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Research Article

Haemato-Biochemical Indices of Growing Rabbits Fed Diets containing Fermented Hunteria umbellata seed meal as a Replacement for Soyabean meal

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Abstract

The rising cost of conventional protein sources in livestock nutrition necessitates the exploration of no-conventional alternatives. This experiment was undertaken to examine the safety and physiological effect of fermented Hunteria umbellata seed meal (FHUSM) as partial replacement for Soyabean meal in rabbit diets using blood indices as primary indicators of health and metabolic status. Fifty growing male rabbits (New Zealand White × Chinchilla) were randomly assigned to five treatment groups (T1-T5). Treatment 1 served as the control while FHUSM replaced soyabean meal at 5, 10, 15 and 20 % in T2 through T5, respectively. All diet were formulated to be isonitrogenous and isocaloric. Feed and clean water was supplied at all times. After a 42 day feeding trial blood was collected for haematological and serum biochemical analysis. Haematological indices, including pack cell volume, haemoglobin and red blood cell counts, showed no significant differences ($p>0.05$) across all inclusion levels, remaining within the standard physiological reference range for rabbits. White blood cells remained stable suggesting no adverse immune reactions to the fermented seeds. Serum biochemical analysis revealed that total protein, albumin, globulin levels were lowest in T1 compared to the other treatments ($p<0.05$). Furthermore, creatinine, urea, alanine aminotransferase and aspartate amino transferase did not ($p>0.05$) did not deviate significantly from the control group suggesting absence of systemic toxicity or organ damage. Based solely on the blood profile, FHUSM is a safe alternative to soyabean meal. The fermentation rendered the seeds safe for consumption, supporting normal haemato-biochemical integrity in rabbits at inclusion levels up to 20 %.

Keywords: Hunteria umbellata, Rabbits, Nutrition, Haematology, Serum, Seeds.

Introduction

The rabbit (Oryctolagus cuniculus) holds significant potential in bridging the animal protein gap in developing countries due to its high fecundity, short generation interval, and ability to utilize fibrous non-conventional feed sources [1]. However, the sustainable production of rabbit is heavy hindered by the rising cost of conventional feed ingredients, particularly, soyabean meal, which is a staple protein source in monogastric nutrition [2, 3]. This economic pressure has led researchers to investigate indigenous, underutilized tropical seed as viable alternatives [3].

Hunteria umbellata popularly known as “Abeere” in West Africa, is a tropical plant whose seeds are traditionally recognized for their medicinal properties [4]. While rich in nutrients, these seeds contain various phytochemicals and anti-nutritional factors that can interfere with nutrient bioavailability and animal physiology [4]. Raw H. umbellata

seeds are known to contain high concentrations alkaloids that could potentially induce hepatic toxicity [5], renal stress or haematological disorder such as anaemia [6]. Processing techniques, such as fermentation, have been proposed as effective, low cost methods to degrade these anti-nutritional factors, thereby enhancing the nutritional value and safety of seeds [6, 7]. Evaluating the blood profile serves as a diagnostic tool to determine the systemic impact and safety of this non-conventional ingredients on the animal's internal environment [8].

Previous studies by [9, 10, 11] have shown that replacing Soyabean meal with Moringa seed at level up to 40 – 50 % maintains normal pack cell volume, haemoglobin and red blood cell counts. Replacing soyabean meal with Delonix regia seeds at levels above 10 – 15 % can lead to a decrease in haeamtological and serum biochemical parameters. Albizia lebbeck seeds are generally efficient at maintaining total protein at a 15 – 20 % replacement rate suggesting good amino acid availability. However, there is little or no information on the effect of replacing Soyabean meal with fermented Hunteria umbellata seeds on the blood profile of rabbits.

The study is justified by an urgent need to identify and validate sustainable, low cost protein alternatives that can reduce the total dependence on soyabean meal in rabbit production. By utilizing a 7 day fermentation process, this experiment explores a biological detoxification method that is easily replicable for farmers potentially transforming a local seed into a high value feed ingredient. The focus on haemato-biochemical indices provides a scientific guarantee for safety [12, 13]. Blood is a major indicator of the health status of an animal, establishing that fermented Hunteria umbellate seed does not negatively alter blood will provide economic relief and livestock sustainability [14].

Materials and Methods

Study Location

The research was conducted at the Non-Ruminant Section of Gandhi College of Agriculture, Rajasthan India. Prior to the commencement of the trial, experimental pens and environment underwent a rigorous sanitation process. Iron cages, drinking troughs and feeders were thoroughly disinfected 2 weeks before the arrival of animals to ensure pathogen free environment.

