Using manufactured Bee Bread as a biotechnological tool to Enhance Growth and Performance in cow calves

Raghda A. Taghian*[1], Eman A. Negm [2]

[1] Animal Production Department, Faculty of Agriculture, Assiut University, Assiut, Egypt. Corresponding author Email: raghda.taghian2024@aun.edu.eg
[2] Physiology Department, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.

ABSTRACT

The goal of this study is to assess and evaluate how manufactured Bee Bread (BB) affects the performance and blood parameters of male and female calves. Twenty healthy cow calves (10 males and 10 females), aged 4–6 months with initial body weights (IBW) of 102.4 kg for males and 98.6 kg for females, respectively, were randomly assigned into two groups, each consisting of 5 males and 5 females. 40g of BB was added to the basal diet three times per week for the treatment group, Whereas the control group received only the basal diet. Animals were weighed monthly, and feed intake (FI) was recorded to calculate the feed conversion ratio (FCR). Blood samples were collected monthly to measure hemoglobin (Hb), total protein (TP), albumin (Alb), globulin (Glob), triiodothyronine (T_3), and thyroxin (T_4). Calves supplemented with BB showed significantly higher final body weight (FBW), body weight gain (BWG), and average daily gain (ADG) ($P < 0.05$) compared to the control group, Furthermore, the addition of BB significantly improved (FCR) and increased daily feed intake (DFI). Calves in the BB group had higher levels of Hb, TP, Alb, Glob, T_3, and T_4 ($P < 0.05$). In conclusion, supplementing the diet of growing cow calves with manufactured Bee Bread enhanced growth performance and improved feed utilization efficiency.

Keywords: Bee Bread, Blood metabolites, thyroid functions, Feed efficiency, Growing calves, Growth performance.

INTRODUCTION

The use of biotechnology is expected to bring a significant transformation in the economic returns from livestock production (Fereja, 2016). According to Getabalew and Alemneh (2019), biotechnology plays a vital role in improving animal feed through three main approaches: enhancing the nutritional value of forage, producing feed additives, and manipulating rumen microbes to improve feed utilization. It also supports the activity and metabolism of gut microbes, which are essential for animal growth and overall well-being. Modern biotechnology offers a wide range of beneficial applications, including environmental protection, alteration of the gut microbiota and the development of balanced diets and feed additives that promote healthy growth and enhance animal wellbeing (Okon et al., 2022). Using natural feed additives is one viable and promising way that biotechnology is being used in livestock husbandry, such as bee products, probiotics, and prebiotics. These supplements help improve the microbial balance in the digestive tract. Unlike antibiotics, which eliminate bacteria, probiotics promote the growth of beneficial strains at the expense of harmful ones (McDonald et al., 2010). Bee products offer a dual advantage: they are nutritionally rich and act as natural probiotics. Among these, BB has drawn interest due to its potential as a feed additive that enhances growth and overall well-being in livestock. In hives, a fermented mixture of bee pollen, honey, and lactic acid bacteria is termed "bee bread." It has a superior nutritional profile compared to raw pollen and is a valuable source of rare nutrients, although produced in limited quantities (Krell, 1996).This concept led to the idea of artificially manufacturing bee bread, simulating the natural process used by bees, producing larger quantities suitable for animal feeding and investigating its impact

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on blood parameters, growth, and performance. Probiotics have been found to enhance immunity, growth rate, and health in livestock species, including pigs, cattle, poultry, and small ruminants (El-Trwab et al., 2016; Alayande et al., 2020) However, their efficacy may differ based on outside variables like the environment and feed composition (Leistikow, et al., 2022). In addition to fighting antibiotic resistance, the cattle industry is depending more and more on probiotics to increase production efficiency (Gilchrist et al., 2007). Animal nutrition has traditionally employed lactic acid-producing probiotics, such as strains of Bifidobacterium, Enterococcus, Streptococcus, Pediococcus, and Lactobacillus (Deng et al., 2022). Bee products are high in vitamins, minerals, and good fats. These include honey, propolis, pollen, bee bread, bee venom, and royal jelly. While, traditionally used by humans as dietary supplements or natural remedies, recent research has explored their potential benefits in animal nutrition and therapy (Madras-Majewska et al., 2015). Studies have shown that bee products positively impact animal health and productivity. They may also serve as alternatives to conventional treatments, especially in cases of antibiotic-resistant infections. Due to their rich nutritional content, bee products could outperform standard mineral-vitamin mixes or complement them during critical breeding periods (Madras-Majewska et al., 2015). Many studies have shown that bee products such as honey, propolis, and royal jelly can significantly enhance animal health and performance by improving immunity, promoting growth, and supporting physiological functions in livestock and poultry (Park & Ikegaki, 1998; Roulston & Cane, 2000; Bansal et al., 2005; Patel et al., 2021; Bengouga et al., 2025).

