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# ENHANCEMENT OF IMMUNITY, ANTIOXIDANT STATUS, METABOLIC ATTRIBUTES AND REPRODUCTIVE HORMONAL PROFILES BY ADDING SOME NATURAL ADDITIVES TO RABBIT DOE'S DIET

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## ABSTRACT

*The aim of the study aimed to find out cheap local bioactive materials that support immunity, antioxidant capacity, and reproductive efficiency of California rabbits. Seventy-two adult rabbit does were equally allocated into six treatments (12, each). All does were fed a basal diet and orally given three ml distilled water as a basal solution during 10 days before mating until 28-days post insemination. Control does (C) received no supplement, G2 (HB) does were given 50mg honeybee/head/day; G3 (DPP) received 50 mg date palm pollen/head/day; G4 (BP) received 200mg bee pollen/head/day; G5 (SY) received three ml of a commercial synbiotic, and G6 (MIX) received a combination of all previous compounds.*

***Results indicated that*** *all treatments improved (P<0.05) the immunity functions and antioxidant biomarkers. Similarly, all treatments increased (P<0.05) plasma protein, albumin, and globulins, However it reduced (P<0.05) liver enzymes activity. Treatments remarkably reduced the bad cholesterol (free and LDL) and triglycerides, but increased HDL compared with control. Furthermore, all treatments elicited more secretion of ovarian hormones, which in turn increased pituitary gonadotrophins (FSH and LH). Contrariwise, all treatments resulted in lower levels of prolactin.*

***In conclusion****, provision of rabbit does during its productive life with bee products, date palm pollen and synbiotic would be a good solution for improving productive and reproductive performance of such animal.*

***Keywords:*** *Bee products, date palm, immunity, probiotic, productivity,*

## INTRODUCTION

The nutritional composition of some medicinal plants is believed to benefit productive, reproductive and performance in mammals; specifically, it has been found that these nutritive compounds are able to treat sexual dysfunction (**Sumalatha *et al,* 2010**). Moreover, the consumers awareness of food safety is expanding rapidly. Public health concerns are emerged over the use of antibiotics as growth promoters in farm animals' feed (**De Marco *et al,* 2015**). Therefore, great concern has been raised to find out natural alternatives for animal feeding strategies (**Hosseini *et al,* 2013**). As a result of the emerging information, the feed industry began searching for alternative feed additives to replace antibiotics in order to provide proper productivity. Antibiotics and other pharmaceuticals have long been widely utilized, with the objective of manipulating gut microbes for enhancing food for animal productivity. Long-term use of some of these drugs has led to the formation of drug-resistant microorganisms, representing a health threat to the consumers leading to a negative impact on the human gut ecology (**Biernasiak *et al,* 2010).**

Synbiotics (SY) are essential ingredients in animal feeding because they include both probiotics and prebiotics (**Gibson and Roberfroid, 1995).** Synbiotic improves host welfare by enhancing the survival and activity of live microbial dietary supplements in the digestive tract, by selectively stimulating the development and/or activating the metabolism of one specie or more of health-promoting bacteria (**Cencic and Chingwaru, 2010).**

Bee pollen (BP) could also be a promising additive since it involves a significant number of bioactive compounds such as carbohydrates, enzymes, vitamins, fatty acids, essential amino acids or carotenoids depending on the botanical and geographical origin of the bee pollen (**Denisow and Denisow-Pietrzyk 2016)**. Additionally, bee pollen contains bioactive components including; polyphenolics, flavonoids, proteins, and essential amino acids **(Carpes *et al,* 2009).** Furthermore, BP contains vitamins, minerals and coenzymes which are recognized for their antioxidant activities and contribute to anti-inflammatory effects **(Attia *et al,* 2011 and Morais *et al,* 2011).**

The variability of metabolites in bee pollen sets this product apart from other bee products and can be used in a variety of medical and therapeutic applications**.** Date palm pollen (DPP) is also known to include variety of compounds, including high total phenolics content, flavonoids, anthocyanins, and seleno-proteins (**Baliga *et al,* 2011**), making it an excellent candidate for antioxidant activity with little negative side effects (**Fallahi *et al,* 2015**). Moreover, honeybee (HB) contains a high amount of metabolizable energy in

the form of glucose and fructose, and also antibacterial activity against microorganisms that inhibit a broad spectrum of bacteria, including aerobes and anaerobes, gram positives and negatives (**Hannan *et al,* 2009**). Seemingly, HB contains low levels of amino acids and vitamins, antioxidant properties, phenolic acids and flavonoids (**Andrade *et al,* 1997**).

