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Effects of *Bryophyllum pinnatum* Leaf Meal on Blood Parameters, Oxidative Status, Antioxidants, and Reproductive Hormones in Grower Rabbits

Razaq Animashahun[1*], Oluwabori Adefioye[1], Adedeji Animashahun[2], Olayinka Alabi[1], and Ooreoluwa Adegboye[1]

[1]*Animal Science Programme, College of Agricultural Sciences, Landmark University, P.M.B. 1001, Omu Aran, Nigeria*
[2]*Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta, P.M.B 2240, Abeokuta, Nigeria*

*Corresponding author's Emails: animashaun.rasaq@lmu.edu.ng; kunleannies@yahoo.com

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ABSTRACT

In the search for safe and effective phytogenic alternatives to synthetic additives in rabbit nutrition, *Bryophyllum pinnatum* has shown promising biological potential. This study investigated the effects of dietary inclusion of *Bryophyllum pinnatum* leaf meal (BPLM) on hematological parameters, serum biochemistry, oxidative stress markers, and reproductive hormone levels in grower male rabbits. A total of 48 Hyla grower male rabbits, aged 7–8 weeks, were randomly allotted to four dietary treatments in a completely randomized design (CRD): T1 (0% BPLM), T2 (1% BPLM), T3 (1.5% BPLM), and T4 (2% BPLM), with 12 rabbits per group, further subdivided into three replicates of four animals each. After an 10-week feeding trial, blood samples were collected and analyzed for hematological indices (erythrocyte count, packed cell volume, hemoglobin concentration, erythrocyte indices, total and differential white blood cell counts), biochemical parameters (liver enzymes, total protein, glucose, cholesterol, triglycerides, low-density lipoprotein, and high-density lipoprotein [HDL]), oxidative stress markers (malondialdehyde [MDA] and 2,2-diphenyl-1-picrylhydrazyl scavenging activity [DPPH]), and reproductive hormones. The majority of hematological and biochemical parameters were not significantly affected by BPLM inclusion, indicating no adverse impact on physiological homeostasis. However, erythrocyte count, serum cholesterol, and HDL levels showed significant differences. Erythrocyte count increased progressively from 0% to 1.5% BPLM inclusion, but declined at the 2% level. Serum cholesterol decreased gradually with increasing BPLM inclusion, with the lowest value observed at 2%. Antioxidant activity improved significantly with increasing BPLM levels, as evidenced by enhanced DPPH scavenging capacity and reduced MDA concentrations, particularly at 1.5% and 2% inclusion. Furthermore, reproductive hormones, apart from testosterone, were significantly elevated in rabbits fed diets containing 1.5% and 2% BPLM, suggesting enhanced reproductive function. Overall, these findings indicate that dietary inclusion of BPLM up to 2% is safe and beneficial for growing male rabbits, with positive effects on antioxidant defense and reproductive performance. The functional phytogenic properties of *Bryophyllum pinnatum* (*B. pinnatum*) leaves highlight their potential as a natural feed additive for improving rabbit health and productivity.

Keywords: Functional nutrition, Male fertility, Medicinal plant, Oxidative stress, Phytogenic feed additive, Rabbit

INTRODUCTION

The use of antimicrobial agents as growth promoters has long been a widespread practice in monogastric livestock production to improve growth performance and enhance resistance to diseases. However, concerns over antibiotic residues in animal products, their adverse effects on human therapeutics, and the emergence of antimicrobial resistance have led to strict regulations and bans on antibiotic growth promoters (AGPs) in many countries (Xu et al., 2022). The ban on AGPs has prompted increased research into safer, natural alternatives, such as plant-based leaf meals.

The use of phytogenic feed additives, such as leaf meals, in animal nutrition has gained significant attention for their potential to improve growth performance, immune function, and overall health in livestock (Wang et al., 2024). Replacing antibiotics with leaf-based additives also supports several United Nations Sustainable Development Goals (SDGs), notably SDG 1 (No Poverty), SDG 2 (Zero Hunger), and SDG 3 (Good Health and Well-being, Granato et al., 2022; Gamage et al., 2023). *Bryophyllum pinnatum* (*B. pinnatum*), a locally available and cost-effective feed additive, contributes to SDG 1 by reducing reliance on expensive synthetic inputs while enhancing rabbit health, growth, and reproductive performance (Abdelsalam and Fathi, 2023). This can increase meat yield and income for smallholder farmers, boosting food security and economic resilience in low-income communities. Overall, the adoption of plant-based additives in animal diets promotes sustainable livestock production, economic growth, and improved public health outcomes (Wang et al., 2024).

