded twice daily throughout experiment period using wall mount thermometer and a wall mount wet-dry bulb hygrometer (temperature ranged between 32:36 ° C, while humidity from 60-70% respectively) Moreover,

Every 2 pairs were housed in a house their dimension was 100x100x250 cm with ad-libitum offer

to feed and water in plastic containers. The composition and chemical analysis of diets used presented in Table (1) which calculated according to (NRC, 1994).

2.2. Experimental procedures and design:-

The experimental period was divided into two periods, the first was the pre-experimental period for one month for acclimatization with allowing free choice mating and formation of pairs to ensure ability of pigeon for reproduction and production (Spudeit et al., 2013) and the second period was experimental period which was during summer season in Egypt for 3 months.

The experimental design as following:-

a) Control group:- 6 pairs of pigeon (2 pairs X 3 replicate) fed basal diet and reared at 22:26° C

b) Heat stress groups: - 18 pairs of pigeon reared in 34:36°C divided into three groups as heat stress group fed basal diet only, propolis group fed basal diet contain 500mg propolis and wheat diet group which fed diet contain wheat only.

2.3. Measured parameters:-

2.3.1) Behavioral Patterns

The behavioral observation was started after one month from introducing pigeons into the experimental room to become more adaptive to the presence of the camera used for behavior recording.

Focal sampling observation was used according to (Altmann, 1974) for 1hr/day for 90 days between 08:00-11:30 hrs. The behavior observed were according to ethogram presented in Table (2) (Fabricius and Jansson, 1963). Averages of the replicates for each behavioral pattern were used for the analysis. A two courtship cycles were observed for recoding the courtship and parental behavior.

2.3.2) Reproductive performance: - (pigeon parental behavior)

Incubation period, (days), egg cycle (interval between two consecutive laid eggs days), egg number, egg weight (g), total fertility % and total hatchability% were recorded.

Brooding behavior (young sitting): Behavior associated with the provision of heat by the parents to young and alternate care of the young by female and male which expressed as average of frequency of male and female parent care to young during 2 cycles (Eisner, 1960).

2.3.3) Net return and Economic efficiency (%):-

1) Net return = Price of pairs of squabs during 90 day – price of feed cost during 90 days

2) Economic efficiency (%) = {Net return \ price of feed cost during 90 days} x 100

3) Relative economic feed efficiency = {EFE of treated group \ EFE of control} x 100

To estimate the former parameters the following data were recorded: feed intake (kg) for 90 days, number of squabs/pair, sale price of squabs (L.E), cost of feed for 90 days (L.E) which calculated by the following formula: Total feed intake (kg)x Cost of feed (L.E).

2.3.4) Carcass traits.

Prior to slaughtering, the birds were fasted for eight hours during which they had access to fresh water. The birds were slaughtered in batches of six (3 males and 3 females) from each treatment.

Birds were individually weighed to the nearest gram get live body weight and slaughtered by severing the carotid artery and jugular veins. Scalding was done in hot water (85°C) immediately after 4 minutes of bleeding. The defeathered birds were dissected by hand and after the removal of the head, carcass was manually eviscerated were determine the percentage of carcass; empty gizzard, liver, spleen, pancreas, thymus, spleen, testis and ovary related to live body weight

2.3.5) Total aerobic microbial counts:-

Intestinal content from the duodenum, jejunum, ileum, and caecum were taken separately and immediately after slaughter in previously weighed screw capped sterile plastic cups. The sealed containers were kept on ice until they were transported to the laboratory for counting of the microbial population.

The fresh mass was mixed with an appropriate volume of sterile a solution of 0.1% peptone solution to prepare 1:10 dilution. Ten-fold serial dilutions up to 10⁷ of each sample were then prepared in 9 ml of 0.1% sterile peptone solution. Counting of total viable aerobes was performed using the spread-plate technique. Total aerobes were cultured on nutrient agar (oxoid) and incubated aerobically at 37 ° c. After incubation, all colonies found were counted. The averages of counts of two plates were recorded. Numbers of colony-forming units are expressed as log colony-forming units per gram of digest content (Awan and Rahman, 2005).

2.3.6) Blood Sampling:-

To avoid any circadian effect on hormones levels like (Testosterone or prolactin) and other blood constituent, the sampling time was fixed and following the circadian cycle on basis of one bird/treatment till end of the experiment.

Lastly, 3 pairs were randomly taken from each treatment, weighed and slaughtered. Blood samples were collected during the bird's exsanguinations as following: about 3 cm³ of blood from each bird was collected in a tube without anticoagulant to separate serum for determination of the biochemical parameters and hormones levels.

