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STIMULATORY EFFECT OF THE BEE POLLEN ADMINISTRATION ON GROWTH AND REPRODUCTIVE PERFORMANCE IN MALE RABBITS

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This study aimed to evaluate the effect of orally bee-pollen (BP) administration as a feed additive on performance of male rabbits during pre- and post-puberty period. Ninety-six weaned New-Zealand White (NZW) male rabbits aged 35 days and weighing 658 g ± 6.30 were divided into four groups (24 in each). All animals were fed the same commercial diet and receiving a water solution containing 0 (control, G_1), 200 (G_2), 300 (G_3) and 400 mg (G_4) bee pollen/kg body weight, respectively, once-daily during the experimental period (184 days).Daily weight gain and daily feed intake were recorded from weaning up to 92 d of age (marketing age). At this time, four rabbits from each group were slaughtered and immediately their genitalia were taken and dissected. Blood samples were collected at 120 and 150 d of age from five bucksof each group. At maturity age reproductive traits were recorded. Results revealed that orally BP administration at the highest level (G_4) significantly ($P\leq0.01$) increased the final body weight, daily weight gain, feed efficiency and plasma testosterone concentration. The earlier puberty age was obtained by 400 mg BP/kg BW administration. Libido, mating activity, advanced-sperm motility, alive spermatozoa and morphological normal spermatozoa were significantly ($P\leq0.05$) increased by orally BP administration, being optimized for 400 mg/kg BW. Histological examination of the testis and epididymis of treated rabbitsrevealed an increased seminiferous tubule diameter with active spermatogenic cells when compared with control group.

***In conclusion**, the addition of 400 mg/kg BW to the growing and mature NZW male rabbit diets improved growth and reproductive performance.*

Key words: Male rabbits, bee pollen, growth performance, reproduction, semen quality.

The scientific literature provides numerous attempts to promote the rabbit production and reproduction using some commercial growth promoters (Ashour *et al.*, 2004; El-Kholy *et al.*, 2012). During the last decade, interest in the study of phenolic compounds has increased greatly, mainly due to the antioxidant capacity of these substances in scavenging free radicals that are harmful to human and animal health (Prelipcean, 2012 and Capcarova *et al.*, 2013). One of those widely used natural supplements is bee pollen. Bee pollen (BP) is an agglomerate of pollen grains from various botanical sources, which are collected by the bees and mixed with nectar and secretion from the hypopharyngeal glands, such as α and β-glycosidase enzymes (Silva *et al.*, 2006). It contains many essential nutritional elements important for growth and development of animals and humans (Orzaez Villanueva *et al.*, 2002 and Haščík *et al.*, 2011). Bee pollen is a rich source of protein (25%); 23 essential amino acids; oil (6%), containing more than 51% polyunsaturated fatty acids of which 39% linolenic (omega-3), 20% palmitic and 13% linoleic acids (omega-6); more than 12 vitamins; 21 minerals (calcium, chlorine, copper, iron, magnesium, iodine, molybdenum, selenium, strontium, stannum, boron, fluoride, vanadium, chromium, phosphorus, potassium, sulfur, aluminum, iron, manganese and zinc); 11 enzymes or co-enzymes; 11 carbohydrates (35–61%) which are mainly glucose, fructose and sucrose; flavonoids and carotenoids; phytosterols (Xu *et al.*, 2009). Carpes *et al.* (2009) and Campos *et al.* (2010) demonstrated that these nutrients of BP are the reason for why BP is used by humans as an alternative food source and/or food supplement. In addition, BP is a product with added value because it can also be used for certain health benefits such as high contents of polyphenolic substances, chiefly flavonoids with antioxidant (Prelipcean, 2012 and Capcarova *et al.*, 2013), and an anti-inflammatory, and / or an antimicrobial agent (García *et al.*, 2001 and Campos *et al.*, 2010) and antifungal (García *et al.*, 2001). In bee pollen, it was isolated and purified the flavonoids demarcating with strong antioxidant ability as quercetin-7-rhamnoside, kaempferol-3-glucoside, isorh-amnetin, kaempferol and quercetin (Maruyama *et al.* 2010). Also, Graikou *et al.* (2011) reported that the pollen is rich in flavonoids and phenolic acids which indicate the observed free radical scavenging activity. Because of its nutrient-rich components, it has been used as a folk medicine for centuries, to alleviate or cure conditions such as cold, heat stress, flu, ulcer, premature aging, anemia, colitis, enteritis and allergic reactions (Šarić *et al.*, 2009).

According to our knowledge, studies on the addition of BP to rabbits under Egyptian conditions during growing period are somewhat limited (Attia *et al.*, 2014). El-Hanoun *et al.* (2007) and Attia *et al.* (2011b) found

that growing rabbits administrated bee pollen at 250 and 500 mg/kg BW significantly increased growth and survival rate during growing period. Attia *et al.* (2011a) showed that oral BP administration at 200 mg/kg BW, twice per week, significantly ($P<0.01$) improved semen quality and increased fertility percentage of rabbit bucks. On the other hand, no mammalian studies have ever examined the long-run effects of sequence BP addition to males from weaning until sexual maturity.