However, it includes enzymes, ascorbic acid, and minerals (**White, 1975**). Thus, honeybees have antimicrobial properties, according to **Molan and Russell (1988).** Therefore**,** the use of natural products as dietary supplements could be a reasonable alternative for enhancing immunity, antioxidant status, metabolic, and hormonal profiles of rabbits raised under shortage of feedstuffs in hot-arid developing countries.

Therefore, the aim of the present study to find out cheap some local bioactive materials that support immunity, antioxidant capacity, and reproductive efficiency of California rabbits.

## MATERIALS AND METHODS

The study was conducted at a private rabbit farm in Qalyubia governorate, where ،ambient indoor temperatures ranged from 19 to 27°C, humidity 43 to 54 %, and the light/dark cycle was 16/8 hours throughout the study term.

***Experimental design:***

In this study, seventy-two Californian does aged 6 months, and weighted of 3250±78.2g were used. Rabbits were randomly allotted into 6 equal treatment groups (12 in each ), and orally received 3 ml distilled water daily for 10 days before mating until 28 days post mating. Control (C) does received distilled water only, however treated does received the tested bioactive compounds.

All does were fed a basal diet (Table 1), and submitted to the following treatments; control (**C**) received no supplement, Synbiotic does (**SY**) received 3ml synbiotic (0.1%),Date palm pollen**(DPP)** does received 50mg per a doe/day, Bee pollen(**BP**) does were given200mg per a doe/day, Honeybee **(HB)** was supplemented with 50 mg per a doe/day, and Mixture **(MIX)** does received 3 ml symbiotic containing 50 mg DPP 0.200 mg BP, and 50 mg HB. The does were artificially inseminated. Insemination was performed with semen collected and pooled from 15 adult Californian males. The chosen

doses of bee pollen (BP) and date palm pollen (DPP) were according to **El-Hanoun *et al* (2007),** while synbiotic dose was according to **Abdel-Raheem *et al* (2012).**

***Housing, feeding and management:***

Rabbits were housed in a naturally ventilated barn equipped with wire galvanized batteries (60 × 55 × 40 cm, L×W×D).

The batteries were equipped with pellet feeders and automatic drinkers. Fresh water was provided in the experiment. The basal experimental diet (Table 1) was designed and pelleted to satisfy the nutritional requirements of rabbits as a whole (**NRC, 1977**).

***Blood sampling:***

Blood samples (5ml) were withdrawn at the end of the trial from marginal ear veins from all does within a treatment in the morning before receiving feed and water, using sterile disposable needles and heparinized sterile tubes. Plasma samples were obtained after centrifugation blood samples at 3000 rpm for 15 min and stored at -20˚C until analyzed.

***Hormonal determinations*:**

Plasma progesterone (P4) was determined according to the method of **Simersky *et al* (2007)** using a commercial ELISA 96-well kit (Diagnostics Test Canada, Inc., Ontario, Canada). The intra-assay coefficient of variation (C.V) was 7.6%.

Plasma estradiol-17ß (E2) was analyzed according to the method of **Ratcliff *et al* (1988)** using enzyme-linked immune absorbent assay (ELISA) kits (Diagnostics Test Canada, Inc., Ontario, Canada). The intra-assay C.V was 7.2%. All samples were analyzed in one assay for each hormone.

Plasma prolactin (PRL) determinations were done according to **Zoli *et al* (2002)** using a commercial ELISA kit (Immuno spec Corporation/ USA). The intra-assay CV was 8.3%.

Plasma FSH determination was done by a commercial ELISA kit (My Bio Source, San Diego, CA, USA). The intra-assay CV was 7.8%. Plasma LH values was determined by a commercial ELISA kit (My BioSource, San Diego, CA, USA). The intra-assay CV was 7.6%.

