THE IMPACT OF BEE POLLEN ON CALVES' HEALTH AND LIVER FUNCTION: A PATHWAY TO ENHANCED LIVESTOCK

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ABSTRACT

This research was focused on the effect of bee pollen (B.P.) on calves' and heifers' physiological health. 20 calves and heifers, ages eight to ten months, were divided into one of two groups. One group received a basic diet plus 30 grams of fresh commercial multifloral B.P. three times a week for three months, while the other group just received the standard diet. Monthly blood samples were used to measure biochemical and hematological characteristics. Results cleared that the treated B.P. group showed a significant enhancement ($P < 0.05$) in glucose (G.L.C.), white blood cells (W.B.Cs) and red blood cells (R.B.Cs) numbers, while there was a noticeable decline ($P < 0.05$) in triglycerides (T.G.), high-density lipoprotein (H.D.L.), low-density lipoprotein (L.D.L.), Total cholesterol (T.C.), very-low-density lipoprotein (V.L.D.L.), alanine aminotransferase (A.L.T.) and malondialdehyde (M.D.A.) concentrations. On the other hand, there is a nonsignificant change between different genders. Overall, there is a clear improvement effect of B.P. supplementation on the general physiological health of calves.

***Keywords**: Bee pollen, blood parameters, liver enzyme, lipid profiles, oxidative stress.*

INTRODUCTION

Consumer expectations for food production have evolved substantially. Food is used as nutrition and to prevent nutrition-related diseases and boost mental, physiological and physical reactions of customers **(Mark-Herbert 2004)**. B.p. is another natural substance that is presently the subject of extensive research, which has significant industrial potential and can be used as a functional meal at reasonable costs. B.p. is one of the primary functional food additives because of its enormous potential for nutritional and biological uses **(Khalifa et al. 2021)**.

A "functional food" is one that serves an extra purpose in promoting welfare or preventing disease by combining one or more of its ingredients, either by demonstrating the synergistic reaction of related or novel substances that may be created. Recently, the market for functional foods, food supplements, and other beverages has grown significantly. Functional foods are dietary components that are intended to provide physiological advantages and/or reduce the risk of chronic illness in addition to their nutritional purposes **(Martirosyan and Singh 2015)**.

From B.p., more than 250 physiologically active compounds with a botanical origin have been identified. Bees collect pollen from herbaceous trees' flowers, which is a good supply of compounds with biological activity. In their back legs, there is a pollen basket where bees carry pollen loads—a mixture of pollen and a tiny bit of saliva or nectar—to the hive. Pollen traps are used to collect pollen loads, which are then dried and used as raw materials for nutritional, cosmetic, and

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medicinal applications **(Rzepecka-Stojko et al. 2015)**.

Plant pollen and bee bread were employed from ancient periods, primarily in Greece, China, and Egypt, even though B.P. pellet and pollen trap forms can be moderately new. Pollen was referred to by the ancient Egyptians as a "life-giving dust" **(El-Seedi et al. 2020)**.

B.p. application in public health is documented in the Bible and ancient Egyptian writings. Because of its active ingredients, which have important health and therapeutic benefits, it is regarded as a nutritional gold mine **(Khalifa et al. 2021).**

The essential elements of B.P. improve several body processes and supply defense opposites to a variety of illnesses **(Khalifa et al. 2021).** After extensive research, recent reviews show that B.P. typically consists of dietary fiber (0.3-20%), total carbohydrates (13-55%), lipids (1-13%) (saturated/unsaturated fatty acids, involving α-linolenic acid), ash (2–6%,) protein (10-40%), and a variety of other substances, including carotenoids, flavonoids, and terpenes. Furthermore, it should be noted that pollen contains many minerals and vitamins, including vitamin E, biotin, provitamin A, niacin, folic acid, and thiamine, as well as vital minerals like zinc, copper, and iron **(Kacemi and Campos 2023).**

MATERIALS AND METHODS

The experiment was applied in the animals Experimental Farm, Department of Animal Production, Faculty of Agriculture, Assiut University, Assiut, Egypt. As stated by the Faculty of Veterinary Medicine's Ethical Committee of Assiut University in Assiut, Egypt (Reference Number 06/2024/0246).

