

## PRODUCTIVE AND REPRODUCTIVE PERFORMANCE OF RABBITS DOES AS AFFECTED BY BEE POLLEN AND/OR PROPOLIS, INULIN AND/OR MANNAN-OLIGOSACCHARIDES

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**Abstract:** The aim of the paper was to compare the effect of prebiotics (inulin and/or mannan-oligosaccharides, MOS) and bee products (bee pollen and/or propolis) on productive and reproductive performance of rabbit does. Seventy nulliparous V-line female rabbits were distributed among 7 groups. The groups were fed the same diet and received no supplements (control group), natural molecules (bee pollen and/or propolis) at 200 mg/kg body weight (BW) or prebiotics (inulin and/or MOS) at 35 mg/kg BW. Productive, reproductive, biochemical and haematological traits were investigated. Bee pollen with propolis significantly increased body weight gain of does 1 wk after mating (3.53%), decreased feed intake (4.49%) and caused larger litter size (39.4%), heavier body weight of litter (17.7%), a greater number of kits born alive (48.7%), higher weight of kits (87.81%) at 28 d of age, higher milk yield (43.6%) and more favourable milk conversion ratio (31.6%). Moreover, bee pollen with propolis had significantly increased plasma total protein (43.1%), albumin (45.7%), globulin (41.0) and progesterone (60.5%), and had a significantly decreased plasma cholesterol (31.1%), aspartate aminotransferase/alanine aminotransferase ratio (20.3%) compared to the control group. Does treated with growth promoters had significantly fewer services per conception (22%) and greater fertility rate (21%) compared to the control group. Inulin with or without MOS significantly increased plasma glucose (49.9 and 50%, respectively) and feed cost (90.2%) compared to the control group. Supplementation of MOS or bee pollen with or without propolis had significantly greater relative economic efficiency (61.9, 55.1 and 27.1%, respectively) than the control group. MOS and bee pollen with or without propolis are able to improve productive and reproductive performance and economic efficiency of rabbit does in comparison to the unsupplemented group.

**Key Words:** rabbit, natural growth promoters, bee pollen, propolis, inulin, mannan-oligosaccharides.

## INTRODUCTION

The use of alternative growth promoters in animal production has increased since the European Community antibiotics ban in 2006. In recent decades different prebiotics have been evaluated, such as oligosaccharides derived from inulin as fructose-oligosaccharides (FOS) and from yeast cell walls such as mannan-oligosaccharides (MOS) or other oligomers. Prebiotics, as they are not degraded in the stomach, reach the intestine unmodified and thus mainly act by supplying a nutrient "ready to use" for intestinal microflora (Bovera *et al.*, 2010). This helps optimise the eubiosis, improves the digestion capacity and increases the animal health status (Attia *et al.*, 2011a). Oligosaccharide-based prebiotics like inulin have been intensively examined as an alternative to antibiotics in growing rabbits (Maertens *et al.*, 2004; Bonai *et al.*, 2010) and rabbit does (Maertens *et al.*, 2013).

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Radwan and Abdel-Khalek (2007) reported that galacto-oligosaccharides and MOS are able to fortify rabbits reared under high ambient temperatures by improving growth and health of growing rabbits. In addition, Navidshad *et al.* (2015) indicated that MOS had a positive effect on the animals' immune system.

Along with prebiotics, a large number of natural molecules were tried out as supplements in animal nutrition as alternatives to antibiotic. Among these, bee products have recently been evaluated for their interesting properties. Bee pollen appears promising because it enhances the immune function of poultry, promotes animal growth, protects intestinal tract health and improves the quality and safety of animal products (Attia *et al.*, 2011a, 2011b). Bee pollen is confirmed as an interesting supplement in rabbits able to improve productive and reproductive performance (Attia *et al.*, 2011b) also under heat stress conditions (Attia *et al.*, 2011a). Bee pollen protects the kidneys and can lower the level of triglycerides, cholesterol, creatinine and blood urea nitrogen in rats (Hu *et al.*, 2003).

Another very interesting bee product is propolis, which has antibiotic properties and may improve growth performance, feed efficiency and feed intake of animals (Sarker and Yang, 2010). These effects may be due to the content of antioxidants, vitamins, minerals, phenolic constituents and enzymes (El-Hanoun *et al.*, 2007).

The aim of this study was to compare the effect of prebiotics (inulin and/or MOS) to that of bee products used as dietary supplements (bee pollen and/or propolis) on the productive-reproductive performance of V-line rabbit does.