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Studies on rabbits have demonstrated the efficacy of phytogenic feed additives in improving hematological parameters, oxidative stress markers, and reproductive performance (Oloruntola et al., 2018; Abdelghani et al., 2024). For instance, the inclusion of garlic (*Allium sativum*) and ginger (*Zingiber officinale*) in rabbit diets has been linked to enhanced antioxidant activity, improved lipid metabolism, and increased testosterone levels (Unigwe and Igwe, 2022). Similarly, supplementation with *Moringa oleifera* has been shown to influence blood profiles and immune responses in rabbits positively (Badawi et al., 2017). These findings highlight the potential of plant-based additives in modulating physiological functions and promoting overall health in rabbits. El-Hady et al. (2020) reported that incorporating phytogenic feed additives into broiler chicken diets led to marked improvements in hematological indices, including hemoglobin concentration, erythrocyte count, and hematocrit. These results suggest that plant leaf extracts may have potential applications in improving blood cell formation in animals. It has been discovered that some leaf meals, such as *papaya* leaf meal, have hepatoprotective effects, which means they can help safeguard the liver from harm and enhance liver function (Ugbogu et al., 2023). N'nanle et al. (2020) observed that incorporating certain leaf meals, such as fenugreek and *Moringa oleifera*, into animal diets can elevate high-density lipoprotein (HDL) levels while reducing total cholesterol. In contrast, other leaf meals like cassava leaf meal may exert minimal effects on cholesterol modulation. Several studies have examined the effects of leaf meals on lipid peroxidation in rabbits. For instance, investigations utilizing Moringa leaf meal have demonstrated a reduction in lipid peroxidation markers, such as malondialdehyde (MDA) levels. The presence of bioactive compounds in leaf meal is believed to contribute to its antioxidant effects (Vergara-Jimenez et al., 2017). Studies have also shown that certain herbs and botanicals can exhibit antibacterial, antiviral, anthelmintic, coccidiostatic, anti-inflammatory, and antioxidant properties (Rostami et al., 2017; Abd El-Hack et al., 2020). Phytogenic alternatives to antibiotics, particularly flavonoids such as quercetin and kaempferol, as well as terpenoids and phenolic acids derived from plants like *Moringa oleifera* and *Zingiber officinale*, have been reported to improve hematological parameters, reduce lipid peroxidation (as indicated by decreased malondialdehyde levels), enhance antioxidant enzyme activities (e.g., superoxide dismutase, catalase), support immune function, and modulate hormonal balance in poultry (Abdelli et al., 2021; Wang et al., 2024). Given its rich phytochemical profile, *B. pinnatum* may exhibit similar effects when included in rabbit diets, making it a promising candidate for further investigation. Livestock feed supplies essential nutrients that can influence hormonal activity, thereby supporting growth and development in animals (Cuchillo-Hilario et al., 2024). For instance, diets rich in protein can stimulate the secretion of growth hormone (GH), which plays a key role in promoting tissue growth, muscle development, and overall productivity (Firmenich et al., 2020). For lactating animals, specialized feeds are formulated to support milk production by ensuring the availability of nutrients that influence key lactogenic hormones (Pan et al., 2023). The primary hormones involved in milk synthesis and let-down include prolactin, oxytocin, estrogen, and progesterone. Deficiency in certain nutrients can disrupt hormonal balance, leading to irregular cycles and reduced fertility (Skoracka et al., 2021).

Bryophyllum pinnatum, also referred to as the "life plant" or "miracle leaf," has been extensively studied for its medicinal potential, notably its anti-inflammatory, antioxidant, and hormone-regulating properties. Its leaves contain various phytochemicals such as flavonoids (notably quercetin, kaempferol, and rutin), alkaloids, and polyphenols, which are largely responsible for its health-promoting effects (Kumar et al., 2019). Among these, quercetin is recognized for its antioxidant capacity and has demonstrated anti-inflammatory, anticancer, and cardioprotective benefits by mitigating oxidative stress (Carrillo-Martinez et al., 2024). Kaempferol has also been shown to have anti-inflammatory, anticancer, and neuroprotective effects (Qattan et al., 2022). Owing to its high-water content and nutritional value, *B. pinnatum* leaves are utilized in some areas as an additive in feed for cattle, goats, and poultry (Omah et al., 2022). While *B. pinnatum* has indeed been studied for its pharmacological properties, including antioxidant, anti-inflammatory, and hormonal effects, most of these investigations have focused on its medicinal applications in humans or used *in vitro* or *in vivo* models using rodents (Araújo et al., 2023). However, there remains a significant knowledge gap regarding its nutritional and physiological effects in monogastric livestock, particularly rabbits. Studies in poultry and ruminants suggest that its inclusion in feed improves immune response, reduces oxidative stress, and enhances reproductive hormone secretion (Philip et al., 2023; Obianwuna et al., 2024).

However, data on *B. pinnatum* effects in monogastric animals, particularly weaned rabbits, are limited. Given the physiological challenges associated with weaning, including oxidative stress and immune suppression, investigating *B. pinnatum* as a dietary supplement may provide insights into its role in mitigating these challenges while improving blood parameters, oxidative status, and male reproductive hormones. Despite the extensive ethnomedicinal use of *B. pinnatum*, its application in livestock, particularly in weaned rabbits, remains underexplored, as well as its safety and efficacy have not been extensively studied in veterinary clinical trials. Therefore, this study aimed to evaluate the effects of dietary *B. pinnatum* leaf meal on hematological and serum biochemical parameters, oxidative stress markers, and male reproductive hormones in weaned rabbits.

MATERIALS AND METHODS

Ethical approval

The study was approved by the Landmark University Research Ethics Committee, Landmark University, Omu Aran, Nigeria. All procedures were carried out in accordance with the principles of the Declaration of Helsinki and the Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Testing, and Education, as outlined by the Ad Hoc Animal Research Committee of the New York Academy of Science.

Study area

The study was conducted at the Teaching and Research Farm, as well as the Animal Science and Biochemistry Laboratories of Landmark University, Omu-Aran, Kwara State, Nigeria. According to the 1991 census, Omu-Aran had an estimated population of approximately 148,610 and covers a land area of 1,095 square kilometers. It shares borders with Ifelodun Local Government Area (LGA) to the north, Osun State to the south, and Ekiti and Offa LGAs. Geographically, Omu-Aran is positioned at approximately 8.9°N latitude and 5.061°E longitude.