The tubes were kept at the room temperature for 30 minutes then stored at a refrigerator for 60-90 minutes and then centrifuged at 3000 round per mint(r.p.m) for 10 mint and the separated serum was transferred to another Epindoorf's tube using micropipette. The sera were kept at -20 °C until processed for further analysis.

a) Biochemical parameters

Total serum proteins, albumin, inorganic phosphorus, calcium, Glucose, cholesterol, and triglyceride level were estimated through colorimetric method using Digital- VIS/ultraviolet spectrophotometer. Moreover, thyroid hormones levels (T₃ Tri-iodothyronine, T₄ thyroxine), Malondialdehyde (MDA), Protein carbonyls, prolactin and testosterone were estimated using ELISA kits by a solid phase enzyme immunoassay.

b) Haematological parameters: (Heterophils: lymphocyte ratio)

A duplicate blood smears were prepared for each bird immediately after slaughter to determine the haematological parameters using previously published laboratory method (Corrons et al., 2004). The differential count of Heterophils and lymphocytes (100 cells/field).

2.4. Statistical analysis:-

The data were analyzed by one way ANOVA procedure using the statistical computer Program SPSS 13.00 Software (SPSS Inc., Chicago, IL, USA) to compare between means, significance was designated as P < 0.05. Means were compared by Duncan's test when a significant difference was detected.

3. RESULTS

Data presented in the table (3) showed that pigeon reared under heat stress condition 34°C (summer season in Egypt) and fed basal diet without any additives had the higher frequency of sexual activity in comparison with the control group which reared less than 22° c. On the other side, an addition of propolis to basal diet during heat stress condition had negative impact on bowing; attaching; nest collection and nest demonstration sexual activity. Moreover, using wheat diet in heat stress condition

was helpful in alleviating the effect of heat stress on male and female sexual activity.

Table (4) explained that pigeons groups fed basal diet or wheat diet and exposed to heat stress condition had the lower egg production or number; egg weight, fertility %, and hatchability %. While had longer incubation period and egg cycle. Moreover, using propolis containing diet during heat stress lead to significant increase in egg weight; fertility % and hatchability%. While had shorter incubation period and egg cycle with heat stress group. The net return and economic efficiency was lower in heat stress group than other groups as shown in Table (5).

Table (6) showed that Pigeon fed basal diet or basal diet contains propolis or wheat diet during heat stress condition had the lower live body; carcass and spleen weight in comparison with control group. Besides that, wheat diet during heat stress lead to decrease in testis and ovary weight and total aerobic count while increase the pancreas and gizzard weight in comparison with heat stress group.

Heat stress condition lead to a significant decrease in total protein, albumin, globulin, Ca, Ph, T₃, T₄, lymphocyte and testosterone levels, also Ca/Ph ratio and T₃/T₄ ratio. While, had a significant increase in prolactin hormone level, oxidative enzymes (Malondialdehyde and protein carbonyls), leptin metabolites (glucose; cholesterol and triglyceride),

heteroplils and heterophils/lymphocyte ratio. On the other hand, adding propolis to diet alleviate the adverse effect of heat stress condition on T₃ level. However, the use of wheat diet during heat stress condition showed no difference from the heat stress condition group (Table 7).

4. DISCUSSION

4.1. Reproductive behavior.

The reproductive behavior of Baladi pigeons (*Columba Livia*) was observed for only once a day, during 60 minutes' observation period for 2 eggs cycle which containing 2 pre-incubation periods (totally 3 months of the summer season in Egypt). This observation as one long daily observation time for 10 consecutive days according to (Fabricius and Jansson, 1963) at any time of the day according to (Scott, 1971). Who reported observations on the normal breeding stock had indicated no qualitative differences in behavior in the daily cycle.

Courtship pattern of the pigeon seem to form three rather distinct groups, which apparently represent sub instincts of the major reproductive instinct as aggressive behavior, represented by (bowing and attack); the 2nd group is sexual behavior proper consisting of (displacement preening, begging, billing, squatting, mounting and copulation.) and the 3rd group is composed of nest demonstration, pushing, collecting of nest material and nest building. (Tinbergen., 1951).

Table (1). Composition and chemical analysis of the experimental diets.

Ingredients (%)	Basal diet	Wheat diet	Propolis diet
Yellow corn	71.00	-----	71.00
Soybean meal	19.50	-----	19.50
Wheat bran	---	94.55	---
Sunflower oil	4.00	---	4.00
Propolis	---	---	0.05
Limestone	3.40	3.40	3.40
Sodium phosphate dibasic	1.45	1.45	1.45
Common salt	0.35	0.35	0.35
Premix*	0.30	0.30	0.30
Total	100.00	100.00	100.00
Price\ ton (L.E)	3500	2500	3500
Calculated values:			
Metabolized Energy, kcal/kg	3199.71	2900	3199.71
Crude protein, %	15.52	12.5	15.52
Calcium, %	1.33	1.33	1.33
Available phosphorus,%	0.40	0.40	0.40
Lysine ,%	0.71	0.61	0.71
Methionine,%	0.30	0.2	0.30