MATERIALS AND METHODS

At Assiut University's Animal Production Department, Faculty of Agriculture, Animal Experimental Farm, Assiut, Egypt, this experiment was carried out. In compliance with Assiut University's Faculty of Veterinary Medicine's Ethical Committee (Reference No. 06/2024/0247). Determining the body performance and specific blood metabolites in male and female calves given artificial Bee Bread (BB) made from date palm pollen was the goal of this investigation.

Formulation and Laboratory Processing of Artificial Bee Bread

Artificial BB was made using a modified version of (Dany, 1988) recipe. Bee pollen, honey, *Lactobacillus bulgaricus, and Streptococcus thermophiles* were used to artificially replicate the natural BB (10 shares of bee pollen: 1.5 shares of honey: 2.5 shares of clean water: 0.25 shares of lactic acid bacteria), Fermentation was carried out in airtight, wide-mouthed, transparent plastic bottles. There was still enough airspace above the culture (20–25% of the total volume). For the first two or three days, the temperature ranged from 32 to 34 degrees Celsius. The temperature was reduced to 20 to 24°C after the first two or three days. Due to the accumulation of lactic acid, the high starting temperature is crucial in halting the growth of unwanted bacteria as soon as feasible. Certain yeasts continue to proliferate, and only lactic acid generates bacteria (lactobacilli). The former quickly comes to rule the entire culture. After two to three days, the temperature should drop since the lactobacilli's ultimate growth should happen slowly. While lactic acid functions as a natural preservative, the finished product may be kept for years.

Animals, Diets and Experimental Design

Twenty healthy cow calves (10 males and 10 females), aged 4–6 months with initial average body weights (IBW) of 102.35 kg for males and 98.62 kg for females, respectively, were randomly assigned into two groups (5 males and 5 females per group). The control group (Con) received the basal diet, while the treatment group was supplemented with 40 g of manufactured Bee Bread (BB), which was thoroughly mixed into the feed ration three times per week. The components and chemical composition of the basal diet are displayed in Table 1. According to the NRC guidelines (NRC. National Research Council., 2007). For three months, the treated group (BB) received 40 g of produced BB three times a week along with the basal diet. The animals

were given their daily rations twice a day at 7:00 a.m. and 5:00 p.m., which were mixed rations containing 3% DM of body weight to suit their nutritional requirements. Fresh water was available all the time.

TABLE 1. The chemical composition and ingredients of the basal diet

Item	Basal diet
Ingredient, (%)	
Wheat straw	25
Yellow corn	25
Wheat bran	25
Decorticated cottonseed meal	22
Limestone	1.5
Sodium chloride	1
Premix mixtures*	0.5
Chemical composition	
DM (%)	90.23
OM (% DM)	88.92
CP (% DM)	14.21
CF (% DM)	17.32
EE (% DM)	2.46
NFE (% DM)	54.93
Ash (% DM)	11.08

200,000 IU of vitamin A, 200 mg of vitamin E, 100,000 IU of vitamin D_3, 10,000 mg of iron, 2,500 mg of copper, 100 mg of molybdenum, 20,000 mg of manganese, 100 mg of cobalt, 800 mg of iron, 20,000 mg of zinc, and 100 mg of selenium are all present in premix mixes.
Dry matter (DM) and organic matter (OM) Crude protein: Crude fiber; ether extract (EE); nitrogen-free extract (NF)

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Growth Experiment

Before starting the 90-day growth experiment, the animals underwent a 15-day preparatory phase to become acclimated to the experimental diet. To determine the total body weight gain (BWG) and average daily gain (ADG), the animals were weighed as Initial body weight (IBW) at the start of the growth trial and then monthly thereafter. Additionally, the feed conversion ratio (FCR, kg feed/kg growth) was computed using the daily feed intake (DFI, as dry matter).

