

INFLUENCE OF USING DATE PALM POLLEN OR BEE POLLEN ON SOME BLOOD BIOCHEMICAL METABOLITES, SEMEN CHARACTERISTICS AND SUBSEQUENT REPRODUCTIVE PERFORMANCE OF V-LINE MALE RABBITS

Saber S. A. Hassan^{1*}; Hossam A. Shahba² and Mohamed M. Mansour¹

¹Department of Animal and Poultry Production, Faculty of Agriculture, Damanhour University, Damanhour, 22516, Al-Behira, Egypt.

²Animal Production Research Institute, Ministry of Agriculture, ARC, Gizza, Egypt

*Corresponding author: Tel: 0201126114161, Fax: 020453316535,

Email: saber.hassan@agr.dmu.edu.eg & saber.shihata77@gmail.com

ABSTRACT: *The present study aimed to compare the impact of oral administration of date palm pollen (DPP) or bee pollen (BP) in suspension form on rabbit bucks' physiological and reproductive performance traits. Fifty V-line male rabbits at 20 weeks of age were divided into five equal groups; Control group was given water (placebo), DPP groups (DPP1, 150 mg/kg BW; DPP2, 300 mg/kg BW), and BP groups (BP1, 200 mg/kg BW; BP2, 400 mg/kg BW). During a 12-week period, supplements were taken three times a week (Sunday, Tuesday, and Thursday). Blood samples (either plasma or serum) were collected at 32 weeks of age to examine the hematological and biochemical constituents. Also, at 28 weeks of age, semen was collected to evaluate some semen quality traits and seminal plasma metabolites.*

Results indicate that white blood cells (WBCs) and lymphocytes increased significantly in the high level of both supplements compared to the control group. Meanwhile, the BP2 group had an increase in red blood cells (RBCs), hemoglobin (Hgb), and Packed cell volume (PCV) and a decrease in urea significantly compared to control without significant difference between treatments. Furthermore, high density lipoprotein (HDL) and testosterone were increased in the treated rabbits, as well as decreased in low density lipoprotein (LDL), very low density lipoprotein (VLDL), and Alanine aminotransferase (ALT) significantly compared to the control. there was an increase in the total antioxidant capacity (TAC) and testosterone concentration in the treated groups compared with the C rabbits. These results indicated that DPP and BP, which were natural additives considered safe as they improved the immune and physiological parameters and reproductive performance of rabbit bucks.

Accordingly, from an economic point of view it could recommend using levels of DPP (150 mg/kg BW) or BP (200 mg/kg BW) to improve the

biochemical, immunological, physiological, and subsequently buck s reproductive performance. **Keywords:** *V-line rabbits, Physiological, Semen quality, Testosterone, Date Palm bee pollen.*

INTRODUCTION

Rabbit meat is considered a healthful food because it is rich in protein. It includes great values of essential amino acids with easy digestibility (Hernandez and Dalle Zotte, 2010; Dalle Zotte and SzendrÓ, 2011), and it also has low fat and cholesterol (Petracci *et al.*, 2009). Numerous studies had established to evaluate the effect of different natural products on improved feed efficiency, growth rate, immunity, and productivity of rabbits (Perić *et al.*, 2009; Dias *et al.*, 2013).

Date palm pollen (DPP) is the meal origin powder of palm plants, which contains a mixture of compounds like phenolic, flavonoids, anthocyanins, and selenoproteins (Baliga *et al.*, 2011), which makes it a source of natural antioxidants with few side effects (Fallahi *et al.*, 2015). Also, various concentrations (20, 40, and 80mg/mL) of DPP aqueous extract has been used as a vital supplementation to enhance male infertility by decreasing free radicals and improving spermatozoa properties (Laghouati *et al.*, 2021). Moreover, using DPP extract has improved sperm characteristics, such as motility, viability, acrosome reaction, and lipid peroxidation (Fallahi *et al.* 2015). Besides, oral administration with 30, 60, and 90 mg/kg/day of oily DPP suspensions has enhanced testosterone production and increased spermatogenesis in rabbit bucks (Al-Samarrai *et al.*, 2017). Also, Laghouati *et al.*, (2021) showed that DPP extract was a suitable addition for sperm dilution *in vitro*. Besides, DPP extracts improved sperm quality and better protection against oxidative damage. Furthermore, DPP was found to support and increase the resistance of some tissues to several harmful pathogens and toxicants due to their high phenolic and flavonoid contents (Campos *et al.*, 1997).

Bee pollen (BP) is an agglomerate of flower pollen grains collected by honeybees mixed with plant juice and bee saliva enzymes, increasing its pharmaceutical effectiveness (Carpes *et al.*, 2008; Leblanc *et al.*, 2009). BP is rich in protein, polyunsaturated fatty acids, provitamins, vitamins, and minerals (Xu *et al.*, 2009; Haščík, *et al.*, 2012). Besides, several studies showed that BP has some pharmaceutical characteristics as antibacterial (Proestos *et al.*, 2005), antifungal (García *et al.*, 2001), antibiotic, and antioxidant (Almaraz-Abarca *et al.*, 2004; Hajkova *et al.*, 2013). Hashem *et al.* (2013) inclusion of propolis in male rabbits' diets during the hot season could be used effectively to mitigate adverse impacts of elevated temperature on semen quality, oxidative status, and hemato-biochemical features. Using a water suspension with 100, 200, and 300

mg/kg/day supplementation of BP for 5 weeks had improved the productive and reproductive performance of rabbits does (Attia *et al.*, 2015). Also, giving the growing rabbits 250 and 500 mg BP/kg body weight increased their growth and survival ratio from weaning up to mature age (El-Hanoun *et al.*, 2007). However, more studies are needed to compare DPP and/or BP on adult male rabbit productivity. Therefore, the objectives of the present study were to evaluate the effect of oral administration of DPP or BP suspensions on the physiological, reproductive, and production performance of V-line male rabbits. It hypothesized that using DPP or BP can improve the physiological and immunological performance, which subsequently leads to enhancement of the reproductive traits of the rabbit bucks.

