Egyptian Journal of Rabbit Science, 27 (1): 43-64(2017)

SEMEN PHYSICAL CHARACTERISTICS, BLOOD PARAMETERS AND SOME PHYSIOLOGICAL ESTIMATES OF RABBIT BUCKS ADMINISTERED WITH BEE POLLEN UNDER UPPER EGYPT CLIMATIC CONDITIONS

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The present study was conducted to evaluate the effect of orally administration of bee pollen (BP) levels at 500 and 1000mg/buck as capsules form on semen physical characteristics as well as some hematological and physiological traits of rabbit bucks exposed to high temperature under Upper Egypt climatic conditions. A total of 30 rabbit bucks (15 V-line & 15 Moshtohor); 52 weeks old, were equally classified into three experimental groups. Bucks in the 1st group (control) were fed on a commercial ration, while those in the 2nd and 3rd groups were fed on the basal diet and orally administered daily with 500 and 1000mg BP/buck, respectively during the experiment, which lasted 56 days.

***The obtained findings revealed that** the means of white blood cells (WBCs), hemoglobin (Hg), Hematocrit (HCT), MCV (%), total protein (TP) of Moshtohor rabbit bucks were significantly increased, while the total feed intake (TFI) and glucose level were significantly decreased than those of the V-line rabbit bucks.*

The means of live sperm (%), mass motility (%), conc./ml (10^6), conc./ej (10^6) and testosterone (ng/ml) in Moshtohor bucks increased significantly ($P<0.01$) by about 4.3, 6.0, 24.0, 28.6 and 16.8%, while reaction time (RT) was significantly decreased by about 20.2% than those of V-line bucks.

The bucks treated with both of 500 and 1000mg BP /rabbit had significantly ($P<0.01$) increased means of final body weight (FBW), red blood cells (RBCs), WBCs, Hg, HCT, MCV, TP and Alb, glucose and TAC than the corresponding means of the control group. While, the treatment with BP significantly ($P<0.05$) reduced TFI and serum cholestrol.

The means of ejaculate volume, live sperms, mass motility, conc./ml and conc./Ej. (10^6) for bucks treated with BP exceeded significantly ($P<0.01$), while the reaction time and sperm abnormalities were significantly ($P<0.01$) decreased as compared with the corresponding figures in the control group.

Similarly, the means of glutathione content (GSH) and testosterone hormone concentration were significantly increased in the treated bucks, while the mean malondialdehyde (MDA) was significantly decreased as compared with the control group.

***Conclusively,** from the obtained results it could be concluded that treating rabbit bucks raised under high temperature with both of 500 and 1000mg/ buck significantly improved the semen characteristics, blood parameters, antioxidant activities, and testosterone hormone.*

Keywords: Rabbit bucks, Bee Pollen, blood proteins, physical semen characteristics.

In Egypt as a subtropical country, there is a wide range between maximal and minimal temperature during summer season, which adversely affects the production and reproduction performance as well as semen quality and some physiological estimates of rabbits (El-Hammady *et al*., 2010 and El-Hanoun *et al.*, 2014). In Upper Egypt, the high temperature coincided with a relatively increased relative humidity represents a heat stress, which controls the breeding season, during the period from the onset of September till the end of April (El-Hammady *et al*., 2010 and Attia *et al*., 2011).

The production and reproduction performance, as well as, feed consumption of rabbits raised in semi-closed buildings under the high prevailing temperature decrease remarkably due to the adverse stressful effect of the heat stress (El-Hammady *et al*., 2010). This is expected since they have no sweat glands (Ondruska *et al*., 2011 and El-Hammady and Abdel-Kareem, 2014). Recently many attempts were performed to ameliorate the adverse effects of high temperature in addition to improving the productive and reproductive performance of rabbits and chickens raised under high ambient temperature by using some natural products as honey bee products including propolis, bee pollen and royal jelly (Elnagar *et al.*, 2010; Attia *et al.*, 2011 and El Saidy *et al.*, 2016). The treatment of farm animals and poultry with antibiotics and other different chemical substances adversely affects their products (meat and milk) through the harmful effects of their residues, which in turn have hazardous effects on human health (Pikkemaat, 2009).

Through the last thirty years, it became important to produce safe animal and poultry products by using natural resources as different feed additives (herbal seed, bee pollen, royal jelly and propolis) which improve their production performance without any adverse effects (El-Neney and El-Kholy, 2014 and Khadr *et al*., 2015).

