

EFFECIENCY OF SOME SUPPLEMENTAL PROTEIN NUTRITION PROCESS IN HONEY BEE COLONIES IN IMPROVED PROPERTIES OF THE HYPOPHARYNGEAL GLANDULAR SECRETION

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Abstract

Quality of the Royal jelly (RJ) is a reflection of the hypopharyngeal glandular (HG) physiological state. Faraway it depends on the feeding protein and lipids qualitative during and after bee workers maturation. Effect of sustain honey bee colonies by some nutrition process on the (HG) secretion were investigated using HPLC analysis. Honey bee colonies fed one of the 4 treatments (Brewer's Yeast, Soybean flour, Date palm pollen (*Phoenix dactylifera*) and Pollen grains from other plants); further control treatment. The results indicated presence a clear amelioration in the RJ quality phenols. 1-The tested Benzoic phenol with Date palm pollen treatment recorded the lowest concentration (2.65mg/100g) in comparable to control one (6.88mg/100g) and lower than others, that is important for public health. 2- The Caffeine phenol restricted the highest record with the pollen grains treatment (3.90mg/100g) in comparable with the control (0.95mg/100g) that crucial for memory. 3- The highest concentration of the Ellagic phenol was recorded with pollen grains treatment (9.55mg/100g). 4- Ferulic phenol recorded higher concentration only with Brewer's Yeast treatment (1.31mg/100g). 5- Higher density of the (RJ) secretion inside the secret vesicles lobules with Date palm pollen followed with Brewer's Yeast treatments was detected. This paper summary to produce high quality royal jelly one must consider food technology for honeybee colonies in feed diets.

INTRODUCTION

Royal jelly (RJ) mainly is a secreted by the hypopharyngeal gland between the sixth and twelve days of honey bees their life. Additional to another of the mandibular glands. RJ is a complex compound contains many components such as proteins, amino acids, vitamins, sugars, and fats which are essential for development of honey bee members (Fabio *et al.*, 2012). A lot of pharmacological activities on the antibacterial, anti-allergic and anti-aging were studied (Park *et al.*, 2012). RJ improve lipoprotein metabolism and reduce serum total cholesterol in the human healthy (Gugo *et al.*, 2007). Diseases and parasites infected honey bee colonies are

considered as one of the most important factors affects on the qualitative and quantitative of Royal jelly properties. Presence a royal jelly from different knowing and unknowing sources with significant differences in their components consider a trouble affair, particularly those exported from abroad with lower quality. In another side the beekeepers in Egypt used the supplemental protein diets to feeding bee colonies without completely studies their side effects on the RJ characters. Amino acid could be a good indicator of the origin the RJ. Most of the active substances within royal jelly lead to its natural properties are still unknown (Schönleben *et al.*, 2007). Therefore, this research is interested to study role of some supplements nutrition process in honey bee colonies (*Apis mellifera* L.) in improved properties of the hypopharyngeal glandular secretion.

MATERIALS AND METHODS

This work was carried out at the Apiculture Dept., Plant Protection Institute, Agriculture Research Center during late summer season, 2015. Fifteen of honey bee colonies equal in their population density were selected for this study. They were divided into five groups. Each group was offered one of the following feeding processes:

Diets (1) consist of (80% sugar powder + 20 % water) for the control.

Diet (2) consist of (30% Brewer's yeast + 50% sugar powder + 20% water).

Diet (3) consist of (30% Soybean flour +50% sugar powder + 20% water).

Diet (4) consist of (30% Pollen grains +50% sugar powder + 20% water).

Diet (5) consist of (30% Date palm pollen (*Phoenix dactylifera*) +50% sugar powder + 20% water).

100g of each the previously all tested diets were fed by patties that directly placed over the brood nest of tested honeybee colonies two /weekly intervals for one month.

I-Royal Jelly phenol Analysis

Artificially queen rearing of the tested honey bee colonies was done for each group. Royal jelly was collected at the 3rd day of queen larvae old then kept at -18° till used. High Performance Liquid Chromatography (HPLC) was used to determine the phenol compounds of the tested royal jelly based on the method of Oszmianski and Lee, (1990).