Experimental design, animals and diets

20 cows consisting of healthy heifers and calves were separated randomly into two groups of five calves and five heifers each (aged eight to ten months). The calves' initial average body weight (I.B.W.) was 136.1 kg, while the heifers were 129.3 kg. The treatment group (B.P.) was given 30 g of fresh commercial multifloral B.p. three times per week for three months with the basal diet, while the control (Con.) one had only the basal diet consisting of 25% wheat straw, 25% wheat bran, 25% yellow corn, 22% decorticated cottonseed meal, 1.5% limestone, 0.5% premix mixtures and 1% sodium chloride on a dry matter (D.M.) basis. **Table 1** shows the basal diet chemicals and components; each animal received its daily ration at a 3% level.

According to the NRC's (2007) recommendations, each animal received their daily rations separately at a level of 3% D.M. of their body weight as complete mixed meals twice per day; 7:00 am and 5:00 pm to satisfy their nutritional needs. Fresh water was accessible throughout the day.

Blood sampling and blood parameters

Two blood samples (approximately 7 ml each) were collected monthly throughout the trial period from all experimental animals via jugular venipuncture. The first was a total blood sample immediately used for R.B.Cs. count and W.B.Cs. estimation by using Symex Automated Hematology Analyzer (S.A.H.A.). The second was transferred to a vial containing Ethylenediaminetetraacetic acid (E.D.T.A.) as an anticoagulant, 3000 round per minute (R.P.M.) centrifugation for 15 minutes to take plasma, every sample was kept at -20°C until analysis.

Plasma lipid peroxide M.D.A. was detected using a reagent kit obtained from Biodiagnostic, Giza, Egypt. Also, plasma lipid profiles were carried out using a spectrophotometer and the colorimetric method with a commercial kit produced by an Egyptian Biotechnology Company in Cairo, Egypt, as T.G. (catalog number: 314002), L.D.L., H.D.L. (catalog number: 266001) and T.C. (catalog number: 230002) with calculating the V.L.D.L. = TG/5. Moreover, liver enzymes A.L.T. (catalog number: 260001), and G.L.C. (catalog number: 250001) were measured.

Statistical analysis

The SPSS statistical program, General Linear Models (G.L.M.), was used to statistically assess all the data gathered from growth trials and blood specimens (SPSS Institute, 2008). The obtained data was analyzed using the following statistical model:

$$Yij = Gj + Ti + \mu + (T \times G)\, ij + \varepsilon ijk,$$

as μ ijk is the residual error, Gi is the fixed effect of genders (female and male), Ti is the fixed effect of treatment (Con. and B.P.), μ is the overall mean, and Yij is the dependent variable (blood parameters).

Table 1. Chemical composition and ingredient of basal diet.

Item	Basal diet
Ingredient, %	
Yellow corn	25
Wheat bran	25
Wheat straw	25
Limestone	1.5
Decorticated cottonseed meal	22
Premix mixtures*	0.5
Sodium chloride	1
Chemical composition	
D.M. (%)	90.23
O.M. (% DM)	88.92
C.F. (% DM)	17.32
C.P. (% DM)	14.21
E.E. (% DM)	2.46
Ash (% DM)	11.08
N.D.F. (% DM)	54.93

* Premix mixtures: content 200 mg vitamin E, 200,000 IU vitamin A, vitamin D3100,000 IU, 2500 mg Cu, 10,000 mg Fe, 100 mg Mo, 100 mg Co, 20,000 mg Mn, 100 mg Se, 20,000 mg Zn and 800 mg I; D.M.: Dry matter; O.M.: Organic matter; C.F.: Crude fiber; C.P.: Crude protein; E.E.: Ether extract; N.D.F.: Nitrogen free extract.

RESULTS

1. *Hematological Parameters (W.B.Cs. and R.B.Cs.)*

Table 2 showed that all biochemical blood indicators were significantly more affected by B.p. addition in calves' and heifers' diets than those received a basal diet alone. Additionally, adding B.p. to calves' and heifers' diets did not differentiate significantly between both genders ($P > 0.05$) in all measured blood parameters except for R.B.Cs. which was significantly more advanced in calves than in heifers ($P < 0.05$). Animals that received a basal diet with B.p. had higher ($P < 0.05$) W.B.Cs. and R.B.Cs. levels than those that received a basal diet alone.

Table 2. Effect of B.P. additive on hematological parameters (W.B.Cs. and R.B.Cs.) levels of growing heifers and calves.

Item	Treatment		Gender		SEM	P-value		
	Con.	B.P.	Male	Female		T	G	T×G
W.B.Cs.	9.79	11.14	10.39	10.53	0.112	<0.001	0.303	0.767
R.B.Cs.	8.78	10.32	9.74	9.36	0.116	<0.001	<0.001	<0.001

Results are expressed as mean ± SEM. Con.: experimental animals received basal diet; B.p.: experimental animals received basal diet + 30 g of B.P. W.B.Cs.: white blood cells ($X10^3$/ µl).; R.B.Cs.: red blood cells ($X10^6$/ µl).