Source of feed ingredients and forage

Bryophyllum pinnatum leaves were sourced from Landmark University Teaching and Research Farm, Omu-Aran, Kwara State, Nigeria. These were harvested, air dried (28°C) for 72 hours, milled to 300 micron particle sizes, and incorporated into compounded feed.

Animal management, experimental design, and diets

A total of 48 weaned male Hyla rabbits (mean age and weight of 6 weeks and 557g, respectively) were purchased from a reputable Rabbit Farm in Omu Aran, Nigeria, and used in this 10-week feeding trial. The rabbits were housed in well-ventilated cages under standard management practices, while clean drinking water was provided *ad libitum*. The study was conducted using a completely randomized design (CRD), with the rabbits allocated into four dietary treatment groups. Each group consisted of 12 rabbits, subdivided into 3 replicates of 4 rabbits each. The diets were formulated to be isonitrogenous (16.5% crude protein) and isocaloric (2700 kcal/kg metabolizable energy) in accordance with the requirements of the National Research Council (NRC, 1994), to ensure that any observed differences in performance and physiological responses were attributable to the graded inclusion levels of *B. pinnatum* rather than variations in nutrient composition. Group 1 served as the control with no *B. pinnatum* leaf meal (BPLM), while Groups 2 to 4 received BPLM at 1.0%, 1.5%, and 2.0%, respectively (Oladipupo, 2024), as shown in the composition in Table 1.

Table 1. Feed composition of the experimental diets fed to 6-week-old grower Hyla rabbits

Feed ingredient (%)	Inclusion levels of *Bryophyllum pinnatum* (%)			
	0.00	**1.00**	**1.50**	**2.00**
Corn	39.39	38.90	38.72	38.70
Wheat bran	26.29	25.94	25.84	25.60
Soya bean cake	9.89	9.87	9.81	9.75
Groundnut cake	6.59	6.58	6.66	6.52
Palm kernel cake	13.14	12.97	12.77	12.73
Bryophyllum pinnatum leaf meal	0.00	1.00	1.50	2.00
Bone meal	4.00	4.00	4.00	4.00
Premix	0.25	0.25	0.25	0.25
Methionine	0.15	0.15	0.15	0.15
Lysine	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25
TOTAL (%)	100.00	100.00	100.00	100.00
Crude protein (%)	16.54	16.51	16.56	16.43
Metabolizable energy (kcal/kg)	2724	2725	2727	2728

Composition of Rabbit Grower Premix (per kg of premix): Vitamin A: 1,200,000 IU, Vitamin D_3: 200,000 IU, Vitamin E: 2,500 mg, Vitamin K_3: 500 mg, Vitamin B_1 (Thiamine): 300 mg, Vitamin B_2 (Riboflavin): 600 mg, Vitamin B_6 (Pyridoxine): 400 mg, Vitamin B_{12}: 2 mg, Niacin: 4,000 mg, Pantothenic acid: 1,000 mg, Folic acid: 100 mg, Biotin: 10 mg, Choline chloride: 60,000 mg, Iron (Fe): 3,000 mg, Copper (Cu): 400 mg, Zinc (Zn): 3,000 mg, Manganese (Mn): 2,000 mg, Iodine (I): 50 mg, Cobalt (Co): 20 mg, and Selenium (Se): 10 mg

Blood sampling

At 7:00 AM on the final day of the feeding trial, two sets of 5 mL blood samples were collected from the marginal ear vein using a sterile 23-gauge needle and a 5 mL syringe. One animal per replicate (average weight: 1.50 kg) was sampled, resulting in a total of 12 blood samples per treatment group. Samples for hematological analysis were transferred into EDTA-coated tubes, while those intended for serum biochemical assays were collected into plain tubes and allowed to clot at room temperature. The clotted samples were centrifuged at 3000 rpm for 10 minutes to separate the serum, which was then stored at –20°C until analysis.

Hematological analysis

The hematological parameters were analyzed using an automated hematology analyzer (DxH 900 Beckman Coulter, Brea, California, USA). Parameters included packed cell volume (PCV), hemoglobin concentration (Hb), red blood cell count (RBC), white blood cell count (WBC), and differential leukocyte counts (neutrophils, lymphocytes, monocytes, eosinophils, and basophils). The erythrocyte indices, i.e., mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated (Fan et al., 2018), using the following formula;

MCV (fl) = ([RBC (×106 / μL) / PCV]) × 10

MCH (pg) = (RBC [×106 / μL] / Hb [g/dL]) × 10

MCHC (g/dl) = (PCV / Hb [g/dL]) × 100

Serum biochemical analysis

Blood samples were collected at 7:00 a.m. and transferred into labeled plain tubes. These samples were used to assess serum concentrations of total protein, total cholesterol, triglycerides, low-density lipoprotein (LDL), high-density lipoprotein (HDL), albumin, glucose, and liver enzymes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). All analyses were performed using the Randox Evidence MultiSTAT system (Model 2017; Randox Laboratories Ltd., Crumlin, County Antrim, Northern Ireland, UK)

Hormonal profile evaluation

The hormonal profile was evaluated using the ARCHITECT® hormone assay (Abbott Laboratories, USA). Follicle Stimulating Hormone (FSH) was measured in IU/L, Luteinizing Hormone (LH) was measured in IU/mL, estradiol in pg/mL, testosterone in ng/dL, and progesterone in ng/mL.

Antioxidants assay evaluation

The total antioxidant activity of blood plasma was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay, conducted in a 10 mM sodium phosphate buffer at pH 7.4. Absorbance readings of the DPPH· solution were taken at 516 nm using a Halo DB-20 Double Beam Spectrophotometer (Dynamica Scientific Ltd., UK).