***Blood biochemical determinations:***

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were accomplished according to **Reitman and Frankel (1957**). Total protein (TP) was determined according to **Song *et al* (2023**), albumin (Alb) was

**Table 1.** Experimental diet composition and chemical analysis.

| Ingredient | % | *Calculated analysis* | |
|---|---|---|---|
| Yellow corn | 6.22 | Crude protein, % | 18.8 |
| Soybean meal, 44% | 22.33 | Crude fiber, % | 13.0 |
| Wheat bran | 23.33 | Ether extract, % | 3.0 |
| Barley | 15.00 | Digestible energy (kcal/kg diet) | 2680 |
| Alfalfa hay | 30.12 | n-6 poly unsaturated FAs% | 0.3 |
| Ground limestone | 1.00 | n-3 poly unsaturated FAs% | 1.03 |
| Dicalcium phosphate | 1.20 | ***Determined analysis (g/kg)*** | |
| Common salt | 0.50 | Dry matter | 897.1 |
| Vit. + min. premix* | 0.30 | Organic matter | 801.4 |
| **Total** | **100.0** | Crude protein | 169.8 |
| | | Crude fiber | 138.5 |
| | | Ether extract | 26.2 |
| | | Nitrogen-free extract | 575.0 |
| | | Ash | 87.9 |

*****Each 3 kg of premix contains: Vit. A: 12, 000,000 IU; Vit. $D_3$: 3, 000,000 IU; Vit. E: 10.0 mg; Vit. $K_3$: 3.0 mg; Vit. $B_1$: 200 mg: Vit. $B_2$: 5.0 mg Vit. $B_6$: 3.0 mg: Vit. $B_{12}$: 15.0 mg; Biotin: 50.0 mg; Folic acid: 1.0 mg; Nicotinic acid: 35.0 mg: Pantothenic acid: 10.0 mg; Mn: 80 g; Cu: 8.8 g; Zn: 70 g; Fe: 35 g; I: 1 g; Co: 0.15g and Se: 0.3g.

quantified according to **Doumas *et al* (1971**), however, globulin (Glo) was estimated by the difference between TP and Alb. Triglycerides (TG) levels were determined using the method of **Fossati and Prencipe (1982)**. Determinations of total cholesterol (TC), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were done following the method of **Birtcher and Ballantyne (2004)**. All biochemical attributes were determined in commercial kits (Human, Liquicolor, Germany).

***Antioxidant activity determination:***

Plasma total antioxidant capacity (TAC) and malondialdhyde (MDA) were determined using commercially available kits using a spectrophotometer (GNW-Model: SM-721) according to the methods reported by **Gawel *et al* (2004).**

***Immunoglobulins determination***

Rabbit IgG and IgM were determined by commercial ELISA kits (Abcam Limited, Cambridge, UK).

The sensitivity of the IgG assay was 0.23ng/mL (**Lopez and Alvariño, 2000)** and the intra-assay CV was 6.5%, however, the sensitivity of the IgM assay was 0.188 ng/ml, and the intra-assay CV was 7.2%.

***Measurement of plasma digestive enzyme:***

Amylase and lipase activities were determined using commercial kits (Human Gesellschaft, Germany) by the use of an automated analyzer (Chem-Well 2900T, USA).

***Statistical analysis:***

All data were subjected to the least square analysis of variance according to **Steel and Torrie (1984 )** using SAS package **(SAS, 2002)**.

The significant differences among treatment groups were tested using Multiple Range Test according to **Duncan (1955).**

## RESULTS AND DISCUSSION

***Immunoglobulins and antioxidants***

As shown in Table 2, all bioactive components and their mixture enhanced ($P<0.05$) both fractions of Ig.

The improvement ranges between 44-90% increases above control. Moreover, TAC as an indicator of the anti-oxidation biomarker, remarkably ($P<0.05$) increased due to treatments.