2. *Lipid profiles (T.G., L.D.L., H.D.L., V.L.D.L., T.C.)*

Related to the Con., treated B.P. animals showed a significant decline in T.G., L.D.L., H.D.L., V.L.D.L. and T.C. plasma concentrations (P < 0.05). Likewise, there was a nonsignificant change between different genders of heifers and calves (P > 0.05) in those parameters **(Table 3).**

Table 3. Effect of B.P. additive on T.G., L.D.L., H.D.L., V.L.D.L. and T.C. levels of growing heifers and calves.

Item	Treatment		Gender		SEM	P-value		
	Con.	B.P.	Male	Female		T	G	T×G
T.G.	23.81	22.49	23.15	23.15	0.097	<0.001	0.959	0.646
L.D.L.	36.42	33.95	35.02	35.35	0.216	<0.001	0.283	0.901
H.D.L.	48.06	45.35	46.71	46.69	0.217	<0.001	0.821	0.451
V.L.D.L.	4.762	4.498	4.63	4.63	0.0194	<0.001	0.959	0.646
T.C.	89.24	83.79	86.37	86.65	0.400	<0.001	0.326	0.655

Results are expressed as mean ± SEM. Con.: experimental animals received basal diet; B.p.: experimental animals received basal diet + 30 g of B.P. T.G.: triglyceride (mg/dl), L.D.L.: low density lipoprotein (mg/dl); H.D.L.: high density lipoprotein; V.L.D.L.: very low-density lipoprotein (mg/dl), and T.C.: total cholesterol (mmol/l).

3. *Plasma liver enzyme (A.L.T.), G.L.C. and M.D.A.*

Blood samples from animals in the treatment B.p. group had a lower level of A.L.T. and M.D.A. (P < 0.05) while the G.L.C. level was higher (P < 0.05) than those in the Con. animals. In comparing A.L.T., G.L.C. and M.D.A. levels of different genders, there was a non-significant difference between both heifers and calves **(Table 4)**.

Table 4. Effect of B.P. additive on A.L.T., G.L.C. and M.D.A. levels of growing heifers and calves.

Item	Treatment		Gender		SEM	P-value		
	Con.	B.P.	Male	Female		T	G	T×G
A.L.T.	23.99	20.41	22.07	22.33	0.264	<0.001	0.249	0.002
G.L.C.	51.28	68.14	59.82	59.59	1.441	<0.001	0.700	0.002
M.D.A.	2.89	2.09	2.50	2.48	0.059	<0.001	0.341	0.659

Results are expressed as mean ± SEM. Con.: experimental animals received basal diet; B.p.: experimental animals received basal diet + 30 g of B.P. M.D.A.: malonaldehyde (nmol/ml); A.L.T.: alanine amino transferase (U/L); G.L.C.: glucose (mg/dl).

DISCUSSION

The current study approved that B.P. has a significant improvement in W.B.Cs. and R.B.Cs. count compared to that of the Con. Group, which agrees with **Mohamed (2018)** whose results showed that B.P. has an effective response on the hematopoietic system in the diabetic rats, which appeared as an increase in R.B.Cs., hemoglobin (H.B.), platelets, W.B.Cs., ferritin and iron concentrations. So, B.p. improves immunity as W.B.Cs. show an important function in supporting the body by antagonizing foreign bodies **(Abdel-Raouf et al. 2018).**

B.P. also significantly decreases the negative influence of iron deficiency, thus demonstrating its antianemic effects. The positive effect of B.P. in hemolytic anemic rats and mice has also been approved B.P. caused hematopoietic system enhancement, so B.P. may have antianemia properties since it contains a lot of iron **(Anna et al. 2015)** and can enhance bone marrow activities, which are important for erythropoiesis **(Orhue et al. 2008).** The components of pollen that promote iron absorption, such as fructose, bioflavonoids, vitamin C, and histidine (free amino acids), can achieve this effect **(Roulston and Cane 2000).** In diabetic rats, bee or palm therapy may prevent R.B.Cs. hemolysis and the oxidation of cell membrane unsaturated fatty **acids (Mohamed et al. 2018).**