## MATERIALS AND METHODS

The doses of propolis and bee pollen used in the trial were according to El-Hanoun *et al.* (2007) and Attia *et al.* (2011a, 2011b), whereas inulin was supplemented according to Eiben *et al.* (2008) and MOS according to the manufacturer's recommendations.

### Animals and treatments

Seventy 5-mo-old nulliparous V-line doe rabbits were distributed into 7 homogeneous groups (10 does/group) in a straight run experimental design. The groups were fed the same basal diet and submitted to the following treatments: the control was unsupplemented; bee pollen group (BP) received bee pollen at 200 mg/kg body weight (BW); propolis group (P) had propolis at 200 mg/kg BW; bee pollen with propolis group (BP+P) received 200 mg/kg BW of each additive; inulin group (In) was supplemented with 35 mg/kg BW of inulin; MOS group received MOS at 35 mg/kg BW and inulin with MOS group (In+MOS) received 35 mg/kg BW of each prebiotic. Supplementations were given orally as water suspension for 3 d/wk (Saturday, Tuesday and Thursday) for 5 wk (1 wk before mating and 4 wk after mating) for 6 consecutive matings. The does were naturally mated for the first time at 5 mo (1 wk after supplementations); after the first time, the does were mated 11 d from the last kindling. Mating was randomly performed with 15 adult males of V-line rabbits, non-treated.

**Table 1:** Ingredients profile and determined composition of the experimental diet (as feed basis).

Ingredients	g/kg	Determined analysis	
Barley	130	Dry matter, %	87.47
Yellow corn	120	Crude protein, %	18.31
Wheat bran	250	Crude Fibre, %	13.6
Soybean meal	220	Ether extract, %	3.01
Clover hay	160	Nitrogen free extract, %	57.25
Clover straw	74.5	Ash, %	10.07
Molasses	22.5	Digestible energy, kcal/kg	2502
Limestone	15.0	Neutral detergent fibre %	32.98
Sodium chloride	3.0	Acid detergent fibre %	16.34
Vitamin and minerals mixture <sup>a</sup>	3.0	Hemicellulose, %	16.64
DL- methionine	2.0		
Total	1000		

Hemicellulose calculated as the differences between neutral and acid detergent fibre.

<sup>a</sup> Each kg of vitamin and mineral contains, Vit. A 2000000 IU; Vit. D 150000 IU; Vit. E 8.33 g; Vit. K 0.33 g; Vit. B<sub>1</sub> 0.33 g; Vit. B<sub>2</sub> 1g; Vit. B<sub>6</sub> 0.33 g; Vit. B<sub>9</sub> 8.33 g; Vit. B<sub>12</sub> 1.7 mg; Pantothenic acid 3.33 g; Biotin 33 mg; Folic acid 0.83 g; Choline Chloride 200 g; Zn 11.7 g; Fe 12.5 g; Cu 0.5 g; I 33.3 mg; Se 16.6 mg and Mn 5 g.

Rabbits were housed in a naturally ventilated building and kept in individual Italian wire galvanised cages (60×55×40 cm) equipped with an internal nest-box. Rabbits were fed *ad libitum* the commercial basal diet (Table 1). Diet chemical composition was determined according to AOAC (2004) procedures, digestible energy was estimated from chemical composition according to the equation proposed by Xiccato (1989), the neutral detergent fibre and acid detergent fibre were determined according to Van Soest and Wine (1967) and Van Soest and Robertson (1980), while the digestible energy was calculated according to NRC (1977). They were given 14 h of light daily. Air temperature during the experimental period ranged from 15.1 to 29.2°C, while relative humidity ranged from 37.8 to 78.7%.

### **Response criteria**

Body weight of does was recorded at the beginning of the trial and one week after each mating. For each mating, feed intake was recorded throughout the pregnancy period (31 d) and milk yield up to 28 d after parturition by the difference between weights of offspring before and after suckling. Productive traits such as number of services per conception, conception rate and litter sizes (total and alive) at birth day, number of kits alive at 28 d of age (survival rate; %) kits body weight (g) at birth and 28 d after parturition were also recorded.

Data after mating were presented as average values of a set of 5 values.

### **Blood biochemical constituents**

Blood samples were collected from the marginal ear veins from 6 does/treatment at day 15 after mating in the morning before feeding. The blood samples were collected in both heparinised and non-heparinised tubes to collect plasma and serum. All biochemical traits of blood plasma (total protein, albumin, creatinine, urea, total lipids, total cholesterol, triglycerides, glucose, alanine aminotransferase – ALT - and aspartate aminotransferase - AST) were determined using commercial kits (Diamond Diagnostics, Egypt) as reported by Attia *et al.* (2009; 2011a; b). Globulin was obtained by subtracting the concentration of albumin from that of plasma total protein.