Therefore, the purpose of the present study was to evaluate the response of some productive and reproductive traits at pre and post puberty of NZW rabbit males affected by oral BP administration. The study also aimed to establish the optimum level of BP for male rabbits.

MATERIALS AND METHODS

Experimental animals and management:

Ninety-six weaned New Zealand White (NZW) male rabbits aged 35 days and weighed 658 g ± 6.30 (mean ±SE) were equally and randomly divided into four groups (24 in each). The first group was used as a control. Animals in the second, third and fourth groups were treated orally administration with 200, 300 and 400 mg BP/kg body weight, once-daily during the experimental period (184 days). The pollen was collected from clover (*Trefoil alexandrinum*) using special pollen traps from bee hives located at Menoufyia Governate, Egypt. Firstly, the BP was ground and this ground was stored at 2–8 °C in desiccators. Microscopic examination demonstrated a purity of greater than 97%, with less than 0.5% foreign BP and less than 0.9% plant parts. The powder of BP was suspended in tap water and mixed vigorously for 5 min. Chemical composition of BP was determined in duplicate according to AOAC (2000) and it contained (% DM): 30.53 crude protein, 4.87 ether extract, 61.01 nitrogen free extract and 1.09 crude fibre. Animals were fed *ad libitum* a commercial pelleted diet according to NRC (1977) recommendations. Ingredients and chemical composition of the diet are shown in Table 1.

All the experimental animals were healthy and clinically free from internal and external parasites and were kept under the same management and hygienic conditions.

Experimental procedure

The averages of daily weight gain (ADG, g/d) and daily feed intake (FI, g/d) were recorded weekly for each rabbit during the growing period (from the weaning age up to marketing age at 92 d). Feed efficiency (FE, g/g) was calculated as a ratio of g gain/g feed. At the end of this period, feeding economic efficiency (EE) was calculated according to the prices of commercial

Table 1: Ingredients and chemical composition of the commercial pelleted diet.

Items	Dietary treatments (CP %)
Ingredients, %	
Yellow corn	5.20
Soybean meal (48%)	6.50
Wheat bran	29.52
Corn gluten meal (60%)	3.50
Clover hay	38.68
Barley	10.40
Molasses	3.00
L-Lysine HCl	0.05
Limestone	1.00
Dicalcium-phosphate	1.50
NaCl	0.35
Premix*	0.30
Total	**100**
Calculated analysis**	
DE, Kcal/ Kg	2520
Crude protein (CP)	17.20
Ether extract (EE)	2.69
NFE	56.08
Ash	9.93
Fiber fractions:	
Crude fiber (CF)	14.10
NDF %	35.76
ADF %	21.66
Hemicellulose	14.00
Cellulose	10.88
ADL%	10.47
Ca %	1.32
Total P %	0.54
Lys %	0.53
Meth %	0.18

*** Each 3 kg of vit and Min in Premix contain**: 6000000IU Vit A, 900000 IU Vit D3 40000mg Vit E,2000mg Vit K, 2000mg Vit.B1, 4000mg Vit.B2, 2000mg Vit. B6, 10mg Vit..B12, 50000mg Niacin, 10000 mg pantothenic acid, 50mg Biotin, 3000mg Folic acid, 250000 mg choline, 50000mg Zn, 8500mg Mn, 50000mg Fe, 50000mg Cu, 200mg I , 100mg Se and 100mg Co.

** According to NRC (1977).

pelleted diet, additives and rabbit meat prevailing during year 2013.

At marketing age, four growing male rabbits from each experimental group were randomly chosen and slaughtered (by bleeding). Genitalia were

taken immediately after the rabbits slaughter, and its dissection was performed. Weights of each of pituitary gland, testes, epididymis and sexual accessory glands were recorded.

Blood samples, about 3 ml, were collected at 120 and 150 d of age between 08.00 and 09.00h from the marginal ear vein of five bucks from each group. Plasma was separated by centrifugation at 3000 r.p.m. for 20 minutes and kept at -20 ^{0}C until hormonal assay. Blood serum testosterone (T) hormone concentration of the rabbit males was determined using RIA kits (Immunotech, A Coulter Co., Czech Republic) according to the manufacturer information. Minimum detectable limit was 0.20 ng/ml and inter- and intra-assay coefficients of variation for T assay were 10.8 and 5%, respectively. All samples were run in duplicate and assayed by the same investigator, who was blind to the experimental situation.

At the age of maturity, weight and age of fifteen rabbit bucks from each group at puberty (first mating) were recorded. Scrotal circumference (n = 10 rabbits per group) was measured as the method described by Boiti *et al.* (2005). Testicular index (length×width×depth) (n=10 rabbits per group) was calculated in cubic centimetres as recorded by Castellini *et al.* (2006). At 5 month of age, semen was collected artificially twice a week from ten bucks from each group during experimental period by means of an artificial vagina as described by Boiti *et al.* (2005). Immediately after collection, semen ejaculate volume (ml), advanced sperm motility (%), alive spermatozoa (%), morphological normal spermatozoa (%), acrosomal damages (%), sperm-cell concentration (N×10^6/ml) and total-sperm output (N×10^6/ejaculate) were estimated according to Boiti *et al.* (2005) and Castellini *et al.* (2006). Libido (sexual desire) was assessed in terms of reaction time in seconds that was estimated just from the time of introducing doe to the buck until the buck start to mount (Castellini *et al.*, 2006). Mating activity (frequency of mating within 15 minutes) of ten bucks was determined using sexually receptive doe.