This research recorded that B.P. group significantly decreased L.D.L., T.G., H.D.L., T.C. and V.L.D.L. levels in comparison with the Con. group. Likewise, supplementing high-fat-fed mice with Brassica campestris B.P. led to a notable reduction in L.D.L., T.G., and T.C. **(Oyarzún and colleagues 2020, Khalifa and colleagues 2021, Yan and colleagues 2021).** The polysaccharide from B.P. Fagopyrum esculentum is another example, since it significantly reduced T.G. and L.D.L. levels **(Zhu et al. 2020).** Another study clarified that diets supplemented with ethanolic extract of B.P. corrected the female mice's liver steatosis and damaging alternations through decreasing the concentrations of the T.C. and the L.D.L. **(Rzepecka-Stojko et al. 2018)**. Also, it was reported that B.P. reduces blood T.C., L.D.L. and T.G. amounts **(Selmanoğlu et al. 2009).**

Numerous studies have demonstrated that B.P. lowers T.C. levels and liver fat accumulation. The alcoholic extracts of numerous multifloral pollens demonstrated notable hepatoprotective properties against experimentally produced free radicals and effectively decreased lipid buildup in liver cells in a mouse hepatic cell line **(Oyarzún et al. 2020).** It decreased T.C. and L.D.L., glycogen deposition and T.G. while simultaneously increasing nucleus size and reducing portal vein blood pressure **(Jarosz et al. 2022).** The ethanolic component of an industrial B.P. sample was also shown to mitigate oxidative stress and restore hepatocyte function in rats poisoned by propionic acid by normalizing a number of molecular indicators and hepatic enzymes **(Al-Salem et al. 2020).**

In addition, **Ahmed and Aseel (2024)** presented a significant reduction in the concentration of L.D.L., V.L.D.L. and TC in the animals treated with B.P., which received carbimazole dosages continuously during the experiment; this might be because B.P. effective in restoring its proportion to the physiological value, raising the concentration of associated hormones such as insulin, testosterone and thyroxine, which increases fat metabolism and lowers fat levels **(Karimi et al. 2020).**

In the current research, group supplied by B.P. has a lower A.L.T. and M.D.A. while increased G.L.C. levels increased than the Con. group received the basal diet only. It could be ascribed to B.P. phenolic chemicals, which alter the density of bacteria and protect the liver. It also lowers oxidative stress and its consequences, such as inflammation, in rat intestines, preventing non-alcoholic fatty liver disorders brought on by a diet that is high in fats **(Cheng et al. 2019, Mohamed et al. 2021).**

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Furthermore, enhancement of serum A.L.T. and aspartate aminotransferase (A.S.T.) approved the induction of hepatic dysfunction. A rise in the activity of A.L.T., a hepatocyte's cytosolic enzyme, indicates a rise in the plasma membrane permeability that is linked to cell death **(Yıldız et al. 2013)**. So, B.P. aids the recovery of the liver damage induced by toxicity, as it decreased the activities of blood A.S.T. and A.L.T. **(Yıldız et al. 2013, Omnia et al. 2014)**. It has been reported that B.P. phenolic structure can scavenge free radicals counter to oxidative injury, which may be the reason for the decline of these hepatic enzymes. The ability of a drug's components to restrict the aromatic activity of cytochrome p-450 by promoting regeneration of the liver is a crucial component of its hepatoprotective activity **(Gil et al. 2000).**

Ahmed and Aseel (2024) approved the effectiveness of B.P. in improving liver enzymes A.L.T. and A.S.T. and lipid profile. In addition to other factors like soil type, meteorological conditions, and bee behavior that alter B.P. chemically, this contradiction may be caused by plant life and geographic origins **(Liolios et al. 2019, Mayda et al. 2020)**.

According to **Almeida-Muradian et al. (2005),** B.P. is composed of 30.8% carbohydrates, which include reducing sugars like G.L.C. and fructose that are used to produce energy. So, B.P. may act as a natural α-glucosidase suppressor **(Daudu 2019).** α-glucosidase and α- amylase, two intestinal enzymes, change polysaccharides into GLC so that it can enter bodily cells **(Shobana et al. 2009)**. By suppressing the production of the sodium-dependent G.L.C. transporter 1 and 2 proteins, Camellia sinensis B.P. extract dramatically reduced G.L.C. transport through the epithelium monolayer and significantly reduced G.L.C. absorption; this effect appeared to be caused by phenolic compounds **(Zhao et al. 2022)**.

In addition, B.P. phenolic chemicals aid electrons to the free radicals, which lessens their destructive action **(Zeb 2020).** Also, B.P. compounds (phenolic and non-phenolic), endeavor to activate antioxidant-active enzymes like glutathione peroxidase (G.P.X.), glutathione reductase (G.S.H.), Superoxide dismutase (S.O.D.) and Glutathione-S-transferase (G.S.T.) **(Mohamed et al. 2018, Flohé et al. 2022). Abdelnour et al. (2019) and Karimi et al. (2020)** suggested that B.P. can lessen the negative effects of carbimazole because of its antioxidant composition, which also preserves the liver, enhances immunity, and promotes side-effect-free wound healing.