Experimental Animals and Management

A total of fifty growing male rabbits (New Zealand White × Chinchilla) with an average initial body weight of 759.1 ± 1.22 g was used for the study. Upon arrival, the animals underwent a two week quarantine period to monitor their health status. Prophylactic treatment consisting of intramuscular injection of antibiotics (oxytetracycline LA) at the rate of 0.5ml/5 kg BW, dewormed with levamisole plus® at the dosage of 0.5 ml/5 kg body weight was also administered. Feed (control diet) was given with clean water at all times. Following the quarantine period, rabbits were stratified by weight and randomly assigned to one of five experimental treatment. Each group consisted of 10 rabbits each serving as a replicate. Experimental diet was compounded to be isocaloric and isonitrogenous with fermented Hunteria umbellata seed meal (FHUM) replacing soyabean meal at: Treatment 1 (control): 0 % FHUSM; Treatment 2: 5 % FHUSM; Treatment 3: 10 % FHUSM; Treatment 4: 15 % FHUSM; Treatment 5: 20 % FHUSM.

The experiment lasted for 42 days excluding the two weeks quarantine period and a Completely Randomized Experimental Design was used. Biosecurity was strictly observed, routine and regular management practices was strictly maintained throughout the period of the experiment. Rabbits were weighed individually at the end of the experimental period on a digital scale. Feed consumed was recorded daily while the body weight was recorded on weekly basis.

Processing of fermented Hunteria umbellata seed

Fresh Hunteria umbellata seed was purchased from an open market in Rajasthan India. The plant seeds were identified and authenticated at Herbarium Unit of Botany Department, Gandhi College of Agriculture, Rajasthan, India. The seeds were screened to remove undesirable materials such as stones and other impurities, after which they were washed with running tap water and cracked manually to increase their surface area for microbial activity. Cracked seeds were placed in a clean plastic container and covered with water (ratio 1:3 w/v) under room temperature for 7 days to promote the growth of lactic acid bacteria. Fermented seeds were sundried for 11 days milled into powder and the powder kept in an air-tight polythene bags until needed for feed formulation and other laboratory analysis.

Blood sample collection

On the last day of the experiment, two sets of blood samples were collected from 5 randomly selected rabbits per treatment via the marginal ear vein using hypodermic syringes into labeled sample. One set of the blood samples (3 mL) was collected into plastic tubes containing ethylene diamine tetra acetic acid (EDTA) for the determination of hematological parameters. The other set of blood samples (5 mL) was collected into EDTA-free plastic tubes. Samples were kept in an ice pack and sent to the laboratory for further evaluation. Haematological parameters which includes, pack cell volume, haemoglobin, red blood cell, white blood cell and their differentials were analyzed using Sysmx XN-V Series (Model XN-1000 V/XN 2000 V, Japan) which measures white blood cell via fluorescence flow cytometry, haemoglobin (Cyanide free SLS method) and Hydrodynamic impedence for the other components. Serum biochemical indices were analyzed using Mindray BS-240 (model BS-240, China) adjusted to a sample volume of 2-5 µL according to the manufacturers

recommendation to ensure precision in results.

Chemical analysis

Analysis of experimental diet was carried out using the standard methods of the Association of Official Analytical Chemists [15]. Phytochemical analysis of fermented Hunteria umbellata seed meal was carried out according to the methods outlined by [16].

Statistical analysis

All data collected on haematological and serum biochemical indices will be subjected to a One-way Analysis of Variance. Significant differences between treatment will be separated using Duncan Multiple Range Test [17] at a 5 % probability level ($p<0.05$).

Result and Discussion

Proximate composition of fermented Hunteria umbellata seed is presented in Table 2. Dry matter was found at 88.89 %, crude protein (42.14 %), crude fibre (3.42 %), ether extract (5.66 %), ash (10.43 %) and 3200.8 Kcal/kg (energy). The result obtained suggests that fermented Hunteria umbellata seed contain higher concentration of protein making it a suitable alternative protein supplement for monogastric. The protein level was lower than 46.33 % recorded for Moringa oleifera seed by [18]. The low ether extract content in fermented Hunteria umbellata seed suggests that it can prevent the risk of coronary heart disease [19]. Fibers in the diet are necessary for digestion and for effective elimination of wastes, and can lower the serum cholesterol [20]. The ash content in fermented Hunteria umbellata seed is higher than those reported by [20], this suggests that the seeds could be good sources of minerals which are needed as a co-factor for enzymatic activities in the body [21]. In this study, fermented Hunteria umbellata seed revealed to be a good source of energy for livestock and is comparable to the result recorded by [21].