Histopathological features :

At the end of 6 month of age, sections of tissue samples from different parts of genitalia (5 µm-thicknesses) from 3 bucks from each group were taken for histopathological examinations. These sections were fixed in Boun's solution then processed by standard technique and stained with haematexylin and Eosin (H and E) and examined with a light microscope (Mescher, 2013).

Housing

This study was carried out in an Industrial Rabbitry, Tohoria village, near Shebeen El-Kanater City, Qalubia Province, Egypt, from May till

October, 2013. Rabbits were housed separately in individual cages (35 × 35 × 60 cm) of conventional universal galvanized wire batteries. All cages were equipped with feeding hoppers, which made of galvanized steel sheets and nipples for automatic drinking. The batteries were located in a well-ventilated building. Averages of ambient temperature (AT, ^{o}C) and relative humidity (RH, %) inside building were determined weekly. Then, the temperature humidity index (THI, units) was calculated using the equation modified by Marai *et al.* (2001) as follow:

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

Where: db ^{o}C = Dry bulb temperature in Celsius, RH= Relative humidity percentage/100.

The values obtained are then classified as absence of 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).

Statistical analysis

Data were statistically analyzed according to SPSS (2013) computer program using the following fixed model :-

$$Y_i = \mu + B_i + e_i$$

Where: Y_i = Observations of the i^{th} rabbit; μ = Overall mean, common element to all observations; B_i = Effect of the treatments (i = 1, 2, 3 & 4) and e_i = Random error component assumed to be normally distributed. Data presented as percentages were transformed to the corresponding arcsine values (Warren and Gregory, 2005) before being statistically analyzed. The differences between means were tested by using Duncans Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Climatic conditions :

Averages of AT, RH and THI during the whole experimental period are shown in Table 2. The THI data clearly indicated very severe of heat stress conditions (more than 30) during weaning and severe heat stress during pre-puberty periods. While during the post-puberty period rabbit bucks exposed to moderate heat stress according to estimated THI value (27.8). It was suggested that the optimal temperature humidity index for the rabbit husbandry is 27.8 (Marai *et al.*, 2002).

Growth performance

Data presented in Table 3, in general, showed that, final body weight (BW) and body weight gain (BWG) values of growing NZW rabbit males were

Table 2: Averages of ambient temperature (AT, °C), relative humidity (RH, %) and temperature humidity index (THI, units) during weaning, pre-and post puberty periods.

Periods	Months	AT (°C)	RH (%)	THI (Units)
Weaning	May 25	30.5 ± 0.29	68.0 ± 0.66	28.9 ± 051
	June	32.8 ± 0.34	70.8 ± 0.48	31.1 ± 0.62
	July 20	32.4 ± 0.44	71.3 ± 0.50	30.8 ± 0.61
	Average	**31.9 ± 0.54**	**70.0 ± 0.53**	**30.3 ± 0.58**
Pre-puberty	July 21	32.0 ± 0.45	70.0 ± 0.48	30.4 ± 0.56
	August	33.4 ± 0.37	71.2 ± 0.54	31.7 ± 0.55
	September	31.0 ± 0.35	70.4 ± 0.66	29.5 ± 0.55
	October 26	29.0 ± 0.44	70.0 ± 0.58	27.6 ± 0.49
	Average	**31.4 ± 0.40**	**70.4 ± 0.57**	**29.8 ± 0.56**
Post-puberty	October 27	29.7 ± 0.43	71.0 ± 0.60	28.3 ± 0.50
	November	28.5 ± 0.40	70.0 ± 0.62	27.2 ± 0.56
	Average	**29.1 ± 0.42**	**71.5 ± 0.661**	**27.8 ± 0.53**

significantly increased ($P \leq 0.05$) with BP inclusion compared to the control group. While, daily FI and FC were significantly ($P \leq 0.05$) decreased with increasing the BP inclusion. These results are in agreement with the results of Attia *et al.* (2011b and 2014) and Dias *et al.* (2013). The decrease in daily feed intake by adding BP may be due to an increase in the efficiency of nutrition absorption and/or nutrients utilization. Due to the lower feed intake, feed conversion rate was lower in treated groups compared to control group. Similar results were observed by El-Kholy *et al*. (2008b).

The trend of final live BW result could be a reflection of FC which was better for the rabbits in treated groups than those in control group. Accordingly, the positive effect of bee pollen on rabbit growth could be attributed to the levels of macro- and micro-nutrients, among which are polyunsaturated fatty acids, minerals, etc. as well as protective agents and phytosterols such as flavonoids, carotenoids and phenolic constituents (Ŝarić *et al.*, 2009 and Attia *et al.*, 2014). In this respect, Wang *et al.* (2007)**.** showed that BP contains a wide spectrum of amino acids, vitamins, hormones, and minerals, as well as enzymes and coenzymes necessary for good digestion and cell growth. In addition, the pronounced improvement in treated groups may be due to enhancement of immunizing function and protection of intestinal tract health (Liu *et al.*, 2010; Attia *et al.*, 2011a, b and 2014 and Hajkova *et al.*, 2013) and /or due to the bactericidal, antimycotic or antifungal properties of bee pollen as reported by García *et al.* (2001) and Campos *et al.* (2010).