MATERIALS AND METHODS

Ethical statement

The present study was carried out at El-Bostan Experimental Station, Faculty of Agriculture, Damanhour University, Al-Behera governorate, Egypt. The current study has been accompanied with the recommendations of the ethical principles of animal research and approved by the Ethical Animal Care and Use Committee of Damanhour University.

Date palm pollen and bee pollen preparation

Dried DPP and BP were purchased from the local market and ground to a fine powder using an electric dry mill; the powders were then stored in well-tied black plastic bags at room temperature (~25° C) until used. As described by Al-Samarrai *et al.*, 2017, and Attia *et al.*, 2015, the DPP and BP treatments were suspended in clean water in shares by weight.

Animals and experimental design

A total number of 50 male V-line rabbits at 20 weeks old were used in the current study. All rabbits were housed individually in a naturally ventilated building, kept in Italian wire galvanized cages (50L × 50W × 40H), and given 16 hr. of light daily including 12 h of natural day light and 4 h of supplementary electric light. The batteries were accommodated with automatic stainless-steel nipple drinkers, feeders for pelleted rations. All rabbits were fed the same basal diet (*ad libitum*), formulated according to NRC (1994) and AOAC (1995), which feed ingredients and calculated chemical proximate analyses are presented in Table (1).

The freshwater was available continuously during the experiment. All animals were kept under similar management (environmental temperature, humidity, light-dark cycles, and lengths) and similar hygienic conditions (vaccinations and health care).

Table 1: feed ingredients and calculated chemical composition of the basal diet

Ingredients	(kg/ton)	chemical composition (g/kg)	
Yellow corn	100.0	Dry matter	903.2
Barley	130.0	Organic matter	804.8
Molasses	30.0	Crude protein	172.4
Clover hay	395.0	Crude fibre	134.6
Wheat bran	150.0	Ether extract	28.00
Soybean meal	175.0	Nitrogen-free extract	569.8
Dicalcium phosphate	8.0	Ash	95.20
Limestone	5.0	digestible energy (kcal/kg)	2464
Sodium chloride	3.0		
Vitamin and minerals mixture*	3.0		
DL-methionine	1.0		

*Provides per kg of diet: Vit.A,1200 IU; Vit.D3, 2500 IU; Vit. E, 10 mg; Vit. K3, 3mg; Vit.B1, 1mg; Vit.B2, 4mg; Pantothenic acid, 10 mg; Nicotinic acid, 20 mg; Folic acid, 1 mg; Biotin, 0.05mg; Niacin, 40 mg; Vit.B6, 3 mg; Vit. B12, 20 mcg; Choline Chloride, 400 mg; Mn, 62 mg; Fe,44 mg; Zn, 56 mg; I, 1 mg; Cu, 5 mg and Se, 0.01mg.

The rabbit bucks were randomly divided into five equal groups (n = 10 each) as follows: Control group (C), which was orally administered with 2 ml of water (placebo) using a 3 cm syringe, date palm pollen groups (second and third), which was orally treated with 2 ml of water suspension of DPP containing 150 mg/kg BW (DPP1) or 300 mg/kg BW (DPP2), respectively by using a 3 cm syringe, and bee pollen groups (fourth and fifth), which orally treated with 2 ml of water suspension of BP containing 200 mg/kg BW (BP1) or 400 mg/kg BW (BP2), respectively by using a 3 cm syringe. During a 12-week period, supplements were taken three times a week (Sunday, Tuesday, and Thursday). The initial body weights at the beginning of the experiment were 3182±29.1, 3141±25.6, 3159±27.4, 3180±23.7, and 3200±13.5 for the C, DPP1, DPP2, BP1, and BP2 groups, respectively without significant differences ($P > 0.5$). All rabbit's weight was recorded at the end of the experiment and average body weight gain was estimated.

Blood biochemical constituents

At 32 weeks of age, five samples of blood of each treatment from the marginal ear vein of the bucks were collected in the morning at 8 o'clock before the regular time of feeding. The blood was collected in clean tubes with or without heparin to collect plasma and serum. The blood samples collected with heparin are used to measure hematological blood parameters like hemoglobin concentration (Hgb) were assessed in fresh blood samples using commercial kits (according to Tietz, 1982). Packed cell volume was carried out by using micro-

hematocrit capillary tubes according to Blaxhall and Daisley (1973). Red blood cells (RBCs) and white blood cells (WBCs) were counted in fresh blood samples according to Hawks and Dennett (1989) utilizing hemocytometer (German Hemocytometer Manufacturers) and counted at 400x objective of a phase-contrast microscope. Differential leukocyte count was estimated according to Schalm, (1986). The phagocyte activity (PA), phagocytic index (PI), Bactericide activity (BA), and lysosome activity (LA) were determined according to Kawahara *et al.*, (1991).

Blood serum was collected by centrifugation at 860 x g for 20 min at 4°C and stored at -20°C until analysis. All biochemical traits of blood (glucose, total protein, albumin, globulins “ α , β and γ ”, total lipids, triglycerides, total cholesterol, high-density lipoprotein “HDL”, low-density lipoprotein “LDL”, very low-density lipoprotein “VLDL”, alkaline phosphatase “ALP” alanine aminotransferase “ALT”, aspartate aminotransferase “AST”, urea, creatinine, total antioxidant capacity “TAC”, malondialdehyde “MDA”) were determined by using commercial kits (Diagnostic Product Company, LOS Angeles, CA). Also, serum testosterone concentrations were determined by radioimmunoassay (RIA) in duplicate 100 μ l aliquots using a commercial kit (Diagnostic Product Company, LOS Angeles, CA). Assay sensitivity was 0.1 ng/ml with a coefficient of variation of < 8 %.

Semen analysis and reproductive performance evaluation

Semen was collected once weekly after 8 weeks of the initiation of the experiment. Ejaculates were collected using an artificial vagina maintained at 45-46°C and a teaser doe. Reaction time (RT), ejaculate volume (EV), sperm concentration (SC), total sperm output (TSO), Advanced motility (AM), total motile sperm (TMS), dead sperm (DS), abnormal sperm (AS), live sperm (LS), and total live sperm (TLS) were measured according to Smith and Mayer (1955) and Blom (1950).

