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Immune Indicators, Growth, Hematological Parameters, Liver Enzymes and Kidney Function of Growing Rabbits as Affected by Bee Pollen and/or Mannan-Oligosaccharides

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ABSTRACT

In this study, we overviewed the influences of orally provided bee pollen (BPO) and Mannan-oligosaccharides (MOS) suspension on the biochemical, hematological and immunity parameters and antioxidant activity of growing rabbits. Rabbits of 35 days of age weight (751.8±68.8 g) were distributed into four groups (20 rabbits per group); control group; bee pollen (200 mg/kg BW), MOS (35 mg/kg BW) and a combination group (BPO+MOS) for eight weeks. MOS treated group with significantly increased PCV in comparison with BPO and BPO with MOS. Group treated with combination BPO with MOS had the highest WBCs in comparison with the other treatments and control. The treated rabbits with MOS significantly increased glucose levels, while the means of cholesterol significantly decreased less than the control group. BPO and MOS had a significantly higher T3 hormone in comparison to the control group. The treated rabbits with BPO and MOS had significantly decreased liver enzymes and urea less than those of the control group. Furthermore, Rabbits treated with BPO and/or MOS also showed decreased malondialdehyde, but increased total antioxidant capacity and phagocytic activity. Conclusively, adding BPO and MOS to growing rabbits enhances cell-mediated immune response, hematological and biochemical parameters reflecting and raising immunity and antioxidative responses.

Keywords: Bee pollen, mannan-oligosaccharides, rabbits, immune, antioxidant.

INTRODUCTION

Commercial rabbit livestock has been drawing much attention because of their rapid growth, small body size, high prolificacy, and meat production. Affirming that, rabbits convert about twenty percent of the diet protein into flesh, which is higher than beef (Alagawany *et al.*, 2016). Due to bacterial propagation in the caecum, which rendered its susceptibility to alimentary diseases where gastrointestinal tract of rabbits has a variety of physiology. In light of this, antibiotics and zinc bacitracin are frequently used in rabbit farms to less the mortality rate in rabbits (Bovera *et al.*, 2011).

Producers of rabbit meat have a significant problem according to the European Union's restriction for using antibiotic growth promoters in animal husbandry. As a result, numerous studies have been conducted to assess the possibility of various feed additives to replace antibiotics in rabbit production (Attia *et al.*, 2010). One of the interestingly noted candidates in nature is bee pollen (BPO). It is a material which naturally produced by plants' pollen in the flowering stage, mixed with the bee digestive secretion enzymes and nectar and stacked by the honeybees. Furthermore, BPO is a rich source of many nutrients like protein, essential amino acids, lipids, polyunsaturated fatty acids (linoleic, palmitic and linolenic acids), vitamins, enzymes, carbohydrates, carotenoids, phenolic, and flavonoids compounds (Xu *et al.*, 2009). Nevertheless, bee pollen has different constituents present in flora in different areas (Nogueira *et al.*, 2012).

Bee pollen has been utilized as medicinal and feed supplement in animal rations because of their growth-enhancing potentiality, antioxidant capacity, and immune-stimulating characteristics (Liu *et al.*, 2016). Furthermore, the presence of phenolic compounds, in particular, flavonoids, has shown to possess antimicrobial, anti-inflammatory, anti-carcinogenic, antiproliferative, and hepatoprotective properties (Yamaguchi *et al.*, 2006; Pascoal *et al.*, 2014).

Mannan-oligosaccharides (MOS) is one of the structural cell wall compartments of *saccharomyces cerevisiae*. The MOS possess anti-microbial activity which is well known for linking pathogens and denying them the attaching to the gut wall, depriving their stabilization and the later colonization and multiplication to disease-causing levels induced by toxins (Patterson and Burkholder, 2003; Spring *et al.*, 2015). The MOS via nitrogenous combination is involved in interacting with the animal's defense and could amend its activity (Che *et al.*, 2012), and it is speculated that they take a part in antioxidant and anti-mutagenic defenses (Krizková *et al.*, 2006). In this respect, Spring *et al.*, (2015) reviewed a lot of papers on applying MOS in poultry diets and enhancing the performance and immunity status, they showed little researches on rabbits which included good responses of performance and mortality.