II- Histological studies

Histological studies of hypopharyngeal glands of honey bee workers were maintained in the laboratory of Pathology Department, Faculty of Veterinary Medicine Beni sueif University; Egypt.

This work was carried out to estimate the internal cellular differences of the hypopharyngeal gland tissue from nursing worker honey bees during naturally queen rearing ten worker honey bees were collected on the queen cells of each the tested honey bee colonies represented different supplemental protein nutrition as follows: The samples (ten head of tested worker honey bees/colony treatment) were immediately immersed in the fixative solution. For histological studies the samples were fixed in 10% formalin solution for 24h, followed by staining with Haematoxylin &Eosin. Samples were dehydrated in a standard ethanol series: 70%, 80%, 90%, 95%, and 100% 10min for each sample. The glands were cleared in histoclear "xylene" for 2 h. All previous processes were carried out by using rotary tissue processor (Leica TP 1020), then were impregnated by placing the samples in 1:1 histoclear "xylene": wax for 1 h in the incubator at 60 °C followed by three changes embedding in pure paraffin wax for 45 min, 1 h and 3 h, respectively at 60°C. The tissue was embedding console system (Leica EG 1150 H). The blocks were cut into 4 µm. Thickness using automatic rotary microtome (Leica RM 2255, Germany).The sections were mounted on chemically clean glass slides without using any adhesive material. For studying general structure of the developmental of hypopharyngeal glands of honeybee workers in sections, the haematoxylin and eosin stains were prepared and used as described by (Drury and Wallington 1980) and (Bancroft and Gamble 2002).

RESULTS AND DISCUSSION

I- Royal jelly phenol analysis

HPLC analysis in the tested Royal jelly detected the following phenols; Benzoic, Ellagic, Caffeine, Ferulic, Cinnamic, Caffeic and Rutin as (mg/100g) of all tested samples.

1- Benzoic phenol

Benzoic phenol is found in both naturally as an antimicrobial agent and as an added preservative in processed food. The high exposures to it can be poisonous and showed different degrees of the toxicity. Extreme toxicity in some animals was due to degenerative changes in liver, kidney and lungs. People at highest risk from exposure to benzoic acid include patients with liver disease. Toxic symptoms include diarrhea muscular weakness. In human benzoic acid is slightly affects on the skin and irritation to the eye (0.2-0.7 percent) with dose 10mg or up to 100mg/day/Five days. Lower doses used to treatment the patients with hyper ammoniaemia affects urea cycle

enzymes to help nitrogen excretion. Benzoic acid exerts strong antimicrobial effects in the gastrointestinal tract of some animal (Kluge *et al.*,2006).

It could be concluded that food of lower doses of the Benzoic phenol benefit people suffering of healthy problems as nitrogen mal-excretion. So, RJ from treated honey bee colonies by the tested supplemental nutrition let to reduce the Benzoic acid concentrations particularly with the Date Palm pollen grains (*Phoenix dactylifera*) (2.65 mg/100g) in comparable to control one (6.88mg/100g) and lower than other tested phenols as shown in Table (1).

2- Ellagic phenol

Ellagic phenol is a naturally occurring plant polyphenols that exhibits antioxidative properties both in vivo and in vitro. Recently dietary polyphenols are increasing attention as potential protectors against a variety of human diseases like cancer and chemotherapy induced toxicity in animal models. Ellagic acid decreased effect of the Malondialdehyde (MDA) resulted of Aluminum residue in food pollution. It also improved Glutathione (GSH) and Glutathione peroxidase (GSHPX) in animal liver (Özkaya *et al.*,2010).

Analysis of the RJ revealed presence a Ellagic phenols in all tested foods except of Soybean flour. Pollen grains treatment recorded the highest concentration of it (9.55mg/100g) than control (3.54mg/100g) and higher than others. That may be decreasing the harmful effects of the pollution in the preserving food and other harmful effects.

