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The Power of Lactobacillus Based Probiotic on Boosting Calf Health

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Abstract

THIS study evaluated the effects of Fermented Bee Pollen (FBP) as a probiotic formulation including *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, fermented with lactic acid, on the general health of male and female calves over a three-month period. Twenty calves and heifers, aged 6 - 8 months, were randomly assigned to two groups**,** one that was given 40 grams of FBP three times/week in addition to a basal diet, while the control group received only the basal diet. Monthly blood samples were collected to assess haematological and biochemical parameters, including glucose (GLC), total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), very-low-density lipoprotein (VLDL), malondialdehyde (MDA), alanine aminotransferase (ALT), and red and white blood cell counts (RBC and WBC). The results showed that calves supplemented with FBP exhibited consistent improvements demonstrated by significant reductions in MDA, TG, LDL, HDL, TC, and ALT levels ($P < 0.05$) compared to the control group. In contrast, the FBP group showed significantly higher levels of GLC., WBC, and RBC ($P < 0.05$). These findings suggest that FBP, as a probiotic based on *Lactobacillus strains*, may enhance immune cell proliferation, metabolic activity, and overall health in calves.

Keywords: Fermented Bee Pollen (FBP), Probiotics in Livestock, Calf Health, Lactobacillus bulgaricus, Haematological, and Biochemical Parameters.

Introduction

Livestock production has become essential to meet the growing food demand, especially in developing countries. However, it poses environmental and public health challenges. To ensure a sustainable agroecosystem, adopting eco-friendly agricultural practices is crucial, with animal husbandry playing a key role. Despite its significance, this sector faces major obstacles such as extreme weather, aridity, drought, and disease outbreaks [1]**.** Nevertheless, there is a growing conflict between environmental preservation and economic growth [2]. In recent years, restrictions on the use of antibiotics as growth promoters in animal feed have expanded globally, driven by rising concerns over antimicrobial resistance and food safety [3] .As a result, probiotics have drawn a lot of attention from around the world as an alternate method of boosting animal growth [4, 5, 6]. Probiotics are defined by FAO/WHO as “live microorganisms that, when administered in sufficient amounts, provide health benefits to the host” [7] They have long been used to enhance productivity in both ruminant and non-ruminant animals, as well as human health. Common probiotic strains include *Enterococcus*, lactic acid bacteria (LAB), *Lactobacilli*, and *Bifidobacteria* [8]. According to recent research, multi-strain probiotic formulations may provide more advantages than single-strain probiotics, particularly when strains complement one another's functions**,** highlighting the importance of strain specificity and disease context in probiotic efficacy [9]. Based on this, our research hypothesizes that a probiotic blend of Bee pollen with *Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus* can be supplemented as a safe growth promoter and alternative to harmful antibiotics. Its effects on calf and heifer health were assessed through blood parameters, oxidative stress markers, and liver function. Probiotics containing *Lactobacilli*, *Enterococcus*, *Bifidobacteria*, and yeasts have been shown to improve growth, productivity, and overall health by modulating gut microbiota [4, 10, 11, 12, 13, 14], with *Lactobacillus*-based probiotics, in particular, are

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promising due to their positive impact on gut health, immune function, and nutrient absorption [**6**].

From a husbandry perspective, Combining Lactobacillus-Streptococcus probiotics into calf diets supports preventive health management by stabilizing the gut microbiome, enhancing nutrient utilization and immunity, and reducing heat stress and behavioural issues. probiotics, particularly yeast-based supplements, can enhance feed efficiency in dairy cows under heat stress by improving digestion and metabolic adaptation [15] and reduced behavioural stress in cows caring for weaned calves [16] .

Bee pollen (BP), the core component of the supplemented probiotics, is widely recognized for its nutritional and therapeutic value in farm animals. It is completely safe for both animals and humans. Honeybees produce BP by collecting diverse floral pollen grains and mixing them with nectar and enzyme-rich saliva, enhancing its bioactive profile[17, 18] .Classified as a safe dietary supplement, BP contains around 250 bioactive compounds, including essential minerals and phenolics. It offers antioxidant, anti-inflammatory, antimicrobial, immune-boosting, and hepatoprotective effects [19]. Additionally, BP is considered a natural growth promoter, vitality enhancer, and a potential antibacterial agent [20, 21, 22] .