The improvement ranges between 61-116% above control values. Basically, the increase of MDA is a strong indicator of the accumulation of reactive oxygen species. Treatment reduced the levels of MDA by 22-33% of the control value. It has been recently established that bee derivatives (i.e. honey, pollens) are crucial components for increasing the immunity and the anti-oxidative status via improvement of intestinal absorption of nutrients and activating digestive enzymes in large animals, poultry and rabbits **(Abdelnour *et al*, 2019 and Abd El-Aziz *et al*, 2023).**

The richness of honeybee and bee pollens in simple sugars, vitamins, carotenoids, essential amino acids, polyunsaturated fatty acids, proteins and minerals imposes vital roles for such compounds.

The immune-competence requires special nutrients such as bee products (honeybee, royal jelly, bee pollens, venom).

**Table 2**. Effect of natural additive on blood plasma immune-globulins and antioxidant indicators of Californian rabbit does

| Treatment groups | IgG (mg/dl) | IgM (mg/dl) | TAC (nmol/ml) | MDA (nmol/ml) |
|---|---|---|---|---|
| Control (C) | 461.0$^{d}$ | 17.0$^{c}$ | 1.04$^{c}$ | 7.15$^{a}$ |
| Honeybee (HB) | 878.0$^{a}$ | 23.0$^{bc}$ | 2.25$^{a}$ | 4.77$^{c}$ |
| Date Palm Pollen (DPP) | 663.0$^{c}$ | 28.0b$^{a}$ | 1.89$^{ab}$ | 5.49$^{b}$ |
| Bee pollen (BP) | 723.0$^{b}$ | 32.0$^{a}$ | 1.68$^{b}$ | 5.32$^{b}$ |
| Synbiotic (SY) | 727.0$^{b}$ | 26.0$^{ab}$ | 1.87$^{b}$ | 4.93$^{c}$ |
| Mixture (MIX) | 858.0$^{a}$ | 21.0$^{bc}$ | 1.79$^{ab}$ | 5.53$^{b}$ |
| **MSE** | **8.73** | **3.49** | **0.35** | **0.56** |
| **P-value** | **0.0001** | **0.0004** | **0.0001** | **0.006** |

a,b.c.d Values with different superscripts in the same column differ significantly (P<0.05). IgG: immunoglobulin G, IgM: Immunoglobulin M, TAC: Total antioxidant capacity, MDA: malondialdehyde.

***Blood biochemical changes:***

As shown in Table 3 illustrates significant (P<0.05) increases in TP, Alb and Glo. Contrariwise, liver enzymes (AST and ALT) decreased (P<0.05) in does that were given the bioactive compounds, especially when a synbiotic was given. The highest values of protein and its fractions accompanied with the lowest liver enzymes were observed in case of synbiotic treatment. Generally, probiotics enhance the gut health leading to increased absorptive capability of the intestinal mucosa to absorb more protein **(Jäger *et al,* 2018).** In conjunction with what mentioned in Table 2, protein and its fraction, globulins play vital roles in the immunity system health and function. Albumin regulates the distribution of the extracellular fluids and acts as a carrier for a variety of bio-chemicals such as bilirubin, fatty acids, hormones and vitamins **(Attia *et al,* 2015).**Additionally, albumin-based antibodies are the main protein components that are synthesized in the hepatic tissues functioning as humoral immune agents. Other research study on sheep concluded significant increases of protein and globulins in lambs provided with probiotic, prebiotic, and synbiotc **(Elliethy *et al,* 2022)**. Furthermore, synbiotic, honeybee, bee pollens, and date palm pollens reduced AST and ALT which means improvement of the liver health. Evidently, the content of flavonoids in date palm pollen are high enough to protect unsaturated fatty acids which strengthen cellular membranes and protect it from oxidation, thus maintain liver integrity and function **(Abdel-Shaheed *et al,* 2021).** Besides, bee pollen was effective to reduce serum urea, AST and ALT when added to the rabbit'

diet leading to the improvements of hepatic and renal functions **(Sˇaric' et al, 2009).** This enhancement was attributed to the richness of bee pollen with antioxidant constituents. In the same manner, **El-Hammady *et al* (2017)** reported increased serum protein and albumin in rabbit bucks provided with bee pollen.