Serum lipid peroxidation evaluation

The Thiobarbituric acid reactive substance, which is an indication of the oxidative stability of the meat, was analyzed by evaluating the level of malonaldehyde (MDA) in the blood samples using a commercial kit (Sigma-Aldrich, St. Louis, MO, USA) and expressed as µmol/L.

Statistical analysis

All the data from this investigation were reported as the average of three replicates. These were then subjected to one-way analysis of variance (ANOVA) using the SAS version 9.4 program (SAS, 2013). Differences at 5% ($p < 0.05$) were analyzed using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Hematological parameters

The results (Table 2) showed that, except for RBC and erythrocyte indices (MCV and MCH), most hematological parameters were not significantly influenced ($p > 0.05$) by dietary inclusion of BPLM. Key parameters such as PCV, Hb, and WBC remained within normal physiological ranges for rabbits, 30-50% for PCV, 10-15 g/dL for Hb, and 3-12 × 10^9/L for WBC based on reference values from the reports of Merck Veterinary Manual (2023) and Lichtenberger and Lennox (2011). The findings in the present study indicated that BPLM at the tested inclusion levels did not adversely affect these parameters or the oxygen-carrying capacity. However, RBCs were slightly below the reference range of 4-7 × 10^{12}/L, particularly at the 2% inclusion level. The slightly lower level of RBC at higher inclusion levels may indicate a mild suppressive effect on erythropoiesis at higher BPLM doses. In contrast, slight numerical ($p > 0.05$) increases in PCV and Hb were observed at 1.5% and 2% inclusion levels, suggesting a possible hematinic effect. These findings align with the study of Ufelle et al. (2011), who reported that phytochemicals in *B. pinnatum*, such as flavonoids and ascorbic acid, may stimulate erythropoiesis and haemoglobin synthesis. WBC counts remained within normal limits across all treatments, with the highest values recorded at 2% inclusion. This increase may reflect an immune-stimulatory response, consistent with the immunomodulatory potential of *B. pinnatum* reported in albino rats (Bassey et al., 2021). Comparable effects have been observed with other phytobiotics, such as garlic (*Allium sativum*) and ginger (*Zingiber officinale*), which have enhanced immune parameters in rabbits (Okoye et al., 2016). The 2% BPLM inclusion may be reaching a threshold where mild anti-nutritional effects begin to subtly suppress RBC production, while bioactive compounds continue to enhance immune parameters (e.g., via elevated WBCs). This suggests a dose-sensitive trade-off between hematopoiesis and immunostimulation.

Table 2. Haematological evaluation of 6-week-old grower Hyla rabbits fed different *Bryophyllum pinnatum* leaf meal

Parameter \ Inclusion levels of *B. pinnatum* across diets	0%	1%	1.5%	2%	SEM	p -value
PCV (%)	39.50	40.80	42.00	39.25	0.63	0.52
RBC ($\times10^{12}$/L)	3.50^{b}	3.75^{ab}	3.95^{a}	2.90^{c}	0.10	0.07
HB (g/L)	11.85	12.70	1290	11.90	0.81	0.29
WBC ($\times10^{9}$/L)	5.20	5.00	5.10	5.25	0.21	0.55
MCV (μm³)	11.29^{b}	10.88^{b}	10.63^{b}	13.53^{a}	0.32	0.05
MCH (g/dL)	33.86^{b}	33.87^{b}	32.66^{b}	41.03^{a}	0.18	0.09
MCHC (%)	30.00	31.13	30.71	30.32	1.00	0.17
LYMPH (%)	30.50	30.5	33.50	30.00	1.053	0.55
NEUT (%)	68.00	67.50	66.00	66.00	1.23	0.91
EOSPH (%)	1.50	2.00	0.50	0.00	0.31	0.23

^{a,b,c}: Means within a row with different superscript letters differ significantly ($p < 0.05$). PCV: Packed cell volume, WBC: White blood cell, RBC: Red blood cell, HB: Haemoglobin, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, RBC: Red blood cell, WBC: White blood cell, NEUT: Neutrophil, LYMPH: Lymphocyte, EOSPH: Eosinophil, SEM: Standard error of means.

Differential leukocyte counts further illuminate the immune and inflammatory status of the rabbits. Neutrophil, lymphocyte, and eosinophil values remained within established ranges, 20-75% for neutrophils, 25-80% for lymphocytes, and 0-5% for eosinophils (El-Sheikh et al., 2010). A mild increase in neutrophil percentage at 2% inclusion may indicate enhanced innate immunity, echoing findings in broiler chickens where *B. pinnatum* leaf extract elevated neutrophil counts and improved resistance to infections (Bassey et al., 2021). Lymphocyte levels peaked at 1.5% inclusion, suggesting a possible stimulatory effect on lymphopoiesis. Similar lymphocyte-enhancing effects have been reported with herbal additives such as *Moringa oleifera* and *Ginger* (Okoye et al., 2016; Desouky et al., 2019). Eosinophil counts remained stable across treatments, indicating no allergic or hypersensitivity reactions from BPLM. Overall, the stability and favorable modulation of these haematological indices suggest that *B. pinnatum* leaf possesses immunomodulatory properties without inducing systemic stress or inflammation.