*Mineral and vitamin premix Heromix broilers (Heropharma Co., Egypt). Each 2.5 kg contain: Vit. A, 1200000 IU; Vit. D3, 300000 IU; Vit. E, 700 mg; Vit. k3, 500 mg; Vit. B1, 500 mg; Vit. B2, 200 mg; Vit. B6, 600 mg; Vit. B12, 3 mg; Vit. C, 450 mg; Niacin, 3000 mg; Methionine, 3000 mg; Pantothenic acid, 670 mg ; Folic acid 300 mg; Biotin, 6 mg; Choline chloride, 10000 mg; Magnesium

sulphate, 3000 mg; Copper sulphate, 3000 mg; Iron sulphate, 10000 mg; Zinc sulphate, 1800 mg; Cobalt sulphate, 300 mg.

Table (2) Behavioral Ethogram.

Behavior	Definition
Courtship behavior	
Male sexual activity	
Head bowing	Thrusting head; neck forward and below the body. fig.(1)
Driving.	The male chases the female very closely fig.(1)
Attacking.	Grasps its opponent by the mandibles, beats it powerfully on the head or neck.
Mounting	Jumps on the back of the squatting female with or without copulation. fig.(1)
Female sexual activity	
Nibbling.	Gently pecks at the male head crown. fig.(1)
Begging.	Gently pecks at the male's bill feathers. fig.(1)
Pushing	The female pushes herself under male body while he sitting at the nest site.
Billing.	Inserts her bill into male opens mouth, and both do pumping and twisting movements of necks. fig.(1)
Squatting.	Horizontal position of body close to ground lowers her head and spreads wings and 'invites' male to mount.
Nest building	The female sitting on nest and taken the twig held by male
Male and Female sexual activity.	
Collection of nest material	The male take signal twig in a horizontal position into its beak, and carries it to the nest and put it in front of female (may done by female)
Nest demonstration.	Male or female bird lies down on the tarsi, repeatedly nods the head deep toward the floor, vibrates the top of the wing that is closest to the partner and do a calling sound
Displacement preening	Male or female bird rapidly touches the scapulars with the bill, which is then quickly withdrawn. This action often produces a cracking noise.
Nest defense.	Pigeon in nest show raised feathers and it look very big, and do pecking and powerful wing beats toward any enemy or invadeours.

Table (3) Effect of heat stress on courtship behavior of pigeons and roles of propolis or wheat in alleviating this effect.

Item	group	Control	Heat stress(HS)	HS +Propolis	HS + wheat
Male sexual activity (act/60 min.)					
Bowing		6 ± 1.3 ^b	9.9 ± 1.1 ^a	6.9 ± 1.2 ^b	9.2 ± 0.6 ^a
Driving		5.6 ± 1.4	6 ± 1.6	5.8 ± 1.1	5.6 ± 0.3
Attacking		1.9 ± 1.1 ^b	4 ± 0.9 ^a	2.3 ± 0.9 ^b	2.8 ± 0.1 ^a
Copulation		2.1 ± 0.1 ^a	1 ± 0.1 ^b	1.6 ± 0.1 ^{a,b}	0.7 ± 0.1 ^c
Material nest collection		1.8 ± 0.2 ^b	2.3 ± 0.1 ^a	1.9 ± 0.5 ^b	2.5 ± 0.1 ^a
Nest demonstration		4.3 ± 0.7 ^b	5 ± 0.3 ^a	4.6 ± 0.2 ^{a,b}	5.3 ± 0.9 ^a
Displacement preening		2.3 ± 0.2	2 ± 0.6	2.1 ± 0.3	1.9 ± 0.4
Nest defense		0.1 ± 0.01	0.2 ± 0.05	0.09 ± 0.001	0.09 ± 0.004
Over all		3 ± 0.7 ^b	4.3 ± 0.9 ^a	3.9 ± 0.5 ^{a,b}	4.04 ± 0.8 ^a
Female sexual activity (act/60 min.)					
Begging		1.8 ± 0.6 ^a	0.9 ± 0.1 ^b	1.2 ± 0.5 ^{a,b}	0.8 ± 0.6 ^b
Billing		2 ± 0.9	1.7 ± 0.1	1.9 ± 0.2	1.8 ± 0.2
Nibbling		3.2 ± 0.9 ^a	2 ± 0.4 ^b	2.8 ± 0.3 ^{a,b}	1.9 ± 0.4 ^b
Pushing		5.2 ± 1.5 ^a	4.2 ± 0.9 ^b	4.8 ± 0.9 ^{a,b}	4 ± 0.8 ^b
Displacement preening		1.1 ± 0.4	0.9 ± 0.5	0.8 ± 0.3	1 ± 0.2
Material nest collection		0.4 ± 0.1 ^b	0.9 ± 0.1 ^a	0.5 ± 0.5 ^b	1 ± 0.1 ^a
Nest demonstration		2 ± 0.7 ^b	3.4 ± 0.3 ^a	2.2 ± 0.2 ^b	3.7 ± 0.9 ^a
Nest building		0.2 ± 0.09	0.4 ± 0.08	0.3 ± 0.01	0.2 ± 0.07
Nest defense		0.06 ± 0.004	0.08 ± 0.004	0.07 ± 0.007	0.06 ± 0.008
Over all		1.97 ± 0.6 ^b	2.14 ± 0.2 ^a	2.02 ± 0.7 ^{a,b}	2.03 ± 0.9 ^{a,b}

^{a,b,c} Means with different superscripts in the same raw differ significantly at (p < 0.05).