Honeybee is not only a rich source of simple sugars [i.e. glucose (32%) and fructose (43%)], but also it is rich in vitamins, flavonoids, antibacterial and antioxidant properties (**Tuksitha *et al,* 2018**). Parallel to the present study findings**, El Rabey *et al* (2013)** revealed that providing male albino rats with 2.5 g/kg body weight natural honeybees significantly improved liver function by reducing AST and ALT activities, and increased serum protein.

Concerning lipid fractions (Table 4), all treatments improved lipid fractions (i.e., reduced LDL and triglycerides and elevated HDL). Only honeybees reduced total cholesterol, however other ingredients tended to decrease total cholesterol. A proposed mechanism of the role of probiotic bacteria on lowering lipid fractions in circulation was stated by **Fukushima and Nakano(1995)** who hypothesized that probiotic bacteria (i.e. *Lactobacilli*, *Bifidobacteria*) pose bile salts hydrolase activity leading to the linkage of cholesterol to the bacteria to produce short-chain fatty acids. Another possible explanation of the reduction of cholesterol and its derivatives is that probiotic microorganisms may inhibit hydroxymethyl-glutaryl co-enzyme A, an enzyme involved in the synthesis of cholesterol **(Abdelhady and El-Abasy, 2015).** Most recently **Kim and Kim (2023)** attributed the cholesterol-lowering effect of dead probiotic bacteria (parabiotic) to three mechanisms; first, binding of probiotic to the dietary cholesterol contributing to its absorption inhibition and promoting its excretion. Second, activating nuclear receptors of enterocytes which modulate cholesterol transport by inhibiting the expression of an influx protein called Niemann-Pick C1-like 1 (NPC1L1) and promoting the expression of an efflux transporter named ATP-Binding Cassette (ABC). Third, reducing the synthesis of lipids in the liver. **Zeedan *et al* (2017)** stated serum total lipids, cholesterol, and triglycerides were diminished in rabbit bucks given different doses of bee pollen. Clearly, the presence of high levels of unsaturated fatty acids (i.e., oleic, linoleic, and linolenic) could play a vital role in preventing the formation of lipid peroxidation, resulting in reduced levels of triglycerides and cholesterol **(Farag and El-Rayes, 2016).** Additionally, the hypo-lipidemic effect of date palm pollen may be due to presence of polyunsaturated fatty acids and phyto-sterols which interfere with cholesterol absorption via enterocytes **(Abbas and Ateya, 2011).**

**Table 3**.Plasma total protein, albumin, globulins, AST, and ALT of Californian rabbit does responses to natural additive treatments.

| Treatment groups | TP (g/dl) | Alb (g/dl) | Glo (g/dl) | AST (U/l) | ALT (U/l) |
|---|---|---|---|---|---|
| Control (C) | $4.40^{c}$ | $3.26^{b}$ | $1.13^{c}$ | 11.33 | $26.33^{a}$ |
| Honeybee (HB) | $5.91^{b}$ | $3.95^{a}$ | $1.96^{a}$ | 15.500 | $18.00^{ab}$ |
| Date palm pollen (DPP) | $5.80^{bc}$ | $4.20^{a}$ | $1.60^{b}$ | 10.67 | $22.67^{ab}$ |
| Bee pollen (BP) | $5.80^{bc}$ | $4.24^{a}$ | $1.57^{b}$ | 13.00 | $21.33^{ab}$ |
| Synbiotic (SY) | $6.20^{a}$ | $4.33^{a}$ | $1.87^{a}$ | 8.68 | $13.68^{b}$ |
| Mixture (MIX) | $4.77^{bc}$ | $3.44^{b}$ | $1.33^{b}$ | 10,00 | $18.33^{ab}$ |
| **MSE** | **0.206** | **0.166** | **0.098** | **3.638** | **4.445** |
| **P-value** | **0.0001** | **0.0001** | **0.0001** | **0.8052** | **0.0208** |

a,b, c. Values with different superscripts in the same column, differ significantly($P<0.05$).ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, TP: Total protein, Alb: Albumen, Glo: Globulin.

***Digestive enzymes***

As shown in Table 4 exhibits the activity of amylase and lipase. All bioactive compounds enhanced ($P<0.05$) the activity of both enzymes. Several studies investigated the effect of probiotic, prebiotic, and bee pollen on the digestive enzymes activity and concluded higher activity than control.