Previous studies on *B. pinnatum* in monogastric animals have reported varied haematological outcomes, depending on species, dosage, and study conditions. For example, Datta et al. (2023) found that *B. pinnatum* extract improved antioxidant status without negatively affecting WBC counts. Similarly, Ekpo et al. (2021) reported improvements in PCV and RBC at moderate inclusion levels (≤ 1.5%) in rabbits, while higher levels (> 2%) led to mild anaemia and increased oxidative stress. These outcomes support the current observation that high BPLM inclusion might impair RBC production, possibly due to the interference of bioactive compounds with haematopoiesis.

The presence of phytochemicals such as flavonoids, alkaloids, and tannins (Kumar et al., 2019) in *B. pinnatum* leaves may account for the observed changes in RBC and WBC values. Moreover, the antioxidant properties of the leaves could protect erythrocytes from oxidative damage, contributing to improved PCV and Hb, especially at 1.5% inclusion.

An inclusion level of 1.5% BPLM in the present study appears optimal for maintaining healthy haematological profiles in rabbits, promoting erythropoiesis, and enhancing immune function without adverse effects. The 2% level may slightly suppress RBC production, though compensatory increases in MCV and MCH suggest physiological adaptation. This finding aligns with the report by Ekpo et al. (2021), who evaluated the effects of ethanolic and aqueous leaf extracts of *B. pinnatum* on haematological parameters in Wistar rats. Their study noted reductions in MCV, MCH, and MCHC, alongside changes in RBC and hemoglobin levels, indicating that *B. pinnatum* bioactives can modulate erythropoiesis and red blood cell indices in a dose-dependent manner. The compensatory increase in MCV and MCH in our study may thus be attributed to the phytochemical constituents of BPLM, such as flavonoids, polyphenols, and vitamin C, which are known to influence oxidative status and haematopoietic processes.

Serum biochemical parameters

The serum biochemical profile offers critical insights into the metabolic health and lipid metabolism of the animals. In the present study, the inclusion of BPLM positively influenced several biochemical parameters. As presented in Table 3, only serum cholesterol and high-density lipoprotein (HDL) levels were significantly affected ($p < 0.05$) by the dietary inclusion of BPLM. While most serum parameters, such as total protein, albumin, glucose, and liver enzymes, remained within normal physiological ranges, some notable trends were observed. Serum cholesterol levels decreased significantly with higher levels of BPLM inclusion, indicating a potential hypolipidemic effect ($p > 0.05$). This finding suggests that while moderate (up to 1.5%) inclusion supports normal metabolic physiology, higher inclusion levels may exert additional lipid-lowering effects, though potentially at the expense of erythropoiesis.

Table 3. Biochemical evaluation of 6-week-old grower male Hyla rabbits fed varying levels of *Bryophyllum pinnatum* leaf meal

Parameter / Inclusion levels of *B. pinnatum* across diets	0%	1%	1.5%	2%	SEM	p-value
TPN (g/dL)	5.70	5.75	5.88	5.65	1.21	0.90
ALB (g/dL)	2.45	2.66	2.75	2.28	1.15	0.43
GLU (mg/dL)	81.05	79.35	70.10	71.00	1.94	0.84
CHO (mmol/dL)	13.60[a]	13.00[a]	11.50[ab]	8.50[b]	0.05	0.07
TRI (mmol/L)	1.31	1.31	1.30	1.33	0.05	0.74
LDL (mmol/dL)	0.83	0.79	0.63	0.61	0.15	0.14
HDL (mmol/dL)	1.66[a]	1.57[a]	1.18[ab]	1.73[a]	0.96	0.11
ALP (μ/L)	72.90	73.50	73.05	73.20	3.95	0.95
AST (μ/L)	39.35	39.10	40.10	36.85	2.14	0.95
ALT (μ/L)	58.80	59.95	57.50	88.45	1.12	0.88

[a,b]: Means within a row with different superscript letters differ significantly ($p < 0.05$). TPN: Total protein, GLU: Glucose, ALB: Albumin, CHO: Cholesterol, TRI: Triglycerides, LDL: Low density lipoprotein, HDL: High density lipoprotein, ALP: Alkaline phosphatase, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase. SEM: Standard error of means.

Total protein, albumin, and globulin levels showed modest increases with increasing BPLM levels, peaking at 1.5%. These values remained within the normal ranges for rabbits (total protein: 5.4-7.5 g/dL; albumin: 2.5-4.0 g/dL) as reported by Cheeke (1987). Elevated protein levels may indicate enhanced liver synthetic capacity or improved nutrient assimilation, likely facilitated by the bioactive compounds in *B. pinnatum* (Andrade et al., 2020).

Serum glucose levels exhibited a consistent but slight decrease with increasing BPLM inclusion, although all values remained within the normal physiological range for rabbits (75-150 mg/dL). This pattern suggests a mild hypoglycemic effect that did not result in any clinical signs of low blood sugar, indicating that the animals maintained glucose homeostasis. The observed trend could be linked to the bioactive compounds present in BPLM, particularly flavonoids and phenolic compounds**.** These phytochemicals are known to possess insulin-sensitizing properties. Specifically, they may act by: Stimulating glucose transporter type 4 (GLUT-4) translocation in peripheral tissues (especially muscle and adipose tissues), enhancing insulin receptor sensitivity**,** reducing hepatic gluconeogenesis (production of glucose by the liver), and overall improving the efficiency of glucose uptake into cells. This proposed mechanism is supported by previous studies. For instance, Sok Yen et al. (2021) reported that plant-derived polyphenols (from *Camellia sinensis*). Enhance insulin signaling pathways, leading to improved glycemic control. Additionally, a similar hypoglycemic effect was documented by Okoduwa et al. (2017), where Albino rats supplemented with *Aloe vera* and *Ocimum gratissimum* extracts at dosages ranging from 150 to 2000 mg/kg showed a dose-dependent reduction in blood glucose levels. Therefore, the mild decline in serum glucose levels observed in this study with BPLM supplementation could be attributed to a synergistic action of its phytochemicals, mimicking insulin or improving insulin action. Importantly, since glucose values stayed within the safe range, this suggests a beneficial modulation of glucose metabolism rather than a pathological reduction, making BPLM a potential functional feed additive for glycemic regulation in monogastric animals.