Therefore, the target of this study is to test the hypothesis that supplementation of bee pollen and mannan-oligosaccharides, as favorable feed additives, could relieve

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the unfavorable back draws of overcrowding on the productive and economic efficiency of growing rabbits.

MATERIALS AND METHODS

Preparation of natural growth promoters:

Bee pollen (BPO) is an Egyptian commercial product in form of granules. The MOS is extracted by *S. cerevisiae* produced by Alltech Inc., Nicholasville, Kentucky, USA. Feed supplements were administered orally for three days each week as a water suspension (Saturday, Tuesday and Thursday).

Animals and experimental design:

Eighty male weaned Alexandria rabbits at 35 d-old of age and weighing (751.8±68.8 g), in a straight-run design were evenly distributed into 4 experimental groups (20 rabbits in each) according to live body weight. Each group included five replicates, four rabbits in each

The experimental groups included the control (1st group) without treatment, while the 2nd, 3rd and 4th groups were orally treated with BPO (200 mg/kg BW), MOS (35 mg/kg BW) and a combination of BPO and MOS, respectively for eight weeks. The groups consumed the same basic diet and water *ad libitum*.

Feeding system:

The diet fed to all groups was prepared conferring to NRC (1977) and the analytical composition of this diet is offered in Table 1.

Table 1. Chemical analysis and composition of the commercial diet used in feeding all groups

Ingredient	g/kg
Yellow Corn	151.0
Soybean meal, 44%	150.0
Wheat bran	250.0
Barley	150.0
Alfalfa hay	270.0
Ground limestone	10.0
Dicalcium Phosphate	12.0
Common salt	4.0
Vit. + Min. Premix*	3.0
Total	1000
Calculated analysis**	
Digestible energy (kcal/kg diet)	2580
Determined analysis (g/kg)	
Dry matter	902.4
Organic matter	912.1
Crude protein	172.4
Crude fiber	138.5
Ether extract	26.2
Nitrogen-free extract	575.0
Ash	87.9
Determined cell wall constituents	
Neutral detergent fiber (NDF)	331.1
Acid detergent fiber (ADF)	160.9
Hemicellulose	170.2

* Each 3kg of rabbits' premix contains; Vit. A : 12,000,000 IU ; Vit. D3 : 2,000,000 IU; Vit. E:10 000 mg; Vit. K3: 2000 mg; Vit. B1: 1000 mg; Vit. B2: 5000 mg Vit. B6: 1500 mg; Vit. B12: 10 mg; Biotin: 50.0 mg; Folic acid: 1000 mg; Nicotinic acid: 30 000 mg; Pantothenic acid: 10 000 mg; Choline Chloride 250 000 mg; Mn: 60 g; Cu: 10 g; Zn: 50 g; Fe:30 g; I: 1 g; Co: 0.10 g and Se: 0.1g; and Antioxidant, 1000 mg to 3000 g.

** According to NRC (1977).

Management and housing:

Rabbits were housed in an open system house in a battery with wired cages of customary dimensions (60 L, 50 W, 40 H cm). A manual feeder was given for each cage, and a system of nipple drinkers operated automatically to provide clean-fresh water continuously. Throughout the experimental period, rabbits were housed in the same sanitary and

environmental conditions and provided 16 hours of artificial light every day.

Data collected:

Individual live body weight: rabbits were individually weighed biweekly in the morning before offering feed.

Blood samples:

At termination time on day 91st (twelve blood samples) were collected from all groups. Samples were taken into heparinized tubes for hematological analysis or in plain tubes for biochemical criteria. Blood samples in plain tubes were left for coagulation then after they were centrifuged at 3000 rpm for 15 minutes to separate serum for biochemical analysis.

Hematological parameters including packed cell volume (PCV%), PCV was measured by hematocrit tubes centrifuged at 4000 rpm for 20 minutes (Lee, 1993). Leukocytic counts (WBCs) was determined using hemocytometer under light microscope at 100x magnification according to Hawkey *et al.*, (1989) where a blood sample was diluted (1:200) with Natt-Herrick's diluent. Then, the diluted sample was placed in a Neubauer improved hemocytometer (Precicolor, HBG. Germany), and then the blood cells were counted using a light microscope (Zuzi, Series 116, Auxilab S.L. Spain).