Concentrations of plasma estradiol-17 $\beta$  (E<sub>2</sub>) and progesterone (P<sub>4</sub>) were assayed by radioimmunoassay (RIA) using DSL-43100 and DSL-3900, respectively (Diagnostic systems Laboratories Inc, TX, USA) according to Abraham (1977). The sensitivity of the E<sub>2</sub> assay is 11.0 pg/mL at 95% confidence level. The intra-assay coefficients of variation ranged from 2.01-5.17% from the mean of 12 replicates each. The coefficient of variation for the inter-assay ranged from 6.59-7.93% of the average of duplicates for 24 separate runs.

The sensitivity of the P<sub>4</sub> assay of 12 replicates of the 0 ng/mL P<sub>4</sub> standard is 0.12 ng/mL. The intra-assay coefficients of variation ranged from 4.8-8.0% of the mean of 10 replicates each. The coefficient of variation for the inter-assay ranged from 9.2-13.1% of average duplicates for 10 separate runs.

### **Statistical analysis**

The statistical analyses model used is two factorial experimental design (feed additives×parity) plus the unsupplemented control) with repeated measurements according to the following model:

$$y_{ijk} = \mu + A_i + \beta_j + (A\beta)_{ij} + e_{ijk}$$

in which  $\mu$ =general mean, A<sub>i</sub>=effect of type of feed additives,  $\beta_j$ =effect of parity number, (A $\beta$ )<sub>ij</sub>=interaction between type of feed additives and parity number, and e<sub>ijk</sub>=random error.

## **RESULTS**

### **Productive performance of does**

Table 2 shows the *in vivo* performance of does throughout the trial. BP and BP+Pro groups had significantly heavier BW 1 wk after mating compared to the other groups and all the other supplemented groups had higher BW (*P*<0.01) after mating than the control.

**Table 2:** Effect of natural growth promoters on body weight parameters and feed intake during pregnancy period of V-line rabbit does (n=10).

Treatment	Initial body	Body weight	Body weight gain	Daily Feed
	weight* (g)	1 wk after mating (g)	1 wk after mating (g)	intake (g)
Control	3680	3855 <sup>c</sup>	174.9 <sup>a</sup>	243.4 <sup>a</sup>
Bee pollen	3687	3988 <sup>a</sup>	300.9 <sup>b</sup>	234.9 <sup>bc</sup>
Propolis	3673	3913 <sup>b</sup>	239.6 <sup>cd</sup>	237.5 <sup>b</sup>
Bee pollen with propolis	3679	3995 <sup>a</sup>	316.5 <sup>a</sup>	231.5 <sup>d</sup>
Inulin	3678	3909 <sup>b</sup>	231.2 <sup>d</sup>	233.9 <sup>cd</sup>
MOS	3667	3908 <sup>b</sup>	241.1 <sup>cd</sup>	237.0 <sup>b</sup>
Inulin with MOS	3672	3915 <sup>b</sup>	242.7 <sup>b</sup>	233.2 <sup>cd</sup>
<i>P</i> -value	NS	0.0001	0.0001	0.0001
SEM	14	14	3.0	0.8

\* at 5 months of age; <sup>a-i</sup> Means within a column at each item bearing different superscripts are significantly different. NS: Not significant. MOS: Mannan-oligosaccharides. SEM: standard error of mean.

Body weight gain of does was the highest in BP+Pro group ( $P<0.01$ ). The other supplemented groups had higher BWG than the control ( $P<0.01$ ), with the exception of Pro and MOS groups which showed no different BWG than the control group and the In group, where BWG was not different from Pro and MOS but lower than the control ( $P<0.01$ ).

Feed intake was highest in the control group. BP+Pro group consumed less feed than BP, Pro and MOS groups ( $P<0.01$ ). On the other hand, there were no significant differences among BP+Pro, In and In+MOS groups.

### Reproductive performance of does

Data presented in Table 3 showed that all the supplements and prebiotics had similar ability to decrease the number of services to achieve pregnancy and to increase fertility than the control group.