Data are represented as means of behavior frequency of 6 pigeon pairs observed for 60 min. per day for 20 days (2 courtship cycles).

Table (4) Effect of heat stress on reproductive performance of pigeons and roles of propolis or wheat in alleviating this effect.

Item	group	Control	Heat stress(HS)	HS + Propolis	HS + wheat
Egg numbers		2 ± 0.6 ^a	1.62 ± 0.2 ^b	1.92 ± 0.3 ^a	1.56 ± 0.3 ^{b,c}
Egg weight (gm)		18 ± 0.16 ^a	16 ± 1.16 ^b	16.9 ± 1.3 ^{a,b}	15 ± 1.58 ^b
Incubation period(days)		17 ± 2.33 ^b	18.4 ± 0.33 ^a	17.4 ± 2.33 ^b	18.5 ± 0.58 ^a
Average young sitting		0.5 ± 0.02 ^b	0.9 ± 0.03 ^a	0.6 ± 0.02 ^{a,b}	0.7 ± 0.05 ^{a,b}
Egg cycle (days)		49 ± 0.9 ^b	51 ± 0.7 ^a	49.2 ± 0.7 ^b	51.4 ± 2.17 ^a
Fertility %		90 ± 3.29 ^a	68 ± 0.29 ^b	83 ± 0.29 ^a	70 ± 0.29 ^b
Hatchability %		85 ± 0.29 ^a	71 ± 0.29 ^b	80 ± 0.29 ^a	68 ± 0.29 ^b

^{a,b,c} Means with different superscripts in the same raw differ significantly at (p < 0.05).

Data are represented as means of performance of 6 pigeon pairs for 2 courtship cycles.

Table (5) Effect of heat stress on net return and economic efficiency of pigeons and roles of propolis and wheat in alleviating this effect.

Item	group	Control	Heat stress(HS)	HS + Propolis	HS + wheat
Average feed intake of pairs during 90 days (kg)		6.2	5.8	5.9	6.8
Cost of feeding (L.E)=TFI X cost\kg diet (3.5 L.E for kg basal diet and 2.5 L.E for kg wheat)		21.7	20.3	20.7	17
Average number of squabs \ pairs (during 90 day)		3.8	2.5	2.9	2.3
Sale price of pairs of squabs (pairs price 35 L.E)		133	87.5	101.5	80.5
Net return (L.E)		111.3	67.2	80.8	63.5
Economical efficiency (%)		410	331	404	373.5
Relative economic efficiency %		-----	80.7	98.5	91.1
TFI means total feed intake.					

Table (6) Effect of heat stress on carcass parameters % and total microbial aerobic count of pigeons and role of propolis and wheat in alleviating this effect.

Item	group	Control	Heat stress(HS)	HS + Propolis	HS + wheat
Average carcass parameters weight (one pigeon)					
L.B.W		350 ± 2.50 ^a	340 ± 1.50 ^b	330 ± 1.3 ^b	315 ± 3.39 ^c
Carcass%		0.82 ± 0.02 ^a	0.77 ± 0.02 ^b	0.78 ± 0.01 ^b	0.68 ± 0.06 ^c
Gizzard %		0.022 ± 0.0002 ^b	0.021 ± 0.0001 ^b	0.022 ± 0.0002 ^b	0.027 ± 0.0001 ^a
Pancreas %		0.003 ± 0.0001 ^b	0.003 ± 0.0003 ^b	0.003 ± 0.0002 ^b	0.006 ± 0.0002 ^a
Liver %		0.0182 ± 0.002	0.0183 ± 0.001	0.0183 ± 0.003	0.0186 ± 0.003
Thymus%		0.004 ± 0.0007	0.003 ± 0.0004	0.003 ± 0.0004	0.003 ± 0.0004
Spleen %		0.001 ± 0.0001 ^a	0.0001 ± 0.0003 ^b	0.0001 ± 0.0002 ^b	0.0006 ± 0.00002 ^c
Testis%		0.006 ± 0.0001 ^a	0.006 ± 0.0003 ^a	0.006 ± 0.0002 ^a	0.003 ± 0.0005 ^b
Ovary %		0.003 ± 0.0003 ^a	0.003 ± 0.0003 ^a	0.003 ± 0.0001 ^a	0.001 ± 0.0003 ^b
Total aerobic microbial count (cfu\ gram)					
Small intestine		6 x 10 ⁶ ± 2.3 ^b	7 x 10 ⁶ ± 2.9 ^a	5 x 10 ⁶ ± 1.2 ^b	8 x 10 ⁵ ± 4.3 ^b
Large intestine		5 x 10 ⁵ ± 2.9 ^b	5 x 10 ⁶ ± 1.5 ^a	4 x 10 ⁶ ± 3.5 ^a	7 x 10 ⁵ ± 1.5 ^b

^{a,b,c} Means with different superscripts in the same raw differ significantly at (p < 0.05).

