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Research Article

Influence Of Fermented *Hunteria Umbellata* Seed Oil on The Hematology, Serum Biochemistry, Immune Response, And Oxidative Status of Growing Rabbits

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Abstract

The search for natural alternatives to synthetic antibiotic growth promoters has led to increased interest in phytogenic feed additives. This study evaluated the effect of fermented *Hunteria umbellata* seed oil (FHUSO) on the hematology, serum biochemistry, immune response, and oxidative status of growing rabbits. A total of 40 growing rabbits were randomly assigned to four dietary treatments (T1–T4). T1 served as the control (basal diet only), while T2, T3, and T4 were fed basal diet supplemented with FHUSO at 10, 20, and 30 mL/kg of diet, respectively. The experiment lasted 60 days. Following the trial, blood samples were collected to analyze hematological parameters, serum proteins, immunoglobulins (IgA, IgG, IgM), and oxidative stress markers (SOD, CAT, GPx, and MDA). Evaluation of the FHUSO revealed 10 major bioactive compounds, including linalool and pinene. Supplementation with FHUSO significantly ($P<0.05$) enhanced hematological indices (PCV, Hb, and RBC) and serum protein profiles in T2–T4 except for creatinine, urea, alanine aminotransferase and aspartate amino transferase ($P>0.05$). Immunological analysis showed increase in IgA, IgG, and IgM levels among rabbits in T2 – T4, suggesting improved mucosal and systemic immunity. Furthermore, FHUSO supplementation significantly boosted the antioxidant defense system, with higher activities of Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPx) in the treated groups. Conversely, Malondialdehyde (MDA) levels were significantly higher in the control (T1) than in the FHUSO-supplemented groups, indicating reduced lipid peroxidation in the latter. In conclusion, the results demonstrate that fermented *Hunteria umbellata* seed oil, rich in bioactive terpenes like linalool and pinene, acts as a potent immunomodulator and antioxidant in growing rabbits. The oil successfully improved the blood profile and mitigated oxidative stress, with the 30 mL/kg inclusion level (T4) showing the most pronounced benefits. FHUSO is therefore recommended as a viable natural nutraceutical for enhancing rabbit health and productivity.

Keywords: *Hunteria umbellata*, Phytogenics, Rabbits, Antioxidants, Immunoglobulins, Linalool, Pinene.

Introduction

The global livestock industry is increasingly turning toward the domestic rabbit (*Oryctolagus cuniculus*) as a sustainable solution to animal protein shortages [1]. Rabbits are uniquely suited for intensive production due to their short generation interval, high prolificacy, and ability to convert non-competitive cellulosic biomass into lean, high-quality meat [2]. However, the transition from traditional backyard rearing to intensive systems has highlighted a critical vulnerability in the "growing phase"—the period between weaning and maturity [3]. During this window, rabbits undergo rapid

metabolic expansion and are highly susceptible to oxidative stress, environmental pathogens, and gastrointestinal disturbances. These physiological stressors often lead to significant mortality rates, which currently represent the primary bottleneck in the economic viability of the rabbit sector [4]. Historically, these health challenges were managed through sub-therapeutic antibiotic growth promoters (AGPs). However, the global emergence of antimicrobial resistance (AMR) and subsequent bans on AGPs have forced researchers to explore natural alternatives. Phytochemicals—plant-derived bioactive compounds—have emerged as the most viable substitutes [5]. Previous studies on essential oils (EOs) in rabbit nutrition have provided a strong foundation for this shift [4]. *Hunteria umbellata*, popularly known as "Abeere" in West Africa, is a medicinal plant whose seeds are a dense reservoir of indole alkaloids, saponins, and flavonoids [6]. While its aqueous extracts have a long history of use in treating inflammation and metabolic disorders, the seed oil remains largely untapped in animal science [7]. The oil contains essential fatty acids and fat-soluble phytochemicals that possess potent antimicrobial and antioxidant properties [8]. However, the utilization of raw seed products is often hindered by anti-nutritional factors (ANFs) such as phytates and tannins [8]. These compounds can form insoluble complexes with essential minerals and proteins, which may inadvertently suppress growth and negatively alter the blood chemistry of the animal [9].