BP+Pro group had greater litter size, body weight of kits at birth and number of kits born alive than the other groups ( $P<0.01$ ). The other groups receiving supplements or prebiotics had greater litter size, body weight of kits at birth and number of kits born alive than the control and the In group showed higher values than the other supplemented groups ( $P<0.01$ ), with the exception of litter size, which is no different from that of the BP group. The number of stillborn kits did not significantly vary among the different groups.

### Effects on offspring performance during days 1-28 of age

Data presented in Table 4 showed that BP+Pro group had the highest growth of kits, milk yield and milk conversion ratio during days 1-28 of age. Control group had the lowest values for these criteria ( $P<0.01$ ). Survival rate of litter from BP+Pro group was higher than the control ( $P<0.05$ ), while no differences were observed among the other groups.

**Table 3:** Effect of natural growth promoters on reproductive performance of V-line rabbit does (n=10).

Treatment	Services/ conception	Fertility (%)	Litter		
			Size	Total body weight at birth (g)	No. born alive
Control	1.77 <sup>a</sup>	69.5 <sup>b</sup>	5.25 <sup>d</sup>	225.4 <sup>a</sup>	4.62 <sup>e</sup>
Bee pollen	1.32 <sup>b</sup>	86.9 <sup>a</sup>	6.47 <sup>bc</sup>	320.4 <sup>c</sup>	5.97 <sup>c</sup>
Propolis	1.45 <sup>b</sup>	81.9 <sup>a</sup>	6.08 <sup>c</sup>	290.3 <sup>d</sup>	5.63 <sup>d</sup>
Bee pollen with propolis	1.35 <sup>b</sup>	84.2 <sup>a</sup>	7.32 <sup>a</sup>	393.4 <sup>a</sup>	6.87 <sup>a</sup>
Inulin	1.35 <sup>b</sup>	85.3 <sup>a</sup>	6.73 <sup>b</sup>	333.0 <sup>b</sup>	6.27 <sup>b</sup>
MOS	1.48 <sup>b</sup>	81.4 <sup>a</sup>	6.15 <sup>c</sup>	291.5 <sup>d</sup>	5.70 <sup>d</sup>
Inulin with MOS	1.35 <sup>b</sup>	84.7 <sup>a</sup>	6.38 <sup>bc</sup>	313.1 <sup>c</sup>	5.88 <sup>cd</sup>
<i>P</i> -value	0.008	0.01	0.0001	0.0001	0.0001
SEM	0.09	3.5	0.11	4.1	0.08

<sup>a-i</sup> Means within a column at each item bearing different superscripts are significantly different; NS: not significant. MOS: Mannan-oligosaccharides. SEM: standard error of mean.

**Table 4:** Effect of natural growth promoters on offspring performance of V-line rabbit does during days 1-28 of age (n=10).

Treatment	Litter			
	Survival rate %	Body weight at 28 day of age (g)	Milk yield/ doe (g)	Milk conversion ratio (g milk/g gain)
Control	78.2 <sup>b</sup>	435.4 <sup>f</sup>	3847.2 <sup>f</sup>	2.72 <sup>d</sup>
Bee pollen	83.6 <sup>ab</sup>	466.5 <sup>c</sup>	4541.7 <sup>c</sup>	2.10 <sup>bc</sup>
Propolis	85.2 <sup>ab</sup>	451.3 <sup>e</sup>	4140.5 <sup>e</sup>	2.03 <sup>abc</sup>
Bee pollen with propolis	87.4 <sup>a</sup>	484.5 <sup>a</sup>	5176.7 <sup>a</sup>	1.86 <sup>a</sup>
Inulin	84.3 <sup>ab</sup>	468.7 <sup>bc</sup>	4573.1 <sup>c</sup>	1.94 <sup>ab</sup>
MOS	82.9 <sup>ab</sup>	455.6 <sup>d</sup>	4225.0 <sup>d</sup>	2.11 <sup>bc</sup>
Inulin with MOS	82.5 <sup>ab</sup>	472.6 <sup>b</sup>	4686.2 <sup>b</sup>	2.20 <sup>c</sup>
<i>P</i> -value	0.02	0.0001	0.0001	0.0001
SEM	1.8	1.5	16.4	0.05

<sup>a-f</sup> Means within a column at each item bearing different superscripts are significantly different. MOS: Mannan-oligosaccharides. SEM: standard error of mean.