Blood Sampling and Blood Parameters

Every month during the trial period, two blood samples (about 7 ml each) were drawn from each experimental animal by jugular venipuncture at 8:00 a.m. First, the Symex Automated Hematology Analyzer (SAHA) was utilized to estimate hemoglobin (g/L) from a total blood sample. To estimate total protein (TP. g/L), albumin (Alb. g/L), globulin (Glob. g/L), A/G ratio, triiodothyronine (T_3, ng/mL), thyroxin (T_4, ng/mL), and T_4/T_3 ratio, the second samlple was moved to a tube without anticoagulant and centrifuged at 3000 rpm for 15 minutes to obtain serum. Diamond Diagnostics (Egypt) provided the kits. Prior to analysis, all samples were stored at -20°C.

Statistical Analysis

The SPSS (Graham, 2008) General Linear Models (GLM) approach was used to examine all data that was gathered. The data was analyzed using the following statistical model:

$$Y_{ij} = \mu + T_i + G_j + (T \times G)_{ij} + \varepsilon_{ijk}$$

Where µijk is the residual error, µ is the general mean, Ti is the treatment effect (Con and BB), Gi is the gender effect (male and female), and Yij is the dependent variable (blood characteristics and growth performance).

RESULTS AND DISCUSSION

Effect of BB on growth performance:

Supplemented male and female calves showed significant improvements in the growth rate, FDI and FCR by supplementing BB to their diet as shown in (Table 2). BB treated calves had higher FBW, total BWG and ADG compared to the controls ($P<0.05$). Similarly, Males had higher FBW, total BWG, and ADG than females ($P<0.05$). (Table2 & Figure 2). Although no statistically significant differences were observed between males and females, DFI showed a notable increase ($P < 0.05$) when BB was supplemented to the basal diet of the treated animals (Table 2 & Fig 1). Moreover, the FCR improved ($P<0.05$) in both male and female calves may be related to increasing in BWG (Table 2 & Fig 3).

TABLE 2. Impact of bee bread (BB) supplementation on growing male and female calves and its feed intake, feed conversion ratio, and growth performance

Item	Treatment		Gender		SEM	*P*-value		
	Con	BB	Male	Female		T	G	T×G
IBW, (kg)	102.33	98.64	102.35	98.62	1.071	0.3	0.3	0.3
FBW, (kg)	170.31	193.68	186.2	177.79	3.445	<0.001	<0.001	0.373
BWG, (kg)	68	95	83.8	79.2	5.59	0.01	0.01	0.01
ADG, (g)	755.3	1056	931.7	879.7	21.27	<0.001	<0.001	0.149
DFI, (kg)	5.11	5.77	5.44	5.43	0.053	<0.001	0.671	0.744
FCR	6.75	5.46	5.95	6.17	0.091	<0.001	<0.001	0.386

Con: experimental animals were fed a basic diet; BB: three times a week, experimental animals were fed a basic diet plus 40 g of dried bee bread; ADG: Average daily growth, DFI: Daily feed intake, FCR: Feed conversion ratio, IBW: Initial body weight, FBW: Final body weight, and BWG: body weight gain.