Material and Methods

In Assiut University, Assiut, Egypt, the study procedure was conducted in the Animal Experimental Farm, Animal Production Department, Faculty of Agriculture. According to the Ethical Committee of the Faculty of Veterinary Medicine at Assiut University (Reference No. 06/2025/0290).

Animal Management

Twenty healthy (6-8 months aged) local or native crossbred cow calves and heifers prevalent in Upper Egypt were randomly divided into two groups (5 calves and 5 heifers each). For three months, the control group was fed a baseline diet Con in compliance with the 2007 NRC guidelines [23], while the treatment group FBP received 40 g of fermented bee pollen , mixed with the diet, three times a week alongside the baseline diet. The diet, on a dry matter basis, included 0.5% premix combinations, 1.5% limestone, 1% sodium chloride, 22% decorticated cottonseed meal, 25% wheat straw, 25% yellow corn, and 25% wheat bran. Animals were fed daily rations at 3% DM of body weight, twice daily at 7:00 and 17:00, to meet their nutritional needs. Fresh water was available throughout the day.

Formulation and Laboratory Processing of Fermented Bee Pollen probiotic

Probiotic treatment FBP was prepared using fresh, commercial bee pollen. Distilled water and bacterial strains of *Lactobacillus bulgaricus subsp. delbrueckii*. Additionally, *Streptococcus thermophilus* was combined with pollen at a ratio of (10 shares fresh bee pollen: 2.5 shares distilled water: 0.25 shares bacteria strains). The mixture was transferred into clean glass jars, leaving approximately 25% of each jar empty to allow for gas expansion during fermentation.

The jars were tightly closed and stored somewhere that is dark at a regulated 25–30°C temperature for a duration of two-three weeks. This period of incubation allows the complete fermentation of lactic acid by the probiotic strains. After the fermentation, the FBP product was considered ready for use and was either applied in its fresh form or after drying for preservation and later application.

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 Red Blood Cells (RBCs.) and White Blood Cells (WBCs.) 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 Malondialdehyde (MDA.)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 Triglycerides (TG.) (catalog number: 314002) ،Low-Density Lipoprotein (LDL.) , High-Density Lipoprotein (HDL.) (catalog number: 266001) and Total Cholesterol (TC.) (catalog number: 230002) with calculating the Very-Low-Density Lipoprotein (VLDL.) = TG/5. Moreover, liver enzymes Alanine Aminotransferase (ALT.) (catalog number: 260001), and Glucose (GLC.) (catalog number: 250001) were measured.

Statistical analysis

All collected data were analyzed using the General Linear Models (GLM) procedure of SPSS (2008). The GLM approach was selected due to its suitability for evaluating the effects of multiple fixed factors and their interaction on continuous dependent variables. The design included equal numbers of calves and heifers per group to enable basic comparisons and interaction analysis.

The statistical model used was:

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

where μ is the overall mean, Ti is the fixed effect of treatment (Con and FBP), Gi is the fixed effect of gender (male and female), ε ijk represents the residual error., and Yij is the target variable (growth and blood characteristics).

Results

FBP enriched with *Lactobacillus* and *Streptococcus* strains significantly enhanced multiple health biomarkers in calves over three months. FBP treatment improved the lipid profile (lower TG, VLDL, LDL, and TC), reduced oxidative stress (lower MDA), and increased hematological parameters (higher GLC, RBCs, and WBCs).

Lipid Profile & Metabolic Health Indicators

TC and VLDL levels were significantly affected by both FBP supplementation and the gender of the animals (Table 1). FBP treated group consistently showed improved lipid metabolism, as evidenced by a significant reduction in TG and VLDL concentrations ($P<0.05$) compared to controls.

Similarly, blood levels of LDL, HDL, and TC were significantly lower in the FBP groups ($P<0.05$) than in the control group. However, no significant differences were observed between male and female groups for these parameters.