### Blood constituents of V-line rabbit does

Data presented in Table 5 showed that groups supplemented with different additives or prebiotics had the highest plasma total protein, albumin and globulin ( $P<0.01$ ). However, all the treatments had higher values of these 3 plasma constituents than the control, while the In and In+MOS groups showed higher values than the other supplemented groups ( $P<0.01$ ). Globulin to albumin ratio of BP group was higher than the other supplemented groups but not different from the control ( $P<0.01$ ). The In and In+MOS groups had higher glucose values than the other groups and were followed by BP, MOS, BP+Pro, Pro and control groups ( $P<0.01$ ). BP group also showed the highest levels of total lipids and all the other supplemented groups were not different for these constituents and had higher values than the control ( $P<0.01$ ). Control group had the highest value of cholesterol followed by Pro, In, MOS and BP together, In+MOS and BP+Pro groups ( $P<0.01$ ).

### Effects on kidney function, hepatocellular leakage and ovarian hormones

Data presented in Table 6 showed that natural supplements or prebiotics resulted in lower plasma urea than the control group and groups supplemented with Pro and BP+Pro had lower values than the others.

Plasma creatinine was higher in all the supplemented group in comparison to the control and Pro, BP+Pro and In+MOS groups had higher values than the others ( $P<0.01$ ). The urea/creatinine ratio was the highest in the control group followed by BP, MOS, In+MOS together and In and both Pro and BP+Pro groups ( $P<0.01$ ). Control group

**Table 5:** Effect of natural growth promoters on blood plasma biochemical constituents of V-line rabbit does (n=10).

Treatment	Total protein (g/dL)	Albumin (mg/dL)	Globulin (mg/dL)	Globulin/albumin	Glucose (mg/dL)	Total lipids (mg/dL)	Cholesterol (mg/dL)
Control	5.18 <sup>f</sup>	2.45 <sup>f</sup>	2.73 <sup>e</sup>	1.12 <sup>ab</sup>	92.1 <sup>f</sup>	431 <sup>c</sup>	84.5 <sup>a</sup>
Bee pollen	6.23 <sup>d</sup>	2.90 <sup>d</sup>	3.33 <sup>c</sup>	1.15 <sup>a</sup>	132.5 <sup>b</sup>	562 <sup>a</sup>	65.8 <sup>d</sup>
Propolis	5.88 <sup>e</sup>	2.82 <sup>e</sup>	3.06 <sup>d</sup>	1.09 <sup>b</sup>	117.9 <sup>e</sup>	504 <sup>b</sup>	71.9 <sup>b</sup>
Bee pollen with propolis	7.41 <sup>a</sup>	3.57 <sup>a</sup>	3.85 <sup>a</sup>	1.08 <sup>b</sup>	126.0 <sup>d</sup>	505 <sup>b</sup>	58.2 <sup>f</sup>
Inulin	6.89 <sup>b</sup>	3.31 <sup>b</sup>	3.60 <sup>b</sup>	1.09 <sup>b</sup>	138.0 <sup>a</sup>	512 <sup>b</sup>	68.4 <sup>c</sup>
MOS	6.40 <sup>c</sup>	3.08 <sup>c</sup>	3.32 <sup>c</sup>	1.08 <sup>b</sup>	130.1 <sup>c</sup>	509 <sup>b</sup>	66.8 <sup>d</sup>
Inulin with MOS	6.83 <sup>b</sup>	3.28 <sup>b</sup>	3.55 <sup>b</sup>	1.08 <sup>b</sup>	138.1 <sup>a</sup>	507 <sup>b</sup>	63.0 <sup>e</sup>
<i>P</i> -value	0.0001	0.0001	0.0001	0.001	0.0001	0.0001	0.0001
SEM	0.04	0.03	0.03	0.01	0.62	43.1	0.3

<sup>a-f</sup> Means within a column at each item bearing different superscripts are significantly different; MOS: Mannan-oligosaccharides. SEM: standard error of mean.

**Table 6:** Effect of natural growth promoters on kidney function and liver enzymes and reproductive hormones of V-line rabbit does (n= 10).