The reproductive performance and fertility assessment of bucks have been carried out according to the International Rabbit Reproduction Group recommendations (IRRG, 2005). Briefly, at 8:00 a.m. bucks of each group were mated to ten receptive nulliparous female rabbits. The mating was done randomly so that the male in any treatment have the similar chances to mate any female in the population. Every doe was transferred to the buck's cage for mating and returned to its cage after copulation. Each doe was subjected to two insemination services within 30 min by the same buck. Total litter size at birth (total and a live) were recorded for three consecutive parities per each doe and the average value was calculated per each buck. Fertility rate was measured by divided the number of kindled does by the number of mated does $\times 100$.

Seminal plasma was collected by centrifugation at 860 x g for 20 min at 4°C and stored at -20°C until analysis. Seminal plasma metabolites included total protein, albumin, globulins “ α , β and γ ”, total lipids, triglycerides, total cholesterol, superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX), TAC, MDA, were measured using commercial kits (Diagnostic Product Company, LOS Angeles, CA). Also, seminal plasma testosterone concentration was determined by RIA in duplicate 100 μ l aliquots using a commercial kit (Diagnostic Product Company, LOS Angeles, CA). Assay sensitivity was 0.2 ng/ml with a coefficient of variation of < 5 %.

Statistical analysis

Statistical analysis was done using the GLM procedure of statistical analysis software of SAS Institute (SAS, 2002) using one-way analysis of variance according to the following statistical model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where: Y_{ij} = an observation, μ = The general overall mean, T_i = The effect of treatment ($i=1 - 5$), and e_{ij} = The experimental random error. Mean treatment differences were obtained by Duncan’s multiple range tests (Duncan, 1955) and values are presented as means \pm SEM. All the analyses were considered to be statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Body weight and weight gain response in rabbits

As shown in Table 2, no differences ($P > 0.5$) were found in the rabbit groups’ final body weights or weight gain.

Table 2: Effect of date palm pollen (DPP) or bee pollen (BP) on body weight and body weight gain of V-line rabbit bucks

Traits	Experimental groups					P-value
	C	DPP1	DPP2	BP1	BP2	
Initial body weight	3182 ± 29.1	3141 ± 25.6	3159 ± 27.4	3180 ± 23.7	3200 ± 13.5	0.496
Final body weight	3782 ± 31.7	3756 ± 71.5	3724 ± 35.3	3780 ± 40.0	3784 ± 40.1	0.867
Body weight gain	600 ± 35.6	615 ± 53.9	565 ± 11.0	600 ± 43.2	584 ± 31.4	0.902

C = Control, DPP1 = 150 mg/kg BW, DPP2 = 300 mg /kg BW, BP1 = 200 mg/kg BW, BP2 = 400 mg/kg BW.

Blood cells contents

The complete blood cells analysis is exhibited in Table (3). As shown, the total RBCs were greater ($P < 0.05$) in treated rabbits except BP1 compared with the C group. Furthermore, the BP2 bucks had the highest Hgb concentration by (21.5%, $P < 0.05$). Also, the PCV ratio in BP2 and DPP2 rabbits were highest ($P < 0.05$) compared with the C group by (20.74% and 17.65%, respectively).

Table 3: Effect of date palm pollen (DPP) or bee pollen (BP) on blood hematological parameters, and immune indices of V-line rabbit bucks

Traits	C	DPP1	DPP2	BP1	BP2	P-value
<i>Red blood cell parameters</i>						
RBCs ($10^6/\text{mm}^3$)	1.63 $\pm 0.02^b$	1.87 $\pm 0.02^a$	1.93 $\pm 0.02^a$	1.77 $\pm 0.05^{ab}$	1.90 $\pm 0.06^a$	0.001
Hgb (g/dl)	10.70 $\pm 0.21^b$	11.70 $\pm 0.21^{ab}$	12.70 $\pm 0.56^{ab}$	12.00 $\pm 0.37^{ab}$	13.00 $\pm 0.37^a$	0.001
PCV (%)	32.30 $\pm 0.76^b$	35.00 $\pm 0.63^{ab}$	38.00 $\pm 1.67^a$	36.30 $\pm 0.92^{ab}$	39.00 $\pm 1.10^a$	0.001
<i>White blood cell parameters</i>						
WBCs ($10^3/\text{mm}^3$)	21.01 $\pm 0.37^c$	21.30 $\pm 0.21^c$	25.70 $\pm 0.21^a$	22.70 $\pm 1.12^{bc}$	24.3 $\pm 0.56^{ab}$	0.001
Lymphocyte (%)	40.01 $\pm 0.97^c$	41.01 $\pm 0.37^{bc}$	43.01 $\pm 0.37^{ab}$	41.01 $\pm 0.31^{bc}$	44.30 $\pm 0.56^a$	0.001
Monocyte (%)	6.30 ± 0.21	6.30 ± 0.21	6.30 ± 0.56	6.70 ± 0.42	6.30 ± 0.21	0.958
Basophils (%)	1.00 ± 0.01	1.00 ± 0.01	0.67 ± 0.21	0.67 ± 0.21	1.00 ± 0.01	0.098
Eosinophils (%)	11.30 $\pm 0.56^{ab}$	11.01 $\pm 0.37^{abc}$	9.33 $\pm 0.21^c$	12.70 $\pm 0.56^a$	10.70 $\pm 0.21^{bc}$	0.001
Neutrophils (%)	41.30 ± 1.69	40.70 ± 0.56	40.70 ± 1.17	39.01 ± 0.63	37.70 ± 0.56	0.055
<i>Immune indices</i>						
PA (%)	20.30 $\pm 0.21^c$	24.00 $\pm 0.73^a$	22.30 $\pm 0.56^{abc}$	21.70 $\pm 0.42^{bc}$	23.00 $\pm 0.37^{ab}$	0.001
PI (%)	1.90 $\pm 0.03^b$	2.17 $\pm 0.04^a$	2.03 $\pm 0.02^{ab}$	2.07 $\pm 0.05^{ab}$	1.97 $\pm 0.06^b$	0.001
BA (%)	41.00 $\pm 0.37^{ab}$	41.00 $\pm 0.37^{ab}$	41.70 $\pm 0.56^a$	41.70 $\pm 0.56^a$	39.30 $\pm 0.56^b$	0.004
LA (%)	0.13 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.662

^{a,b,c} Means within the same row with different superscripts are significantly different at ($P < 0.05$). C = Control, DPP1 = 150 mg/kg BW, DPP2 = 300 mg /kg BW, BP1 = 200 mg/kg BW, BP2 = 400 mg/kg BW. WBCs = white blood cells, RBCs = Red blood cells, Hgb = Hemoglobin, PCV= Packed cell volume, PA= Phagocyte activity; PI= Phagocytic index; BA= Bacteriocidal activity; LA= Lysosome activity.