Oxidative Stress & Liver Functions

MDA & ALT Levels showed significantly decreased in the FBP group ($P<0.05$) compared to those in the control group (Table 2). Although there are no observable gender differences.

Haematological Parameters

The treated group FBP had significantly higher GLC levels, WBCs, and RBCs counts than the control group ($P < 0.05$). However, none of the parameters showed any appreciable gender differences (Table 3).

Discussion

Our results showed that FBP supplementation significantly enhanced the lipid profile, with TG, LDL, VLDL, and TC levels being significantly lower ($P < 0.05$) in FBP-treated group compared to controls. These findings are in line with the FAO report on Probiotics in Animal Nutrition [24] , which reported similar reductions in TG and TC levels in poultry, swine, and ruminants following probiotic supplementation. Likewise, previous studies [25] documented improvement in the lipid profile and metabolic efficiency in livestock fed multiple strains probiotics.

Recent studies confirm that the lipid-lowering effects of probiotics in ruminants are linked to enhanced bile salt hydrolase (BSH) activity and gut microbiota modulation. For example, *Lactobacillus* strains with high BSH activity have demonstrated significant cholesterol-lowering abilities in vitro and in vivo, supporting their use in animal nutrition [26,27,28] or higher short-chain fatty acid (SCFA) production, which prevents the liver from synthesizing fatty acids [29]. Perhaps since probiotics have been shown to promote lipid metabolism and decrease intestinal lipid absorption [30]. De Rodas et al. [31] recommended additional mechanisms to explain the reduction in the cholesterol levels after using probiotics, including disruption of enterohepatic bile salt circulation, Cholesterol assimilation in the gut, Propionic acid production, and conversion of cholesterol to coprostanol. Al-Saiady [32] found that feeding *Lactobacillus acidophilus and L. plantarum* to calves significantly lowered serum cholesterol. Similarly, [33] observed enhancements in TG and cholesterol levels with probiotic supplementation.

Even though these findings, the exact mechanisms remain partially unclear and may involve hormonal regulation of microbial populations and lipid-processing enzymes.

Additional blood indicators including the albumin/globulin ratio, TC, TG, HDL, LDL, VLDL, and haematological markers remained within normal ranges in pre-weaning calves, supporting the safety of the probiotic combination as documented in several studies [34, 35] . Additionally, following probiotic treatment, lower TG levels in cattle were reported [36, 37] . But according to some research [38, 39] there were no statistically significant changes in cholesterol levels, suggesting that host variables, dosage, and probiotic strains may all influence the reaction. Overall, our findings align with existing research suggesting that probiotics could improve lipid profile and potentially serve as a natural alternative to pharmacological lipid-lowering agents.

Oxidative Stress Marker (MDA) & Liver Function indicator

MDA is a key indicator of oxidative stress and lipid peroxidation, and is considered a valid marker of oxidative damage [40, 41]. The probiotic treatment particularly formulations containing *Lactobacillus plantarum* demonstrated a strong antioxidative effect [42, 43]. This may be attributed to the high glutathione peroxidase content, superoxide dismutase expression, and reactive oxygen species scavenging ability of *Lactobacillus plantarum*, which could explain the observed increase in antioxidant capacity. Numerous studies have shown the antioxidant capacity of probiotics. In our study, FBP supplementation with *Lactobacillus* and *Streptococcus* strains may have improved MDA levels, suggesting enhanced antioxidant capacity and reduced oxidative stress. This is consistent with studies showing increased antioxidant enzyme activity in calves given *Lactobacillus*[44].

Additionally, *Lactobacillus* and *Bifidobacterium* have been shown to enhance antioxidant enzyme activity [45, 46].Liver enzymes such as ALT and AST are key indicators of hepatic health and amino acid metabolism. Therefore, in our study, the decrease in ALT levels observed in animals treated with FBP may indicate a potential liver-protective effect of the treatment.