3- Caffeine phenol

Methylxanthine caffeine present in many common beverages during more extensively consumed by the world population. Beside a stimulate effect on the heart respiratory system and numerous behavioral effects. Moderate doses of it induce behavioral stimulant effects that suggest Central nervous system (CNS) stimulation but higher doses can suppress behavioral activity and even performances associated with learning and memory.

RJ analysis revealed presence a higher concentration of the Caffeine with the Pollen grains treatment (3.90mg/100g) in comparable with the control (0.95mg/100g) and higher than others as shown in Table (1). That may be causes higher activity of the respiration system or behavior effects in the consumers. Braz, (2002), reported that Caffeine acid improved memory retention at the doses (0.3-10 mg/kg) of some animals.

4- Ferulic phenol

Ferulic (FA) is a hydroxycinnamic acid classified as a phenolic compound. It protects plants from attack of microorganisms and provides antioxidant properties for animals. FA is a phenolic compound with anabolic effects (Crus *et al.*, 2014). Lower concentration of it was recorded with Pollen grains treatment (0.95 mg/100g), While the higher concentration was detected with Brewer's Yeast treatment (1.31mg/100gm).

5-Cinnamic phenol

Cinnamic phenol is a widely distributed phenyl propound component naturally occurring in plants and is mainly found in Cinnamomum cassia BLUME and Panax ginseng. Cinnamic acid was recently reported to exert a tyrosinase inhibitory effect. Treatment with 100 ppm of cinnamic acid resulted in a significant reduction of melanin production and reduce tyrosinase expression. It exhibited depigmenting activity on the UV-B- induced hyperpigmentation of brown guinea pig skin. It may be act as a skin whitening agent via inhibition of tyrosinase activity and expression within melanocytes (Lui *et al.*, 2006). Analyses of the RJ recorded the highest concentration of Cinnamic phenol particularly with the Pollen grains (2.94mg/100g than control 1.36mg/100g).

6- Caffeic and Rutin Phenols.

Limit detection of Caffeic and Rutin phenols was 0.3 µg/ml.

From the results obtained it could be concluded that RJ secretion after feeding honey bee colonies by Date palm (*Phoenix dactylifera*) treatment recorded the lowest concentration of the benzoic phenol benefit in public health. While pollen grains treatment recorded the higher values of the Caffeine phenol can be effects on the behavioral stimulant of CNS than the lower doses, also it recorded higher concentration of the Ellagic phenol higher than others. The highest concentration of the Cinnamic phenol was detected with the Pollen grains. While the higher concentration of Ferulic phenol was higher with Brewer's Yeast treatment.

Table 1. Phenolic concentrations of the Royal jelly (mg/100g) at different feeding treatments.

Item	Benzoic	Ellagic	Caffeine	Ferulic	Cinnamic	Caffeic	Rutin
Control	6.88	3.54	0.95	1.28	1.36	< LOQ	< LOQ
Brewer's yeast	3.31	3.64	2.64	1.31	1.83	< LOQ	< LOQ
Soybean flour	3.68	ND	1.99	1.05	1.66	< LOQ	< LOQ
Pollen grains	4.75	9.55	3.90	0.59	2.94	< LOQ	< LOQ
Date Palm pollen	2.65	3.49	1.20	0.82	1.23	< LOQ	< LOQ

ND: Not determined.

Limit detection (LOQ) of Caffeic and Rutin was 0.3 µg/ml.

II--Histological studies

Hypopharyngeal gland of the honey bee workers in non treated and treated honey bee colonies by tested the supplemental protein nutrition were dissected out to clarify their effect on the glandular structure tissue particularly secrete vesicle globules. There was a crowded in the glandular lobules with the Brewer' yeast (Fig.2) than those recorded with control one (Fig.1) and other glandular lobules (Figs.3,4,5). The secrete vesicles lobules showed the little secretion and higher vacuoles with control and with Soybean flour treatment (Figs.6,7,8). Whereas with Date palm pollen treatment the higher density secretion of the (RJ) was detected (Fig.10) followed with pollen grains treatment (Fig.9).