The present study results indicated that treating rabbits with DPP and BP had improved the hematological variables, which are in agreement with the previous results of Abuoghaba *et al.*, (2017), El-Neney and El-Kholy, (2014), Khalil *et al.*, (2015), and Taghian, *et al.* (2017). Treatment with both 250 and 500 mg BP/buck of rabbit bucks raised in hot, humid environments showed significant improvement in the RBCs, lymphocytes, and neutrophils parameters (Abuoghaba *et al.*, 2017). Also, BP significantly increased RBC and WBC counts in rabbits compared with control (Neney and El-Kholy, 2014). In addition, treatment with BP significantly increased the levels of RBCs, neutrophils, and lymphocytes in Baladi rabbit bucks (Khalil *et al.*, 2015). The increased total WBCs, lymphocytes, neutrophils, and RBCs count and Hgb concentrate for treated bucks may be due to the vital role of DPP and BP in enhancing the immune functions (Abuoghaba *et al.*, 2017). Besides, the improvement of the RBCs ratio increased the packed cell volume ratio represented by PCV, PA, PI, and BA. Furthermore, the positive effect of DPP and BP on hematological variables can be attributed to the presence of a lot of nutrient subjects (antioxidants, vitamins, mineral, essential fatty acids, amino acids, enzymes, etc.) components in the DPP and BP which can improve the nutrient value of the feed as well as feed digestibility and absorption (Leja *et al.*, 2007; Šarić *et al.*, 2009; Taghian, *et al.*, 2017), which accordingly improved the hematological variables of the treated rabbits.

Blood metabolites parameters

Within the treated rabbits, the glucose concentration was highest ($P < 0.05$) in DPP1 bucks (77.0 ± 0.36 mg/dl) compared with their counterparts (Table 4). Regarding the protein profiles, no differences ($P > 0.05$) were found in the total protein and globulin concentrations between the rabbit groups. Meanwhile, the albumin concentration was lowest (3.20 ± 0.03 g/dl, $P < 0.05$) in the C bucks compared with DPP and BP groups. However, the α -globulin level was highest ($P < 0.05$) in the BP1 group (1.27 ± 0.04 mg/dl) compare with the other rabbit groups. However, the β -globulin level was lowest ($P < 0.05$) in the DPP2 bucks (0.86 ± 0.02 mg/dl) compare with their counterparts except BP1 group (Table 4). In considering the lipid profiles, the total lipid concentration was lowest ($P < 0.05$) in the treated rabbits compare with the C bucks. Also, the triglyceride level was highest ($P < 0.05$) in the C group (122.70 ± 2.79 mg/dl) compared with the other groups. Besides, the LDL and VLDL concentrations were highest ($P < 0.05$) in the C rabbits compared with DPP and BP groups. Contrariwise, the HDL level was highest ($P < 0.05$) in treated bucks compared with the C group (Table 4). Concerning the liver enzymes, there was a decrease ($P < 0.05$) in the ALT levels

Table 4: Effect of date palm pollen (DPP) or bee pollen (BP) on serum biochemical parameters of V-line rabbit bucks

Variables	C	DPP1	DPP2	BP1	BP2	P-value
<i>Glucose and protein constituents</i>						
Glucose (mg/dl)	76.01 ±0.73 ^{ab}	77.01 ±0.36 ^a	75.01 ±0.36 ^{bc}	74.31 ±0.91 ^{bc}	74.01 ±0.73 ^c	0.024
Total Protein (g/dl)	6.10 ±0.03	6.30 ±0.09	6.40 ±0.07	6.40 ±0.07	6.40 ±0.07	0.258
Albumin (g/dl)	3.20 ±0.03 ^c	3.43 ±0.07 ^b	3.53 ±0.04 ^{ab}	3.43 ±0.02 ^b	3.60 ±0.03 ^a	0.001
Globulin (g/dl)	2.90 ±0.03	2.87 ±0.05	2.87 ±0.08	2.97 ±0.09	2.80 ±0.03	0.567
α-globulin (mg/dl)	1.13 ±0.02 ^c	1.17 ±0.02 ^{bc}	1.23 ±0.02 ^{ab}	1.27 ±0.04 ^a	1.17 ±0.02 ^{bc}	0.013
β-globulin (mg/dl)	1.00 ±0.03 ^a	1.00 ±0.03 ^a	0.86 ±0.02 ^c	0.90 ±0.03 ^{bc}	0.96 ±0.04 ^{ab}	0.033
γ-globulin (mg/dl)	0.76 ±0.05	0.70 ±0.06	0.76 ±0.11	0.80 ±0.09	0.66 ±0.02	0.724
<i>Lipid profile</i>						
Total Lipids (g/dl)	4.60 ±0.03 ^a	4.40 ±0.73 ^b	4.30 ±0.03 ^{bc}	4.37 ±0.09 ^b	4.13 ±0.02 ^c	0.001
Triglyceride (mg/dl)	122.70 ±2.79 ^a	111.70 ±1.05 ^b	110.01 ±1.83 ^b	111.7 ±2.79 ^b	110.01 ±1.83 ^b	0.005
Total cholesterol (mg/dl)	155.01 ±2.39	152.01 ±2.28	149.71 ±2.01	153.01 ±1.59	152.01 ±1.26	0.378
HDL (mg/dl)	35.70 ±0.91 ^c	39.01 ±1.10 ^b	41.01 ±0.73 ^{ab}	41.3 ±0.92 ^{ab}	41.7 ±0.56 ^a	0.003
LDL (mg/dl)	95.01 ±1.32 ^a	90.70 ±1.17 ^b	86.71 ±1.48 ^c	89.30 ±0.76 ^{bc}	88.31 ±0.76 ^{bc}	0.004
VLDL (mg/dl)	24.3±0.56 ^a	22.3 ±0.21 ^b	22.0 ±0.37 ^b	22.3 ±0.56 ^b	22.0 ±0.36 ^b	0.005
<i>Liver functions</i>						
ALP (U/L)	9.00 ±0.63	8.33 ±0.84	9.33 ±0.55	9.67 ±0.84	8.67 ±0.55	0.753
ALT (U/L)	59.70 ±0.76 ^a	57.70 ±0.55 ^b	56.30 ±0.21 ^{bc}	57.30 ±0.55 ^{bc}	55.70 ±0.21 ^c	0.009
AST (U/L)	48.01 ±0.96	48.01 ±0.36	45.70 ±1.48	45.70 ±1.28	43.30 ±0.84	0.053
<i>Kidney functions</i>						
Urea (mg/dl)	22.01 ±0.36	20.70 ±0.92	20.70 ±0.21	21.00 ±0.36	20.00 ±0.36	0.088
Creatinine (mg/dl)	1.27 ±0.06	1.27 ±0.06	1.20 ±0.10	1.23 ±0.02	1.27 ±0.04	0.933
<i>Antioxidant status</i>						
TAC (mmol/l)	2.10 ±0.01 ^c	2.15 ±0.01 ^{ab}	2.15 ±0.01 ^{ab}	2.13 ±0.01 ^b	2.16 ±0.01 ^a	0.004
MAD (nmole/ml)	11.70 ±0.21	10.70 ±0.56	11.30 ±0.21	11.7 ±0.56	10.0 ±0.37	0.062
<i>Male hormone</i>						
Testosterone (ng/dl)	2.43 ±0.04 ^c	2.67 ±0.07 ^{ab}	2.77 ±0.02 ^a	2.57 ±0.02 ^b	2.77 ±0.02 ^a	0.001