Among these safe and beneficial natural additives are the honey bee products (Attia *et al*., 2011). As known, the bee pollen is an agglomerate of flower pollen grains, gathered by honey bees and mixed with plant nectar and bee saliva enzymes, which transforms its composition and consequently improves its therapeutically efficiency (Carpes *et al*., 2008 and Leblanc *et al*., 2009). The chemical analysis of bee pollen indicated that it contains about 25-30% proteins, 35-65% carbohydrates, 51% polyunsaturated fatty acids, 39% linolenic acid, 20% palmitic acid, and 13% linoleic acid and more than 12 vitamins, 28 minerals, 59 trace elements, 11 enzymes or coenzymes, (Xu *et al*., 2009; Attia *et al*., 2011 a & b and Haščík *et al*., 2012). The findings of many researchers indicated that the bee pollen tended to have some therapeutically characteristics as antifungal (García *et al*. 2001), antibiotic (Almaraz-Abarca *et al.* 2004), antibacterial (Proestos *et al*. 2005), antidiarrheal and antioxidant (Hajkova *et al.*, 2013). The findings of Carpes *et al*., (2007) revealed that the pollens which exhibited the highest scavenging capacity and antioxidant activity tend to contain the highest levels of flavonoids and phenolic acid derivatives. The findings of Attia *et al*., (2011a & 2014) indicated that supplementing the ration of rabbits with some levels of bee pollen increased their body weight gain and survival rate, while it reduced the feed intake and consequently improved the feed conversion ratio of the offspring up to 12 weeks of age.

Regarding the effect of bee pollen on the semen quality, Wang *et al*., (2002) found that the treated breeder cocks, exposed to heat stress with at 1 and 1.5% pollen levels had significantly increased ($P \leq 0.05$) sperm quality, activity and density than those of the control. Also, Abou El-Naga (2014) found that semen volume, sperm concentration, and livability were significantly ($P \leq 0.05$) improved of Norfa cockers treated with 1 or 2% BP supplementation than those of the control. Similarly, Liu *et al*., (2009) found that supplementing the ration of hy- line breeder cocks with 0.5, 1, 1.5, 2 and 2.5% bee pollen improved significantly their reproductive performance and efficacy. Also, the findings of Attia *et al.*, (2011b) indicated that supplementing the ration of NZW rabbits with 100, 200 and 300mg BP/kg BW increased remarkably the concentrations of plasma glucose, total protein, and albumin. Similar results were found by Kughn (2010), who

stated that Yangzhou cocks treated with pollen positively affected serum total protein, albumen, and globulin as compared with the control.

Therefore, the current study was conducted to evaluate the impact of daily treating the experimental rabbit bucks with 500 and 1000 BP/buck in capsulated form on productive traits, blood constituents, physical semen characteristics and testosterone hormone.

MATERIALS AND METHODS

The experimental work was performed at the Rabbit Farm, Department of Poultry Production, Faculty of Agriculture, Assiut University, Egypt. The experiment was initiated from April 2016 and terminated on June 2016. The Moshtohor line was developed as a native Egyptian rabbit by crossing Spanish V-line female rabbits with males of Sinai Gabali (Iraqi *et al.*, 2008).

Experimental animals and management

The present study was conducted to evaluate the effect of administration of bee pollen (BP) on physical semen characteristics, some hematological and physiological traits of rabbit bucks exposed to high temperature in Assiut governorate, Egypt.

For this purpose, thirty bucks, 15 for both of V-line and Moshtohor, 52 weeks old were divided into three equal groups. In the first group, bucks were fed on a commercial ration and served as control. In the second and third groups bucks were fed the basal control diet and administered daily orally with 500 and 1000mg bee pollen/buck, respectively. Bucks were fed *ad-libitum* on a basal diet containing 17.0% crude protein, 12.5% crude fiber, 2.99% fat, 0.6% minerals mixture and 2500Kcal/kg digestible energy. Fresh tap water was available all the time throughout the experimental period. Bucks were individually housed in wire galvanized battery cages with dimensions (50L×50W×40H) and raised under the same managerial conditions. Bucks were daily exposed to continuous (16L: 8D) lighting hours.

Environmental conditions in the rabbit farm

Ambient temperature (^{o}C) and relative humidity (%) were recorded inside the rabbitry all-over the day by using a thermo-hygrograph. The averages of minimal and maximal ambient temperature, as well as relative humidity, were determined as shown in Table 1.

The maximal temperature was determined as the average of five measurements at 10 AM, 12 Noon, 2, 4 and 6 PM, while the minimal temperature was determined at 8 and 10 PM, 12 Mid night, 2 and 4 AM, respectively.

Table 1. Ambient temparature (AT/ºC), relative humidity (RH/%) and temperature humidity index (THI/units) all-over the experimental period

Weeks	Date	Minimum			Maximum			Average		
		AT (ºC)	RH (%)	THI (units)	AT (ºC)	RH (%)	THI (units)	AT (ºC)	RH (%)	THI (units)
1	23-30/4	26.4	75.5	25.49	34.2	62.6	31.90	30.30	68.85	28.76
2	30/4-7/5	26.2	68.4	25.04	35.2	60.6	32.66	30.70	64.50	28.91
3	7-14/5	28.2	66.6	26.77	39.1	62.2	36.20	33.65	64.40	31.52
4	14-21/5	30.4	60.5	28.44	40.4	58.6	37.06	35.40	59.55	32.77
5	21-28/5	28.5	66.5	27.04	40.4	58.8	37.08	34.45	62.65	32.13
6	28/5-4/6	28.8	64.6	27.22	41.3	60.4	37.99	35.05	62.50	32.65
7	4-11/6	30.6	60.4	28.61	41.5	58.4	38.00	36.05	59.40	33.32
8	11-18/6	30.8	58.8	28.71	42.2	60.8	38.82	36.50	59.80	33.75

The temperature humidity index (THI) was calculated using the equation according to (Marai *et al*., 2001):

$$THI = db\ ^{o}C-[(0.31-0.31\times RH) \times (db^{o}C-14.4)],$$

Where, db ^{o}C = dry bulb temperature and RH %= relative humidity. The THI values were classified as follow: absence heat stress (<27.8), moderate heat stress (27.8-28.8), severe heat stress (28.9-29.9) and very severe heat stress (>30.0) as presented in Table 1.