Serum biochemical parameters were analyzed using ready kits (Diamond Diagnostics, Egypt) after the following methods. Concentration of total proteins (Doumas *et al.*, 1981) and albumin (Doumas *et al.*, 1971), while the concentration of albumin was subtracted from the concentration of total protein to obtain globulin.

Serum concentrations of creatinine (Fabiny and Ertingshausen, 1971), urea (Sampson *et al.*, 1980), total lipids (Manirakiza *et al.*, 2001), total cholesterol (Allain *et al.*, 1974), triglycerides (Ziegenhorn, 1975), and glucose (Hyvarinen and Nikkila, 1962) as well as activity of alkaline phosphatase (ALP) (Belfield and Goldberg, 1971), alanine amino transferase (ALT), and aspartate amino transferase (AST) were measured (Reitman and Frankel, 1957). Also, thyroid hormones, triiodothyronine (T3) and tetraiodothyronine (T4) were determined in blood serum according to Young *et al.*, (1975).

Total antioxidant capacity was determined according to Koracevic *et al.*, (2001). Level of malondialdehyde (MDA, µmol/L) was analyzed according to Placer *et al.*, (1966).

Determination of phagocytic activity (PA) and phagocytic index (PI):

Phagocytic activity was determined according to Hustedt *et al.*, (2021). One milliliter of citrated blood was mixed with 50 µg of the *Candida albicans* culture, shaken in a water bath at 23 to 25 degrees Celsius for three to five hours, and then stained with Giemsa solution. By calculating the percentage of macrophages with intracellular yeast cells among a random sample of 300 macrophages and expressing the result as a percentage of PA, the phagocytic index was calculated. In the phagocytic cells, the number of phagocytized organisms was counted and called PI.

PA = Phagocytic cells in percentage having yeast cells.

PI = Number of yeast cell phagocytes/number of phagocytic cells.

Statistical analysis:

All analyses were performed using the GLM procedure of Statistical Analysis System package (SAS) Version 2002 software (Statistical Analysis System, SAS

Institute Inc., Cary, NC, USA) and statistical significance was set at $p < 0.05$. Data were subjected to one-way analysis of variance (ANOVA) using a model that included treatment and animal as possible source of variation. Duncan multiple range test was used to test the significance of variance between the means of the studied parameters.

RESULTS AND DISCUSSION

Growth performance

From data presented in Table 2, it could be seen that the treated rabbits with MOS had significantly ($P \leq 0.05$) raised body weight more than those of the control. At 63 days of age combinations of BPO and MOS had significantly increased rabbit body weight as compared to control. Moreover, among all treatments MOS showed the highest body weight gain at 77 days of age and at the end of the experiment. Abdel-Hamid and Farahat (2016) found that dietary MOS supplementation elevated immunity, improved the health and weights of rabbits. Ewuola *et al.*, (2011) and Oso *et al.*, (2013) reported that MOS addition resulted in higher final weight.

Table 2. Effect of bee pollen and/or MOS on body weight (gm) of growing rabbit during period 35-91 d of age.

Item	Control	BPO	MOS	BPO + MOS	SEM	P-value
Body weight						
Day 35	578.9	568.3	593.9	649.4	18.05	0.3451
Day 49	830.6	928.3	953.9	1001	37.47	0.3215
Day 63	1264 ^b	1373 ^{ab}	1446 ^a	1466 ^a	47.11	0.0116
Day 77	1658 ^b	1713 ^{ab}	1863 ^a	1687 ^b	47.03	0.0024
Day 91	1910 ^b	2035 ^{ab}	2094 ^a	1897 ^b	49.75	0.0127

^{ab} Means contained by the same row have different superscript letters are significant at ($p \leq 0.05$).

Hematological parameters

Table 3 puts under the spotlight the influence of BPO and MOS on blood hematological criteria of growing rabbits aged 91 days. Group treated with MOS significantly raised PCV percentage in comparison with BPO and BPO with MOS. Group treated with a combination BPO with MOS exhibited the highest WBCs when it's compared with all treatments including the control.