^{a,b,c} Means within the same row with different superscripts are significantly different at (P < 0.05).

C = Control, DPP1 = 150 mg/kg BW, DPP2 = 300 mg/kg BW, BP1 = 200 mg/kg BW, BP2 = 400 mg/kg BW. HDL= High density lipoprotein, LDL = Low density lipoprotein, VLDL = Very low-density lipoprotein, ALP = Alkaline phosphatase, ALT = Alanine amino transferase, AST = Aspartate aminotransferase, TAC = Total antioxidant capacity, MAD = Malonaldehyde.

of the treated groups compared with the C rabbits. Likewise, the AST and urea levels of BP2 bucks were lowest numerically compared with the other groups (Table 4). Moreover, there was an increase ($P<0.05$) in the TAC concentration in the treated groups in particular BP2 group compared with the C rabbits (Table 4).

In addition, the plasma testosterone levels had increased ($P<0.05$) in treated rabbits compared with the C bucks. Besides, the DPP2 and BP2 bucks had the highest ($P<0.05$) levels of testosterone compared with the C bucks by 13.99% (Table 4).

The current study results indicated that rabbits treated with 300 mg DPP/kg body weight have a greater blood glucose level. The increased glucose levels might be due to the positive effect of the previous treatment on increased glucose availability, especially for two biochemical and physiological body functions. On the other hand, results show that the bucks treated with DPP and BP had higher total protein and albumin concentrations than those of the control. These are in the contract of the results obtained by Abuoghaba *et al.*, (2017), who indicate that treated rabbits with BP had improved the blood total protein and albumin levels. Also, improvement of the ratio of the total protein in the treated groups could be due to the better crude protein digestibility, which increases the amino acids in the circulation. However, Attia *et al.*, (2014) indicate that albumin and globulin levels of growing rabbits were insignificantly affected by BP supplementation in the diet. While, the current results show that α -globulin levels were highest in the BP1 group compared with the other rabbit groups. Also, the β -globulin level was lowest in the DPP2 bucks compared with their counterparts, suggested that maybe rabbit's immunity had improved, which reproduces healthier liver efficacy in synthesizing enough globulins for immunologic action.

The present study results show that treated bucks with DPP and BP cause improvement in their lipid profile throughout decreasing the total lipids, triglyceride, LDL, and VLDL levels and increased HDL concentration. The achieved findings are in agreement with previous reports of El-Neney and El-Kholy (2017), who demonstrated that treated New Zealand White (NZW) male rabbits with BP at 200, 500 and 700 mg/kg BW led to a reduction in plasma total lipids as well as cholesterol. The improvement of the lipid metabolism in treated bucks might be due to the influence of phospholipids and linolenic fatty acid in the BP (Xu *et al.*, 2009). Also, these may be due to high volatile unsaturated fatty acid contents and flavonoid compounds in the DPP, which play a vital role as potent antioxidants (Saleh *et al.*, 2021). Additionally, unsaturated fatty acids have a positive effect on preventing the accumulation of lipid peroxidation products (Abuoghaba *et al.*, 2017).

Regarding the liver enzymes, the present consequences revealed that treated bucks with DPP or BP improved their liver function, exemplified by decreased plasma ALT. These results agree with the finding of Abuoghaba *et al.*, (2017) who showed that treated bucks with BP had significantly lowered ALT than those of the control bucks. Similarly, treated rabbit's doe with BP significantly decreased concentrations of ALT in serum than those of the control (Hedia *et al.*, 2007). Furthermore, the present study suggests that treating rabbits' bucks with DPP or BP hasn't any adverse impact on the liver tissues and their functions. In the same trend, the current results show that treated bucks with DPP or BP improved the kidney function represented by decreased urea concentration in the blood. These could be due to the higher metabolic rate and the improved digestibility and immune activity in treated rabbits. In rabbit bucks treated with 250 and 500 mg BP/buck (Aboghaba *et al.*, 2017), and in laying hens treated with 1.25, 2.5, and 5.0 g DPP/kg, proliferative, developmental, and differentiation of intestinal cells were enhanced, improving the conditions of intestinal microbial activity. That might be due to the bioactive potent antioxidant component in the DPP and BP.