Treatment	Kidney function			Liver Enzymes			Hormones		
	Urea (mg/dL)	Creatinine (mg/dL)	Urea/ Creatinine ratio	AST (IU)	ALT (IU)	AST/ALT (%)	E <sub>2</sub> (ng/mL)	P <sub>4</sub> (ng/mL)	E <sub>2</sub> /P <sub>4</sub> ratio
Control	26.5 <sup>a</sup>	1.26 <sup>c</sup>	21.3 <sup>a</sup>	33.0 <sup>a</sup>	62.6 <sup>a</sup>	52.7 <sup>a</sup>	0.154 <sup>b</sup>	0.400 <sup>e</sup>	0.385 <sup>a</sup>
Bee pollen	21.4 <sup>b</sup>	1.61 <sup>b</sup>	13.4 <sup>b</sup>	23.6 <sup>c</sup>	54.0 <sup>c</sup>	43.6 <sup>b</sup>	0.200 <sup>a</sup>	0.600 <sup>b</sup>	0.333 <sup>c</sup>
Propolis	18.2 <sup>e</sup>	1.64 <sup>a</sup>	11.2 <sup>e</sup>	23.7 <sup>c</sup>	53.2 <sup>c</sup>	44.7 <sup>b</sup>	0.200 <sup>a</sup>	0.538 <sup>d</sup>	0.375 <sup>a</sup>
Bee pollen with propolis	18.1 <sup>e</sup>	1.66 <sup>a</sup>	11.0 <sup>e</sup>	23.1 <sup>c</sup>	54.9 <sup>b</sup>	42.0 <sup>c</sup>	0.200 <sup>a</sup>	0.642 <sup>a</sup>	0.314 <sup>c</sup>
Inulin	19.8 <sup>cd</sup>	1.64 <sup>a</sup>	12.2 <sup>d</sup>	25.0 <sup>b</sup>	55.6 <sup>b</sup>	45.0 <sup>b</sup>	0.200 <sup>a</sup>	0.600 <sup>b</sup>	0.333 <sup>c</sup>
MOS	20.3 <sup>c</sup>	1.58 <sup>b</sup>	12.9 <sup>c</sup>	25.3 <sup>b</sup>	55.7 <sup>b</sup>	45.5 <sup>b</sup>	0.196 <sup>a</sup>	0.554 <sup>c</sup>	0.356 <sup>b</sup>
Inulin with MOS	19.4 <sup>d</sup>	1.58 <sup>a</sup>	12.3 <sup>d</sup>	24.5 <sup>bc</sup>	55.4 <sup>b</sup>	44.2 <sup>b</sup>	0.200 <sup>a</sup>	0.600 <sup>c</sup>	0.333 <sup>c</sup>
P-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
SEM	0.2	0.008	0.16	0.3	0.3	0.5	0.002	0.004	0.006

<sup>a-d</sup> Means within a column at each item bearing different superscripts are significantly different. E<sub>2</sub>: Estradiol-17 $\beta$ . P<sub>4</sub>: Progesterone. AST: Aspartate aminotransferase. ALT: Alanine aminotransferase. MOS: Mannan-oligosaccharides. SEM: standard error of mean.

showed the highest values of liver enzymes (AST, ALT and AST/ALT) ( $P < 0.01$ ) and few differences were observed among the supplemented groups for these enzymes.

E<sub>2</sub> hormone was the lowest in the control group ( $P < 0.01$ ) and no differences were observed among the supplemented groups. P<sub>4</sub> was the highest in BP+Pro group ( $P < 0.01$ ) and, with the exception of MOS and In+MOS, all the supplemented groups had higher values than the control. The E<sub>2</sub>/P<sub>4</sub> ratio in control and Pro groups was higher than the others ( $P < 0.01$ ) and MOS group had higher values than BP, BP+Pro, In and In+MOS groups.

## DISCUSSION

Bee pollen and/or propolis improved productive performance of does and growth traits of their offspring. The combination of bee pollen and propolis had a stronger effect than bee pollen or propolis administered alone on BWG of doe 1 wk after mating, litter size, growth of kits, number of kits born alive and milk yield. El-Hanoun *et al.* (2007) and Attia *et al.* (2011b) reported that propolis and bee pollen significantly increased milk yield, especially at 200 mg /kg BW/d.

**Table 7:** Effect of natural growth promoters on feed intake, reproductive performance and kits at weaning of V-line rabbit does along the entire period of the trial.

Treatment	Feed intake (g/doe)	Kits at weaning /doe (n)
Control	7302 <sup>a</sup>	3.58 <sup>d</sup>
Bee pollen	7047 <sup>bc</sup>	4.95 <sup>c</sup>
Propolis	7125 <sup>b</sup>	4.77 <sup>c</sup>
Bee pollen with propolis	6945 <sup>d</sup>	5.97 <sup>a</sup>
Inulin	7017 <sup>cd</sup>	5.27 <sup>b</sup>
MOS	7110 <sup>b</sup>	4.68 <sup>c</sup>
Inulin with MOS	6996 <sup>cd</sup>	4.82 <sup>c</sup>
P-value	0.0001	0.0001
SEM	15	0.5

<sup>a-d</sup> Means within a column bearing different superscripts are significantly different. EE: Economic efficiency. REE: Relative economic efficiency as a percentage of the control. MOS: Mannan-oligosaccharides; EGP: Egyptian pound (1 Egyptian pound=0.128 USD). SEM: standard error of mean.