From all results obtained it could be concluded that Date palm pollen treatment indicated improved in the secrete vesicle globules tissue and higher density of the royal jelly secretion than other tested protein nutrition in same time it showed specific properties of the RJ phenol components followed with the pollen grains treatments.

Zohra and Talat, (2008) reported that RJ quality analysis is not related to the glandular lobule size. That agreement with our results whereas the treatment with Brewer's yeast treatment recoded higher lobule size of the Hypopharyngeal gland whereas their secrete vesicle globules showed higher vacuoles and little secretion of the RJ. So, bigger Size of the HG lobules is not reflects on the secretion density or quality. In spite of the hypopharyngeal glandular lobules with Date palm pollen treatment showed moderate size may they showed the higher density of the RJ secretion. That affirm the (HJ) lobule size is not indictor to the quantities secretion of the RJ. Therefore, it must be not depends on the HG lobule size as sign or indicator on the higher secretion or quality of the royal jelly secretion.

Borguini *et al.*, (2012) reported that RJ presented relatively low antioxidant potent. The antioxidant is due to phenolic content, protein and fatty acids. Exposure some animals to electromagnetic fields (EMF) induces harmful effects on testis and reproductive activities. Administration of Date palm pollen before exposure to EMF improved the sperm count, viability, motility and testosterone level and prevent the sperm abnormality (Baharara *et al.*, 2015). Martos *et al.*, (2008) and Kohichi *et al.*, (2014) reported that the biological activates of honey, propolis and Royal jelly are mainly attributed to the phenolic compounds such as flavonoids exhibit a wide range of biological activity including antiviral, anti inflammatory, antiallergic and inhibit oxyenase. Phenolic compounds produce physiological effect beneficial to health. Bees fed sugar syrup alone had lower protein concentrations and smaller hypopharyngeal gland.

There was a relationship between diet protein levels and immune responses (Hoffman, 2010). Abd El Azim *et al.*, (2015) Identified a six phenols as Caffeic acid, Gallic acid, Coumaric acid, Chlorogenic acid and Quercetin in Date palm pollen (*Phoenix dactylifera*) (DPP). In addition DPP has been used for long time as a traditional Egyptian herbal medicine for improving male and female fertility. From these results it could be advice to use Date Palm Pollen (*Phoenix dactylifera*) to improving properties of the hypopharyngeal glandular secretion.