Previous research has shown that essential oils, such as those derived from eucalyptus and thyme, have demonstrated the ability to stabilize erythrocyte membranes, thereby maintaining optimal Packed Cell Volume (PCV) and hemoglobin levels during periods of heat or metabolic stress [10, 11]. Research on *Origanum vulgare* has shown that plant-derived oils can significantly upregulate endogenous antioxidant enzymes, such as Superoxide Dismutase (SOD), while simultaneously reducing Malondialdehyde (MDA), a hallmark of lipid peroxidation [12, 13]. However, there is a lack of empirical data regarding the safe inclusion levels of fermented *Hunteria umbellata* seed oil. High concentrations of raw alkaloids could potentially induce hepatic or renal toxicity, manifesting as abnormal fluctuations in the blood profile [14]. In its raw form, the seed oil may contain secondary metabolites that interfere with the rabbit's delicate redox balance or suppress immune-competence during the high-risk growing phase [15].

This study proposes the use of fermentation as a bioprocessing tool to unlock the full nutritional and therapeutic potential of *Hunteria umbellata* seed oil. Fermentation by beneficial microbes (e.g., *Lactobacillus* spp.) is a proven method for the enzymatic degradation of anti-nutritional factors and the bioconversion of complex phytochemicals into more bioavailable forms [15]. The research will help to establish safety benchmarks, maximize bioavailability and promote livestock sustainability.

Materials and Methods

Experimental location and ethical approval

This study was carried out in the Rabbitry Unit of Teaching and Research Farm, Gandhi College of Agriculture, Rajasthan India in the Month of March to June, 2024. The area has a mean annual rainfall of 1020mm; temperature range of 29.4 – 32.6°C and mean relative humidity range of 60.55 %. The research ethics and guidelines of the Animal Nutrition and Biochemistry Department of the institution approved the conduct of the experiment (Ref/GHA/2023/008)

Collection, processing and extraction of *Hunteria umbellata* seed

Fresh, mature and healthy seeds of *Hunteria umbellata* was sourced from an open market in Rajasthan and authenticated at the Botany Department, Gandhi College of Agriculture, Rajasthan (Ref. No. BF2003). After being washed and manually cracked, the seeds underwent a 7 day submerged fermentation in water (at a ratio of approximately 1:2 seeds to water); the fermented water was subsequently drained and the oil was extracted via hydrodistillation using Clavenger apparatus. The chemical profile of the resulting oil was determined using Agilent Technologies 7890A GC system (USA) coupled with a 5997B mass selective detector (MSD). The system utilized an HP-5ms Ultra inert capillary column (30 m × 250 µm × 0.25 µm) and Agilent Chem Station software for data acquisition. Technical specifications included a temperature programmable oven ranging from ambient to 450 °C, a split/splitless capillary injector operating with high purity helium carrier gas and an electron ionization source with a mass range of 1.6 – 1050 µ to facilitate accurate identification via the NIST Mass Spectral Library.

Experimental animals and their management

A total of 40 growing rabbits with an average weight of 741± 1.85 g (Chinchilla × New Zealand White) were procured from a reputable source in Rajasthan. Upon arrival, rabbits underwent a two week quarantine period to monitor their health status. During this phase, they received prophylactic treatment and were maintained on basal diet formulated to meet the nutritional requirement of animal according to NRC standards [16] presented in Table 2. Following the quarantine period, the rabbits were stratified by weight and randomly assigned to the four dietary groups (5 bucks and 5 does /group) in a feeding trial that lasted twelve (12) weeks. Animals were housed individually in a wired cage equipped with drinkers and feeders which was properly washed and disinfected two weeks before the commencement of the experiment. The experimental treatment includes, treatment 1 (T1) basal diet only which also served as the control, treatment 2, 3 and 4 received basal diet supplemented with fermented *Hunteria umbellata* seed oil at 50, 100 and 150 mL /kg diet respectively. Animals were fed thrice between 7:00 H, 12:00 H and

16:30 H, water was made available at all times and a completely randomized experimental design was adopted. Feed intake was recorded daily as the difference between the feed served and the left over. Weight was taken weekly per replicate using a digital weighing scale.