Table 3: Effect of orally bee-pollen administration on growth performance and economic efficiency of growing NZW male rabbits during growing period

Items	Bee-Pollen levels (mg/kg BW)			
	0 (Control, G_1)	200 (G_2)	300 (G_3)	400 (G_4)
Body weight (g):				
Initial body weight at 35 d (g)	659±6.23	658±7.31	657±6.32	658±7.13
Final body weight at 92 d (g)	1684±6.70^{d}	1715±10.02^{c}	1942±11.08^{b}	1970±13.00^{a}
Daily weight gain (g)	18.30±0.15^{c}	18.88±0.22^{c}	22.95±0.18^{b}	23.43±0.25^{a}
Feed intake (g):				
Daily feed intake (g)	84.02^{a} ±20.00	81.25^{b}±17.30	75.43^{c} ±14.50	74.02^{d}±10.20
Feed efficiency (g/g)	4.59^{a}±0.07	4.30^{b}±0.03	3.29^{c}±0.04	3.16^{d}±0.05
Economic efficiency:				
Total feed intake /rabbit (kg)	4.705	4.550	4.224	4.145
Price bee-pollen	-	0.17	0.22	0.25
Price/kg diet (LE)[1]	2.02	2.02	2.02	2.02
Total feed cost/rabbit (LE)[2]	09.50	09.36	08.75	08.62
Price/kg body weight (LE)	25.00	25.00	25.00	25.00
Selling price (L.E/ head)	25.63	26.43	32.13	32.80
Net revenue (LE)	16.13	17.07	23.38	24.18
Economic efficiency (EE)[3]	1.70	1.82	2.67	2.81
Relative economic efficiency (REE, %)	100	107	157	165

Means within the same row bearing different letter superscripts (a, b, c) are significantly different (P≤0.05).

[1]The price was calculated on the base of ingredients price through the experimental period; L.E = Egyptian pound.

[2]Total feed cost/rabbit (LE) = Total feed intake /rabbit (kg) x Price/kg diet (LE) + Price bee-pollen during the experimental Period.

[3]Economic efficiency (EE) = Net revenue/Total cost, Price of 150g bee-pollen =19.50 LE.

Data concerning economic evaluation also summarized in Table 3. The present results indicated an improvement in net revenue and relative economic efficiency (REF) for rabbits received different levels of BP (G_2, G_3 and G_4) compared to control (G_1) group. The highest values of net profit and relative economic efficiency due to BP administration were observed in G_4, being 24.18 L.E and 165%, respectively. Similar results were obtained by Attia, *et al.* (2014) who showed that rabbits treated with BP had high value of EE and REE (1.4 and 127.3, respectively) than in the control group. These results showed that BP administration had a positive effect on the economic efficiency.

Finally, these results provide an effective approach to improve the productive performance of male NZW rabbits at a higher revenue profit of each head through short-term of BP administration.

Body weight and some anatomical parameters

Results in Table 4 showed that absolute weights of testes, epididymis, accessory glands and pituitary glands and relative sexual-accessory glands weight were increased significantly ($P \leq 0.05$) with BP administration. However, relative weights of testis, epididymis and pituitary glands were not affected by BP administration. These results are not similar with those observed in mature male rats, in which the inclusion of 60 mg/per animal/ per day over a 30-day period of bee pollen decreased the relative weight of the epididymis (Selmanoğlu *et al*., 2009).

Plasma testosterone concentration

Table 5 showed that, values of plasma testosterone concentration were higher significantly ($P \leq 0.05$) in orally administrated growing NZW rabbits with 300 or 400 mg BP/kg than those in control group. These values are still within the normal ranges. Plasma testosterone concentration value in 300 mg Bee-pollen group (G_2) did not differ than that of control (G_1) group. The trends resulting from adding Bee-pollen in the present study are in agreement with the findings of Attia *et al.*(2011a) and Selmanoğlu *et al.* (2009).

The increase in plasma testosterone concentration in treated groups may be reflected mainly on the increase in sexual accessory glands activity with treatments (Table 4), Omotuyi *et al.* (2010) reported the ability of flavonoids found in BP to increase testosterone levels in rabbits.