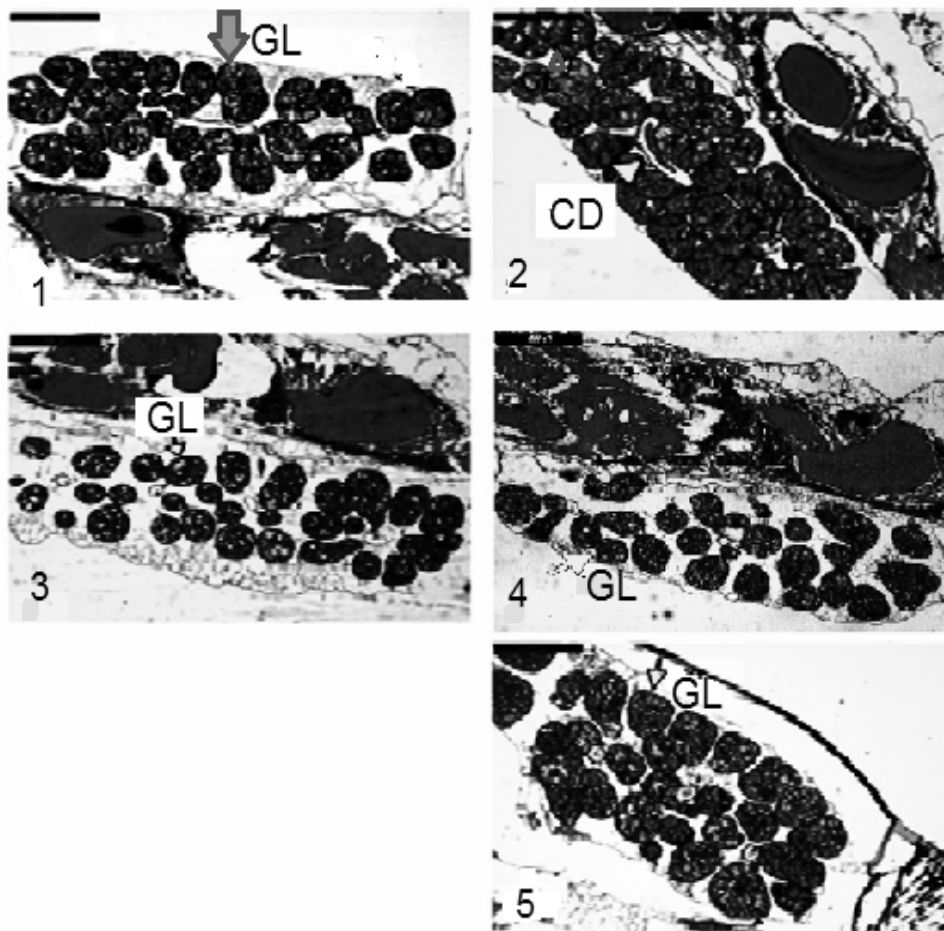


Fig.1: Hypopharyngeal gland lobules as control.

Fig.2: Same in treated with Brewer's yeast.

Fig.3: Same in treated with Soybean flour.

Fig.4: Same with pollen grains treatment.

Fig.5: Same with Date palm pollen treatment.

CD : Common duct. **GL:** Glandular lobules.

Scale bar:----- 20 μ m. (x-200).

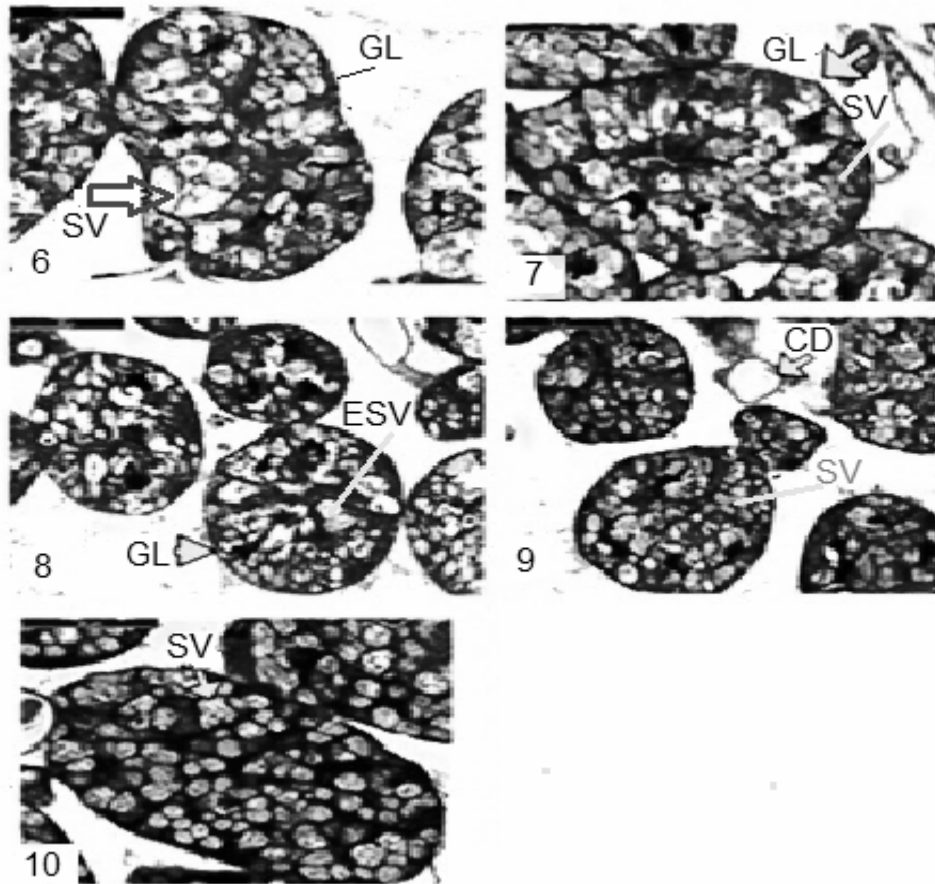


Fig.6: Glandular lobule of the HG as control.

