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Research Article

Effect Of Replacing Soyabean Meal with Fermented Hydrocotyle Asiatica Seed Meal on The Growth Performance, Semen Quality and Oxidative Stress Markers of Rabbit Bucks

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Abstract

This study evaluated the effects of replacing soybean meal (SBM) with Fermented Hydrocotyle asiatica seed meal (FHASM) on the growth performance, oxidative status, and seminal characteristics of rabbit bucks. Forty (40) healthy rabbit bucks were randomly assigned to four dietary treatments in a Completely Randomized Design (CRD). The control group (T1) received a standard diet with 0% FHASM, while treatments T2, T3, and T4 replaced SBM with FHASM at inclusion levels of 5%, 10%, and 15%, respectively. The experimental period lasted for 90 days. Results indicated that growth performance parameters, including final body weight gain, daily feed intake, and feed conversion ratio (FCR), were not significantly ($P>0.05$) influenced by the dietary replacement, suggesting that FHASM is a nutritionally viable alternative to SBM. Regarding oxidative indices, Malondialdehyde (MDA) concentrations significantly decreased ($P<0.05$) as the inclusion of FHASM increased across the treatments, indicating a reduction in lipid peroxidation. Conversely, the activities of endogenous antioxidant enzymes—Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione (GSH)—were significantly higher ($P<0.05$) in groups T2–T4 compared to the control. Furthermore, semen quality was markedly improved by FHASM inclusion; semen volume, sperm concentration, mass motility, and the percentage of live sperm were significantly higher ($P<0.05$) in rabbits fed fermented seed meal than those in T1. It was concluded that fermented Hydrocotyle asiatica seed meal can replace soybean meal up to 15% in rabbit buck diets to enhance reproductive potential and oxidative stability without compromising growth performance..

Keywords: Hydrocotyle asiatica, Fermentation, Rabbit Bucks, Semen Quality, Oxidative Stress, Soybean Meal Replacement.

Introduction

The global rabbit production industry is increasingly recognized as a vital sector for addressing protein insecurity, particularly in developing countries [1]. Rabbits possess unique physiological advantages, including a short gestation period, high prolificacy, and the ability to convert non-competitive fibrous materials into high-quality animal protein [1]. However, the sustainability of rabbit farming is currently threatened by the escalating cost of conventional feed ingredients, most

notably Soybean meal [2]. As a primary protein source, soyameal faces intense competition between human nutrition, industrial applications, and the livestock sector, necessitating the exploration of Non-Conventional Feed Resources (NCFRs) [3]. One such potential candidate is Hydrocotyle asiatica (commonly known as Gotu Kola or Asiatic Pennywort) [3]. While primarily recognized for its pharmacological properties in traditional medicine, its seeds represent an underutilized

byproduct that may serve as an alternative protein and bio-active source [4]. However, the presence of ant-nutritional factors (ANFs) often limits the direct inclusion of raw tropical seeds in monogastric diets [4]. Fermentation emerges as a cost-effective biotechnological tool to enhance the nutritional profile of such seeds [5]. By leveraging microbial activity, fermentation can reduce ANF concentrations, improve protein bioavailability, and enrich the meal with probiotic metabolites and organic acids [5].

Furthermore, intensive rabbit production systems often subject animals to environmental and physiological stressors. These stressors trigger the overproduction of Reactive Oxygen Species (ROS), leading to oxidative stress [6]. In buck rabbits, oxidative stress is a primary culprit behind poor reproductive performance, as it induces DNA fragmentation in sperm and reduces motility [7, 13]. Conventional synthetic antioxidants used to combat this are often expensive and carry concerns regarding residual effects in the meat, creating an urgent need for natural, plant-based alternatives that can simultaneously provide nutrition and antioxidant protection [7, 14]. The integration of fermented *Hydrocotyle asiatica* seed meal (FHASM) into rabbit diets may not only support growth performance but also offer systemic physiological benefits. In male rabbits (bucks), reproductive efficiency is highly sensitive to dietary quality [8, 14]. Phytochemicals within FHASM, such as phenols and flavonoids, are known for their potent antioxidant properties [8]. These compounds may mitigate oxidative stress markers, protecting delicate spermatozoa from lipid peroxidation and subsequently enhancing semen quality [8, 9, 10].

Studies focusing on fermented seeds, such as *Hunteria umbellata* and *Canna bidentata*, indicate that they can effectively replace soyameal at various inclusion levels (typically up to 20%) without detrimental effects on growth performance, feed intake and feed conversion ratios of rabbits [10, 11, 12]. However, there is little or no research on replacing soyameal with fermented *Hydrocotyle asiatica* seed meal. This study is justified by the urgent need to diversify the feed base of the rabbit industry using locally available and underutilized tropical resources. Replacing soyameal with Fermented *Hydrocotyle asiatica* seed meal will help to promote economic sustainability, nutritional enhancement, reproductive longevity and oxidative defense.