Table 4: Effect of orally bee-pollen administration on body and internal genitalia organs weights of growing NZW male rabbits[1]

Items	Bee-Pollen levels (mg/kg BW)			
	0 (Control, G_1)	**200 (G_2)**	**300 (G_3)**	**400 (G_4)**
Live body weight, g (BW)	1468.8±57.02^{b}	1556.5±27.04^{ab}	1668.5±59.6^{a}	1683.8±37.21^{a}
Testis weight:				
Absolute, g	4.05±0.06^{c}	4.65±0.06^{b}	5.05±0.06^{a}	5.15±0.06^{a}
Relative, % BW	0.28±0.015	0.30±0.003	0.30±0.013	0.31±0.08
Epididymis weight:				
Absolute, g	0.65±0.01^{d}	0.72±0.01^{c}	0.80±0.01^{b}	0.83±0.01^{a}
Relative, % BW	0.04±0.002	0.05±0.001	0.05±0.002	0.05±0.001
Sexual-accessory glands weight:				
Absolute, g	2.07±0.05^{b}	3.02±0.03^{a}	3.05±0.06^{a}	3.15±0.06^{a}
Relative, % BW	0.14±0.009^{b}	0.19±0.004^{a}	0.18±0.005^{a}	0.19±0.07^{a}
Pituitary glands weight:				
Absolute, g	2.65±0.06^{c}	2.75±0.06^{c}	2.95±0.06^{b}	3.15±0.06^{a}
Relative, % BW	0.18±0.012	0.18±0.006	0.18±0.010	0.19±0.006

Means within the same row bearing different letter superscripts (a, b, c) are significantly different ($P \leq 0.05$). [1]n = 4 per treatment.

Table 5: Effect of orally bee-pollen administration on some reproductive traits of NZW buck rabbits[1]

Items	Bee-Pollen levels(mg/kg BW)			
	0 (Control, G_1)	200 (G_2)	300 (G_3)	400 (G_4)
Testosterone, ng/ml	3.13±0.39[a]	4.16±0.32[ab]	5.07±0.39[b]	4.84±0.40[b]
Weight at puberty, g	2762±21.26	2760±20.86	2764±21.93	2738±63.06
Age at puberty, days	161.1±1.37[b]	155.3±1.10[a]	152.5±1.13[a]	152.7±1.45[a]
Scrotal circumference, cm	6.96±0.08	7.13±0.07	7.23±0.11	7.29±0.11
Testicular index, cm^3	4.43±0.20[c]	5.72±0.17[b]	6.14±0.16[ab]	6.28±0.17[a]

Means within the same row bearing different letter superscripts (a, b, c) are significantly different (P≤0.05).

[1]n = 15 per treatment except for testosterone where n = 20, and scrotal circumference and testicular index where n = 10

[2] Average value of analysis recorded at 120 and 150 d.

Reproductive performance:

Administration of BP caused significant (P≤0.05) decrease in age at puberty and the percentages of change as compared with control were 3.6, 5.3 and 5.2% for G_2, G_3 and G_4, respectively (Table 5). The results are agreement with those obtained by El-Kholy *et al.* (2008a and 2014). This result might be related to the maximum final body weight obtained with these BP levels. In addition, this result may be due to the effect of BP to improve growth traits and testosterone concentration which lead to fast maturity age. Similar results were obtained by Meshreky *et al.* (2005) who found that age at puberty was related to testosterone concentration. Also, Castro *et al.* (2002) mentioned that testosterone is needed to initiate spermatogenesis at puberty and for the maintenance of this process in the adult. El-Sherbiny (1994) found that the onset of puberty involves appearance of first spermatozoa in the caudal epididymis of the male rabbits. Moreover, the testicular index was significantly (P≤0.05) increased as levels of BP increased (Table 5). Testicular size is the best primary assessment of spermatogenesis, since the tubules and germinal elements account for approximately 98% of the testicular mass (Sherines and Howards, 1978). Also, testicular index reflects spermatogenesis and testosterone production (El-Mougy *et al.*, 1991). Bee-pollen inclusion had no effect on weight at puberty and scrotal circumference.

The effect of BP treatment on reproductive parameters of buck rabbits was so clear, where treatment caused significant (P<0.05) increase in mating activity, ejaculate volume, advanced-sperm motility, alive spermatozoa, morphological normal spermatozoa, sperm-cell concentration and total-

sperm output (Table 6). The present results showed that the highest increases in these parameters were obtained in G_4 being 51.1, 63.7, 17.7, 8.1, 33.4 and 76.9 %, meanwhile the lowest increases were obtained in G_2, being 41.7, 38.4, 10.4, 3.8, 24.5 and 39.7%. While, BP administration caused significant ($P<0.05$) decreases in acrosomal damage values in G_2 and G_3 and G_4. The percentage of change as compared with control was 10.3, 21.9 and 24.1% for G_2 and G_3 and G_4, respectively (Table 6). The percentages of morphological normal spermatozoa increased significantly ($P<0.05$) with administration of BP. An increase in ejaculate volume occurred may be due to the increase in sexual accessory glands weight in treated rabbits (Table 4), since the accessory glands and spermatogenesis are controlled by testosterone concentration which was higher in treated rabbits. In addition, testosterone is required for the maturation of male germ cells and sperm production and quality (Walker, 2009 and Attia *et al.* 2011a). On the other hand, a decrease in sperm concentration in control group may be due to degeneration of germinal epithelium and partial atrophy in the seminiferous tubules (El-Sherry *et al.*, 1980) which was confirmed by histological photomicrograph result of control testis (Plate 1).