Fig.7: Same in treated with Brewer's yeast.

Fig.8: Same in treated with Soybean flour

Fig.9: Same with pollen grains treatment.

Fig.10: Same with Date palm pollen treatment.

CD: Common duct.

GL: Hypopharyngeal Glandular lobule.

SV: Secrete vesicle globule.

ESV: Empty secrete vesicle globule.

Scale bar: _____ 50 μ m- (x-400).

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فاعلية بعض نظم التغذية البروتينية لطوائف نحل العسل فى تحسين خصائص الإفراز الغدى لغدة الغذاء الملكى

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ترجع جودة الغذاء الملكى الى الحالة الفسيولوجية لغدد الغذاء الملكى فى شغالات نحل العسل ، والى تتوقف الى حد كبير على نوع التغذية البروتينية والدهنية أثناء وبعد مراحل التطور العمري لشغالات نحل العسل، وفى هذا البحث تم دراسة تأثير بعض أنواع التغذية البروتينية على جودة الغذاء الملكى عن طريق التحليل بواسطة جهاز HPLC ، وكذلك على الحالة الفسيولوجية لشغالات نحل العسل المنتجة للغذاء الملكى(النحل الحاضن) عن طريق عمل قطاعات هستولوجية فى نسيج غدد الغذاء الملكى .

تم اختبار أربعة أنواع من التغذية البروتينية فى تغذية طوائف نحل العسل وهى (الخميرة الجافة، دقيق فول الصويا ، حبوب اللقاح و طلع النخيل) بالإضافة الى معاملة المقارنة . أظهرت النتائج وجود تحسن ملحوظ فى مستوى تركيزات فينولات الغذاء الملكى مع بعض أنواع نظم التغذية البروتينية:-

- ١ . حقق Benzoic phenol أقل التركيزات (2.65 مجم / ١٠٠جم) مع معاملة حبوب لقاح طلع النخيل بالمقارنة بالكنترول (6.88 مجم/١٠٠جم) وأقل عن باقى المعاملات الأخرى وهذا الخفض مهم للصحة العامة.
- ٢ . سجل Caffeine phenol أعلى مستوى من التركيزات (3.90مجم/١٠٠جم) مع معاملة حبوب لقاح النباتات الأخرى مقارنة بالكنترول(0.95 مجم / ١٠٠جم) وباقى المعاملات وهذا التركيز العالى مهم لتقوية الذاكرة .
- ٣ . حقق Ellagic phenol أعلى مستوى من التركيزات (9.55 مجم/١٠٠جم) مع معاملة حبوب اللقاح أيضا وأعلى عن الكنترول (3.54مجم/١٠٠جم) وباقى المعاملات كذلك.
- ٤ . حقق Ferulic phenol تركيز عالى فقط مع معاملة الخميرة الجافة (١,٣١مجم/١٠٠جم).
- ٥ . وعند معاينة القطاعات الهستولوجية فى نسيج غدد الغذاء الملكى وجدت زيادة ملحوظة فى الإفراز الغدى من الغذاء الملكى داخل الفصوص الإفرازية لغدة الغذاء الملكى أمكن رصدها خاصة مع معاملة حبوب لقاح طلع النخيل يليها حبوب لقاح النباتات الأخرى. يمكن أن نستخلص أن كلا من نظم تغذية طوائف نحل العسل خاصة بحبوب لقاح طلع النخيل أو حبوب اللقاح الخاصة بالنباتات الأخرى أدت إلى تحسن ملحوظ وزيادة فى الإفراز الغدى و مستوى تركيزات الفينولات فى الغذاء الملكى لصالح الصحة العامة مما يكون له مردود اقتصادى هام.