LBW means live body weight.

Table (7) Effect of heat stress on biochemical parameters of pigeons and role of propolis and wheat in alleviating this effect.

Item	group	Control	Heat stress(HS)	HS + Propolis	HS + wheat
Average biochemical analysis of blood					
Total protein (mg/dl)		3.12 ± 0.6 ^a	2.78 ± 0.4 ^b	2.98 ± 0.2 ^a	2.71 ± 0.1 ^b
Albumin (mg/dl)		1.75 ± 0.2 ^a	1.55 ± 0.3 ^b	1.68 ± 0.1 ^a	1.51 ± 0.2 ^b
Globulin (mg/dl)		1.37 ± 0.3 ^a	1.23 ± 0.5 ^b	1.30 ± 0.7 ^a	1.20 ± 0.2 ^b
A/G ratio		1.28 ± 0.6	1.26 ± 0.2	1.29 ± 0.4	1.26 ± 0.9
Calcium (mg/dl)		12.3 ± 1.1 ^a	10.6 ± 1.4 ^b	11.8 ± 1.4 ^a	9.9 ± 0.19 ^c
Phosphorus(mg/dl)		9.3 ± 0.6 ^a	7.3 ± 0.3 ^b	8.9 ± 0.7 ^a	4.3 ± 0.34 ^c
Ca/ph. ratio		1.32 ± 0.1 ^c	1.45 ± 0.3 ^b	1.33 ± 0.4 ^c	2.3 ± 0.3 ^a
Hormonal level					
Average T ₃ levels		13.8 ± 1.4 ^a	9.2 ± 1.3 ^b	10.8 ± 1.5 ^{a,b}	8.9 ± 1.9 ^b
Average T ₄ levels		25.6 ± 3.4 ^a	19.3 ± 1.42 ^b	20.9 ± 3.4 ^b	22.9 ± 2.1 ^{a,b}
T ₃ \ T ₄ ratio		0.53 ± 0.02 ^a	0.48 ± 0.04 ^b	0.52 ± 0.07 ^a	0.39 ± 0.08 ^c
Testosterone (nmol/L)		2.8 ± 0.2 ^a	1.8 ± 0.3 ^b	2.4 ± 0.3 ^a	1.6 ± 0.5 ^b
Prolactin (nmol/L)		29.4 ± 2.79 ^b	35.4 ± 1.79 ^a	22.1 ± 0.001 ^b	39.5 ± 1.44 ^a
Blood oxidative markers					
Oxidative enzymes					
Malondialdehyde (μmol/L)		1.3 ± 0.04 ^c	2.4 ± 0.6 ^a	1.9 ± 0.4 ^b	2.9 ± 0.7 ^a
Protein carbonyls (nmol/L)		0.9 ± 0.09 ^c	1.9 ± 0.9 ^a	1.1 ± 0.2 ^b	2.2 ± 0.6 ^a
Leptin metabolites					
Glucose (mg\dl)		204 ± 4.2 ^b	230 ± 3.6 ^a	212 ± 5 ^b	235 ± 2.8 ^a
Cholesterol (mg\dl)		165 ± 3.3 ^b	178 ± 4.8 ^a	170 ± 3 ^b	183 ± 3.9 ^a
Triglyceride (mg\dl)		160 ± 3.7 ^c	172 ± 3.3 ^a	169 ± 4 ^b	175 ± 4.8 ^a
Blood hematology					
Heterophils (%)		47 ± 2 ^b	52 ± 3 ^a	49 ± 3 ^b	51 ± 3 ^a
Lymphocytes (%)		53 ± 3 ^a	48 ± 1 ^b	51 ± 1 ^a	49 ± 1 ^b
H/L ratio		0.89 ± 0.04 ^b	1.08 ± 0.08 ^a	0.96 ± 0.06 ^b	1.04 ± 0.07 ^a

^{a,b,c} Means with different superscripts in the same raw differ significantly at (p < 0.05).