The results of sperm concentrations and motility are in agreement with Fields *et al.* (1979) who observed in young bulls that sperm concentration was positively correlated with motility and testicular size. Progressive motility of spermatozoa is an important parameter in the fertilization process *in vivo* and *in vitro* (Nikolettos, 1999). He added that it can be used as a single and independent predictor for successful fertilization. The decrease in acrosomal damages in treated groups can be attributed to the antioxidant activity of BP due to flavonoids which can protect the plasma membrane that surrounds the acrosome and the tail. Thus, the improvements in semen quality could be attributed to increasing oxidative stability. Accordingly, it seems that BP may display an indirect role in rabbit spermatogenesis.

The significantly higher sperm count obtained in the treated groups was correlated well with the numerous spermatogonia observed in the seminiferous tubules of the BP treated rabbits compared to the control as mentioned later (plates 3-8). Moreover, the increase of sperm count could be due to increase in spermatocyte which resulted from increase in proliferation of stem cells or increase in spermiogenesis as large masses of seminiferous tubules epithelium appeared to be sloughing or bulging into the tubule lumen as observed in the treated groups photomicrographs as mentioned later in plates 3-8.

Table 6: Effect of orally bee-pollen administration on libido, mating activity and physical semen characteristics of NZW buck rabbits[1]

Items	Bee-Pollen levels(mg/kg BW)			
	0 (Control, G_1)	200 (G_2)	300 (G_3)	400 (G_4)
Sexual desire –libido-, sec.	39.28±1.53^{a}	33.64±1.03^{b}	29.88±1.06^{c}	27.64±0.90^{c}
Mating activity[2]	3.48±0.18^{b}	4.93±0.19^{a}	5.16±0.19^{a}	5.26±0.19^{a}
Semen-ejaculate volume, ml	0.513±0.01^{c}	0.710±0.01^{b}	0.798±0.03^{a}	0.840±0.03^{a}
Advanced-sperm motility, %	65.59±1.66^{b}	72.39±2.07^{a}	76.43±1.60^{a}	77.18±1.98^{a}
Alive spermatozoa, %	73.03±0.27^{b}	75.83±1.79^{ab}	77.53±1.78^{ab}	79.86±1.83^{a}
Morphological normal spermatozoa, %	77.16±1.83^{b}	81.16±1.65^{ab}	82.86±1.55^{a}	83.39±1.56^{a}
Sperm-cell concentration, $N \times 10^6$ / ml	458.35±21.52^{b}	570.79±24.05^{a}	599.44±23.96^{a}	611.45±22.86^{a}
Total-sperm output, $N \times 10^6$ / ejaculate	290.00±14.15^{c}	405.23±19.16^{b}	473.56±21.66^{a}	513.20±24.87^{a}
Acrosomal damages, %	15.49±0.53^{a}	13.89±0.58^{b}	12.09±0.55^{c}	11.76±0.58^{c}

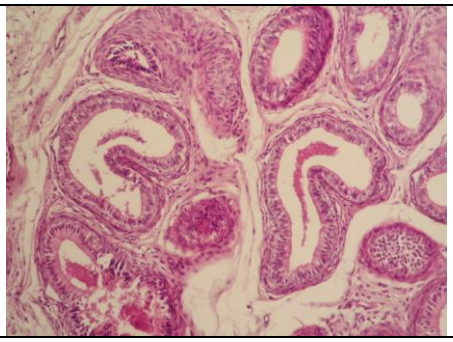
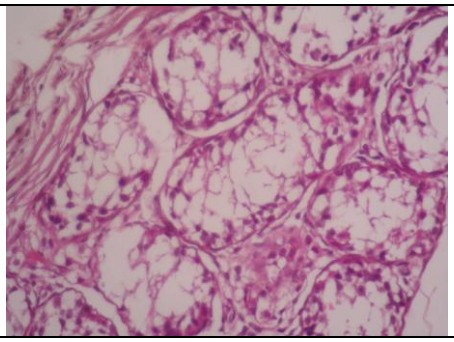
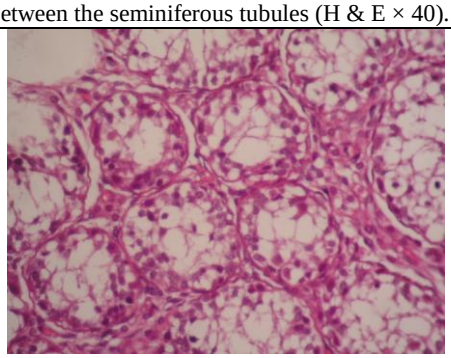
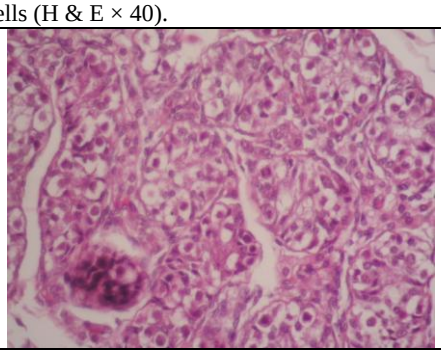
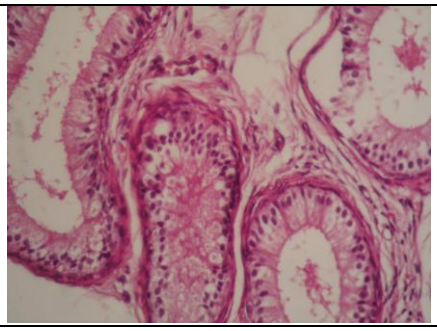
Means within the same row bearing different letter superscripts (a, b, c) are significantly different ($P \leq 0.05$).