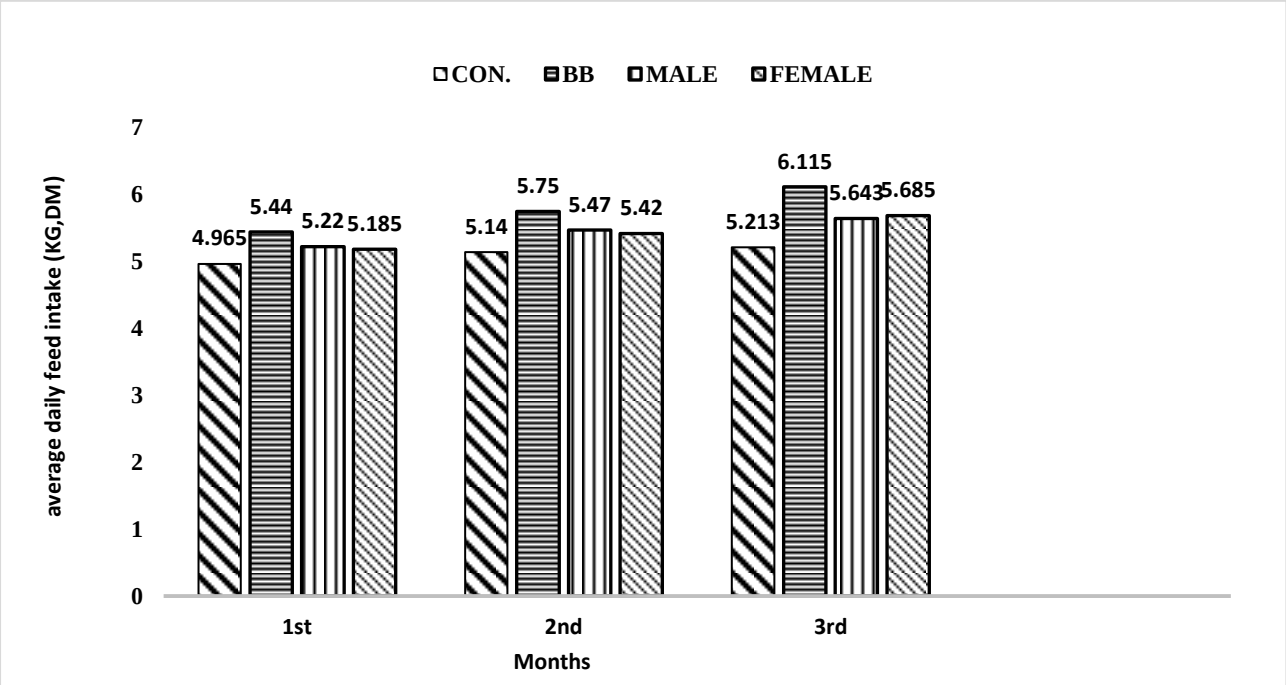

Fig 1. Average daily feed intake of growing male and female calves that affected by BB supplementation.

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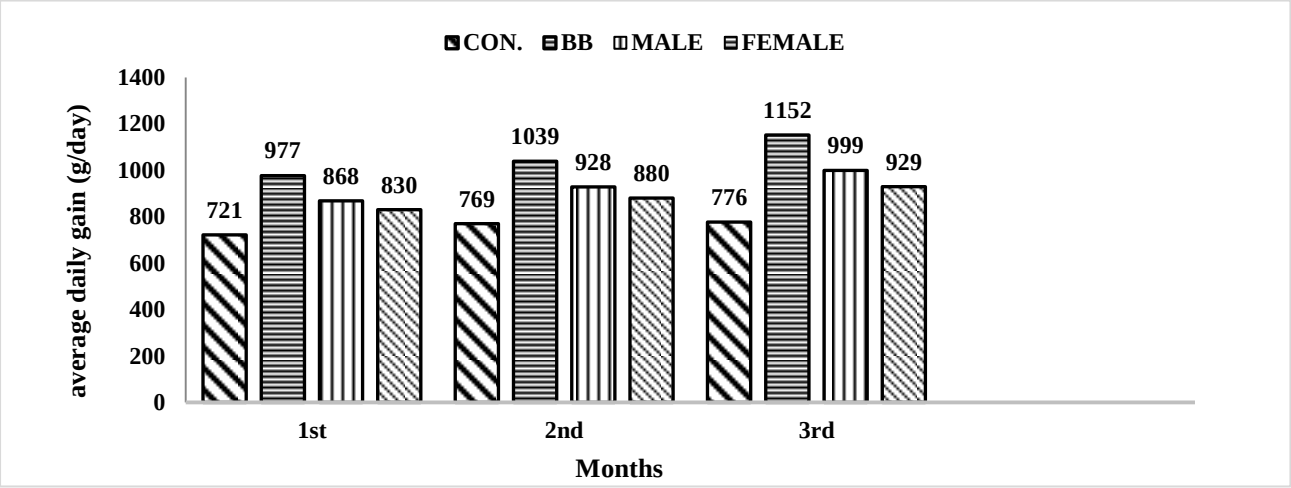

Fig 2. Average daily gain of growing male and female calves that affected by BB supplementation.