**Yang *et al* (2005)** mentioned that intake of probiotics and prebiotics as a synbiotic mixture by rats significantly improved the ecosystem of the intestinal tract by increasing the activity of digestive enzymes; lipase, lactase, sucrase, and iso-maltase.

The higher figures of most metabolites in the current study could be attributed to the elevated activities of digestive enzymes which improve nutrient availability to the animal **(Wang and Gu, 2010)**.

Besides, provision of rabbits with bee pollen increased activity of lipase, amylase and protease than controls **(Zeedan *et al,* 2017).** Concurrently, **Taghian *et al* (2017)** described the beneficial role of date palm pollen to its higher contents of amino acids and/or its antibacterial activity. Altogether, these actions led to increased nutrient digestibility and growth rate **(El-Speiy *et al*, 2024).**

**Table 4**. Blood plasma lipid fractions and digestive Enzymes of Californian rabbit does as affected by natural additive treatments

| Treatment groups | TC (mg/dl) | HDL (mg/dl) | LDL (mg/dl) | TG (mg/dl) | Amylase (U/l) | Lipase (U/l) |
|---|---|---|---|---|---|---|
| Control (C) | 46.00$^{a}$ | 14.57$^{c}$ | 7.9$^{a}$ | 117.67$^{a}$ | 130.66$^{c}$ | 140.25$^{c}$ |
| Honeybee (HB) | 37.5$^{b}$ | 26.01$^{b}$ | 3.49$^{b}$ | 40.00$^{c}$ | 149.05$^{b}$ | 158.40$^{bc}$ |
| Date pollen (DPP) | 44.00$^{ab}$ | 27.9$^{a}$ | 4.68$^{b}$ | 57.00$^{bc}$ | 172.4$^{b}$ | 184.50$^{b}$ |
| Bee pollen (BP) | 45.67$^{ab}$ | 28.87$^{b}$ | 3.67$^{b}$ | 65.67$^{bc}$ | 169.00$^{b}$ | 175.25$^{b}$ |
| Synbiotic (SY) | 43.25$^{ab}$ | 27.13$^{a}$ | 3.25$^{b}$ | 64.33$^{bc}$ | 164.5$^{b}$ | 224.30$^{a}$ |
| Mixture (MIX) | 42.33$^{ab}$ | 25.80$^{b}$ | 3.73$^{b}$ | 67.33$^{bc}$ | 149.95$^{b}$ | 179.90$^{b}$ |
| **MSE** | **2.819** | **0.888** | **2.902** | **20.935** | **21.539** | **14.362** |
| **P-value** | **0.0043** | **0.0001** | **0.002** | **0.0001** | **0.0001** | **0.0001** |

a, b, c. Values with different superscripts in the same column differ significantly (P<0.05).
TG: Triglycerides, TC: Total cholesterol, HDL: High-density lipoprotein, LDL: low-density lipoprotein, TG: triglycerides.

***Reproductive hormones responses to natural additive treatments***

As shown in Table 5, revealed that all treatments significantly (P<0.05) elevated E17β, P4, FSH, LH, and E2/P4 ratio. Contrarily, all treatments reduced (P<0.05) prolactin levels, where synbiotic and mixture resulted in the lowest prolactin levels, followed by honeybee. The improvement of the E2 secretion in does that were given bee derivatives (i.e., honeybee, and bee pollen) ranges between 61-74% over the control values. Moreover, the increase of progesterone ranges between 35-61% over control, with a highest value resulting from bee pollen. In accordance with this finding, **El-Komy *et al* (2021)** documented15% E2 increase when rabbit does were provided with bee venom.

Many research studies on the effect of honeybee and other bee products (*i.e.*, royal jelly, bee pollen, bee venom) on sex steroid hormones have been published, attributing its effect to the high content of quercetin, caffeic acid, and phenolic compounds in these products (**Zaid *et al,* 2010; Kolesarova *et al,* 2011; Mosavat *et al,* 2014 and Banihani, 2019).**

Gonadotrophin hormones (FSH and LH) were also improved remarkably as a function of bioactive compound treatments. As shown in Table 5, the ranges of increase over control for FSH and LH were 49-71%, and 20-33%, respectively. The highest increases of both hormones were evidenced in case of the mixture provision. The augmented effect of the mixture of such bioactive compounds could probably attribute to the facilitative roles of the

components on the level of pituitary cells **(Karmali *et al,* 2018)**. Likewise, a significant increase of FSH was documented in women supplemented with probiotic **(Szydłowska *et al,* 2021).** Furthermore, **Rahman *et al* (2023)** concluded that provision of probiotic to polycystic ovarian women enormously increased FSH.