Flavonoids is the most crucial component among the polyphenols present in B.P. Flavonoids antioxidative qualities are affected by the C3 and C2 double bonds in the ring C of the flavonoid structure. The compounds can scavenge hydroxyl radicals because of a carbonyl group at the C4 position. The compounds' ability to prevent lipid peroxidation is due to the existence of the OH at the C3 position in the ring C. The more hydroxyl groups there are, the more capable the substance is of scavenging hydroxyl radicals **(Rzepecka-Stojko et al. 2015)**.

Low molecular weight compounds, such as polyphenolic and ascorbate (vitamin C) compounds, are the very crucial antioxidants presented in B.P. Carotenoids and tocopherols (vitamin E), on the other hand, are hydrophobic antioxidants **(Kuźnicki 2006).** Vitamin E's antioxidative properties include its capacity to stop lipid peroxidation and its reaction with organic free radicals and R.O.S. Moreover, carotenoids can suppress lipid peroxidation, particularly in L.D.L., quench singlet oxygen, and lower organic free radicals **(Rzepecka-Stojko et al. 2015).**

B.P. and black seed additives added to Friesian calves' diet during lactation and post-weaning times could enhance blood W.B.Cs., R.B.Cs., H.B., albumin, total protein, globulin, while reduced A.S.T. and A.L.T. levels, led to an enhancement of blood biochemical, immunity and hematological responses **(Abdel-Raouf et al. 2018).** Moreover, **(Zhang et al. 2022)** showed that B.P.-exposed mice had the ability to significantly reduce the T.G., L.D.L., A.L.T., A.S.T., and M.D.A.

CONCLUSION

The current study's findings supported the idea that B.P. might be used as a feed additive in animal cattle production. Its improvement of hematological state, lipid profiles, G.L.C. level, liver function and oxidative stress markers suggests that it may have the capacity to improve calf welfare and performance in an environmentally friendly manner.

AUTHOR CONTRIBUTIONS

Eman A. Negm: methodology, conceptualization, visualization, validation, writing, data curation, review and editing. Raghda A. Taghian: methodology, visualization, data curation, formal analysis, validation and supervision.

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STATEMENT OF CONFLICT OF INTEREST

The authors reveal no conflicts of interest.

DATA AVAIL ABILITY STATEMENT

The corresponding author can provide the datasets created and examined in this study upon reasonable request.

ANIMAL WELFARE STATEMENT

The research followed the international and national regulations for animal management and was approved by Assiut University in Egypt with approval number 06/2025/0289.

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الملخص العربي

تأثير حبوب لقاح النحل على صحة العجول ووظائف الكبد: طريق نحو تعزيز إنتاجية الثروة الحيوانية

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ركز هذا البحث على تأثير حبوب لقاح النحل (B.P.) على الصحة الفسيولوجية للعجول والعجلات. تم تقسيم ٢٠ عجلًا وعجلة، تتراوح أعمارهم بين ثمانية وعشرة أشهر، إلى مجموعتين. تلقت المجموعة الأولى نظامًا غذائيًا أساسيًا بالإضافة إلى ٣٠ جرامًا من حبوب لقاح النحل التجارية متعددة الأزهار الطازجة ثلاث مرات أسبوعيًا لمدة ثلاثة أشهر، بينما تلقت المجموعة الأخرى النظام الغذائي القياسي فقط (كنترول). تم اخذ عينات دم شهرية لقياس الخصائص البيوكيميائية والدموية. أظهرت النتائج أن المجموعة المعالجة بواسطة حبوب لقاح النحل قد أوضحت تحسنًا ملحوظًا معنويا في تركيز الجلوكوز، وكريات الدم البيضاء، وكريات الدم الحمراء، بينما كان هناك انخفاض في تركيزات الدهون الثلاثية ، والبروتين الدهني عالي الكثافة، والبروتين الدهني منخفض الكثافة، والكوليسترول، والبروتين الدهني منخفض الكثافة جدًا، والانين أمينوترانسفيراز، والمالونديالديهايد. من ناحية أخرى، لم يكن هناك تأثير تبعا لجنس الحيوان بشكل عام، هناك تأثير واضح لمكملات حبوب لقاح النحل على الصحة الفسيولوجية العامة للعجول.