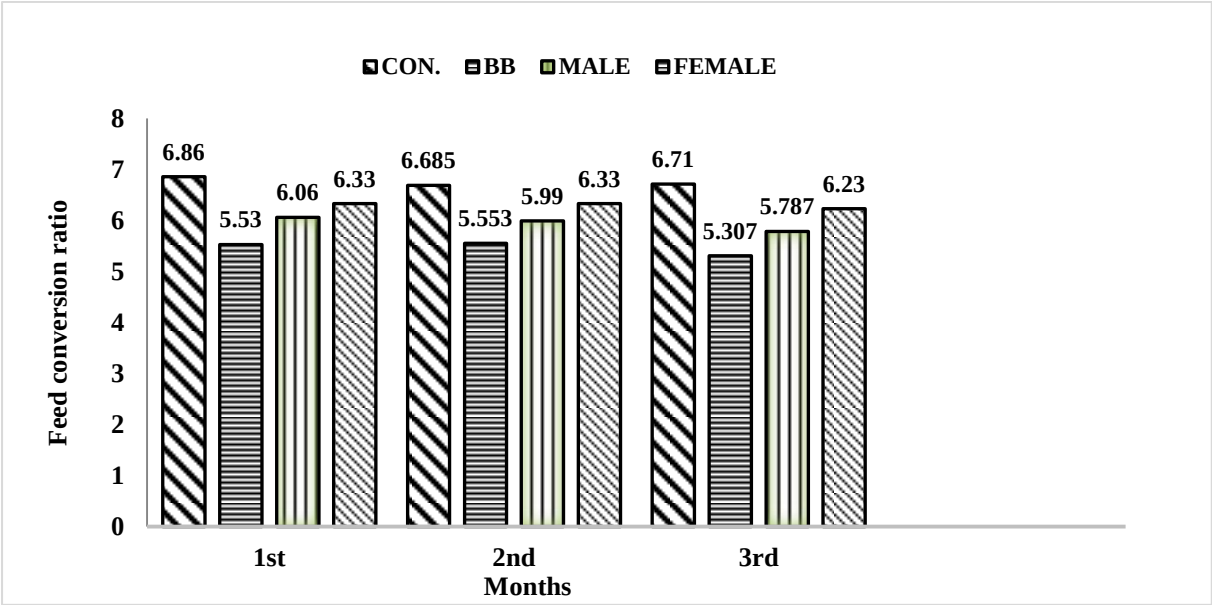

Fig 3. Feed conversion ratio of growing male and female calves that affected by BB supplementation.

Variations in BW are commonly used as markers of an animal's growth efficiency and nutritional condition. In this study, final body weight FBW and average daily gain ADG of male and female calves were considerably higher (P<0.05) in the BB-treated group than in control group. These results are in line with those of Hussein (2018), who found that lambs supplemented with 20 g seaweed powder, 1 g alpha-amylase, *Saccharomyces cerevisiae*, and *Lactobacillus sporogenes* demonstrated significant (P<0.05) increases in body weight in both sexes when compared to control. In addition, both Abas et al. (2007) and Ismaiel et al. (2010) reported similar findings, indicating that the ADG of lambs was elevated by yeast culture. Furthermore, Hussein (2014), Abdel-Salam et al. (2014), and Mohamed (2018) agreed with us when noted that probiotic-supplemented Najdi lambs outperformed the control group in growth rate GR, ADG, and TWG by a substantial margin (P<0.05). Saleem et al. (2017) found that probiotic supplementation improved growth performance in Saidi