[1]n = 10 per treatment

[2]No. of mated during 30 minutes.

Histopathological examination of male genitalia:

The results of histological photomicrographs of the testis and epididymis sections of treated and untreated rabbits are shown in Plates 1–8. At 6 months of age, the testicles of control group showed that most layers of germinal epithelium lining the basement membrane were absent and notice edema in between the seminiferous tubules. Also, the epididymis showed hyperplasia of the epithelial tissue of some tubules with presence of few numbers of spermatozoa in their lumen and few cases showed degenerated spermatozoa and spermatid giant cells. Testes of males treated by BP were highly active, showing accumulated sperms in seminiferous tubules with increased size of the interstitial cells when compared to control. Also, the testicles of treated groups showed presence of normal seminiferous tubules underdoing spermatogenic cycle composed of spermatogonia,

	
Cross section in testis of control male rabbits at 6 months of age, showing absence of most layers of germinal epithelium and notice edema in between the seminiferous tubules (H & E × 40).	Epididymis of control male rabbits at 6 months of age, showing hyperplasia of the epithelial of some tubules with presence of few numbers of spermatozoa in their lumen (H & E × 40).
	
Cross section in testis of G_2 male rabbits at 6 months of age, showing atrophy of seminiferous tubules with absence of most layers of germinal epithelium and vaculation of cytoplasm of sertoli cells (H & E × 40).	Epididymis of G_2 male rabbits at 6 months of age, showing mature spermatozoa in the lumen accompanied by spermatid giant cells (H & E × 40).
	
Cross section in testis of G_3 male rabbits at 6 months of age, showing more development and the number of spermatozoa increased in the seminiferous tubules (H & E × 40).	Epididymis of G_3 male rabbits at 6 months of age, showing presence of large numbers of spermatozoa (H & E × 40).

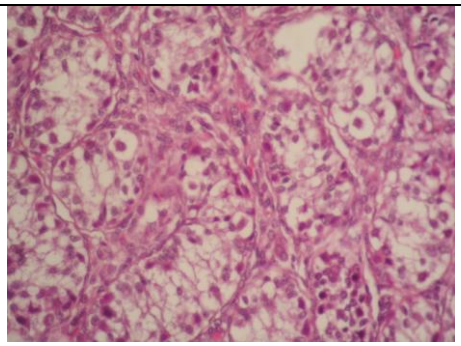
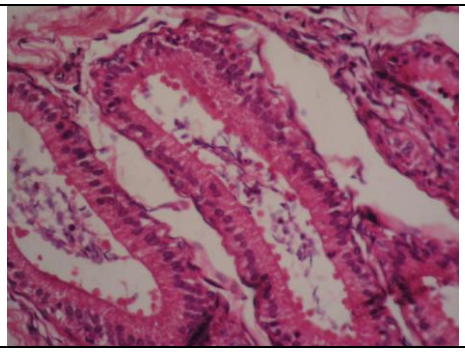
	
Cross section in testis of G_4 male rabbits at 6 months of age, showing well mature seminiferous tubules with a complete spermatogenic cycle	Epididymis of G_4 male rabbits at 6 months of age, showing presence of large numbers of spermatozoa (H & E × 40).

Plate 1-8: Histological photomicrographs of the testis and epididymis sections of treated and untreated rabbits .

spermatocytes, round spermatids and elongated spermatids consistent with general spermatogenesis. In rabbits treated with 300 and 400 mg BP/kg, number of mature spermatozoa was almost equal but increased compared to control ($G_{1)}$ and 200 mg BP/kg (G_2). This observation was consistent with the increase in semen quality parameters of bucks in G_3 and G_4 treatment groups as mentioned before (Table 6).

The result concerning the histopathological examination of testis and epididymis for untreated bucks may be discussed from the view which demonstrated by Aitken and Roman (2008) who showed that the high rates of cell division inherent in the spermatogenesis process imply correspondingly high rates of mitochondrial oxygen consumption by the germinal epithelium are vulnerable to oxidative stress. In addition, seminal oxidative stress (OS) develops as a result of an imbalance between ROS generating and scavenging activities (Sikka, 2001)**.** Spermatozoa are particularly susceptible to seminal-OS induced damage because their plasma membranes contain large quantities of polyunsaturated fatty acids (Alvarez and Storey, 1995) and their cytoplasm contains low concentrations of scavenging enzymes (De Lamirande and Gagnon, 1995; Sharma and Agarwal, 1996). Conversely, the antioxidant administration (as Bee pollen) may counteract the oxidative stress created in the testes. Hence, these antioxidants compensate for the loss of sperm cytoplasmic enzymes as the cytoplasm is extruded during spermiogenesis, which, in turn, diminishes endogenous repair mechanisms and enzymatic defenses. Also, the main active ingredients of BP are primarily phytoestrogens including isoflavones, flavonols and lignans, otherwise known as plant hormones, compounds with well-documented hormonal benefits for male (Moon *et al.*, 2006). Also, the

phenolic components of BP were reported to exhibit high levels of antioxidant and radical scavenging activity (Graikou *et al.*, 2011).