Moreover, **Tamemy and Amen (2019)** stated that the provision of rabbit females with date palm pollen appreciably elevated both FSH and LH. They attributed this enhancing effect to the flavonoids that play vital antioxidant and synthesis roles in the mammalian cells. Due to the richness of date palm pollen in phytoestrogens, sterols, carotenoids, and flavonoids, this confers a reproductive health action to this product **(Shahin, 2014).**Seemingly, in rabbits, as in rats and mice, GnRH surge only occurs when estrogen peak and the circadian rhythm clock signals coincide leading to LH surge **(Chappell, 2005).** These together are supported by the concurrent improvement in fertility and the higher litter sizes obtained in the current study in does that were offered bioactive-fortified diets.

Concerning honeybees (HB), **Hashem *et al* (2021)** reported that honeybees exhibit varieties of nutraceutical compounds (i.e., flavonoids and phenolics) that influence the reproduction functions by encouraging gonadal steroidogenesis, resulting in higher secretion of E2 and P4 in females, and higher androgens in the males.

This action on gonads is most probably effective to elicit the gonadotrophin (FSH and LH) secretion from anterior pituitary. Besides, other research attempts have shown great impact of bee pollen (BP) on the ovarian function and local sex steroid hormones secretion **(Kolesarova *et al,* 2011; Kolesarova *et al,* 2013 and Attia *et al,* 2015).** Similar finding to the current result with respect to the reduction of prolactin as a response to the supplementation with bee products was reported previously **(Aoki *et al,* 2012).**

Likewise, date palm pollen was previously found to reduce levels of prolactin **(Al-Samarrai *et al,* 2017).** Besides, **Jalalvand *et al* (2021)** documented a significant reduction of prolactin in women supplied with probiotics bacteria (*Elaeagnus angustifolia* L.). Thus, evidently such bioactive compounds mainly directed their actions on the doe's reproductive traits **(El-Speiy *et al,* 2024).**

**Table 5**. Plasma reproductive hormones of Californian rabbit does affected by natural additive treatments

| Treatment groups | E17β (ng/ml) | $P_4$ (ng/ml) | E2/P4 Ratio | FSH (ng/ml) | LH (ng/ml) | Prol (ng/ml) |
|---|---|---|---|---|---|---|
| Control(C) | $0.166^{b}$ | $0.410^{c}$ | $0.405^{c}$ | $55.26^{c}$ | $46.22^{c}$ | $0.355^{a}$ |
| Honeybee (HB) | $0.268^{a}$ | $0.554^{ab}$ | $0.484^{b}$ | $82.28^{b}$ | $55.38^{b}$ | $0.140^{c}$ |
| Date pollen (DPP) | $0.293^{a}$ | $0.625^{a}$ | $0.469^{b}$ | $88.79^{b}$ | $58.39^{a}$ | $0.290^{b}$ |
| Bee pollen (BP) | $0.289^{a}$ | $0.660^{a}$ | $0.439^{b}$ | $89.51^{b}$ | 59.96a | $0.295^{b}$ |
| Synbiotic (SY) | $0.274^{a}$ | $0.610^{a}$ | $0.449^{b}$ | $86.52^{b}$ | $58.45^{a}$ | $0.108^{c}$ |
| Mixture (MIX) | $0.297^{a}$ | $0.562^{ab}$ | $0.528^{a}$ | $94.45^{a}$ | $61.27^{a}$ | $0.109^{c}$ |
| **MSE** | **0.002** | **0.004** | **0.006** | **0.009** | **0.002** | **0.0954** |
| **P-value** | **0.0001** | **0.0001** | **0.0001** | **0.0001** | **0.0001** | **0.0056** |

a, b, c. Values with different superscripts in the same column differ significantly (P<0.05). E17β: Estradiol 17β; P4: Progesterone; LH: luteinizing hormone; FSH: follicle stimulating Hormone; Prol: Prolactin.