Ingredients	Treatment 1 (control)	Treatment 2 (5 % FHUSM)	Treatment 3 (10 % FHUSM)	Treatment 4 (15 % FHUSM)	Treatment 5 (20 % FHSUM)
Maize	40.00	40.00	40.00	40.00	40.00
Fermented Hunteria umbellata meal (FHUM)	0	2.00	4.00	6.00	8.00
Wheat bran	14.00	14.00	14.00	14.00	14.00
Palm kernel meal	20.00	20.00	20.00	20.00	20.00
Soybean meal	20.00	18.00	16.00	14.00	12.00
Bone meal	5.00	5.00	5.00	5.00	5.00
Methionine	0.20	0.20	0.20	0.20	0.20
Lysine	0.20	0.20	0.20	0.20	0.20
Growers Mineral-Vitamin Premix	0.25	0.25	0.25	0.25	0.25
Salt	0.35	0.35	0.35	0.35	0.35
Total	100.0	100.0	100.0	100.0	100.0
Determined analysis					
Crude protein	17.46	17.31	17.22	17.19	17.15
Crude fibre	13.28	11.23	11.18	11.17	11.16
Crude fat	1.92	2.04	2.06	2.08	2.10
Calcium	1.51	1.55	1.55	1.55	1.55
Phosphorus	0.87	0.88	0.88	0.88	0.88
Methionine	0.61	0.63	0.63	0.63	0.63
Lysine	0.85	0.88	0.88	0.88	0.88
Methionine+ Cysteine	0.89	0.92	0.92	0.92	0.92

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 1: Ingredient and chemical composition of experimental diet

Components	Fermented Hunteria umbellata seed
Dry matter	87.89
Crude protein	42.14
Crude fibre	3.42
Ether extract	5.66
Ash	10.43
Energy (Kcal/kg)	3200.8

Table 2: Proximate composition of fermented *Hunteria umbellata* seed

Constituents (%)	Fermented Hunteria umbellata seed
Phenols	14.78
Tannins	6.09
Alkaloids	3.17
Flavonoids	12.05
Saponins	2.64
Phytate	0.18

Table 2: Phytochemical components of fermented *Hunteria umbellata* seed

Phytochemical composition of fermented Hunteria umbellata seed suggests that it contained some medicinal components such as phenols (14.78 %), flavonoids (12.05 %), tannins (6.09 %), alkaloids (3.17 %), saponins (2.64 %) and phytate (0.18 %) in their order of abundance (Table 3). Higher concentration of phenolic compound and flavonoids suggests that Hunteria umbellata seed possess antioxidant, immune-modulatory and antimicrobial properties [22]. These compounds also have the ability to neutralize the activities of free radicals in the body [23]. The other phytochemical compounds have numerous therapeutic properties, such as, antifungal, anti-cancer, anti-tumor, antidiarrheal, anti-helminthic, hypolipidemic, antidiabetic, hepato-protective, gastro-protective amongst others [24, 25]. As previously noted, the phytochemicals in fermented Hunteria umbellata seed anti-bacterial activities against some Gram-positive and Gram-negative bacteria [25].

All the haematological parameters were within the normal physiological ranges for healthy rabbits [25] (Table 4). This outcome suggests that feeding animals graded levels of fermented Hunteria umbellata seed did not negatively affect their immune system [25]. Red blood cell count, pack cell volume and haemoglobin values which varied from 5.09 – 5.83 (x 106/µL), 31.72 – 33.36 % and 12.56 – 13.35 g/dL were similar (p>0.05) among the treatments. The pack cell volume (pcv) is in line with the observations of [26] who noted that pcv values of growing rabbits fed diets containing graded levels of Canna indica seed meal ranged from 28.00 – 35.00 %. This suggests that feeding rabbit's fermented Hunteria umbellata seeds did not affect the activity of iron in the blood decrease in pcv count is a sign of anaemia [26]. According to [27], hepatic disorder and respiratory stress can contribute to low concentration of haemoglobin and red blood cell count in animals. However, red blood cell and haemoglobin count recorded in this study was within the normal range 4.00 – 7.00 (× 106/µL) and 10.00 – 15.00 g/dL reported by [28]. White blood cell, lymphocytes, basophils and eosinophil values which varied from 10.55 – 10.95 (× 106/µL), 65.23 – 66.93 %, 0.12 – 0.16 % and 3.08 – 3.28 % were not influenced (p>0.05) by the treatment. However, values recorded were within the normal range referenced by [29]. According to [21] white blood cell play an important role in the production of antibodies which prevents the body from disease. [22] recorded a white blood cell range of 8.90 – 20.55 (× 106/µL) in weaner rabbits fed diet containing different levels of Phyllantus amarus. Lymphocytes are involved in antibody production, as they are reactive cells in inflammation and delayed hypersensitivity while basophils play a key role in the elaboration of histamines and heparin in circulating blood [24].