Parameters measured

Haematological and serum biochemical assessment

At the end of the study, six rabbits (3 bucks /3 does) in each treatment were chosen randomly for blood collection. Two sets of blood samples were collected from each animal via the marginal ear vein using hypodermic syringes into sample bottles. One set of the blood samples (5 mL) was collected into plastic tubes containing ethylene diamine tetra acetic acid for the determination of hematological parameters. The other set of blood samples (5 mL) was collected into EDTA-free sample bottles. Blood samples for haematology was analyzed using: IDEXX ProCyte Dx® Auto Haemo-Analyzer (Model: ProCyte Dx, USA) which operates via: Laser flow cytometry, Optical fluorescence, and Laminar Flow Impedance technique to ensure precision in results. Serum biochemical indices were examined using Rayto RT-9200 Serum Biochemistry Analyzers (China) operated under optical systems of: 340, 405, 505, 546, 578, 620, 670 nm and subjected at a temperature (25°C, 30°C, 37°C) respectively according to the manufacturers instruction.

Immunological analysis

At the end of the study, six rabbits (3 bucks /3 does) in each treatment, blood used for immunological analysis was from those collected for haematological studies. Immunoglobulins were analyzed using Elecsys® Immunoglobulin Assays (Model/Platform: cobas e 402 / e 801 analytical units, Germany) which operates via Electrocluraiminescence immunoassay. Sample volume was adjusted to 10–20 µL per test to ensure precision in results.

Oxidative stress analysis

At the end of the study, six rabbits (3 bucks /3 does) in each treatment, blood used for oxidative stress analysis was from those collected for haematological studies. Samples were analyzed with Randox RX Series (Automated Analyzers) RX Series (RX monaco, RX daytona+, United Kingdom) adjusted to 12 wavelengths ranging from 340 nm to 800 nm and 170 to 270 photometric tests per hour.

Statistical analysis

All data collected were be subjected to a One-way Analysis of Variance. Significant differences between treatment will be separated using Duncan Multiple Range Test at a 5 % probability level ($p < 0.05$).

Results and Discussion

Table 1 reveals the chemical composition of fermented Hun-

teria umbellata seed oil. Limonene had the highest concentration of 753.5 mg/g followed by α -pinene (206.2 mg/g), terpinene-4-ol (97.03 mg/g), β -caryophyllene (86.22 mg/g), α -terpineol (45.11 mg/g), 1,8-cineole (20.96 mg/g), β -citronella (11.42 mg/g), cis-linalool oxide (7.96 mg/g), α -cadinol (5.04 mg/g), p-cymene (4.90 mg/g), humulane-1,6-dien-3-ol (3.09 mg/g), trans-linalool oxide (2.54 mg/g), α -terpinolene (2.33 mg/g) and cubenol (1.77 mg/g) respectively. These compounds have medicinal and therapeutic properties which have been linked to anti-inflammatory and tissue protective effects [17, 18]. Higher concentrations of Limonene and α -pinene indicates that fermented *Hunteria umbellata* seed oil possess antimicrobial and antioxidant properties [18], it can also stabilize cells, support cardiovascular function and reduce oxidative damage [19, 20]. A synergy between these compounds can support digestion and hinders the proliferation of pathogens in the gut of animals [21].