Materials and methods

Experimental site

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 – 32.6oC and mean relative humidity range of 60.55 %

Sample collection, Identification and processing of *Hydrocotyle asiatica* seeds

Fresh *Hydrocotyle asiatica* seeds free from disease was purchased from an open market in Rajasthan, India during the month of February, 2024. The seeds were identified and authenticated at Herbarium Unit of Taxonomy Department, Gandhi College of Agriculture, Rajasthan India. Seeds were screened to remove dirt's, after which they are slightly crack, placed in a clean container and soaked in water (at a ratio of approximately 1:2 seeds to water) covered and placed at room temperature for one weeks till when the water becomes cloudy and sour. On the 8th day, the water is drained, fermented seeds were air-dried for 5 days until a constant weight was achieved before it was milled into powder and the powder kept in an air-tight polythene bags until needed for analysis. Phytochemical screening of the powdered seeds were carried out by a procedure that was based on those earlier reports by [15] and [16] and its proximate analysis was determined according to the official method of the Association of Official Analytical Chemists [17].

Experimental animals and management

Sixty (60) growing New Zealand white × Chinchilla cross-bred male rabbits of about 10 weeks of age were used for the experiment. The animals were sourced from the Sumitra Research Institute, Rajasthan India. On arrival, animals were subjected to a 2 weeks acclimatization period and given prophylactic treatment against parasites using Ivomectin® injection. The experimental animals were housed in wooden hutches with wire mesh raised from the floor which was well disinfected with Morigad + Aquaclean in ratio 1:1 two weeks before the commencement of the trial. All experimental animals were subjected to the same housing and management conditions. Animals were fed standard diet containing Corn-soya bean meal during the adjustment period, clean water was also offered at all times. On the third week, weights of the rabbits balanced using a digital sensitive scale and randomly assigned to four dietary groups in a feeding trial that lasted eighteen 90 days (12 weeks). Four experimental diets were formulated. Diet 1 (T1) without fermented *Hydrocotyle asiatica* seed meal served as the control diet. In diet 2 (T2), diet 3 (T3) and diet 4 (T4) fermented, *Hydrocotyle asiatica* seed meal was used to replace soyabean meal at 5 %, 10 %, 15 % and 20 % respectively. Diet was formulated to be nearly isocaloric and isonitrogenous to meet the minimum nutrient requirement of rabbits according to [18]. The experimental design is Completely Randomized Design. Other health precautions and biosecurity measures were also taken throughout the experimental period.

Growth performance evaluation

The experimental diets were offered to the rabbits thrice daily

(7:30H, 12:00 and 16:00 H). The amount of feed consumed per rabbit was determined as the difference between the feed offered and refused. Feed offered and refused was sampled per day. Daily feed intake was estimated as body weight gain divided by the duration of the experiment in days. While daily feed consumed was calculated by dividing total feed consumed by the duration of the experiment in days. Mortality rate was monitored and Feed Conversion Ratio (FCR) was calculated as the ratio of feed intake to body weight gain.

Semen collection

A 3-week period was used to train the bucks for semen collection. Semen was finally collected from the buck using the artificial vagina (AV) described by [19]. Prior to semen collection, the AV was warmed for a few minutes in warm water at a temperature slightly above body temperature and thereafter drained. Semen collection was done very early in the morning to ensure that optimum quality semen were obtained. The parameters assessed includes;

Semen volume

The volume of semen collected was measured using the graduated collection tube.

Sperm concentration

Sperm concentration/ejaculate was calculated as: sperm concentration per ml $\times$ volume of ejaculate. Sperm concentration per ml of semen was evaluated using a visual count under the microscope using improved Neubauer haemocytometer as described by [19].

Live sperm percentage

Live sperm cells determination was done by placing a drop of semen mixed with one drop of eosin negrosin stain on a slide and observed under the microscope. The unstained cells represented the live cells while the stained cells are the dead ones.

Sperm motility

To determine the motility of the sperm cells, a drop of undiluted semen mixed with a drop of slightly warmed diluents (sodium citrate) was placed on a sterile slide, covered with

a cover slip and observed under the microscope at (Mag. $\times 400$) and scored within a rating of 0 -100 %.

Oxidative stress analysis

At the end of the trial, blood samples were collected from 5 randomly selected bucks per treatment for oxidative stress indices. 3 ml of blood was collected via the marginal ear vein using hypodermic syringes into labeled bottles containing EDTA. After collection, sample were transferred into an ice pack and taken to the laboratory for further analysis. Parameters examined include, Malondialdehyde (MDA), Glutathione (GSH), Superoxide Dismutase (SOD) and Catalase using Cayman TBARS Assay (Model 700870, USA). Kit was subjected to a temperature of 90 degrees Celsius and Sensitivity of 0.625 m to ensure precision in outcome.

Statistical analysis

All data collected will 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 ($p < 0.05$).