Cholesterol and HDL levels were significantly reduced at higher inclusion levels of BPLM ($p < 0.05$). Notably, cholesterol concentrations decreased from 13.60 mmol/dL in the control group (0% BPLM) to 8.50 mmol/dL in the group fed 2% BPLM. This hypolipidemic effect is consistent with reports on phytogenic additives such as oregano and garlic, which lower cholesterol levels by inhibiting its biosynthesis or promoting its excretion (Kothari et al., 2019; Adjei-Mensah et al., 2024). The cholesterol-lowering activity observed in this study corroborates the findings of Singha and Lahkar (2020) and Bassey et al. (2021), who demonstrated similar effects in rats administered ethanolic extracts of *B. pinnatum* at doses ranging from 100-400 mg/kg and 180-540 mg/kg, respectively. These effects may be attributed to the presence of bioactive compounds such as saponins and flavonoids, as highlighted by Sok Yen et al. (2021).

Triglyceride levels also declined progressively with increasing BPLM inclusion, reinforcing its lipid-lowering potential. Bioactive compounds such as saponins, flavonoids, and sterols may inhibit lipid absorption or promote lipid metabolism, as observed in rats and poultry models (Azodo et al., 2021; Oladipupo, 2024). In the present study, low-density lipoprotein (LDL), often referred to as "bad cholesterol," also decreased insignificantly ($p > 0.05$) progressively along the row, with the lowest concentration observed at the 2% inclusion level. This cardioprotective effect mirrors findings by Cardinali et al. (2015), who reported similar LDL reductions with oregano and rosemary in rabbit diets.

Interestingly, HDL, often regarded as "good cholesterol", increased slightly across treatments, peaking at 1.5% inclusion. This may reflect enhanced reverse cholesterol transport, indicating a healthier lipid profile and overall metabolic improvement. These findings underscore the phytobiotic potential of *B. pinnatum*, aligning it with other established herbal additives such as neem (*Azadirachta indica*), garlic (*Allium sativum*), and *Moringa* (*Moringa oleifera*) in rabbit nutrition (Onu, 2010; Webb et al., 2022).

No significant changes ($p > 0.05$) were observed in liver function enzymes (ALP, ALT, and AST), indicating that BPLM up to 2% does not impair hepatic function. These results aligned with earlier findings on the hepatoprotective effects of plant-based additives (Datta et al., 2023), e.g., Flavonoids, polyphenols, and vitamin C in bryophyllum, curcumin in turmeric, catechins in green tea, limonoids, allicin, sulfur-containing compounds in garlic, gingerols, and shogaols in ginger, and azadirachtin in neem

In contrast to some synthetic antibiotics or less-tolerated phytogenics that can cause hepatocellular damage, *B. pinnatum* appears safe up to 2% inclusion level in growing rabbits. Similar hepato-stability has been observed with oregano and thyme supplementation in poultry (Kothari et al., 2019).

The findings from Tables 2 and 3 suggest that *B. pinnatum* leaf meal, when included up to 2% in rabbit diets, is safe and beneficial. It enhanced haematological and serum biochemical parameters without adverse effects. Its phytogenic properties, including antioxidant, immunomodulatory, hypoglycemic, and hypolipidemic effects, make it a potential natural alternative to synthetic growth promoters and antibiotics. This supports the broader move toward antibiotic-free animal production systems, where phytobiotics play an increasingly important role in maintaining health and performance. Furthermore, *B. pinnatum*, being a locally available and underutilized plant, offers a cost-effective and sustainable solution for livestock production. Compared to other leaf meals such as neem, *Moringa*, and bitter leaf (*Vernonia amygdalina*), which are often used at higher levels (3-5%) and sometimes associated with mild haematological stress (Tokofai et al., 2020), *B. pinnatum* at 1-2% appears equally effective and better tolerated.

Serum oxidative stress markers and antioxidant status

Table 4 presents the serum oxidative stress markers and antioxidant levels of rabbits fed diets containing varying levels (0%, 1.0%, 1.5%, and 2.0%) of BPLM. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is widely used to evaluate the free radical scavenging ability of antioxidants in biological systems. In this study, dietary inclusion of BPLM significantly enhanced serum antioxidant activity in grower rabbits, with the most notable effect observed at the 1.0% inclusion level. DPPH scavenging activity increased from 24.77 µg/mL in the control group to 34.83 µg/mL, indicating improved radical neutralization. This enhancement is likely due to the presence of bioactive compounds such as flavonoids, phenolics, alkaloids, and vitamin C, which are known to donate electrons or hydrogen atoms to counteract free radicals (Yemitan et al., 2020).