Effect of varying levels of fermented *Hunteria umbellata* seed oil on haematological parameters of growing rabbits is presented in Table 3. All the haematological parameters were within the normal physiological ranges for healthy rabbits [22]. Pack cell volume value was lower ($p < 0.05$) in T1 (29.45 %) than T2 (33.46 %), T3 (33.71 %) and T4 (33.93 %) while haemoglobin value ranged from 10.44 – 13.98 g/dL, red blood cell [(5.72 – 7.18 ($10^6/\mu\text{L}$))], white blood cell [(9.77 – 12.67 ($10^3/\mu\text{L}$))], lymphocytes (50.12 – 68.76 %), neutrophils (27.09 – 35.74 %) and monocytes (5.85 – 7.28 %). Haemoglobin, red blood cell and white blood cells were higher ($p < 0.05$) in T2 – T4 than in T1. The significant rise in red blood cell counts, hemoglobin concentration, and packed cell volume in T2, T3, and T4 compared to the control indicates that *Hunteria umbellata* seed oil (FHUSO) stimulates erythropoiesis [22]. Fermentation reduces anti-nutritional factors that typically bind minerals. This increases the availability of iron and copper, essential for hemoglobin synthesis [15]. Compounds like pinene and linalool are known for their antioxidant properties [18]. They protect the erythrocyte membrane from lipid peroxidation, extending the lifespan of circulating red blood cells and maintaining their structural integrity [20]. Linalool acts as an anti-inflammatory and antioxidant agent, reducing systemic stress in the rabbits and allowing more energy to be diverted toward growth and hematological maintenance [23]. An increase in white blood cell counts and differentials suggests an enhanced "immunomodulatory" effect. Bioactive compounds in FHUSO can stimulate the production of antibodies and the activity of macrophages, providing the growing rabbits with a more robust defense mechanism against subclinical infections [24]. Haemoglobin, red blood cell and white blood cell count recorded in this experiment was within the normal range 9.00 – 16.00 g/dL, 5.00 – 12.00 ($10^6/\mu\text{L}$) and 8.00 – 22.00 ($10^3/\mu\text{L}$) cited by [24, 25, 26]. Pack cell volume was within 25.00 – 36.00 % reported by [27] when green coffee powder was supplemented in the diet of rabbits. Effect of varying levels of fermented *Hunteria umbellata* seed

oil on serum biochemical parameters of growing rabbits is presented in Table 4. All the serum biochemical indices recorded in this study were within the normal physiological ranges for healthy rabbits [27]. Total protein value was higher ($p<0.05$) in T2 (6.68 g/dL), T3 (6.72 g/dL) and T4 (6.76 g/dL) than T1 (5.83 g/dL). Albumin value varied from 2.89 – 3.31 g/dL, globulin (2.85 – 3.45 g/dL), glucose (135.6 – 143.5 mg/dl) and cholesterol (61.08 – 79.67 mg/dl) were significantly ($p<0.05$) influenced except creatinine (1.04 – 1.09 mg/dl), urea (37.23 – 38.13 mg/dl), alanine aminotransferase (67.96 – 68.19 U/L) and aspartate amino transferase (27.01 – 27.71 U/L) ($p>0.05$). The increase in serum protein and albumin suggests that FHUSO improves the efficiency of amino acid absorption and protein synthesis in the liver. This is crucial for "growing" rabbits, as albumin serves as the primary transport protein for hormones and nutrients [28]. Higher globulin levels suggests a higher concentration of immunoglobulins. This correlates with the increased white blood cell counts, suggesting that the oil acts as a natural growth promoter and immune enhancer. Linalool has been studied for its ability to modulate metabolic pathways. In this context, it may improve the secretion of digestive enzymes or alter the gut microbiota favorably, leading to better nutrient uptake from the basal diet [29]. Glucose level recorded in this study was within 120 – 200 mg/dl cited by [30] when green tea extract was supplemented in the diet of growing rabbits. Creatinine and urea values suggests that feeding graded fermented *Hunteria umbellata* seed oil did not interfere with the activities of kidney [31]. Alanine aminotransferase and aspartate amino transferase values were within 41.00 – 85.00 U/L and 18.00 – 35.00 U/L recorded by [31]. This result goes further to show the absence of hepatic dysfunction in the body of rabbits [27].