Table 3. Effect of bee pollen and/or MOS on some hematological parameters of growing rabbit at 91 d of age

	Control	BPO	MOS	BPO + MOS	SEM	P-value
PCV, %	31.0 ^{ab}	28.7 ^c	33.0 ^a	29.7 ^{bc}	0.469	0.0019
WBC, $\times 10^3$ cell/mm ³	23.0 ^b	23.7 ^b	21.7 ^c	26.3 ^a	0.402	0.0001
Lymphocytes, %	44.70	45.0	45.0	46.3	0.308	0.2359
Monocytes, %	9.00	9.67	9.67	10.0	0.179	0.2610
Basophils, %	0.30	0.33	0.33	0.67	0.102	0.6072
Eosinophils, %	10.70	11.0	10.3	10.0	0.199	0.3386
Neutrophils, %	35.30	34.0	34.7	33.03	0.451	0.3162

^{abc} Means contained by the same row have different superscript letters are significant at ($p \leq 0.05$); BPO= Bee Pollen; MOS= Mannan-oligosaccharide; PCV= packed cell volume; WBC= White blood cells.

Dias *et al.*, (2013) discovered that supplementing rabbits with BPO could enhance the cellular immunity responses by improving the speed in which an antibody is produced and the immunological system. The hereby findings agree with Elnany and Elkholy (2014), who said that the RBCs and WBCs counts in rabbits treated with BPO were significantly ($P \leq 0.05$) more when compared to those of the control group. These results showed that the interrelationship among the rabbit line and treatments exhibited no significant influence on the hematological traits. Supplementation with BPO, MOS and their mix boost

the body defense, and this was revealed in the raised leucocytic count and lymphocytic infiltration entails a valuable immune response with higher disease resistance. Similar findings were got by (Falção-E-Cunha *et al.*, 2010) who found that prebiotics may reduce mucosal pathogens binding enhancing rabbit immunity. Marín-García *et al.*, (2021) discovered that oligosaccharides dietary addition stimulates the immunity response of rabbits.

Table 4 indicates the impact of BPO and MOS on the blood biochemical constituents of growing rabbits. The concentrations of serum glucose in rabbits were significantly influenced only by MOS and BPO as compared to the control group. However, the levels of albumin and globulin, total lipids and triglycerides were not influenced by treatments. MOS and BPO treatments showed a significant increase in glucose and T3 when compared with the other treatments and control. However, cholesterol concentration was significantly decreased only by BPO and MOS treatment.

Table 4. Effect of bee pollen and/or MOS on blood biochemical constituents of growing rabbit at 91 d of age

Serum biochemical	Control	BPO	MOS	BPO + MOS	SEM	P-value
Glucose, mg/dl	73.3 ^c	77.7 ^b	80.3 ^a	71.3 ^c	0.844	0.0001
Total protein, g/dl	4.90	5.27	5.17	5.23	0.067	0.2144
Albumin, g/dl	2.90	3.17	3.07	3.37	0.076	0.1827
Globulin, g/dl	2.02	2.10	1.80	1.87	0.071	0.4735
Thyroid hormones						
T3, ng/dl	2.27 ^b	2.42 ^a	2.57 ^a	2.24 ^b	0.036	0.0007
T4, ng/dl	1.53	1.37	1.33	1.33	0.035	0.1374
T3/T4 ratio	1.48 ^b	1.81 ^a	1.93 ^a	1.71 ^a	0.049	0.0035
Lipid profile						
Total Lipids, mg/dl	105.7	105.3	102.0	104.3	0.661	0.1965
Cholesterol, mg/dl	230 ^a	227 ^a	228 ^a	214 ^b	1.728	0.0002
Triglycerides, mg/dl	183	184	179	183	0.872	0.2841

^{abc} Means contained by the same row have different superscript letters are significant at $P \leq 0.05$; BPO= Bee Pollen; MOS= Mannan-oligosaccharide; T3= triiodothyronine; T4 = Tetraiodothyronine