Studied traits

***Productive performance*:** During the experimental period, rabbits live body weight, total body weight gain and total feed intake (g) were recorded.

***Hematological parameters and blood biochemical analysis*:** The blood samples, 3ml each were collected from the marginal ear vein at 10.0 AM in both heparinized and no heparinized tubes to measure the biochemical analysis at $56^{th,}$ 58^{th} and 60^{th} weeks of age i.e. after 4, 6 and 8 weeks of bee pollen administration. Blood serum was separated by centrifugation for 15 minutes at 3000 r.p.m and stored frozen (-20°C) in plastic vials until biochemical analysis. Non-coagulated blood samples were used to count red blood cells (RBCs, 10^6), white blood cells (WBCs, 10^3) and WBC's subclasses percentages (lymphocyte, neutrophils, eosinophils, basophils and

monocytes), in addition hemoglobin concentration (Hg, g/100ml) as well as hematocrit (HTC, %) value according to Drew *et al.* (2004).

Levels of serum total protein (TP) and albumin (Alb) were measured by using commercial kits according to the methods of Armstrong and Carr (1964) and Doumas *et al.* (1971), while serum globulin values were obtained by subtracting albumin values from the total protein values.

Cholesterol level in the serum was measured by using specialized commercial kits (Diamond Diagnostic, Egypt). Plasma total antioxidant capacity (TAC/mmol/L) was measured according to Erel (2004).

The Malondialdehyde (MDA/nmol/L) and glutathione contents (GSH/mg/L) concentrations in both serum and seminal plasma were determined using HPLC according to the methods described by Karatepe, (2004) and Jayatilleke and Shaw, (1993).

Semen samples:

Semen samples were weekly collected from the bucks at 10 AM, by using an artificial vagina and a teaser female rabbit. Reaction time for each buck was determined using the stopwatch as the time elapses up to the buck gives semen ejaculate. Semen volume after removing gel excretion was measured per ml by using a graduated collection tube with the nearest 0.1 ml and placed at 37ºC in a water bath according to Breederman *et al.* (1964). The sperm concentration was microscopically examined and measured by using a hemocytometer slide, while the sperm live and abnormality percentages were assessed by the method described by Blom, (1983). The ejaculate samples were centrifuged at 5000 rpm for 10 min to separate seminal plasma and determine MDA and GSH. The recovered seminal plasma fraction was further centrifuged at 10000 rpm for 15 minutes at 4ºC and stored at -20ºC until analysis. Plasma testosterone (ng/ml) hormone concentration was measured by specialized commercial kits according to the method described by Tietz, (1995).

Statistical analysis:

The obtained data were statistically analyzed by using GLM, produced by the statistical analysis systems (SAS, 2004). Duncan's new multiple ranges tests (Duncan, 1955) were used to determine significant differences between treatment means. The following linear model was applied:

$$Yij = \mu + B_i + T_j + B_iT_j + e_{ij}$$

Where, Y_{ij}= Observation measured**,** μ= Overall mean, B_i= Effect of rabbit line (i = 1, 2), Tj= Effect of BP treatment (j = 1, 2 and 3), BT_{ij}= Interaction between rabbit line and treatment, Eij= Random error component was normally distributed assumed.

RESULTS AND DISCUSSIONS

Productive traits:

The findings presented in Table 2 showed that the total feed intake of Moshtohor bucks reduced significantly ($P<0.01$) than those of the V-line, while body weight and total weight gain were not significantly influenced. The relatively increased feed intake of V-line bucks could be attributed to the genetical effect, which reflects pronounced variations in performance and behavior between different lines.

With regard to bee pollen treatment, the obtained findings indicated that the final body weight (g) and total weight gain (g) for bucks treated with 500 and 1000mg BP/buck increased insignificantly than those of the control. Total feed intake (g) for treated bucks were significantly ($P<0.01$) decreased by about 4.4 and 8.5% as compared with the control group. This improvement in feed intake could be attributed to the beneficial effects of the flavonoids in bee pollen, which improved the digestion and absorption of protein and enhanced its utilization. The improved performance of bucks may be due to the improved crude protein digestibility, which in turn improved the intestinal absorptive capacity and the utilization of nutrients and protein.

These results agree with El-Hanoun *et al.,* (2007), found that NZW rabbit does orally treated with propolis at 100, 200, and 300 mg/kg BW increased significantly the live body weight than those in control group. In contrast, the result of Branislav Gálik *et al.,* (2016) showed that the body weight gain and feed conversion ratio of female rats were significantly ($P<0.05$) affected by treatment with bee pollen as compared to the control group.