Effect of varying levels of fermented *Hunteria umbellata* seed oil on immune response of growing rabbits is presented in Table 5. Immunoglobulin A, G and M values varied from 0.56 – 1.21 mg/dL, 0.82 – 1.38 mg/dL and 0.71 – 0.97 mg/dL were influenced ($p<0.05$) by the treatments. Values obtained in this study follow similar trend, as rabbits which received fermented *Hunteria umbellata* seed oil (T2 – T4) had higher ($p<0.05$) concentrations compared to T1. The elevation of

Immunoglobulin A (IgA), IgG, and IgM in the groups supplemented with fermented *Hunteria umbellata* seed oil (T2–T4) indicates a robust activation of both the mucosal and systemic immune systems. Because *Hunteria umbellata* contains a complex profile of bioactive compounds enhanced by the fermentation process the increase is likely due to the following physiological and biochemical drivers [31]. IgA is the primary antibody involved in mucosal immunity, particularly in the gastrointestinal tract. The fermented oil likely improves the integrity of the intestinal barrier. Pinene and other terpenes possess antimicrobial properties that "cleanse" the gut of pathogenic bacteria while favoring beneficial flora [32]. Results obtained in this study indicates that fermented *Hunteria umbellata* seed oil is essentially "priming" the rabbits' immune systems, making them more resilient to environmental stressors and pathogens compared to the control group (T1), which relies solely on a basal diet without these supplemental bioactives [33].

Table 6 reveals the effect of varying levels of fermented *Hunteria umbellata* seed oil on oxidative stress indices of growing rabbits. Malondialdehyde, superoxide dismutase, catalase and glutathione peroxidase were influenced ($p<0.05$) by the treatments. Values obtained ranged from 1.97 – 3.96 (nmol/mL), 32.55 – 43.21 (U/mL), 10.22 – 15.67 (U/mL) and 25.63 – 34.32 ($\mu\text{mol/L}$) respectively. The higher levels of glutathione peroxidase, catalase, and superoxide dismutase in the supplemented groups (T2–T4) alongside lower malondialdehyde levels indicate that the fermented *Hunteria umbellata* seed oil effectively shifts the rabbits' physiological state from oxidative stress to antioxidant homeostasis [33]. The rise in glutathione peroxidase and catalase levels suggests the rabbits are better equipped to handle the H_2O_2 produced by superoxide dismutase [34]. These enzymes neutralize H_2O_2 into water and oxygen, preventing the formation of the highly destructive hydroxyl radical ($\cdot\text{OH}$). Malondialdehyde is a primary biomarker of lipid peroxidation. Rabbit cell membranes are rich in polyunsaturated fatty acids (PUFAs), which are highly susceptible to "oxidative rancidity." The antioxidants in the seed oil (especially the limonene and linalool) incorporate into the lipid bilayer of the cells [34, 35].

Bioactive compounds	Reaction time (min)	Concentration (mg/g)
Limonene	13.24	753.5
β -Caryophyllene	13.80	86.22
α -Terpineol	15.67	45.11
α -Pinene	16.80	206.2
p-Cymene	16.97	4.90
Terpinene-4-ol	24.11	97.03
1,8-Cineole	25.06	20.96
α -Terpinolene	25.87	2.33
α -Cadinol	30.53	5.04

Cubenol	30.81	1.77
β-Citronella	32.27	11.42
Cis-linalool oxide	33.08	7.96
Trans-linalool oxide	33.54	2.54
Humulane-1,6-dien-3-ol	33.86	3.09

Table 1: Chemical composition of fermented *Hunteria umbellata* seed oil

Ingredients	g/kg DM
Maize	405
Wheat bran	100
Palm kernel meal	200
Soybean meal	200
Bone meal	20
Methionine	10
Lysine	10
Growers Mineral-Vitamin Premix	25
Salt	30
Total	1000
Chemical composition	
Dry matter	879
Crude protein	168
Crude fibre	131
Ether extract	21
Ash	80
Organic matter	920
Energy (Kcal/kg)	2500.2

Each 2.5 kg contain: 10,000 IU Vit. A; 6000 IU Vit. D3; 4000 mg Vit. E; 2000 mg Vit. K3; 2000 mg Vit. B1; 4000 mg Vit. B2; 2000 mg Vit. B6; 10 mg Vit. B12; 50 mg Biotin; 100 mg Pantothenic acid; 500 Niacin; 30 mg Folic acid; 250 mg Choline; 850 mg Mn; 500 mg Zn; 500 mg Fe; 200 mg I; 100 mg Se, 50 mg

Table 2: Ingredient and chemical composition of the basal diet (g/kg of DM)