The present study showed that blood testosterone concentration in treated rabbits was higher than the control bucks. This result agrees with the previous reports of Al-Samarrai *et al.*, (2017) who indicated that treating adult male rabbits with 30, 60, and 90 mg/kg/day of oily DPP for 4 weeks significantly increased blood testosterone levels. However, the differences of blood testosterone concentrations were insignificant in rabbit bucks treated with 250 and 500 mg BP/buck (Aboghaba *et al.*, 2017). The improvement in testosterone level might be due to the positive effect of DPP on the testicular function of male rabbits (Al-Samarrai *et al.*, 2017). Also, the enhancement of testosterone concentration in treated rabbits may be due to the great subjects of BP, principally in phospholipids, vitamins, minerals, and antioxidant factors (Šarić *et al.*, 2009). Furthermore, treated rabbits with DPP cusses increased in the blood level of Luteinizing hormone (LH) (Al-Samarrai *et al.*, 2017), which caused a rise of testosterone production by Leydig cell in rabbit's testes.

Semen properties and fertility traits

The semen characteristics are shown in Table (5). As presented, the sperm advanced motility percentage in the DPP1 was highest ($P<0.05$) compared with the C group only by (6.90%). TMS of the groups DPP1 and BP2 were significantly better than the control group by (28.5% and 25.4%, respectively). Besides, the DPP2 bucks had the highest live sperm ratio ($P<0.05$) compared with the C group by (5.34%). Nevertheless, the dead sperm was higher ($P<0.05$) in C bucks compared with their counterparts (Table 5). Accordingly, the total litter size at birth and alive were higher ($P<0.05$) in treated groups compared with the C rabbits (Table 5).

Table 5: Effect of date palm pollen (DPP) or bee pollen (BP) on semen characteristics and fertility traits of V-line rabbit bucks

Variables	C	DPP1	DPP2	BP1	BP2	P-value
Reaction time (sec)	9.60 ±0.62	8.07 ±0.52	8.66 ±0.37	8.13 ±0.56	8.54 ±0.71	0.322
Ejaculate volume (ml)	0.57 ±0.03	0.66 ±0.04	0.62 ±0.06	0.64 ±0.03	0.67 ±0.05	0.365
Sperm concentration (10 ⁶ /ml)	276.5 ±14.10	289.0 ±13.80	290.0 ±13.50	293.6 ±12.20	287.2 ±14.6	0.922
TSO (10 ⁶ /ml)	157.7 ±8.45	189.6 ±8.69	177.5 ±7.36	181.3 ±7.70	188.6 ±9.94	0.051
Advanced motility (%)	72.50 ±1.54 ^b	77.50 ±1.14 ^a	76.40 ±1.07 ^{ab}	76.00 ±1.01 ^{ab}	76.20 ±1.07 ^{ab}	0.048
TMS (10 ⁶ /ml)	114.6 ±6.88 ^b	147.3 ±7.76 ^a	135.4 ±5.74 ^{ab}	138.1 ±6.54 ^{ab}	143.7 ±7.93 ^a	0.014
Live sperm (%)	80.50 ±0.84 ^b	83.60 ±0.78 ^{ab}	84.80 ±0.84 ^a	83.10 ±0.85 ^{ab}	82.90 ±0.81 ^{ab}	0.004
Dead sperm (%)	7.33 ±0.41 ^a	5.79 ±0.37 ^b	5.38 ±0.34 ^b	5.93 ±0.39 ^{ab}	6.57 ±0.40 ^{ab}	0.006
Abnormal sperm (%)	12.20 ±0.65	10.60 ±0.60	9.80 ±0.74	11.01 ±0.56	10.60 ±0.27	0.059
TLS (10 ⁶ /ml)	127.0 ±7.21 ^b	158.9 ±8.11 ^a	150.5 ±6.11 ^{ab}	150.7 ±6.87 ^{ab}	156.4 ±8.42 ^a	0.024
TLSB (kits/ doe)	7.09 ±0.39 ^b	8.12 ±0.18 ^a	8.62 ±0.21 ^a	8.29 ±0.24 ^a	8.79 ±0.24 ^a	0.001
TLSBL (kits/ doe)	6.82 ±0.40 ^b	7.82 ±0.15 ^a	8.39 ±0.14 ^a	8.14 ±0.25 ^a	8.50 ±0.17 ^a	0.001
Fertility (%)	63.40 ±7.73	88.40 ±7.26	88.4 ±7.26	76.7 ±12.20	86.7 ±13.30	0.285

^{a, b} Means within the same row with different superscripts are significantly different at ($P < 0.05$).

C = Control, DPP1 = 150 mg/kg BW, DPP2 = 300 mg/kg BW, BP1 = 200 mg/kg BW, BP2 = 400 mg/kg BW. TSO = Total sperm output, TMS = Total motile sperm, TLS = Total live sperm, TLSB = Total litter size at birth, TLSBL = Total litter size at birth a live.

The improvement of semen characteristics of treated bucks in the current study is in agreement with previous results of Laghouati *et al.* (2021), who indicated that various concentrations (20, 40, and 80mg/mL) of DPP aqueous extract improved sperm characteristics, such as motility, viability, acrosome reaction, and lipid peroxidation as well as increased spermatogenesis. Improved sperm quality might be due to provided better protection against oxidative damages (Laghouati *et al.*, 2021). Likewise, BP contains a noticeable source of compounds with health-protective potential and antioxidant activity (Hajkova *et al.*, (2013). Thus, BP could play a vital role in improved sperm characteristics and proposed defense

antioxidative damages. Furthermore, amino acids, vitamins, and trace elements of BP are nutritionally beneficial for improving sperm quality and the environmental conditions for the spermatogenesis process. Besides, the semen quality improvement in the current study is related to the enhancement of testosterone level, which caused increasing active spermatogenesis with a significant rise in the number of mature sperms (Andrew *et al.*, 2009; Fouad *et al.*, 2014).

Seminal plasma biochemical constituents

As shown in Table (6), Regarding the protein profiles, the total protein and albumin concentrations were higher ($P < 0.05$) in the seminal plasma of treated groups compared with the C rabbits. Furthermore, the DPP1 buck's seminal plasma had the highest globulin concentration (0.27 ± 0.01 g/dl, $P < 0.05$) compared with the other groups.