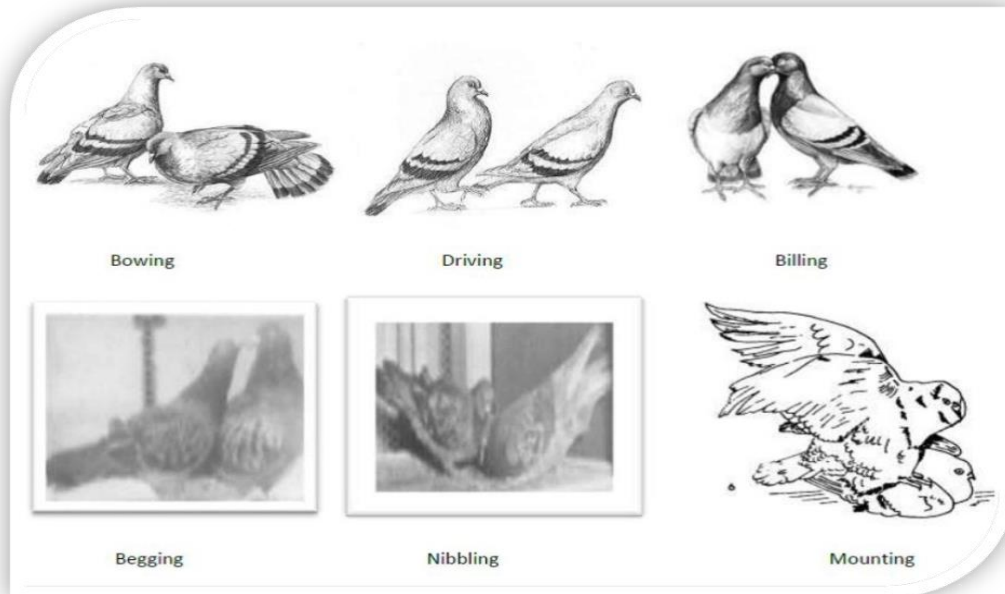


Figure (1). Show courtship behavior pattern.

The significant increase in male sexual activity under heat stress condition may be related to heat stress increase corticosterone level (Star et al., 2008) which reflected mainly in increasing frequency of the aggressive part of courtship behavior (bowing and attaching) beside that, increasing the frequency of nest formation part (nest demonstration and nest material collection) of both male and female pigeon this increase may be related to the effect of heat stress on increasing prolactin hormone level. (Rozenboim et al., 2004).

On the other hand, Heat stress condition lead to decrease copulation frequency of male sexual activity this may relate to the effect of heat stress in decreasing testosterone level (Rozenboim et al., 2004) also, heat stress affected the libido of the male for sexual activity. (Phillips et al., 1989)

Despite that, there is significant decrease in copulation and testosterone level in male pigeon during heat stress condition there was an increase in sexual activity of male which can be explained by the high increase effect of heat stress on an aggressive part such as bowing which consider the main sexual behavior of the male. Finally, the male activity pattern don't reflect the good sexuality or good health of male as it also may be accompanied by testicular or physiological changes but the main expression or showed factor was reproductive production parameters or parental behavior.

The significant increase in male sexual activity in the wheat group in comparison with control one may be related to wheat contain 6-methoxy-2-benzoxazolinone (6-MBOA), a phenol compound that stimulates reproduction in certain small wild herbivorous mammals (Lopez et al., 2011).

Propolis in basal diet during heat stress condition lead to the significant decrease effect on bowing; attaching; nest collection and nest demonstration in comparison with heat stress condition with basal diet only. This may due to Propolis reduced the response of the hypothalamic-pituitary-adrenal axis to various stressors in mice and rats and propolis attenuated the increase in blood corticosterone levels, reduced the c-fos immune reactive neurons and increased glucocorticoid receptor expression in the hippocampus (Lee et al., 2013). Thus facing corticosterone increasing level which causing increase bowing and attaching and also the

increased prolactin which increases nest demonstration and nest collecting.

4.2. Reproductive performance

The significant decrease in egg production, egg weight, fertility % and hatchability % due to heat stress was in agreement with the work of (Mashaly et al., 2004; Clark and Sarakoon, 1967; Muiruri and Harrison, 1991) respectively. This results most likely due to heat stress lead to decrease in feed consumption and reduce digestibility of different components of the diet causing to reducing the available nutrients for egg production (Bonnet et al., 1997). Furthermore, it decreased plasma protein concentration (Zhou et al., 1998) and decreased plasma calcium concentration (Mahmoud et al., 1996), both of which are required for egg formation. On the other side, the body temperature (especially male) is negatively correlated with fertility and sperm-egg penetration. Therefore, it is possible that the elevated body temperature of heat stressed birds may instigate the decrease in fertility and sperm-egg penetration. (Christopher et al., 1995)

Heat stress had lower net return and economic efficiency this may due to its lead to decreased feed intake is very likely the starting point of most detrimental effects of heat stress on production, leading to decreased body weight, feed efficiency, egg production and quality (Deng et al., 2012). This data found in table (3)

The significant increase in incubation and egg cycle period in heat stress group fed wheat (low protein diet) was in agreement with the previous finding of (Abou Khashaba et al., 2009), which may due to the length of the egg cycle depend on the activity of parents to rear of their squabs and environmental conditions (light, warm, and nutrition) (Abou Khashaba et al., 2009) thus, increase in heat stress condition it may due to, high ambient temperature terminated egg laying and increased plasma PRL levels and the incidence of incubation behavior. (Rozenboim et al., 2004).