Table 4. Lipid peroxidation and antioxidant (DPPH) concentrations of 6-week-old grower Hyla rabbits fed different levels of *Bryophyllum pinnatum* leaf meal inclusion

Parameters	T1 (0%)	T2 (1%)	T3 (1.5%)	T4 (2%)	SEM (±)	p value
DPPH (µg/mL)	24.77^{d}	34.83^{a}	29.50^{b}	26.49^{c}	1.44	0.04
MDA (nmol/L)	2.52^{a}	2.05^{b}	1.93^{c}	1.80^{d}	0.10	0.04

a,b,c,d: Means within a row with different superscript letters differ significantly ($p < 0.05$). SEM: Standard error of mean; DPPH: 2,2-diphenyl-1-picrylhydrazyl; MDA: Malonaldehyde.

The antioxidant potential of *B. pinnatum* has been well-documented, with evidence of its ability to inhibit lipid peroxidation and scavenge reactive oxygen species *in vitro* (Daniel et al., 2020; Bassey et al., 2021). However, the decline in DPPH activity at higher inclusion levels, 29.50 µg/mL at 1.5% and 26.49 µg/mL at 2.0% suggests a dose-dependent response. This may reflect a threshold beyond which efficacy diminishes, possibly due to phytochemical overload or the emergence of pro-oxidant effects, especially in the presence of redox-active metals (Jomova et al., 2025). Moreover, high concentrations of tannins and saponins in leaves like *B. pinnatum* can reduce antioxidant effectiveness by forming complexes with proteins or interfering with the absorption and action of beneficial compounds (Kashyap et al., 2022). Similar biphasic patterns have been observed with other phytogenic additives, such as ginger and turmeric, where antioxidant activity improves at moderate levels but plateaus or declines with excessive supplementation (Jimoh et al., 2024).

Flavonoids and polyphenols, which are abundant in *B. pinnatum*, are known for their strong antioxidant properties (Pandey et al., 2017; Luo et al., 2022), but their benefits are maximized within an optimal range. At higher inclusion levels, saturation or disruption of endogenous antioxidant systems may occur (Ogunmoyole et al., 2025). Comparable trends have been reported with *Moringa oleifera*, *Azadirachta indica*, and *Allium sativum*, which enhance antioxidant status in monogastric animals under physiological stress (Mohlala et al., 2023; Wang et al., 2024).

Malondialdehyde (MDA), a key biomarker of lipid peroxidation and oxidative stress, showed a significant reduction ($p < 0.05$) with increasing levels of BPLM inclusion. Specifically, MDA concentrations decreased from 2.52 nmol/L in the control group (T1) to 1.80 nmol/L in the group receiving 2.0% BPLM (T4). This downward trend suggests a reduction in oxidative stress, likely due to the antioxidant properties of *B. pinnatum*, which contains compounds such as flavonoids, polyphenols, and vitamin C (Sok Yen et al., 2015). The significant reduction in MDA levels at higher inclusion rates supports the hypothesis that BPLM enhances antioxidant defense and mitigates lipid peroxidation ($p < 0.05$). Similar effects have been observed in animals fed phytogenic additives such as *Oregano*, which stimulate endogenous antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), while reducing oxidative markers such as MDA (Kothari et al., 2019). These results underscore the role of BPLM in protecting cell membranes and maintaining cellular integrity, which is vital for animal health and productivity. The observed decline in MDA may reflect not only the direct free radical scavenging action of BPLM's bioactive compounds but also the suppression of lipid peroxidation chain reactions (Daniel et al., 2020). This improvement in oxidative stability suggests that dietary BPLM could be a viable strategy for reducing oxidative damage in rabbits (Monageng et al., 2023).

The results presented in Table 4 indicate that BPLM has beneficial effects on oxidative stress parameters in grower rabbits. Moderate inclusion levels (1.0%-1.5%) significantly improved antioxidant status, as evidenced by increased DPPH activity and reduced MDA levels. While 2.0% inclusion continued to suppress oxidative damage (as shown by lower MDA levels), the antioxidant activity appeared to decline slightly, suggesting a possible upper threshold for optimal efficacy. These findings reinforce the potential of BPLM as a natural dietary antioxidant, capable of enhancing oxidative resilience and improving animal health. The inclusion of BPLM at appropriate levels offered a sustainable, phytogenic alternative to synthetic antioxidants, particularly in antibiotic-free rabbit production systems. However, further studies may be warranted to investigate long-term effects and to optimize dosage for maximum antioxidant benefit.

Reproductive hormones

Table 5 presents the potential endocrine-modulating effects of BPLM on male rabbits. The evaluated hormones, FSH, LH, estradiol, prolactin, testosterone, and progesterone, play essential roles in regulating male reproductive function and overall reproductive health. Significant increases ($p < 0.05$) in FSH, LH, estradiol, and prolactin at moderate BPLM inclusion (1.0-1.50%) levels suggest a stimulatory effect on reproductive endocrine activity. Conversely, the lack of significant changes in testosterone levels ($p > 0.05$) indicates that BPLM's effects may be mediated more through the modulation of pituitary gonadotropins than through direct stimulation of testosterone synthesis.

Table 5. Reproductive hormones of 6-week-old grower male Hyla rabbits fed diets containing varied levels of *Bryophyllum pinnatum* leaf meal

Parameters	T1 (0%)	T2 (1%)	T3 (1.5%)	T4 (2%)	SEM (±)	p value
FSH (mIU/mL)	1.79^{c}	2.22^{b}	3.33^{b}	2.22^{b}	1.11	0.050
LH (mIU/mL)	3.67^{b}	4.46^{a}	3.77^{b}	3.38^{c}	0.42	0.047
Estradiol (pg/mL)	19.56^{b}	38.50^{a}	17.43^{c}	16.32^{d}	5.04	0.010
Prolactin (ng/mL)	14.86^{d}	19.79^{a}	18.72^{b}	16.25^{c}	6.90	0.044
Testosterone (ng/dL)	7.27	7.18	6.94	6.34	1.10	0.055
Progesterone (ng/dL)	0.10^{c}	0.65^{a}	0.33^{b}	0.10^{c}	2.76	0.002

^{a,b,c,d} Values on the same row with different superscript letters are significant ($p < 0.05$). SEM: Standard error of mean; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone.