Also, the achieved findings showed that the total feed intake (g) for treated bucks were significantly ($P<0.01$) decreased by about 4.4 and 8.5% as compared with the control group. This improvement in feed intake could be attributed to the beneficial effects of the flavonoids in bee pollen, which improved the digestion and absorption of protein and enhanced its utilization. These results are in agreement with the findings of Abou El-Naga (2014), which indicated that the feed intake of treated Norfa chickens with 1 or 2% BP decreased significantly ($P\leq0.05$) than those of the control.

The final body weight, total weight gain and total feed intake of rabbit bucks were insignificantly affected by the interaction between rabbit line and BP treatment.

No mortality cases were recorded in the different groups all-over the experimental period, which lasted 56 days.

Table 2. Productive traits of rabbit bucks of rabbit bucks affected by line, BP treatment and their interaction.

Traits→ Rabbit line ↓ Treatment ↓		Initial body weight (g)	Final body weight (g)	Total weight gain (g)	Total feed intake (g)
Effect of rabbit line (B)					
V-Line		3067.66	3221.66	154.00	12435.67^{a}
Moshtohor		3101.33	3264.66	163.33	12218.20^{b}
SEM		40.29	36.15	19.42	59.26
Significance		**NS**	**NS**	**NS**	****
Effect of BP treatment (T)					
Control (C)		3027.00	3176.00^{b}	149.00	12456.30^{a}
500mg (T1)		3074.50	3227.50^{ab}	153.00	12230.00^{b}
1000mg (T2)		3152.00	3326.00^{a}	174.00	12294.50^{ab}
SEM		49.34	44.28	23.78	72.58
Significance		**NS**	*****	**NS**	*****
Effect of interaction (B×T)					
V-Line	**Control**	2964.00	3128.00	164.00	12510.00
	500mg	3049.00	3197.00	148.00	12373.00
	1000mg	3190.00	3340.00	150.00	12424.00
Moshtohor	**Control**	3090.00	3224.00	134.00	12402.60
	500mg	3100.00	3258.00	198.00	12087.00
	1000mg	3114.00	3312.00	158.00	12165.00
SEM		69.79	62.62	33.64	102.65
Significance		**NS**	**NS**	**NS**	**NS**

$^{A, b, c}$ Means with different superscripts in the same column for every factor are significantly different (P< 0.05).
NS= Not significance, * = P < 0.05 and ** = P < 0.01.

Hematological parameters

Data presented in Table 3, showed that the Moshtohor rabbit bucks had significantly higher counts of WBCS (10^3) as well as higher levels of Hg, HTC and MCV, respectively than those of V-line. However, RBCs (10^6) for Moshtohor rabbit bucks was insignificantly higher than those of V-line bucks. This improvement may be due to the improved hepatic functions, which positively reflected on the physiological responses of the body. These findings agree with Khalil *et al*., (2015), who found that the RBCs of Baladi rabbit bucks increased significantly (P<0.01) than those of NZW bucks.

Regarding BP treatment, the obtained results revealed that the means of WBCs (10^3), RBCs (10^6), Hg (g/dl), HTC (%) and MCV (%) were significantly (P<0.01 or 0.05) increased for treated bucks than those of the control group.

The increased Hg, HTC, and RBCs of treated bucks could reflect the beneficial role of bee pollen in enhancing immunological functions. This means that the treatment with bee pollen increased the mean of RBCs, which consequently improved both of the hematocrit and hemoglobin levels. These findings are in agreement with those of Song *et al.*, (2005), who reported that the treated rations of broilers with adequate levels of BP improved the cellular immunological responses through enhanced production of antibodies and increased acquired immunity. Similarly, the results of El-Neney and El-Kholy (2014) indicated that the RBC's and WBC's counts increased significantly ($P \leq 0.05$) in the supplemented rabbits with BP than those of the control. The obtained results showed no significant effects on the hematological traits due to the interaction between rabbit line and BP treatment.

Table 3. Hematological parametres of rabbit bucks affected by line, BP treatment and their interaction.

Traits→ Rabbit line ↓ Treatment ↓		**RBC (10^6)**	**WBC (10^3)**	**Hg (g/dl)**	**HCT (%)**	**MCV (%)**	**MCH (%)**	**MCHC (%)**
Effect of rabbit line (B)								
V-Line		4.15	4.54^{b}	11.38^{b}	27.16^{b}	69.10^{b}	31.93^{b}	40.77
Moshtohor		3.93	5.38^{a}	12.32^{a}	33.48^{a}	80.39^{a}	34.32^{a}	38.53
SEM		0.10	0.19	0.16	0.83	1.93	0.82	1.53
Significance		NS	**	**	**	**	*	NS
Effect of BP treatment (T)								
Control (C)		3.72^{b}	4.41^{b}	11.49^{b}	28.16^{b}	70.33^{b}	33.22	38.52
500mg (T1)		4.20^{a}	4.85^{b}	12.11^{a}	32.07^{a}	73.50^{b}	31.98	39.51
1000mg (T2)		4.20^{a}	5.61^{a}	11.96^{ab}	30.74^{ab}	80.41^{a}	34.17	40.92
SEM		0.13	0.23	0.19	1.02	2.37	1.01	1.88
Significance		**	**	*	*	**	NS	NS
Effect of interaction (B×T)								
V-Line	**Control**	3.67	3.66	10.75	24.97	68.40	31.37	40.62
	500mg	4.39	4.37	11.71	29.00	67.25	30.31	38.75
	1000mg	4.38	5.58	11.70	27.53	71.65	34.12	42.92
Moshtohor	**Control**	3.76	5.16	12.23	31.34	72.27	35.07	36.41
	500mg	4.01	5.34	12.52	35.15	79.75	33.67	40.27
	1000mg	4.03	5.65	12.22	33.96	89.17	34.23	38.92
SEM		0.18	0.33	0.27	1.44	3.35	1.43	2.66
Significance		NS	NS	NS	NS	NS	NS	NS