Moreover, the increase in incubation period and egg cycle during heat stress condition may be due to higher ambient temperature which increased plasma prolactin level which terminated egg laying and increased incidence of brooding behavior (Buntin et al., 1991).

Pigeon fed diet containing propolis during heat stress condition showed the significant increase in egg number; egg weight, fertility %, and hatchability %. While had a significant decrease in the incubation period and egg cycle.

The significant increase in propolis group was in agreement with (Galal et al., 2008; Abdel-Kareem and El-Sheikh, 2015).

A highest egg production rate for pigeon fed propolis during heat stress could be attributed to the propolis, which contains digestive enzymes (glucose oxidase, catalase and peroxidase), in addition to the pronounced contents of the essential and aromatic oils that may be associated with the improved digestibility of the different nutrients (Khojasteh and Shivazad, 2006) which lead to increase in nutrient absorption need to egg formation. Moreover, the significant decrease in the incubation period and egg cycle comparing to heat stress group may due to the antioxidant activity of propolis (Vidda-Martos et al., 2008) which alleviate the negative effect of heat stress on reproductivity of pigeon. Moreover it contains flavonoids which act similar to anabolic agents with estrogenic effect (Ziaran et al., 2005).

The significant decrease on egg production, egg weight; fertility % and hatchability % in the wheat group when compared to the control was in agreement with (Kjaer et al., 2007) while, the non-significant effect of wheat on egg production of pigeon when compared to heat stress group was agreed with finding of (Lázaro et al., 2003 ; Liebert et al., 2005) as well as,

The significant decrease in fertility % and hatchability % of wheat group disagreed with (Abou Khashaba et al., 2009) this may be attributed to wheat has higher non-starch polysaccharides concentrations as arabinoxylan (ranging from 57 to 80 g/kg DM) which are thought to tangle up within the gastrointestinal tract and hinder digestion causing reduce nutrient digestibility and bird performance as well as the lower bioavailability of biotin (Bryden et al., 1991) and lower carotenoid contents (Surai and Sparks, 2001) all this causes leading to insufficient minerals as phosphorus and vitamins (A, B, E) which reflected mainly by lowering fertility% and hatchability%. Besides that, the egg weight decrease may be due to low protein and energy level in wheat on which egg weight depends on (Morris and Gous, 1988).

4.3. Economic effect

Wheat group had more economic efficiency than heat stress group this may due to the lower price of wheat in comparative with basal diet price. This disagreed with the finding of (Liebert et al., 2005) this may be due to amino-acid intake: a lack in lysine, methionine, and tryptophan (45%) reduced their feed intake by 60 % (Picard et al., 1993). which decrease feed intake and nearly same for production level so that, good net return.

4.4. Carcass traits and total aerobic microbial counts

The significant decrease in live body weight; carcass weight during heat stress and wheat was agreed with the previous finding of (Sahin et al., 2006; Bennett and Classen, 2003) respectively. This result may be due to reducing feed intake of bird which accompanied with lower testis and ovary weight in the wheat group. While, the significant increase in pancreas and gizzard weight in the wheat group was in agreement with the finding of (Williams et al., 2008; Nir et al., 1990) respectively. This result may be due to inclusion of large particles in the diet increased the retention time in the gizzard. Moreover, the retention time in the gizzard had been shown as linked to the relative weight of this organ (Verdal et al., 2011), besides that, a heavier pancreas and gizzard weight could be positively correlated with an increased digestive ability of the chicken. (Nir et al., 1990)

In addition to that, pigeon exposed to heat stress and fed wheat diet had lower level of total microbial aerobic count in small and large intestine this result was in agreement with (Elad et al., 2014 ; Bjerrum et al., 2005) respectively. This result may be due to wheat feeding in poultry reduces the pH of the gizzard which acts like barrier organ and prevents pathogenic bacteria from entering the distal digestive tract and consequently reduces their population in the ileum. Besides that, higher gizzard functionality may also play a positive role in the control of bacterial populations in the gut and on gut health (Bjerrum et al., 2005). On the other hand, the significant decrease in total microbial aerobic count in propolis group was in agreement with previous finding of (Abdel-Mohsein et al., 2014) which may be due to the antibacterial effect of propolis. (El-Bassuny, 2009). Besides that, the non significant effect of propolis on liver; gizzard and spleen weight was in agreement with (Mahmoud et al., 2017) which may be due to propolis had anon significant effect on Live body weight of pigeon during this experiment.