A combination of inulin and MOS outperformed the effect of both for BWG of does and milk yield, and the effect of only MOS was for growth of kits at day 28 of age. These results showed that inulin had a stronger effect than MOS for most productive and litter traits and, when combined with MOS, improved productive performance and litters traits than MOS alone.

Comparing the combined effect of bee pollen with propolis with that of inulin with MOS, the results revealed that bee pollen with propolis was more efficient than FOS with MOS. It should be mentioned however, that all natural growth promoters were equally potent for improving reproductive traits (number of services per conception and conception rate) of V-line doe rabbits. It must be emphasised that the improved productive performance of V-line doe rabbits was concurrent with significant lowering of feed intake, showing improved feed utilisation. On the other hand, the improvements in litter traits were accompanied by increasing milk yield and enhancing survival rate and milk conversion ratio (MCR) of litters. As for does, the decrease in feed intake of kits may relate to the increase in nutrient availability. Attia *et al.* (2011a) found that growing males of NZW rabbits supplemented with bee pollen had a lower feed intake compared to the control group. In fact, nutrients such as minerals and water-soluble vitamins in bee pollen and propolis could accelerate the metabolism of nutrients and increase energy availability (Attia *et al.*, 2014). Increasing energy availability negatively affects feed intake (Attia *et al.*, 2009).

The comparison among bee pollen, propolis, inulin and MOS revealed that inulin was the most effective single agent. However, all agents are equally potent for improving the number of services per conception and fertility rate. These results are similar to those reported by El-Hanoun *et al.* (2007) and Attia *et al.* (2011a;b) who revealed that the highest fertility rate was observed in rabbits treated with medium levels of bee pollen (200 mg/kg BW).

The positive effect of bee pollen on productive traits of doe rabbits could be attributed to the high macro and micro-nutrient contents (including polyunsaturated fatty acids, minerals, etc.) as well as of protective agents and phytosterols such as flavonoids, carotenoids and phenolic constituents (Leja *et al.*, 2007; Šarić *et al.*, 2009). Bee pollen constituents in mice pass directly from the stomach into the blood stream within two hours after ingestion and are found in the blood, cerebral spinal fluids and urine (Markham and Campos, 1996), showing high absorption and availability of nutrients for animals. Bee pollen was found to enhance immunising function, promote animal growth, protect intestinal tract health and improve animal the quality and security of products (Attia *et al.*, 2011a;b). It was reported that diet supplemented with 1.5% bee pollen could improve the tissue's structure of the digestive organ, thus increasing the body's digestion and absorption functions and significantly improving reproductive performance and plasma concentration of reproductive hormones (Attia *et al.*, 2011a). In addition, bee pollen improved growth and FCR of rabbits and bucks and dams of NZW rabbits (El-Hanoun *et al.*, 2007; Attia *et al.*, 2011b).

The positive effect of propolis on growth performance observed with V-line rabbit does could be attributed to its antibacterial (Scazzochio *et al.*, 2006), antiviral (Gekker *et al.*, 2005), antifungal (Dobrowolski *et al.*, 1991 and Sforcin *et al.*, 2001), antiparasitic (Freitas *et al.*, 2006), anti-inflammatory (Dobrowolski *et al.*, 1991), immunomodulatory (Dimov *et al.*, 1992) and antioxidant (Krol *et al.*, 1990) effects.

Similar to the negative effect of propolis on feed intake, El-Hanoun *et al.* (2007) showed that NZW rabbit does orally supplemented with propolis (0, 100, 200, and 300 mg/kg BW) resulted in significantly decreased daily feed intake compared to control, while increasing BW and improving FCR. In addition, Yousef *et al.* (2010) reported that propolis supplementation increased BW of NZW male rabbits compared to the control group.

Number of kits born alive and survival rate of kits at day 28 of age was significantly improved due to various natural growth promoters or prebiotics. These results are similar to those reported by Attia *et al.* (2011a; b) who, showed that bee pollen (200 and 300 mg/kg BW) improved survival rate of rabbits.