Constituents	T1 (0 %)	T2 (10 mL)	T3 (20 mL)	T4 (30 mL)	SEM
Packed cell volume (%)	29.45b	33.46a	33.71a	33.93a	0.92
Haemoglobin (g/dL)	10.44b	13.52a	13.67a	13.98a	0.04
Red blood cells (106/ µl)	5.72b	7.07a	7.16a	7.18a	0.01
White blood cells (103/µl)	9.77b	12.34a	12.53a	12.67a	0.03
Lymphocyte (%)	50.12b	68.55a	68.61a	68.76a	2.17
Neutrophil (%)	35.74b	27.18a	27.12a	27.09a	0.07
Neutrophil/Lymphocyte ratio (%)	0.71a	0.40b	0.40b	0.39b	0.01
Monocytes (%)	5.85b	7.17a	7.23a	7.28a	0.02

^{a,b} Means in the same row with different superscript are significantly (P< 0.05) different

Table 3: Effect of varying levels of fermented *Hunteria umbellata* seed oil on haematological parameters of growing rabbits

Parameters	T1 (0 %)	T2 (10 mL)	T3 (20 mL)	T4 (30 mL)	SEM
Total protein (g/dL)	5.83b	6.68a	6.72a	6.76a	0.02
Albumin (g/dL)	2.98b	3.27a	3.29a	3.31a	0.01
Globulin (g/dL)	2.85b	3.41a	3.43a	3.45a	0.01
Glucose (mg/dl)	135.6b	140.8a	142.3a	143.5a	9.82
Cholesterol (mg/dl)	79.67a	62.11b	62.18b	61.08b	3.45
Creatinine (mg/dl)	1.09	1.04	1.06	1.08	0.01
Urea (mg/dl)	37.23	38.07	38.11	38.13	1.81
Alanine aminotransferase (U/L)	68.19	67.96	68.08	68.10	2.05
Aspartate amino transferase (U/L)	27.71	27.09	27.03	27.01	0.09

^{a,b} Means in the same row with different superscript are significantly (P< 0.05) different

Table 4: Effect of varying levels of fermented *Hunteria umbellata* seed oil on serum biochemical parameters of growing rabbits

Parameters (mg/dL)	T1 (0 %)	T2 (10 mL)	T3 (20 mL)	T4 (30 mL)	SEM
Immunoglobulin A	0.56b	1.12a	1.18a	1.21a	0.01
Immunoglobulin G	0.82b	1.31a	1.32a	1.38a	0.01
Immunoglobulin M	0.71b	0.92a	0.95a	0.97a	0.02

^{a,b} Means in the same row with different superscript are significantly (P< 0.05) different

Table 5: Effect of varying levels of fermented *Hunteria umbellata* seed oil on immune response of growing rabbits

Parameters	T1 (0 %)	T2 (10 mL)	T3 (20 mL)	T4 (30 mL)	SEM
Malondialdehyde (nmol/mL)	3.96a	2.13b	2.02b	1.97b	0.01
Superoxide dismutase (U/mL)	32.55b	41.18a	42.55a	43.21a	2.35
Catalase (U/mL)	10.22b	15.18a	15.23a	15.67a	0.94
Glutathione peroxidase (μmol/L)	25.63b	31.12a	33.08a	34.32a	2.80

^{a,b} Means in the same row with different superscript are significantly (P< 0.05) different

Table 6: Effect of varying levels of fermented *Hunteria umbellata* seed oil on oxidative stress indices of growing rabbits

Conclusion

The observed increase in hematological and serum parameters in rabbits supplemented with fermented *Hunteria umbellata* seed oil (FHUSO) suggests that the bioactive compounds within the oil exert potent erythropoietic, immune-boosting, and metabolic-enhancing effects. The presence of major bioactive compounds like linalool and pinene, combined with the fermentation process, likely enhances the bioavailability of nutrients and secondary metabolites. FHUSO has also demonstrated its ability to scavenge the activities of free radical. It was concluded that FHUSO can be supplemented in the diet of growing rabbits at up to 30 ml/kg without compromising the health status of animals.

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