[A, b, c] Means with different superscripts in the same column for every factor are significantly different ($P < 0.05$). Number of samples = 90, NS= Not significance, * = $P < 0.05$ and ** = $P < 0.01$. WBC= White blood cells (103), RBC= Red blood cells (106), HCT= Hematocrite, Hg= Hemoglobin

Blood biochemical analysis:

Data in Table 4 showed that the total protein concentration were significantly (P<0.01) affected by rabbit line, while the albumin and globulin levels were not influenced. The fluctuated blood proteins in V-line and Moshtohor bucks could reflect the hepatic functions in both lines. These findings agree with of Abdel-Hamid and Farahat (2015), who stated that the blood protein concentrations varied highly significant (P<0.01) between the different lines.

The means of glucose (g/dl) in V-line rabbit bucks increased significantly (*P*<0.01), while the means of cholesterol and total antioxidant capacity were not affected as compared with those of Moshtohor bucks. These variations in glucose level could reflect the increased glucose utilization, which increased the secretions of catabolic and anabolic enzymes, improving the metabolic rate in rabbit bucks.

Referring to BP treatment, the obtained results showed that the rabbit bucks treated with BP had the highest total protein (P<0.01) and albumin (P<0.05) concentrations than those of bucks in the control. This significant increase may be attributed to the improved crude protein digestibility, which in turn increased the amino acids supplementation for bucks.

These findings agree with the results of Elnagar *et al.,* (2010), who reported that the serum total protein in growing rabbits, which were orally given 200, 400 and 800 mg RJ/kg BW once a week increased significantly than those of the control group. Similarly, the findings of Attia *et al.,* (2014) indicated that albumin and globulin concentrations were insignificantly influenced by BP supplementation in the diet of growing rabbits.

The obtained results indicated that the treated bucks with 500 and 1000mg BP/buck increased significantly (P<0.01) the glucose (g/dl) level and the total antioxidant capacity, while the cholesterol (mg/dl) concentration decreased significantly (P<0.001) than those of the control.

The increased serum glucose level in the treated bucks with BP may be attributed to the high level of carbohydrates present in BP, which consequently support and raise the glucose level in the blood, needed to cover the physiological requirements of the body. The lower cholesterol concentration in the treated bucks with BP may be attributed to the role of BP in lipid metabolism, which prevents the accumulation of lipid peroxidation products.

These findings agree with those of Branislav Gálik *et al.,* (2016), who found that the serum total protein and glucose concentrations were significantly (P<0.05) increased in rats treated with bee pollen as compared with those of the control.

Table 4. Blood biochemical analysis of rabbit buck affected by line, BP treatment and their interaction.

Traits→ Rabbit line ↓ Treatment ↓		Blood proteins			Glucose (g/dl)	Cholesterol (mg/dl)	TAC (mm/l)
		Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)			
Effect of rabbit line (B)							
V-Line		7.59[b]	4.25	3.45	123.70[a]	71.97	0.66
Moshtohor		7.84[a]	4.39	3.34	119.27[b]	75.53	0.57
SEM		0.06	0.05	0.06	0.99	2.07	0.04
Significance		**	NS	NS	**	NS	NS
Effect of BP treatment (T)							
Control (C)		7.51[b]	4.18[b]	3.32	110.45[c]	83.55[a]	0.45[b]
500mg (T1)		7.80[a]	4.38[a]	3.42	123.50[b]	70.25[b]	0.64[a]
1000mg (T2)		7.85[a]	4.40[a]	3.45	130.50[a]	67.45[b]	0.75[a]
SEM		0.07	0.06	0.08	1.22	2.50	0.05
Significance		**	*	NS	**	**	**
Effect of interaction (B×T)							
V-Line	**Control**	7.40	4.17	3.23	107.90	84.30	0.40
	500mg	7.63	4.27	3.36	123.50	71.00	0.63
	1000mg	7.76	4.33	3.23	126.40	71.30	0.69
Moshtohor	**Control**	7.62	4.20	3.42	113.00	82.80	0.51
	500mg	7.96	4.48	3.48	123.50	69.50	0.65
	1000mg	7.95	4.48	3.47	134.60	63.60	0.82
SEM		0.10	0.09	0.11	1.72	3.59	0.07
Significance		NS	NS	NS	*	NS	NS

[A, b, c] Means with different superscripts in the same column for every factor are significantly different (P< 0.05). Number of samples = 60, NS= Not significance, * = P < 0.05 and ** = P < 0.01. TAC= Total antioxidant capacity

The increased total antioxidant capacity in the plasma of bucks treated with BP may be due to the increased the absorption of vitamins, amino acids and trace elements from the bee pollen, which consequently improved the proliferation, development, and differentiation of the intestinal cells and microbial activity conditions. These findings are in agreement with those of Attia *et al.,* (2014), who found a significant increase in the total antioxidant capacity of rabbits fed a commercial ration supplemented with BP as compared to control group.