On the other hand, the variations among male groups in structure and development of testes and epididymis may be due to the differences in testosterone hormone levels as mentioned before (Table 4). However, Sun *et al.* (1990) demonstrated that testosterone hormone is responsible for spermatogenesis and all stages of spermatogenesis are stimulated by testosterone. Walker (2009) showed that testosterone appear to act on the testicular Sertoli cells to promote the structure integrity of the seminiferous tubules, the production of spermatozoa and androgen binding protein. Also, Castro *et al.* (2002) found that plasma testosterone level was correlated significantly with each of seminiferous tubular diameter, number of sertoli cells per tubular cross-section, ratios between germ cells and sertoli cells and ratios among germ cells.

However, it is ideal that BP administration (in the highest levels) may increase activity and development of testicular tissue in rabbits. The result revealed that BP administration positively affect gonad developments (increased proliferation of many types of testis cells involved in spermatogenesis) in rabbit bucks and similar results were reported in study with rabbits supplemented with vitamin E as antioxidant by Amao *et al.*(2012). These indicate that BP administration enhanced primary reproductive organ of male rabbits.

Conclusively, from these results it could be concluded that these improvements achieved in treated bucks of the present study could be explained on the basis that BP with its nutritional elements affects positively the growth rate of the whole body and this leads to an early maturation of the pituitary, which directly affects the growth and function of the testes and finally the performance of the males. From the economic point of view, 400 mg BP/kg BW is recommended for male rabbits.

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التأثير التحفيزي لإضافة حبوب اللقاح على النمو والأداء التناسلي لذكور أرانب النيوزيلندي الأبيض

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تهدف هذه الدراسة الي تقييم اضافة حبوب اللقاح عن طريق الفم علي أداء ذكور الأرانب قبل وبعد البلوغ والنضج الجنسي. تم أستخدم ستة وتسعون ذكر أرنب نيوزيلندي أبيض عمر الفطام ٣٥ يوم بمتوسط وزن ٦٥٨± ٦,٣٠ جم وتم تقسيمهم بطريقة عشوائية إلى أربع مجموعات (٢٤ ذكر لكل مجموعة) وأستمرت التجربة لمدة 184 يوم. المجموعة الأولى عبارة عن مجموعة ضابطة (مقارنة) والثلاث مجموعات الآخرى تم تجريع الحيوانات بثلاث مستويات من حبوب اللقاح المذابة في الماء وهي على الترتيب ٢٠٠ و٣٠٠ و٤٠٠ مجم حبوب لقاح لكل كجم وزن حي. وتم تسجيل زيادة الوزن وإستهلاك العلف اليومي من الفطام حتى عمر ٩١ يوم (عمر التسويق). وفي هذا العمر أُتيح لعدد ١٠ ذكور من كل مجموعة أن يصلوا للنضج

الجنسي. تم تجميع عينات الدم عند عمر ١٢٠ و ١٥٠ يوم من خمسة ذكور لكل مجموعة. وسجلت الصفات التناسلية في عمر النضج الجنسي .

أوضحت النتائج أن إضافة حبوب اللقاح عند أعلي مستوياته (٤٠٠ مجم/كجم وزن حي) كان له تأثيراً معنوياً (عند مستوى ١%) لزيادة وزن الجسم النهائي وزيادة الوزن اليومي والكفاءة الغذائية. وكذلك زيادة تركيز هرمون التستسترون في البلازما عند ١٢٠ و١٥٠ يوم من العمر. وكان الحد الأدني لعمر البلوغ واضحاً بإستخدام نسبة ٤٠٠ مجم/كجم وزن حي مقارنة بمستويات الإضافة الآخرى وكذالك مجموعة المقارنة.. وكان النشاط الجنسي لذكور الأرانب والحركة التقدمية والشكل المورفولوجي الطبيعي للحيوانات المنوية مرتفعة معنوياً (علي مستوى احتمال ٥%) بزيادة حبوب اللقاح وكانت أقصاها عند إضافة ٤٠٠ مجم/كجم وزن حي. كما أظهرت نتائج القطاعات الهستولوجية للخصية والبربخ في الأرانب المعاملة بحبوب اللقاح زيادة في قطر الأنابيب المنوية مع نشاط في عملية تكوين الحيوانات المنوية مقارنة بمجموعة المقارنة.

التوصية: نستنتج من هذه الدراسة أن إضافة حبوب اللقاح لذكور الأرانب النامية حفز الأداء الإنتاجي لها مما أدى إلى تأثير إيجابي على الأداء التناسلي للذكور البالغة. ومن الناحية الاقتصادية يوصى بإضافة حبوب اللقاح بمستوى ٤٠٠ مجم/كجم وزن حي لذكور الأرانب.