Parameters	Control (T1)	T2 (5 % FHUSM)	T3 (10 % FHUSM)	T4 (15 % FHUSM)	T5 (20 % FHUSM)	SEM
RBC (× 10^6/uL)	5.09	5.21	5.46	5.67	5.83	0.25
Hb (g/dL)	12.56	13.04	13.15	13.27	13.35	0.17
Pack cell volume (%)	31.72	32.08	32.17	33.24	33.36	2.81
WBC (×10^3/uL)	8.55	8.63	8.71	8.81	8.95	0.06
Lymphocytes (%)	65.23	66.17	66.85	66.91	66.93	3.56
Basophils (%)	0.12	0.13	0.11	0.16	0.15	0.04
Eosinophils (%)	3.08	3.11	3.15	3.26	3.28	0.29

[a,b] Means in the same row with different superscript are significantly (P< 0.05) different; T1 (0 % FHUSM); T2 : 5 % FHUSM; T3: 10 % FHUSM; T4: 15 % FHUM; T5: 20 % FHUSM; SEM: standard error of mean

Table 4: Haematological indices of weaner rabbit fed graded levels of fermented Hunteria umbellata seed meal

Parameters	Control (T1)	T2 (5 % FHUSM)	T3 (10 % FHUSM)	T4 (15 % FHUSM)	T5 (20 % FHUSM)	SEM
Total protein (g/dL)	5.99[b]	6.67[a]	6.82[a]	6.86[a]	6.96[a]	0.34
Albumin (g/dL)	3.04[b]	3.53[a]	3.61[a]	3.63[a]	3.68[a]	0.22
Globulin (g/dL)	2.95[b]	3.14[a]	3.21[a]	3.23[a]	3.28[a]	0.35
Creatinine (mg/dL)	1.02	1.08	1.10	1.15	1.24	0.25
Urea (mg/dL)	0.17	0.19	0.18	0.15	0.16	0.48
ALT (IU/L)	61.23	63.21	63.47	63.52	63.68	0.75
AST (IU/L)	16.82	16.93	17.12	17.51	17.63	0.97

[a,b] Means in the same row with different superscript are significantly (P< 0.05) different; T1 (0 % FHUSM); T2 : 5 % FHUSM; T3: 10 % FHUM; T4: 15 % FHUSM; T5: 20 % FHUSM; SEM: standard error of mean; Alanine aminotransferase (ALT) and aspartate amino transferase (AST)

Table 5: Serum biochemical indices of weaner rabbit fed graded levels of fermented Hunteria umbellata seed meal

Serum biochemical indices of weaner rabbit fed graded levels of fermented Hunteria umbellata seed meal (Table 5). All serum biochemical parameters were with the normal reference range for healthy rabbits suggesting the absence of metabolic disorder [29]. Total protein, albumin, globulin value which varied from 5.99 – 6.96 g/dL, 3.04 – 3.68 g/dL and 2.95 – 3.28 g/dL were significantly ($p<0.05$) affected by the treatment. Total protein value of rabbits fed T1 was within 3.00 – 6.00 g/dL reported by [30] while those in T2 – T5 was within 4.00 – 8.50 g/dL referenced by [32]. The value of albumin and globulin was within established range (2.00−4.00 g/dL) and (2.50 – 4.50 g/dL) reported by [33]. Globulin conveys essential minerals which helps to fight infections in the body of rabbits [22]. Urea and creatinine values aligns with the reports of [26]. This suggests that the kidney is effectively filtering nitrogenous waste and maintaining glomerular filtration rates [25]. Alanine aminotransferase (ALT) and aspartate amino transferase (AST) values were within 50.00 – 75.00 IU/L and 15.00 – 30.00 IU/L cited by [32]. This outcome indicates the absence of liver damage making detoxification efficient in the body of rabbits [28, 35].

Conclusion

In conclusion, replacing Soybean meal with fermented Hunteria umbellata seed meal up to 20 % inclusion level is physiologically safe for growing male rabbits. The absence of significant deviations in Pcv, Hb and RBC indicates that the experimental diet did not interfere with the oxygen carrying capacity of the blood. White blood cell and its differentials across all treatments confirm that the processed seed did not contain residual toxins sufficient to trigger an anti-inflammatory response or suppress the immune system. Serum parameters recorded suggests absence of metabolic disorder. Fermented Hunteria umbellata seed can safely replace soyameal in rabbit diets without detrimental effect on the animal's health status.

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