The interaction between rabbit line and BP treatment showed insignificant differences in blood proteins, cholesterol and total antioxidant capacity, while the level of glucose was significantly (P<0.01) differed.

Semen physical characteristics

The findings in Table 5 indicated that the reaction time (sec.), live sperms (%), mass motility (%), Conc./ml (10^6) and Conc./Ej. (10^6) of Moshtohor rabbit bucks improved significantly ($P<0.001$) than those of the V-line. This improvement of Moshtohor bucks may be due to the higher testosterone concentration than that of V-line. These findings agree with those of Iraqi *et al*., (2012), who found that the mean ejaculate volume of V-line bucks decreased insignificantly than Moshtohor bucks. In contrast, they added that the sperm cell concentration ($x10^6$) in V-line rabbit bucks increased significantly than that of the Moshtohor. Also, the higher live body weight of the mature rabbit bucks had a relative retarding effect on the sexual libido and semen characteristics, than the bucks having lower body weight (Rodríguez-De Lara *et al.*, 2015).

Regarding the treatment with BP, the achieved findings showed that the reaction time (sec.) in the treated groups decreased significantly than that of the control. The decreased reaction time in treated groups could be considered as indications of the higher sexual activity and the more secretion of testosterone hormone. These findings are in agreement with those of El-Hanoun *et al.* (2014), which indicated that the sexual libido was significantly reduced in V-line rabbit bucks, which received RJ at 100 and 150 mg/kg than that of the control group. Also, Khadr *et al*., (2015) found that the reaction time (RT) of male NZW rabbits treated with both of royal jelly and honey decreased significantly ($P<0.05$) as compared with those of the control group. Similarly, the findings of Elnagar (2010) showed that the sexual libido and semen characteristics of rabbit bucks administrated with royal jelly or/ and honey increased significantly than those of the control.

The ejaculate volume (ml), live sperms (%), mass motility (%), Conc./ml (10^6), Conc./Ej. (10^6) and sperm abnormalities (%) in the treated groups improved significantly than those of the control. The significant improvement in sperm concentrations in the treated groups may be attributed to increased LH hormone concentration, which plays an important role in promoting the secretion of testosterone hormone from Leydig cell, which results in stimulating the germinal cells to produce more sperms. These results agree with those of El-Mougy *et al.*, (2015). Similar results were observed by Karacal and Aral (2008), who found higher sperm concentrations in male mice treated with royal jelly than that of the control group. Also, the findings of El-Kelawy and Aboulnaga (1995) showed that the ejaculate volume as well as both of sperm motility and concentrations of male rabbits manipulated with testosterone, increased significantly than that of the control.

In the present experiment, the daily treatment with both of 500 and 1000 mg BP/ buck minimized significantly the abnormal sperm percentages. These results agree with those of Karacal and Aral (2008), who stated that the lower abnormal sperm percentages were obtained in male mice treated with RJ, while the higher percentage was recorded in the control group. Also, the results of Wang *et al.*, (2002) showed that the sperm quality, activity, and density were significantly increased ($P<0.05$) in breeder cocks exposed to heat stress and manipulated with pollen at 1 and 1.5% levels than those of the control.

The interaction between rabbit line and BP treatment had insignificant effects on all semen characteristics except both of concentration per ml and concentration per ejaculate.

Table 5. Pysical semen charachteristecs of rabbit bucks affected by line, BP treatment and their interaction.