Besides, the α and β -globulin levels were highest ($P < 0.05$) in the seminal plasma of DPP2 bucks (0.09 ± 0.01 , and 0.08 ± 0.01 mg/dl, respectively) compared with their counterparts. However, the DPP2 bucks had the lowest ($P < 0.05$) concentration of the seminal plasma γ -globulin (Table 6). On the other hand, the total lipid concentration was highest ($P < 0.05$) in the seminal plasma of the treated rabbits compared with the C bucks. However, no differences ($P > 0.05$) founded between the rabbit groups for triglyceride and total cholesterol parameters (Table 6). Concerning the antioxidant parameters in the seminal plasma, the SOD levels had increased ($P < 0.05$) in treated rabbits compared with the C bucks. Also, the DPP2 and BP2 groups had the highest ($P < 0.05$) concentration of the catalase in the seminal plasma compare with the C bucks by (16.67% and 15.67%, respectively). Besides, the level of the seminal plasma GPX was highest ($P < 0.05$) in the DPP1, and DPP2 bucks compared with the C group by (38.68% and 41.32%, respectively). Furthermore, the MAD concentration had increased ($P < 0.05$) in treated rabbits compared with the C bucks (Table 6). Likewise, the testosterone levels had increased ($P < 0.05$) in treated bucks compared with the C group (Table 6).

In the same trend, treat bucks with DPP or BP in the present study had improved the seminal plasma biochemical components' level like total protein, albumin, globulin, total lipid, antioxidant parameters and testosterone concentration. Propolis has been successfully used to reduce thiobarbituric acid-reactive substance levels and activate antioxidant enzymes such as superoxide dismutase and catalase against free radicals (Hashem *et al.*, 2013). Also, some *in vitro* findings have shown the positive effect of DPP extract supplementation to sperm extender in preserving and maintaining semen quality during cryopreservation in humans' males, buffalo bulls, and stallions (Al-Dujaily *et al.*, 2012; El-Sheshtawy *et al.*, 2014; El-Sisy *et al.*, 2018; Mohamed and Talal 2020). In human sperm, Al-Dujaily *et al.* (2012) observed that *in vitro* sperm motility was

Table 6: Effect of date palm pollen (DPP) or bee pollen (BP) on seminal plasma biochemical parameters of V-line rabbit bucks

Variables	C	DPP1	DPP2	BP1	BP2	P-value
Protein constituents						
Total Protein (g/dl)	0.43 ±0.02 ^c	0.60 ±0.01 ^a	0.57 ±0.02 ^a	0.50 ±0.01 ^b	0.50 ±0.01 ^b	0.00 1
Albumin (g/dl)	0.25 ±0.01 ^c	0.33 ±0.01 ^b	0.39 ±0.02 ^a	0.31 ±0.01 ^b	0.34 ±0.01 ^b	0.00 1
Globulin (g/dl)	0.19 ±0.02 ^b	0.27 ±0.01 ^a	0.18 ±0.01 ^b	0.19 ±0.01 ^b	0.16 ±0.01 ^b	0.00 1
α-globulin (mg/dl)	0.09 ±0.01 ^{ab}	0.08 ±0.01 ^{ab}	0.09 ±0.01 ^a	0.08 ±0.01 ^{ab}	0.07 ±0.01 ^b	0.00 4
β-globulin (mg/dl)	0.07 ±0.01 ^b	0.07 ±0.01 ^{ab}	0.08 ±0.01 ^a	0.07 ±0.01 ^{ab}	0.06 ±0.01 ^b	0.00 1
γ-globulin (mg/dl)	0.03 ±0.01 ^b	0.12 ±0.01 ^a	0.01 ±0.01 ^c	0.03 ±0.01 ^b	0.02 ±0.01 ^b	0.00 1
Lipid profile						
Total Lipids (g/dl)	0.10 ±0.01 ^b	0.20 ±0.01 ^a	0.20 ±0.01 ^a	0.20 ±0.01 ^a	0.20 ±0.01 ^a	0.00 1
Triglyceride (mg/dl)	36.70 ±5.27	33.30 ±4.59	33.30 ±3.80	33.3 ±4.59	33.30 ±5.87	0.98 7
Total cholesterol (mg/dl)	28.70 ±2.74	28.30 ±2.69	28.70 ±1.87	26.70 ±2.43	26.00 ±2.22	0.91 5
Antioxidant status						
SOD (IU/g)	8.60 ±0.07 ^c	8.83 ±0.16 ^{bc}	9.13 ±0.18 ^{ab}	9.20 ±0.22 ^a	9.20 ±0.07 ^a	0.00 7
Catalase (IU/g)	30.00 ±0.36 ^b	32.00 ±0.73 ^{ab}	35.00 ±0.96 ^a	33.30 ±0.92 ^{ab}	34.70 ±1.38 ^a	0.00 1
GPX (mg/L)	3.80 ±0.13 ^b	5.27 ±0.43 ^a	5.37 ±0.65 ^a	4.80 ±0.63 ^{ab}	4.73 ±0.29 ^a b	0.02 0
TAC (mmol/L)	1.43 ±0.02	1.45 ±0.03	1.42 ±0.01	1.42 ±0.06	1.43 ±0.01	0.95 4
MAD (nmole/ml)	5.26 ±0.02 ^c	5.34 ±0.02 ^a	5.34 ±0.01 ^a	5.30 ±0.01 ^b	5.34 ±0.01 ^a	0.00 1
Male hormone						
Testosterone (ng/dl)	0.13 ±0.02 ^b	0.23 ±0.02 ^a	0.27 ±0.01 ^a	0.23 ±0.02 ^a	0.27 ±0.01 ^a	0.00 1

^{a,b,c} Means within the same row with different superscripts are significantly different at ($P < 0.05$).