The findings above are in alliance with those of Elnany and Elkholy (2014), who stated that the means of cholesterol and total lipids for the male growing New Zealand White rabbits, which were supplemented on daily basis with 200, 300 and 400mg BPO/kg BW, reduced significantly ($P < 0.05$) when compared to those of the control group. The obtained results agree with those of Xu *et al.*, (2009), who ascribed the lowered levels of cholesterol and lipids to the influence of phospholipids and linolenic fatty acid in BPO, which were about 1.19%. Serum triglyceride levels were decreased significantly with supplementing BPO and MOS combination in rabbit diets. Similar results were noted by using MOS (Sudha *et al.*, 2009; Ooi and Liong, 2010). They hypothesized the effect as; posing bile salt hydrolase activity and precipitation of cholesterol by some microorganisms like *Lactobacillus* and *Bifidobacterium* fed on MOS, integration of cholesterol or binding to bacteria and making of short-chain fatty acids by probiotic bacteria.

Also, the results listed in Table 3 uncovered that the differences in the concentrations of serum T3 ($P = 0.0007$) and T3/T4 ratio in rabbits were significantly more in MOS or BPO groups, while the levels of T4 hormone were not influenced in all groups. This increase may mirror the better predicted metabolic functions, due to the increased T3 concentration, which antagonizes the hypothyroid state, induced by the adverse stressful conditions. Data in this study

are in grouping with data of Elnagar *et al.*, (2010), which indicated that the T3 hormone concentration in growing rabbits, orally given royal jelly once a week at 200, 400 or 800 mg/kg BW markedly, raised as compared to control group.

Table 5. Effect of bee pollen and/or MOS on liver and kidney functions of growing rabbit at 91 d of age

Item	Control	BPO	MOS	BPO + MOS	SEM	P-value
Liver function						
AST, U/L	60.7 ^a	60.3 ^a	54.7 ^b	55.3 ^b	0.766	0.0007
ALT, U/L	72.7 ^a	67.3 ^c	69.0 ^{bc}	72.0 ^{ab}	0.722	0.0164
AST/ALT ratio	0.754 ^b	0.895 ^a	0.879 ^a	0.770 ^b	0.015	0.0001
Alkaline phosphatase, U/L	10.7	12.3	11.7	12.7	0.415	0.3542
Kidney function						
Urea, mg/dl	26.0 ^a	22.0 ^b	23.3 ^b	21.0 ^b	0.573	0.0051
Creatinine, mg/dl	1.10	1.17	1.23	1.37	0.047	0.2313
Urea/creatinine ratio	25.0 ^a	19.5 ^{ab}	19.5 ^{ab}	15.7 ^b	1.198	0.0393

^{abc} Means contained by the same row have different superscript letters are significant at (p ≤ 0.05); AST= Aspartate aminotransferase; ALT= Alanine aminotransferase.

From data presented in Table 5, it could be seen that the treated rabbits with BPO and MOS had significantly (P ≤ 0.001) raised AST, ALT levels and urea more than those of the control group. This could indicate that supplementing the rabbits with BPO and/or MOS has no negative effects on the liver and renal tissues and their functions. Data here in arrangement with data of Zeedan *et al.*, (2017), who discovered that the means of AST and ALT concentrations in the serum of female rabbits treated with BPO reduced considerably as compared to the control group. Urea levels and urea/creatinine ratio were significantly decreased by supplementing BPO and MOS or their mixture in rabbits as compared to the control group, related findings were reported by (Abd El-Aziz *et al.*, 2020; Abo Ghanima *et al.*, 2020); (Alkhalf *et al.*, 2010) and (Assar and El-Abasy, 2015) in broiler chickens.

The influences of BPO and MOS on the antioxidant indices and phagocytosis at 91 days of old growing rabbits are in Table 5. These results indicate a rise in the total antioxidants capacity (TAC) and a reduction of malondialdehyde for rabbits supplemented with BPO and/or MOS (p = 0.0001) as compared to the control group (Table 5). There was a marked increase in phagocytic activity in treated groups as compared to the control group.