Similar to the positive effect of propolis on number of rabbits born live and survival rate, El-Hanoun *et al.* (2007) demonstrated that young rabbits produced from rabbit does treated by bee propolis at (100, 200 and 300 mg/kg BW) had a greater survival rate after weaning up to marketing age compared to young rabbits produced from untreated does. They added that NZW rabbit does supplemented with (200 mg/kg BW) of propolis had significantly greater litter size and growth. In addition, rabbit does supplemented with bee propolis had significantly greater ( $P \leq 0.05$ ) litter size, survival percentage and growth of kits. The positive effect of propolis on survival rate could be due to its antimicrobial effects, as propolis was found to be effective against *Staphylococcus aureus* and *S. epidermis* bacteria in chickens



under *in vitro* conditions (Lotfy, 2006), while increasing the numbers of beneficial bacteria (*lactic acid*) in jejunum (Tekeli *et al.*, 2010), and this accompanied by decreasing numbers of harmful bacteria (*Coliform* and *Escherichia coli*). In addition, Tekeli *et al.* (2010) showed that broiler supplemented separately with *Zingiber officinale* and propolis extract and their combinations significantly improved villi length ( $P < 0.05$ ) compared to control.

The effect of bee pollen on plasma metabolites and ovarian hormones may be due to its vitamins, minerals, phospholipids, balanced nutrient profiles (Leja *et al.*, 2007) and copper contents, which play a role in reproduction (Leja *et al.*, 2007; Xu *et al.*, 2009) and antioxidant factors of bee pollen (Quian *et al.*, 2008; Šarić *et al.*, 2009).

In accordance with our results, Attia *et al.* (2011a;b) and Ceglecka (1991) found that rats supplemented with 60 mg of pollen extracts per kg BW significantly decreased liver-enzyme levels compared to control. The cytotoxic effect as indicated by liver damage in the control rats increased their plasma cholesterol by 104% and triglycerides by 37%, whereas these increases were all but prevented in rats given pollen extract.

The decrease in plasma cholesterol could be due to phospholipids and polyunsaturated fatty acids, particularly linolenic fatty acid, which represented 1.19 % in bee pollen (Xu *et al.*, 2009). Meanwhile, the increase in plasma total lipids could be explained by the increase in plasma oestrogen level and/or the polyunsaturated fatty acids of bee pollen.

The increase in plasma protein and albumin may be due to increasing availability of protein and/or amino acids in bee pollen (Bell *et al.*, 1983). The increase in plasma glucose level due to bee pollen supplementation is within the normal limits. Quian *et al.* (2008) and Attia *et al.* (2009) reached similar conclusions due to glucose supplementation during summer months.

The effect of propolis on plasma glucose, total lipids, cholesterol and creatinine are in agreement with those reported by Sforzin *et al.* (2002) who reported that rats supplemented with propolis did not induce kidney damage as indicated by blood urea and creatinine concentration. In addition, El-Hanoun *et al.* (2007) reported that propolis significantly decreased  $E_2$ . On the other hand,  $P_4$  levels were significantly greater only with a medium (200 mg) dose of propolis than in control group. The later effect could explain the increase in plasma  $E_2$  observed in the propolis supplemented-group.

Similarly, Kolankaya *et al.* (2002) found that propolis significantly decreased cholesterol and triglycerides in rats. In addition, Fuliang *et al.* (2005) revealed that ethanol extract propolis and water extract propolis (1 mL/100g BW) decreased blood glucose, total cholesterol and triglycerides in plasma of fasting rats and this directly related to the influence of propolis on lipid metabolism (Matsui *et al.*, 2004).

In line with the present findings, El-Hanoun *et al.* (2007) demonstrated that propolis at low and medium doses (100 and 200 mg/kg BW, respectively) caused significant increases in plasma glucose, total protein and globulin contents compared to the control group, while decreasing total lipids and urea and liver enzymes (AST and ALT) compared to control. They attributed the improvement in liver and kidney functions in rabbit does to the higher biological activity and nutritive values contents in bee propolis, which could prevent lipid peroxidation. The effect of propolis on AST and ALT enzymes are similar to those reported by Nirala *et al.* (2008) and Newairy *et al.* (2009), who indicated that propolis induced reduction of the activity of AST and ALT concentration in plasma of rats. However, El-Kott and Owayss (2008) showed that oral propolis extract (150 mg/kg BW) administered as a supplement to old male mice for 8 wk did not significantly affect liver enzymes AST, ALT and ALP compared to the control group.

## CONCLUSION

MOS and bee pollen with or without propolis improved productive and reproductive performance of rabbit does in comparison to the unsupplemented group. This indicated that MOS or bee pollen could be used as a supplement in rabbits.

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