Traits→ Rabbit line ↓ Treatment ↓		Reaction time (Sec.)	Ejaculate volume (ml)	Live sperms (%)	Mass motility (%)	Conc./ ml (10^6)	Conc./ Ej. (10^6)	Sperm Ab. (%)
Effect of rabbit line (B)								
V-Line		13.61^a	0.79	71.70^b	60.66^b	227.83^b	180.57^b	19.43
Moshtohor		10.86^b	0.84	74.95^a	64.54^a	300.00^a	252.83^a	18.99
SEM		0.28	0.02	0.70	0.63	5.18	7.55	0.56
Significance		**	NS	**	**	**	**	NS
Effect of BP treatment (T)								
Control (C)		15.52^a	0.72^b	65.18^c	56.94^b	229.49^c	167.70^c	22.11^a
500mg (T1)		10.96^b	0.84^a	76.06^b	65.81^a	258.64^b	217.78^b	17.27^b
1000mg (T2)		10.24^b	0.87^a	78.75^a	65.06^a	303.62^a	264.62^a	18.25^b
SEM		**0.34**	**0.03**	**0.86**	**0.78**	**6.35**	**9.24**	**0.69**
Significance		**	**	**	**	**	**	**
Effect of interaction (B×T)								
V-Line	**Control**	16.55	0.67	62.37	55.00	204.07	138.68	23.47
	500mg	12.45	0.82	75.87	63.87	241.20	195.27	16.35
	1000mg	11.85	0.87	76.87	63.12	238.22	207.76	18.47
Moshtohor	**Control**	14.50	0.76	68.00	58.87	254.90	196.72	20.75
	500mg	9.47	0.87	76.25	67.75	276.07	240.29	18.20
	1000mg	8.62	0.88	80.62	67.00	369.02	321.48	18.02
SEM		**0.49**	**0.04**	**1.21**	**1.10**	**8.98**	**13.08**	**0.97**
Significance		**NS**	**NS**	**NS**	**NS**	**	*	**Ns**

[A, b, c] Means with different superscripts in the same column for every factor are significantly different ($P< 0.05$). Number of semen samples = 300, NS= Not significance, * = $P < 0.05$ and ** = $P < 0.01$. Conc./ml= Concentrate per ml, Conc./Ej.= Concentrate per ejaculate (10^6) and sperm Ab.= Sperm abnormalities (%)

Hormonal assay:

Table 6 shows the effects of bee pollen level on malondialdehyde, glutathione and testosterone hormone in rabbit bucks. The highest malondialdehyde concentrations were recorded in the serum and seminal plasma, while the lowest glutathione concentrations were found in Moshtohor than in V-line bucks without significant differences.

Table 6. Antioxidant enzyme activities and testosterone hormone affected by rabbit line, BP treatment and their interaction.

Traits→ Rabbit line ↓ Treatment ↓		**Malondialdehyde (nmol/ml)**		**Glutathione content (nmol/ml)**		**Testosterone (ng/ml) (90)**
		Serum	**Seminal plasma**	**Serum**	**Seminal plasma**	
Effect of rabbit line (B)						
V-Line		5.26	1.68	86.29	45.43	6.80^{b}
Moshtohor		4.49	1.60	87.66	46.13	8.17^{a}
SEM		0.29	0.08	1.35	0.85	0.17
Significance		**NS**	**NS**	**NS**	**NS**	**
Effect of BP treatment (T)						
Control (C)		6.18^{a}	1.82	82.16^{b}	40.10^{c}	5.06^{c}
500mg (T1)		4.28^{b}	1.58	88.50^{a}	46.80^{b}	8.22^{b}
1000mg (T2)		4.16^{b}	1.52	90.28^{a}	50.45^{a}	9.18^{a}
SEM		0.35	0.10	1.65	1.04	0.21
Significance		**	**NS**	**	**	**
Effect of interaction (B×T)						
V-Line	**Control**	6.73	1.86	83.20	39.40	4.40
	500mg	4.52	1.63	86.60	46.40	8.35
	1000mg	4.52	1.55	89.09	50.50	7.65
Moshtohor	**Control**	5.63	1.78	81.12	40.80	5.72
	500mg	4.04	1.53	90.40	47.20	8.09
	1000mg	3.80	1.49	91.47	50.40	10.71
SEM		0.50	0.14	2.34	1.47	0.29
Significance		**NS**	**NS**	**NS**	**NS**	**

[A, b, c] Means with different superscripts in the same column for every factor are significantly different (P< 0.05). NS= Not significant, * = P < 0.05 and ** = P < 0.01. Number of MDA and GSH samples = 60, while testosterone samples = 90

The testosterone hormone of Moshtohor rabbit bucks was significantly increased than that of the V-line. This increase could be attributed to its improved sexual activity as compared with that of V-line, which shows that V-line bucks were more sensitive to the high ambient temperature throughout the experimental period. These results agree with those of EL-

Kamash *et al*., (2000), who stated that the remarkable variations in the testosterone hormone concentrations between the different lines could be attributed to the marked differences in the activity of accessory sex glands, which reflect on the ejaculate volume.

With regard to the treatment with bee pollen, the obtained results indicated that the malondialdehyde and testosterone hormone concentrations of the treated bucks rabbit were significantly increased ($P<0.01$), while the glutathione concentration was significantly ($P<0.01$) decreased. The improved malondialdehyde, glutathione and testosterone hormone concentrations in rabbit bucks treated with BP at 500 and 1000mg/buck could be attributed to its high content of phospholipids, antioxidant factors, vitamins and minerals (Saric´ *et al*., 2009), which could improved both of fertility and semen quality (Xu *et al*., 2009 and Attia *et al*., 2011).

These results agree with those of Kughn (2010), who found that the administered rations with pollen can benefit to secrete FSH, LH, and testosterone in the serum of Yangzhou cocks. Similarly, Liu *et al*., (2009) found that the hy-line breeder cocks supplemented with bee pollen at 0.5, 1, 1.5, 2 and 2.5% increased the concentrations of plasma reproductive hormones, luteinizing hormone and testosterone concentration than those of the control group.