Recent studies have shown that supplementation with *Lactobacillus plantarum* in ruminants can enhance antioxidant activity, upregulate hepatic antioxidant enzymes, and reduce serum lipid peroxidation, which may contribute to improved liver function and lower ALT levels [47]. Additionally, research on bee pollen supplementation in livestock has demonstrated significant decreases in ALT and AST levels, indicating a hepatoprotective effect [48].

Haematological Parameters (Glucose, WBCs, RBCs)

Plasma glucose is a key indicator of physiological status. In our study, GLC. levels were significantly higher ($P < 0.05$) in animals treated with FBP probiotics compared to controls. Similarly, a substantial increase in GLC. levels was observed in both probiotic-supplemented and control group [49]. According to similar findings, calves given live yeast culture exhibited greater GLC concentrations [50, 51] . In our study, the increase in GLC with FBP supplementation remained within the normal range and may indicate greater energy availability for physiological functions. Similar results were observed by [52] who investigated the effects of probiotic and yeast cell wall supplementation on metabolic markers and liver function in dairy calves during the transition period.

WBC count is a reliable indicator of immune functions in animals. Some studies are consistent with the findings of this research regarding the increase in WBC count following probiotic supplementation, For instance [53 &32], documented noticeably higher WBC counts in calves and heifers fed probiotic-supplemented behind basal diets compared to control group, confirming the observed increase in WBC count with probiotic supplementation. The significant rise in WBCs could be attributed to increased immune cell production, which is essential for protecting physiological systems from illness [54] . Nevertheless, not all research has produced reliable findings. For instance, probiotic treatment had no significant effect on the WBC counts of developing pigs and calves, according to [55, 56]. These differences could be caused by variances in animal species, doses, probiotic strains, or experimental setups.

Due to its immunoregulatory properties, BP is considered a potent growth promoter with therapeutic benefits [57] . WBCs play a key role in defending the body against pathogens, and the observed increase in WBCs in treated calves may reflect enhanced T-cell activity. While B-cells produce antibodies, T-cells target infected or abnormal cells. Additionally [58], reported that BP supplementation improved overall health by increasing PCV, Hb, RBCs, and leukocyte counts, especially lymphocytes. Similarly, lambs fed BS-supplemented diets showed notable increases in Hb, RBCs, lymphocytes, eosinophils, and PCV[59].

RBC counts were considerably higher in the FBP group, As observed by [60], who found that the probiotic-supplemented animals showed an increasing in Hct levels & RBC counts as compared to the control group. These improvements may be linked to enhanced iron metabolism via gut microbiota modulation. Additionally, in comparable circumstances, [61, 36] observed enhanced metabolic state and energy balance.

Conclusion

The findings of the current study suggest that fermented bee pollen may serve as a promising probiotic feed supplement in animal husbandry. Its use appears to positively influence calf health by potentially enhancing oxidative stress response, lipid and glucose metabolism, liver function, and immune parameters. These results indicate that fermented bee pollen could contribute to improved calf health and performance in a sustainable and environmentally friendly manner, though further research is needed to confirm its therapeutic applications.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

The Ethical Committee of the Faculty of Veterinary Medicine at Assiut University in Assiut, Egypt, gave its permission to the animal study protocol (Reference No. 06/2025/0290).

TABLE 1. Effect of FBP probiotic supplements on lipid profile of calves

	Treatment		Gender		SEM	*P*-value		
Item	**Con**	**FBP**	**Male**	**Female**		**T**	**G**	**T×G**
TG	23.85	22.20	23.19	22.85	0.135	<0.001	<0.001	0.364
LDL	36.21	33.41	34.74	34.88	0.240	<0.001	0.638	0.071
HDL	48.25	46.06	47.19	47.12	0.171	<0.001	0.558	0.118
VLDL	4.77	4.44	4.64	4.57	0.027	<0.001	<0.001	0.364
TC	89.24	83.99	86.67	86.56	0.398	<0.001	0.700	0.386

* Con: experimental animals received basal diet; FBP: experimental animals received basal diet + 40 g of probiotic three times/week; TG: triglycerides (mg/dL), LDL: low-density lipoprotein (mg/dL), HDL: High-density lipoprotein:(mg/dL), VLDL: very-low-density lipoprotein:(mg/dL), TC: total cholesterol(mg/dL).