***In conclusion***, to improve the productive and reproductive traits of a rabbits raised in developing countries, where lack of feedstuffs prevails, the animal owners must utilize the available bioactive compounds as diet additives. This approach would economically benefit the rabbit raisers and sustain the business for animal protein provision. Also, a multidisciplinary study must be focused to investigate the role (s) of each active constituent of such compounds on the productivity of domestic animals.

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# تعزيزالمناعة وحالة الأكسدة والتمثيل الغذائى ومستوى هرمونات التناسل بأضافة بعض الاضاقات الطبيعية لعليقة إناث الأرانب

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يهدف البحث الى إيجاد مواد طبيعية محلية تعزز المناعة وحالة الأكسدة والتناسل فى إناث الأرانب الكاليفورنيا. تم توزيع ٧٢ أنثى بالغة على ٦ معاملات تجريبية عشوائيا (١٢ أنثى بكل معاملة). كل الإناث غُذيت على العليقة الأساسية كما تم تجريعها فمويا ٣ مل من الماء المقطر (تحتوى على الإضافة) قبل التلقيح ب ١٠ أيام وحتى اليوم الـ٢٨ بعد التلقيح بشكل مستمر. مجموعة المقارنة (الكونترول ) لم تتلقى أى إضافات، بينما تم تجريع المجاميع الأخرى من الثانية حتى السادسة الكميات الأتية بالترتيب: ٥٠ مجم عسل نحل، ٥٠ مجم طلع النخل، ٢٠٠ مجم حبوب لقاح النحل، ٣ مل سينبيوتيك، أو خليط من كل المواد السابقة. وقد أوضحت النتائج أن جميع المعاملات أدت إلى تحسن معنوى بمستوى ٥% للمناعة وحالة الأكسدة، كما لوحظت زيادة معنوية بمستوى ٥% فى محتوى بلازما الدم من كل من البروتين الكلى والألبيومين وكذلك الجلوبيولين، بينما إنخفضت مستويات إنزيمات الكبد، أيضا أدت جميع المعاملات لإنخفاض الكوليسترول منخفض الكثافة (الضار) والدهون الثلاثية بينما زاد مستوى الكوليسترول عالى الكثافة مقارنة بالكنترول. كما أدت المعاملات إلى زيادة مستوى هرمونات التناسل المفرزة من المبيض (البروجستيرون والاستراديول) وتلك المفرزة من النخامية (FSH, LH) كما أدت جميع المعاملات إلى خفض مستوى هرمون البرولاكتين مقارنة بالكنترول.
**الاستنتاج**: إضافة كل من منتجات النحل أوطلع نخيل البلح أوالسينبيوتيك او الخليط في مياه شرب إناث الأرانب أدت لتحسين المناعة والصفات الإنتاجية.
**التوصية:** نظراً لنقص المواد العلفية في الدول النامية، بالإضافة إلى قسوة المناخ، فإن هذه العوامل مجتمعة تشكل تحدياً كبيراً أمام مربي الأرانب للحصول على الإنتاج الاقتصادي. علاوة على ذلك، وكما هو معروف فإن الفراء الكثيف الموجود على جسم الأرنب يشكل عبئا أكبر على إنتاجيته. ومن أجل تعزيز الصفات الإنتاجية والإنجابية للأرانب، يجب على مربي الأرانب استخدام المركبات النشطة بيولوجيا المتوفرة كإضافات غذائية، وخاصة تلك التي استخدامها في الدراسة. وهذا النهج من شأنه أن يفيد مربي الأرانب اقتصاديًا ويدعم زيادة انتاج البروتين الحيواني. يجب أن تركزعلى دراسات متعددة التخصصات لتحديد دور (أدوار) كل مكون نشط من هذه المركبات على الإنتاجية الحيوانية.
**الكلمات المفتاحية:** منتجات نحل العسل، طلع النخيل، المناعة، البروبيوتيك، الكفاءة الانتاجية، الأرانب.