Table 6. Effect of bee pollen and/or MOS on antioxidant indices and phagocytosis of growing rabbit at 91 d of age

	Control	BPO	MOS	BPO + MOS	SEM	P-value
Antioxidant status						
Malondialdehyde, nmol/ml	1.80 ^a	1.23 ^b	1.60 ^a	1.13 ^b	0.079	0.0024
TAC, µm/ml	424 ^c	443 ^b	436 ^b	457 ^a	3.119	0.0001
Phagocytes						
Phagocytic activity, %	21.3 ^c	24.0 ^b	25.7 ^a	23.0 ^b	0.403	0.0001
Phagocytic index	1.27	1.37	1.30	1.30	0.019	0.3604

^{abc} Means contained by the same row have different superscript letters are significant at (p ≤ 0.05); TAC= Total antioxidant capacity.

The elevated TAC in the treated rabbits could be ascribed to the increase of absorbed vitamins, amino acids and trace elements from BPO (Attia *et al.*, 2017), which as a result enhanced the proliferation, development, and differentiation of the intestinal cells, consequently improving the conditions of intestinal microbial activity by the help of the nutritive value of mannan-oligosaccharides as a nutritive

polysaccharide for intestinal beneficial microflora. These results are in agreement with (Kocot *et al.*, 2018; El-Deep *et al.*, 2020).

CONCLUSION

In conclusion, the existing study provides insight on the impact of BPO and MOS or their mixture of growing rabbits and clarifies their capacity to enhance cell-mediated immune response, hematological and biochemical parameters mirroring liver and kidney functions and antioxidant status. Additionally, supplementation with BPO and MOS improved the body weight of rabbits. Relied on the valuable trials achieved on rabbits' healthiness and livestock production, further studies are needed to exploit these helpful possessions with these specific formulations.

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الدلائل المناعية، وقياسات الدم، وإنزيمات الكبد ووظائف الكلى للأرانب النامية المتأثرة بحبوب لقاح نحل العسل و/أو المنان أوليجوسكريدز

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المخلص

في هذه الدراسة، قمنا بدراسة تأثير تجريب حبوب لقاح نحل العسل والمنان أوليجو سكاريدز (الموس) على القياسات الكيميائية والطبيعية والمناعة ومضاد للأكسدة للأرانب النامية. تم تقسيم 80 أرانب تسمين عمر 35 يوماً بمتوسط وزن (68.8 ± 751.8 جم) إلى أربع مجموعات (20 أرانباً لكل مجموعة)؛ مجموعة الكنترول؛ مجموعة حبوب لقاح نحل العسل (200 ملجم/كجم من وزن الجسم)، مجموعة الموس (35 ملجم/كجم من وزن الجسم) ومجموعة مختلطة من كل من حبوب لقاح نحل العسل والموس على التوالي لمدة ثمانية أسابيع. زاد حجم كرات الدم الحمراء مع مجموعة حبوب لقاح نحل العسل و/أو مجموعة الموس. وزاد عدد كرات الدم البيضاء مع مجموعة حبوب لقاح نحل العسل مع الموس مقارنة بالمجموعات الأخرى والكنترول. أدت معاملة الأرانب بالموس إلى زيادة مستوى الجلوكوز، بينما انخفض مستوى الكوليسترول عن مجموعة الكنترول. زاد هرمون الـ T3 مع مجموعة حبوب لقاح نحل العسل والموس مقارنة بالكنترول. أظهرت الأرانب المعاملة بحبوب لقاح نحل العسل والموس انخفاضاً ملحوظاً في إنزيمات الكبد (AST, ALT) واليوريا مقارنة بالكنترول. علاوة على ذلك، أظهرت الأرانب المعاملة بحبوب لقاح نحل العسل و/أو الموس انخفاض في مستوى المالونديالدهيد وازدادت القدرة المضادة للأكسدة الكلية ونشاط البلعمة. وبالتالي نستنتج، أن إضافة حبوب لقاح نحل العسل والموس للأرانب النامية يعزز الاستجابة المناعية الخلوية، وصفات الدم الطبيعية ولكيميائية والتي تعكس وظائف الكبد والكلى وزيادة مستويات الاستجابات المناعية ومضادات الأكسدة.

الكلمات الدالة: حبوب لقاح نحل العسل، الموس، أرانب، مناعة، مضاد الأكسدة.