C = Control, DPP1 = 150 mg/kg BW, DPP2 = 300 mg/kg BW, BP1 = 200 mg/kg BW, BP2 = 400 mg/kg BW. HDL= High density lipoprotein, LDL = Low density lipoprotein, VLDL = Very low-density lipoprotein, SOD = Superoxide dismutase, GPX = Glutathione peroxidase, TAC = Total antioxidant capacity, MAD = Malonaldehyde.

improved when 20% of DPP extract is added to the extender. Also, it has been shown by El-Sheshtawy *et al.* (2014), that aqueous extract of DPP added to Tris citrate-fructose extender (with or without egg yolk) improved sperm motility in bulls, providing a good capacity for the preservation of chilled sperm at 30 mg/mL and frozen-thawed sperm at 30, and 50 mg/mL. Moreover, the addition of 20 mg of pollen grain extract per mL of modified INRA-82 extender improved the chilling and freezing process of Arabian sperm (El-Sisy *et al.*, 2018). Additionally, Mohammed and Talal (2020) found that using DPP extract at 0.04 mg/mL extender in Holstein bulls improved sperm motility both during thawing and 1, 2, and 3 months following cooling. DPP beneficial effect on spermatozoa could be attributed to its antioxidant properties (El-Sisy *et al.*, 2018), and its powerful free radical scavenging capacity (El-Kashlan *et al.*, 2015). Similarly, the BP is rich in polyphenolic substances, flavonoids, phytosterols, and other health-promoting elements, which show the presence of free radical hunting and antioxidants activity (Carpes *et al.*, 2007; Campos *et al.*, 2010). Thus, BP could play an essential role in enhancing the seminal plasma medium, which carries, protect, and nourish spermatozoa after ejaculation up to fertilization. Thus, treating bucks with DPP or BP in the present study had improved the fertility traits represented by the total litter size at birth and alive than those of the control group.

CONCLUSION

Based on the current study results, it could be concluded that treating V-line adult male rabbits with DPP or BP had improved the blood hematological, biochemical variables, and sperm quality and seminal plasma biochemical components. Thus, the positive effect of the previous treatments, especially for the biochemical, immunological, and physiological body functions, resulted in enhancement of the buck's reproductive performance. However, no clear difference was noticeable between both treatments. Accordingly, from an economic point of view, it could recommend using DPP (150 mg/kg BW) or BP (200 mg/kg BW) as a growth promoters' addition for the V-line adult male rabbits without any adverse effect. Also, further studies with more rabbits and flocks are needed to confirm this result and determine the optimal concentration and the possible impact of DPP or BP on the rabbit buck's productivity.

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تأثير استخدام حبوب لقاح النخيل أو حبوب لقاح نحل العسل على المكونات الكيميائية الحيوية للدم، وخصائص السائل المنوي والأداء التناسلي لذكور أرناب الفيلاين

صابر شحاته حسن^١؛ حسام عبد المنعم محمد شهبه^٢؛ محمد محسن منصور^١

^١ قسم الإنتاج الحيواني والداجني، كلية الزراعة، جامعة دمنهور، دمنهور، 22516، البحيرة، مصر.

^٢ معهد بحوث الإنتاج الحيواني، وزارة الزراعة، مركز البحوث الزراعية، الدقي، 12816، الجيزة، مصر.

أجريت الدراسة الحالية بهدف مقارنة تأثير تجريع المعلق المائي لحبوب لقاح النخيل أو حبوب لقاح النحل عن طريق الفم على الأداء الفسيولوجي والتناسلي في ذكور أرناب الفيلاين. تم استخدام عدد 50 ذكر عند عمر 20 أسبوع وقسمت إلي خمس مجموعات متساوية (10 ذكور/ معاملة) كالأتي: مجموعة التحكم تم اعطاءها دواء وهمي عبارة عن 2 مل ماء، مجموعتين حبوب لقاح النخيل تم اعطاؤها 2 مل ماء تحتوي على 100 ملجم/ كجم من وزن الجسم و 300 ملجم/ كجم من وزن الجسم علي التوالي، ومجموعتين حبوب لقاح النحل تم اعطاؤها 2 مل ماء تحتوي على 200 ملجم/ كجم من وزن الجسم و 400 ملجم/ كجم من وزن الجسم علي التوالي. خلال 12 أسبوع (فترة التجربة) كان يتم تجريع الذكور 3 مرات أسبوعياً (الأحد، الثلاثاء، والخميس من كل أسبوع). جمعت عينات الدم في الأسبوع 32 لفحص مكونات الدم الكيميائية والحيوية. وأيضاً، عند عمر 28 أسبوع تم جمع السائل المنوي مرة واحدة أسبوعياً لتقييم جودة السائل المنوي والمكونات الكيميائية الحيوية للبلازما المنوية.

وأشارت النتائج إلى: أن خلايا الدم البيضاء وكذلك الخلايا الليمفاوية قد زادت بشكل ملحوظ عند المستوى المرتفع لكل المكملات مقارنة بمجموعة التحكم. بينما مجموعة المستوى المرتفع من حبوب نحل العسل أدت إلى زيادة في خلايا الدم الحمراء والهيموجلوبين وحجم كرات الدم وانخفاض معنوياً مستوى اليوريا مقارنة مع مجموعة التحكم دون أي اختلافات معنوية بين المعاملات. علاوة على ذلك، زادت الدهون عالية الكثافة والتستستيرون في الأرانب المعاملة، وانخفضت الدهون منخفضة الكثافة والدهون المنخفضة الكثافة جداً والألانين أمينو ترانسفيراز بشكل ملحوظ عند المقارنة مع مجموعة الكنترول. كما أنه زادت القدرة الكلية لمضادات الأكسدة وتركيز هرمون التستستيرون في كل المعاملات مقارنة بمجموعة الكنترول. نستنتج من هذه النتائج أن حبوب لقاح النخيل أو حبوب لقاح النحل تعتبر إضافات طبيعية آمنة كما أنها حسنت الصفات المناعية والفسولوجية والأداء التناسلي لذكور الأرانب، **التوصية:** ومن وجهة النظر الإقتصادية، يمكن التوصية باستخدام مستويات حبوب النخيل بمعدل 150 ملجم/ كجم من وزن الجسم أو حبوب نحل العسل بمعدل 200 ملجم/ كجم من وزن الجسم، لتحسين صفات الدم الطبيعية والبيوكيميائية والصفات المناعية والفسولوجية، وبالتالي الأداء التناسلي لذكور الأرانب.

الكلمات الدالة: أرانب الفي لاين ، جودة السائل المنوي، التستستيرون، حبوب لقاح النخيل، حبوب لقاح نحل العسل.