lambs by raising BWG, TWG, and GR in the post-weaning phase. In contrast to our findings, Taghian et al. (2017) found that rams fed diets containing 30 g/day of Date palm pollen, Bee pollen, and BB three times a week had significantly higher ($P<0.05$) FCR, TFI, DFI, and FBW than control group. Our results in this study are in conflict with those of Baranowski et al. (2007), Titi et al. (2008), and Whitley et al. (2009), who found no impact of yeast supplementation on the growth rate of lambs and goat offspring. While our findings are consistent with those of Ghazanfar et al. (2015), who noted enhanced growth rates in dairy heifers fed Saccharomyces cerevisiae, and Timmerman et al. (2005), who showed higher BWG in calves treated with probiotics. In the other hand, Soliman et al. (2016) noticed that lambs given probiotic-supplemented meals had a noticeably higher TFI than lambs given prebiotics or control diets. This difference was probably caused by using different bacterial strains. Our results are in line with those of other studies by Ismaiel et al. (2010), Chiofalo et al. (2004), Antunović et al. (2005), and Whitley et al. (2009), who also found that probiotics improved ruminant dry matter intake, nutrient utilization, weight gain, and FCR. BB is rich in antioxidants, vitamins, minerals, essential fatty acids, amino acids, and enzymes. Additionally, its protected bioactive compounds such as flavonoids, carotenoids, and phenolics can enhance feed value, digestibility, and nutrient absorption. These properties may explain BB's positive impact on growth performance, (Leja et al., 2007; Saric et al., 2009). Another possible explanation for the probiotic's impact on BWG is enhanced cellulolytic activity, which improves fiber breakdown and microbial protein synthesis, hence increasing the supply of post-ruminal amino acids (Erasmus et al., 1992). Bee pollen also has growth-promoting, energy-boosting, and even antibacterial properties (Almaraz-Abarca et al., 2004). Its nutritional and medicinal benefits as an antioxidant, anti-aging agent, and immune-enhancing supplement have been widely recognized (Almaraz-Abarca et al., 2004; Battaa & El-Kholy, 2014).A notable observation in this study is that the improved growth efficiency in BB-supplemented animals was closely associated with increased feed intake and better FCR, this is in line with findings by Krehbiel et al. (2003) and Antunović et al. (2006), who reported that probiotic-supplemented beef cattle had higher BWG and FCR. According to Fiems et al. (1993) who added *Saccharomyces cerevisiae* to the diet increased BWG in calves by 9.5% and in developing adult cattle by 7.8%. Numerous researchers have found improved FCR using phytogenic growth promoters. According to Zawadzki et al. (2011) found that propolis extract enhanced weight gain and FCR in feedlot bull diets. Since BP is poorly digested, Rimpler (2003) postulated that breaking down its walls could improve digestibility and bioavailability. This could be the reason why artificial BB performed better in this investigation. Lactic acid, produced during the fermentation of BP with honey by *Lactobacillus bulgaricus and Streptococcus thermophilus*, helps break down pollen walls and release their nutrients. This process enhances the digestibility of the final BB product and enriches it with additional nutrients. Interestingly, bees use BB exclusively to feed their larvae. Therefore, the performance-enhancing effects of probiotics may also be linked to improved rumen development, such as an increased propionate-to-acetate molar ratio which reduces hydrogen availability for methane production, thereby lowering emissions Retta (2016). Additionally, higher propionate levels increase the production of

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glucose in the liver which increases lactose synthesis substrates and boosts energy

efficiency (Stein et al., 2006). Lastly, Wang et al. (2024) showed that probiotic supplementation enhances feed efficiency and growth performance, as evidenced by higher BW and ADG and lower FCR as a result of higher DMI.

Effect of BB on blood parameters and Plasma Protein Profile:

Hematological analysis of blood samples showed that Hb was affected by both BB addition and the gender of the animal (Table 3). Animals fed a basal diet plus BB had

higher Hb levels than animals fed a basal diet alone (P<0.05). Additionally, adding BB to male and female calves' diets enhanced most of the blood biochemical indicators, which did not differentiate significantly between both genders. Male and female calves in the BB group had higher levels of TP, Alb, and Glob (P<0.05) than those in the control group. There were also notable gender differences in the A/G ratio, as well as significant differences in TP and Alb levels (P<0.05).