The obtained results showed insignificant effects on malondialdehyde and glutathione content of rabbit bucks due to interaction (B×T), while the testosterone hormone concentration was significantly ($P<0.01$) influenced.

Conclusively, it could be concluded that administering rabbit bucks with daily 500 and 1000mg BP/buck could be highly recommended to improve semen characteristics, blood parameters, antioxidant activities, and testosterone hormone as well as the production performance of rabbit bucks under Upper Egypt climatic conditions especially by small producers.

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الخصائص الطبيعية للسائل المنوى، صفات الدم وبعض التقديرات الفسيولوجية لذكور الارانب المعاملة بحبوب اللقاح تحت الظروف المناخية بصعيد مصر

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أجريت هذه الدراسة لتقييم تأثير معاملة ذكور الأرانب يومياً بحبوب اللقاح عند مستوى ٥٠٠ و ١٠٠٠ ملجم/ ذكر في صورة كبسولات على الخصائص الطبيعية للسائل المنوي، وتم دراسة بعض المقاييس الهيماتولوجية و الفسيولوجية لذكور الأرانب المعرضة لدرجات الحرارة المرتفعة تحت الظروف السائدة بصعيد مصر.

اشتملت هذه الدراسة على عدد ٣٠ ذكر أرنب بواقع (١٥ V-line و١٥ مشتهر)، عند عمر ٥٢ اسبوع، وقسمت بالتساوي إلى ثلاث مجموعات تجريبية. ولقد تم تغذية ذكور المجموعة الأولى على عليقة تجارية واعتبرت مجموعة الكنترول، بينما غذيت مثيلاتها في المجموعتين الثانية والثالثة على نفس العليقة مع إعطائها حبوب اللقاح عند مستوى ٥٠٠ و ١٠٠٠ ملجم/ ذكر في صورة كبسولات على مدار التجربة التي استمرت ٥٦ يوم.

أوضحت النتائج وجود زيادة معنوية بعدد كرات الدم البيضاء ، و مستوي الهيموجلوبين ، الهيماتوكريت والبروتين الكلى لذكور أرانب المشتهر ، بينما قلت معنوياً الكمية الكلية للغذاء المستهلك ومستوى الجلوكوز بالمقارنة بذكور أرانب الـ V-line.

كما أوضحت النتائج وجود زيادة معنوية (عند مستوى ١%) بنسبة الحيوانات المنوية الحية ، والحركة الكلية ، تركيز الحيوانات المنوية بالمل ، تركيز الحيوانات المنوية بالقذفة وتركيز هرمون التستستيرون بذكور أرانب المشتهر بحوالي ٤,٣، ٦,٠، ٢٤، ٢٨,٦ و ١٦,٨% ، بينما انخفض الوقت اللازم للتلقيح معنوياً بحوالي ٢٠,٢% وذلك بالمقارنة بذكور أرانب الـ V-line.

ازداد معنوياً (عند مستوى ١%) متوسط وزن الجسم النهائي ، عدد كرات الدم الحمراء ، عدد كرات الدم البيضاء ، الهيموجلوبين الهيماتوكريت ، البروتين الكلى ، الألبيومين ، والجلوكوز و الكفاءة الكلية لمضادات الأكسدة بذكور الأرانب المعاملة بحبوب اللقاح بمستوى ٥٠٠ و ١٠٠٠ ملجم مقارنة بذكور مجموعة الكنترول. وعلى العكس، نتج عن المعاملة بحبوب اللقاح انخفاض في كمية الغذاء المستهلك ومستوى الكوليستيرول.

ولقد ازداد معنوياً (عند مستوى ١%) متوسط حجم القذفة ، ونسبة الحيوانات المنوية الحية ، والحركة التقدمية ، تركيز الحيوانات بالمل و بالقذفة في ذكور الأرانب المعاملة بحبوب اللقاح، بينما قلت الفترة اللازمة للتلقيح ونسبة الحيوانات المنوية الشاذة بالمقارنة بمثيلاتها بمجموعة الكنترول.

وبالمثل فقد ازداد تركيز الجلوتاثيون وهرمون التستستيرون معنوياً في الذكور المعاملة بحبوب اللقاح، بينما انخفض معنوياً تركيز malondialdehyde بالمقارنة بمجموعة الكنترول.

ومن النتائج السابقة يمكن أن نخلص إلى أن معاملة ذكور الأرانب بحبوب اللقاح عند مستوى ٥٠٠ أو ١٠٠٠ملجم/ذكر تحت درجات الحرارة المرتفعة قد أدى إلى تقليل كمية الغذاء المستهلك، وتحسين مقاييس الدم، خصائص السائل المنوي، نشاط مضادات الأكسدة بالإضافة الى هرمون التستستيرون.

التوصية: أوصت الدراسة بمعاملة ذكور الأرانب بـ ٥٠٠ ملجم حبوب لقاح/ ذكر يوميا لتحسين خصائص السائل المنوي و بعض المقاييس الفسيولوجية للارانب المرباة تحت درجات الحرارة المرتفعة.