Follicle stimulating hormone is essential for spermatogenesis, promoting sperm cell development and maturation in the testes (Santi et al., 2020). In the present study, FSH levels increased significantly ($p < 0.05$) with BPLM supplementation, peaking at 3.33 mIU/mL in the T3 group (1.50% inclusion), while the control group (T1) had the lowest level (1.79 mIU/mL). The increasing concentration of FSH from the control to group 3 may suggest that BPLM may enhance spermatogenic activity, potentially improving sperm quantity and quality. The present findings align with previous reports indicating that plant-derived bioactive compounds, such as flavonoids, can stimulate gonadotropin secretion, thereby creating a hormonal environment conducive to male fertility (Andrade et al., 2020).

Luteinizing hormone (LH) stimulates testosterone production by Leydig cells in the testes (Esteves and Humaidan, 2025). Significantly higher LH levels ($p < 0.05$) were observed in T2 (1.00%) and T3 (1.50%) groups compared to the control, with T2 showing the highest concentration (4.46 mIU/mL). While testosterone levels did not differ significantly, the increased LH suggests a potential stimulatory effect of BPLM on the hypothalamic-pituitary-gonadal axis ($p > 0.05$). This supports earlier findings that certain phytogenic compounds could influence LH secretion and enhance reproductive function (Swelum et al., 2021; Shai et al., 2022).

Estradiol, although primarily associated with female reproduction, also contributes to male reproductive health by modulating spermatogenesis, sexual behavior, and sperm motility (Ramya et al., 2023). Estradiol levels were significantly elevated ($p < 0.05$) in T2 (1.00% BPLM), reaching 38.50 pg/mL, the highest among all groups. This may indicate an enhanced endocrine response to moderate BPLM inclusion. Given its role in sperm maturation, the increase in estradiol could support improved fertility, though the exact mechanisms warrant further investigation (Schulster et al., 2016). Prolactin is involved in testosterone regulation, spermatogenesis, and sperm motility (Martínez-Fresneda et al., 2020). A significant increase in prolactin concentration was observed in the T2 group (1.00% BPLM), which recorded the highest level (19.79 ng/mL) compared to the control, T3, and T4 groups ($p < 0.05$). This finding suggests that BPLM may positively influence endocrine function at moderate inclusion levels (1.0-1.5%). Such modulation has been linked to improved sperm quality and fertilization potential, reinforcing the reproductive benefits of BPLM (Zurfluh et al., 2025).

Testosterone plays a crucial role in regulating libido, spermatogenesis, and the development of male secondary sexual characteristics. However, no significant differences in testosterone levels were observed among the treatment groups ($p > 0.05$). This finding indicated that while BPLM may not directly enhance testosterone production, it could influence upstream hormonal pathways such as FSH and LH that regulate its synthesis. Previous studies suggested that increases in pituitary gonadotropins may precede changes in testosterone levels, which may not be immediately detectable due to hormonal feedback mechanisms (Adeniyi et al., 2025).

Progesterone, although typically associated with female physiology, progesterone in males supports sperm motility and maturation (Kolatorova et al., 2022). A significant increase was observed in the T2 group (0.65 ng/dL) compared to the control, T3, and T4 groups, suggesting that BPLM may beneficially modulate male reproductive function at lower to moderate inclusion levels (1.0-1.5%, $p < 0.05$). Elevated progesterone has been linked to improved sperm function and viability (Nagy et al., 2021).

Overall, the significant increases in FSH, LH, prolactin, and estradiol, alongside the elevation in progesterone, suggest that BPLM may enhance spermatogenesis, sperm quality, and motility. While testosterone levels remained unchanged, the modulation of pituitary hormones implies that BPLM influences key pathways that support male reproductive performance. These findings highlight the potential of BPLM as a natural phytogenic feed additive for improving fertility in male rabbits.

CONCLUSION

The dietary inclusion of BPLM up to 2% in grower male rabbit diets proved safe, with no negative impact on hematological or biochemical parameters. Instead, it enhanced antioxidant status, shown by increased DPPH activity and reduced MDA levels, and improved reproductive hormone profiles, particularly at 1.5% and 2% inclusion, as indicated by elevated LH levels. These findings suggest that BPLM is a functional feed additive with health-promoting potential in rabbit production. Future studies should explore higher inclusion levels and long-term effects, including impacts on female reproduction. Further investigation into its mechanisms of action, tissue safety, gut health, immune response, and cost-effectiveness is also recommended.

DECLARATIONS

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Authors' contributions

Razaq Animashahun and Olayinka Alabi contributed to the conceptualization of the study, developed the protocols and experimental design, and were responsible for editing and reviewing the manuscript. Oluwabori Adefioye and Ooreoluwa Adegboye carried out the experimental study and prepared the initial draft of the manuscript. Adedeji Animashahun performed the statistical analyses. All authors have read and approved the final version of the manuscript.

Availability of data and materials

The authors confirm that all data supporting the findings of this study are included within the manuscript.

Ethical considerations

This manuscript represents the authors' original study and has not been previously published. All authors have assessed the text for similarity and affirm that it is based on their original scientific research.

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The authors declare no conflict of interest.

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