TABLE 3. Effect of Bee Bread (BB) supplement on blood hemoglobin, biochemical and serum thyroid hormonal parameters of growing male and female calves:

	Treatment		Gender		SEM	*P*-value		
Item	**Con**	**BB**	**Male**	**Female**		**T**	**G**	**T×G**
Hb,(g/L)	10.63	11.63	11.29	10.97	0.077	<0.001	0.041	0.982
Total Protein, (g/L)	٥.5٥	6.37	4.85	5.31	21.27	<0.001	<0.001	0.149
Albumin(٤ g/L)	2.90	3.26	2.47	2.95	0.053	<0.001	0.671	0.744
Globulin,(g/L)	٢.65	3.11	2.38	2.38	0.051	<0.001	0.558	0.232
Albumin/Globulin ratio	1.31	1.07	1.09	1.29	0.012	0.685	0.892	0.346
T_3, (ng/mL)	1.40	1.74	1.61	1.53	0.091	<0.001	<0.001	0.386
T_4, (ng/mL)	91.81	93.57	92.73	92.66	3.445	<0.001	<0.001	0.373
T_4/T_3 ratio	1.52	1.85	1.73	1.64	21.27	<0.001	<0.001	0.149

Con: experimental animals received basal diet BB: experimental animals received basal diet + 40 g of Dried Bee Bread three times a week Hb: hemoglobin; T3: triiodothyronine; T4: thyroxin.

Following BB supplementation, Hb levels in both Male and female calves significantly increased, according to blood analysis from the current study. Additionally, most blood biochemical parameters showed elevated values, with notable gender differences observed in Hb levels. These results are in line with those of Yasmin et al. (2021), who found that probiotic-supplemented groups had significantly higher levels of TP, Glob, and RBC. Similarly, probiotic-treated heifers had greater Hb and RBC counts ($P<0.05$) than control, according to Ghazanfar et al. (2015). Additionally, probiotic-fed crossbred calves showed increased PCV and Hb content (Al-Saiady, 2010; Dar et al., 2017). El-Sayed and Mousa (2020) found that probiotic supplementation led to significant increases ($P < 0.05$) in hemoglobin, PCV, and RBC count in lambs. These results reflect direct physiological changes. Meanwhile, Kander (2004) proposed that such effects may be due to probiotics enhancing vitamin B synthesis and iron absorption, which support blood formation. Comparable results were obtained by Abdel-Raouf et al. (2018) in Friesian calves given black seed and bee pollen during the weaning process, showing increased Hb, TP, Alb, and Glob levels. Mohamed (2018) highlighted bee pollen's therapeutic role in stimulating the hematopoietic system, with notable increases in Hb, RBCs, ferritin, iron, albumin, globulin, and total protein—likely due to its rich iron content and compounds that aid iron absorption and bone marrow activity. Significant changes were observed in TP, Alb, and Glob levels, while the A/G ratio remained unaffected. These results are consistent with Hussein (2018), who found that probiotic supplementation significantly ($P<0.05$) raised plasma levels of Glob and TP, but had no effect on Alb or the A/G ratio. Better protein digestibility through protease activity and changed amino acid profiles as a result of increased microbial protein synthesis could be the causes of the rise in plasma protein (Williams, 1989; Abdel-Khalek et al., 2000). Similar results were seen in goats by (Abu El-Ella & Kommonna, 2013), who found that supplementing yeast culture raised TP levels. However, Abdel Rahman et al. (2012) discovered that while yeast culture did not significantly alter TP and Glob in sheep, it did raise Alb concentration. Probiotic-supplemented lambs showed a significant ($P<0.05$) decrease in TP and Alb, according to Hossein-Ali et al. (2014). In Saidi lambs, Saleem et al. (2017) discovered no discernible changes in TP, Alb, or Glob during the post-weaning phase. Additionally, Tu et al. (2014) found no observable differences in TP, Alb, or Glob. between Holstein calves fed BP and control. However, gender differences were observed in TP levels, with females showing significantly higher values, consistent with AL-Hadithy and Badawi (2015) in Awassi sheep. According to Carlos et al. (2015), there were no discernible gender variations in Alb, Glob., or the A/G ratio in Morada Nova sheep. Taghian, R.A (2017) reported that supplementation with DPP, BP, and BB increased plasma TP and Glob concentrations in treated lambs, indicating that the higher protein levels could be the result of these supplements' increased supply of protein and amino acids.