TABLE 2. Effect of FBP probiotic supplement on Oxidative Stress & Liver Function of calves

	Treatment		Gender		SEM	*P*-value		
Item	**Con**	**FBP**	**Male**	**Female**		**T**	**G**	**T×G**
MDA	2.93	2.01	2.47	2.48	0.064	<0.001	0.765	0.646
ALT	24.07	20.48	22.17	22.38	0.264	<0.001	0.380	0.005

Con: experimental animals received basal diet; FBP: experimental animals received basal diet + 40 g of probiotic three times/week; MDA: Malondialdehyde (nmol/mL), ALT: alanine aminotransferase (U/L)

TABLE 3. Effect of FBP probiotic supplement on Haematological Parameters of calves

	Treatment		Gender		SEM	*P*-value		
Item	**Con**	**FBP**	**Male**	**Female**		**T**	**G**	**T×G**
GLC.	42.33	61.13	52.90	50.57	1.468	<0.001	0.002	<0.001
WBCs	6.67	10.97	8.34	9.30	0.359	<0.001	0.032	0.945
RBCs	8.84	10.35	9.68	9.52	0.114	<0.001	0.074	0.821

Con: experimental animals received basal diet; FBP: experimental animals received basal diet + 40 g of probiotic three times/week; GLC: Glucose (mg/dL), WBC: White blood cells ($\times10^3/\mu L$), RBC: Red blood cells ($\times10^6/\mu L$).

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قوة البروبيوتيك المعتمد على اللاكتوباسيلوس في تعزيز صحة العجول

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الملخص

هدفت هذه الدراسة إلى تقييم تأثير حبوب اللقاح المخمرة (FBP) كمزيج بروبيوتيك يحتوي على بكتيريا Lactobacillus bulgaricusو Streptococcus thermophilus، والتي تم تخميرها باستخدام حمض اللاكتيك، على الصحة العامة للعجول الذكور والإناث خلال فترة ثلاثة أشهر. تم تقسيم عشرين عجلًا وعجلة، تتراوح أعمارهم بين 6 إلى 8 شهور، بشكل عشوائي إلى مجموعتين: مجموعة تلقت 40 جرامًا يوميًا من حبوب اللقاح المخمرة بالإضافة إلى النظام الغذائي الأساسي، ومجموعة أخرى تلقت النظام الغذائي الأساسي فقط. تم قياس المؤشرات الدموية والكيميائية الحيوية مثل الجلوكوز(GLC.) ، الكوليسترول(TC.) ، الدهون الثلاثية(TG.) ، البروتين الدهني منخفض الكثافة (LDL.)، البروتين الدهني عالي الكثافة (HDL.) ، البروتين الدهني منخفض الكثافة جدًا(VLDL.) ، المالونديالديهيد (MDA.)، إنزيم ألانين أمينوترانسفيراز(ALT) ، وعدد خلايا الدم الحمراء والبيضاء (RBC) و(WBC)، وذلك باستخدام عينات دم شهرية. أظهرت النتائج أن العجول التي تغذت على FBP تحسنت صحتها بشكل ملحوظ، كما يتضح من الانخفاض الكبير في مستوياتMDA ، TG، LDL، HDL، الكوليسترول، و ALTفي مجموعة FBP مقارنةً بالمجموعة الضابطة .($P < 0.05$) وعلى العكس، كانت مستويات الجلوكوز، وخلايا الدم البيضاء والحمراء في مجموعة FBP أعلى بشكل ملحوظ ($P < 0.05$) مقارنةً بالمجموعات الضابطة. وتشير النتائج إلى أنFBP ، كمكمل بروبيوتيك قائم على بكتيريا Lactobacillus ، قد يعزز من تكاثر الخلايا المناعية، والنشاط الأيضي، والصحة العامة للعجول.

الكلمات الدالة: حبوب اللقاح المخمرة، البروبيوتيك في الثروة الحيوانية، صحة العجول، المؤشرات الدموية والكيميائية الحيوية.