4.5. Biochemical parameters

Heat stress leads to significant decrease in total protein, albumin, Ca, phosphorous, T_3 , globulin and Testosterone levels was in agreement with previous finding of (Nadia 2003; Samara et al., 1996; Rozenboim et al., 2004) respectively. While,

The significant increase in prolactin, Malondialdehyde; protein carbonyls glucose; cholesterol and triglycerides levels was in agreement with the finding of (Rozenboim et al., 2004; Al-Azraqi, 2008) respectively.

The significant decrease of Ca level during heat stress may be due to reduced calcium intake as well as the conversion of vitamin D_3 to its metabolically active form, 1, 25 Dihydroxyl D_3 , which is essential for the absorption and utilization of Ca as well as alterations in acid-base balance, the status of Ca^{2+} and the diminished ability of duodenal cells to transport calcium could be critical factors on egg production (Mahmoud et al., 1996). Besides that, the significant decrease in total protein and albumin levels may be due to heat stress lead to decrease in feed intake (Gharib et al., 2008) which accompanied by decrease in protein and nutrient uptake. While the reduction in T_3 level may be due to T_3 hormone was calorogenic hormone in broiler chicks that decrease at a high environmental temperature and in low nutrition status (Decuypere and Buyse, 2005). Moreover, The increase of Malondialdehyde; protein carbonyls level may be due high ambient temperature depletes antioxidants and induces oxidative stress. (Decuypere et al., 2002). In addition to corticosterone increasing during heat stress (Star et al., 2008) leading to increasing concentrations of glucose and triglyceride (Yuan et al., 2008).

The significant increase effect of propolis in T_3/T_4 ratio, lymphocyte, albumin, total protein and globulin levels in the propolis group was in agreement with (Mahmoud et al., 2017; Abdel-Kareem and El-Sheikh, 2015) respectively. While significant reduction in Malondialdehyde level; cholesterol; Heterophils and H/L ratio was in agreement with the finding of (Attia et al., 2014; Abdel-Kareem and El-Sheikh, 2015; Mahmoud et al., 2017) respectively. In chickens, the Heterophils are phagocytic cells whose main function is the protection against invading micro-organisms, whereas the primary function of lymphocytes is involved in cell-mediated and humoral immunity.

Heterophils increase and lymphocytes decrease when they are stressed so that the ratio

between them is a good index of response to a stressor (Gross and Siegel, 1985). Thus the H/L ratio is a recognized measure for stress in birds (Maxwell, 1993), which has become a valuable tool in stress research, especially when combined with the convenience and repeatability of automated blood cell counts.

The significant reduction in H/L ratio in propolis group may be due to increase the lymphocyte level and decrease Heterophils level may be due to its antibacterial, antiviral and antifungal effect on the immune system (El-Bassuony, 2009). as well as, the decreasing leptin metabolites (glucose; cholesterol and triglyceride); protein carbonyls and Malondialdehyde may be due to the antioxidant, antibacterial, immunomodulatory and/or anti-inflammatory functions of propolis (Bankova et al., 2014), which improving birds immunity and health status by reducing the negative effects of heat stress. Reduced enzyme activity could indicate that propolis is able to reduce tissue damage and prevent the leakage of enzymes through cellular membranes due to its antioxidative function (Newairy and Abdou, 2013).

In addition to that, the increase in total protein could be attributed to improvement in synthesis and digestion of crude protein. Also, the increase in globulin level could be attributed to the improved immunity of bird, through the better efficacy of the liver to synthesize enough globulins for immunological action. Moreover, Blood cholesterol and triglyceride levels were significantly reduced in propolis fed group (Galal et al., 2008; Attia et al., 2014). This decrease in the cholesterol level could be attributed to the fact that propolis might not interfere with HDL-c synthesis but its mode of action might be induced by inhibiting cholesterol biosynthesis through inhibiting of HMG-CoA reductase, the rate-limiting enzyme that mediates the first step in cholesterol biosynthesis (Albokhadaim, 2015). Also, it may be due to its anti-oxidizing properties, where propolis is considered to improve lipid metabolism, liver morphological structures and biological functions (Babińska et al., 2013).

The significant decrease in testosterone level in heat stress condition may be due to glucocorticoids have been designated as the main hormones of the stress because their level sharply rises in response to stressful situations. One of their effects is the decrease of the Leydig cells' testosterone production which

finally affects the male's libido and fertility (Phillips et al., 1989; Orr et al., 1994).

5. CONCLUSION

It can be concluded that the addition of propolis to basal diet during the heat stress condition could be useful to alleviate the negative impact of heat stress, however, the wheat diet had a good net return and economic efficiency which can be used during the heat stress condition as it had no bad effect on productive or reproductive performance of pigeon.

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Ethics approval and consent to participate

We have got an ethical approval from hygiene Department, Faculty of veterinary medicine, Assuit University, Egypt. Which according to the ethical rules for handling the experimental animals. All authors read and approved the final manuscript.

Availability of data and materials.

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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