Effect of BB on Thyroid function:

The concentrations of T3 and T4 in the blood samples of animals in the BB group were significantly higher ($P<0.05$) than those in the control group. Additionally, the T3/T4 ratio was elevated ($P<0.05$), with no notable differences observed between males and females. The current study also revealed increased levels of T_3 and T_4 hormones, along with an improved T_3/T_4 ratio. Significant gender differences ($P<0.05$) were observed, favoring male calves. These results are consistent with the findings of Taghian, R.A. (2017), who reported elevated plasma levels of TP, Glob, glucose, T3, and T4, along with decreased concentrations of cholesterol and triglycerides in rams supplemented with DPP, BP, and BB. This aligns with findings by (Tata, 2011; Mohamed, 2018; Yavuz et al., 2019), who

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demonstrated that BP supports thyroid function by increasing T_3 and T_4 levels, which are closely linked to metabolic activity and growth regulation. Kassab et al. (2017) suggested that increased thyroid hormone secretion in probiotic-treated groups may result from enhanced metabolism of carbohydrates, fats, and proteins, and increased intake of total digestible nutrients (TDN), reflecting improved energy metabolism. This is supported by earlier studies from (Ichiki 2010; Kassab and Hamdon, 2014), who supported the idea that calorie intake and thyroid hormone levels are positively correlated.

Conclusion

The findings of this study indicate that supplementing animal diets with manufactured Bee Bread (BB) improves growth performance, feed efficiency, and overall health status. This highlights BB as a promising natural alternative to conventional feed additives. Furthermore, BB represents a direct application of biotechnology in animal production, functioning as both a probiotic and a prebiotic. This dual role is attributed to its unique composition, which includes bee pollen as the primary ingredient, various strains of lactic acid bacteria, and exposure to fermentation processes—all of which enhance its nutritional value and biological effectiveness.

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إستخدام خبز النحل المصنع كأداة بيوتكنولوجية لتحسين معدل النمو وأداء عجول وعجلات الأبقار

رغدة عادل تغيان [١]، إيمان أحمد نجم [٢]

[١]قسم الإنتاج الحيواني، كلية الزراعة، جامعة أسيوط، مصر.

[٢] قسم الفسيولوجيا، كلية الطب البيطري، جامعة أسيوط، مصر.

الهدف من هذه الدراسة هو تقييم مدى تأثير خبز النحل المصنع (BB) على أداء ومقاييس الدم للعجول الذكور والإناث تم توزيع عشرين من العجول من الذكور والإناث السليمة صحياً (تتراوح أعمارهم فى بداية التجربة بين ٤-٦ أشهر بوزن (١٠٢.٤ كجم و٩٨.٦ كجم للعجول الذكور والإناث على التوالي) بشكل عشوائي إلى مجموعتين (٥ ذكور و٥ إناث لكل مجموعة).

تغذت المجموعة الكنترول على النظام الغذائي الأساسي، بينما تم أضافة ٤٠ جم من BB مع النظام الغذائي الأساسي للمجموعة المعاملة ثلاث مرات في الأسبوع. تم وزن الحيوانات شهريًا وتم تسجيل كمية العلف المستهلك لحساب معدل التحويل الغذائي .تم جمع عينات الدم شهريًا لتقدير الهيموجلوبين والبروتين الكلى والألبيومين والجلوبيولين وثلاثي أيودوثيرونين (T_3) والثيروكسين(T_4). كان الوزن ومتوسط الزيادة اليومية للحيوانات التي حصلت على BB أعلى من تلك الموجودة في المجموعة الكنترول. كذلك، أدت إضافة BB إلى زيادة ($P<0.05$) معنوية في كمية المأكول من العلف (DFI)، وتحسن معدل التحويل الغذائي (FCR) أيضاً تحسناً معنوياً، تحسنت مستويات كل من الهيموجلوبين، والبروتين الكلي، والجلوبيولين، والألبيومين، ومستويات T_3وT_4 في دم الحيوانات في مجموعة BB مقارنةً بحيوانات مجموعة الكنترول. وأخيراً، يمكن ان نستنتج من هذه التجربة أن إضافة خبز النحل المصنع إلى النظام الغذائي للعجول الذكور والإناث وسيلةً لتعزيز النمو، وتحسين معدل التحويل الغذائي.

الكلمات الدالة: خبز النحل المصنع، ميتابولزم الدم،وظائف الغدة الدرقية، كفاءة التغذية، العجول النامية، أداء النمو.