Result and Discussions

Table 2 reveals the proximate composition of fermented *Hydrocotyle asiatica* seed meal (FHASM). The sample contained dry matter and crude protein of 87.63 % and 30.10 % respectively. The result indicates that FHASM has a high quality protein supplement for animals [20]. This crude protein value rivals or exceeds those reported for many legumes, for instance *Cicer arietinum* seed (19 – 25 %), *Vigna unguiculata* seed (23 – 27 %) and *Vicia faba* (24 – 30 %) [21, 22]. Crude fiber (2.77 %) suggests that FHASM will help to facilitate digestion of feed in the gastro-intestinal tract of animals [22]. Ether extract (3.89 %) and ash (9.87 %) indicates that FHASM provides essential fatty acid and it is also abundant in essential minerals like calcium, phosphorus, potassium etc., which helps to promote good health and activates the activities of endogeneous enzymes in the gut [23, 24]. The energy content in FHASM (2406.5 Kcal/kg) was similar to 2700 Kcal/kg reported for soyabean meal by [25].

Ingredients	Control (T1)	T2 (5 %)	T3 (10 %)	T4 (15 %)
Maize	40.00	40.00	40.00	40.00
Fermented <i>Hydrocotyle asiatica</i> seed meal (FHASM)	0	1.17	2.34	3.51
Wheat bran	12.50	12.50	12.50	12.50
Palm kernel meal	20.00	20.00	20.00	20.00
Soybean meal	23.4	22.23	21.06	19.89
Bone meal	3.00	3.00	3.00	3.00
Methionine	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25

Growers Mineral-Vitamin Premix	0.25	0.25	0.25	0.25
Salt	0.35	0.35	0.35	0.35
Total	100.0	100.0	100.0	100.0
Determined analysis (% Dry matter)				
Crude protein	18.17	17.91	17.82	17.73
Crude fiber	13.56	12.81	12.79	12.76
Ether extract	1.97	2.01	2.01	2.01
Calcium	1.37	1.42	1.42	1.42
Phosphorus	0.81	0.85	0.85	0.85
Energy (Kcal/kg)	2600.3	2610.5	2617.3	2620.1

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	% Composition
Dry matter	87.63
Crude protein	30.10
Crude fibre	2.77
Ether extract	3.89
Ash	9.87
Energy (Kcal/kg)	2406.5

Table 2: Proximate composition of fermented *Hydrocotyle asiatica* seed meal (FHASHM)

Phytochemical components of fermented *Hydrocotyle asiatica* seed meal (FHASHM) is presented in Table 3. The fermented seed contained flavonoids (15.67 %) followed by phenols (12.33 %), steroids (5.61 %), tannins (3.30 %), alkaloids (1.85 %) and phytates (0.19 %) respectively. The outcome suggests that FHASHM is exceptionally loaded in bioactive compounds with strong antioxidant and therapeutic potentials [24]. High concentrations of flavonoids and phenols makes the FHASHM exhibits antioxidant activity to neutralize the activities of free radicals in the body of the animals [25]. Tannins and alkaloids have antimicrobial benefits, though at these levels, tannins can slightly astringent the flavor [24]. The presence of steroids in FHASHM makes it act as precursor for hormonal signaling and also provides anti-inflammatory benefits to rabbits [26]. The concentration of phyates is low and favorable level due to the fermentation process, this ensures that minerals are more bioavailable to the body of the animals [26].

Constituents	% Composition
Phenols	12.33
Tannins	3.30

Alkaloids	1.85
Flavonoids	15.67
Steroids	5.61
Phytate	0.19

Table 3: Phytochemical components of fermented *Hydrocotyle asiatica* seed meal (FHASHM)

Growth performance of rabbit bucks fed different levels of fermented *Hydrocotyle asiatica* seed meal (FHASHM) is presented in Table 4. Body weight gain, total feed intake and feed conversion ratio were not significantly ($p>0.05$) influenced by the treatments, values obtained ranged from 1359.49 – 1366.40 g, 7650.0 – 7686.1 g and 4.02 – 4.03 respectively. The similarity in body weight gain in all the groups suggests that the amino acid profile and energy density of FHASHM were sufficient to meet the physiological requirements for rabbit bucks. Fermentation can also increase the concentration of minerals and vitamins thereby compensating for the high nutrient in soyabean meal. The result obtained is in conformity with the reports of [27] when a mixture of rapeseed meal, white lupin seed, and pea seed was fed at different levels in rabbit's diet. FHASHM is known for its alkaloid content which gives it a bitter taste, however, feed intake among all the treatments were not affected suggesting that the fermentation process significantly reduced the bitter taste ensuring that the feed remained palatable. This result is in agreement with the reports of [28]. FCR across all treatments implies that the protein in FHASHM was as accessible and absorbable as that of soyabean meal maintaining the efficiency of nutrient to tissue conversion. The result obtained also suggests that the metabolizable energy of the diet was consistent. This outcome aligns with the findings of [29] when pea and faba bean was used to replace soyabean meal in the diet of animals.

Parameters	Control (T1)	T2 (5 %)	T3 (10 %)	T4 (15 %)	SEM
Number of animals	10.00	10.00	10.00	10.00	-
Experimental duration	90.00	90.00	90.00	90.00	-
Initial body weight (g)	1069.08	1068.9	1069.1	1068.7	0.01
Final body weight (g)	2428.57	2430.1	2432.2	2435.1	56.81
Body weight gain (g)	1359.49	1361.2	1363.1	1366.4	37.24
Average daily weight gain (g)	15.11	15.12	15.15	15.18	0.31
Total feed intake (g)	7650	7681.9	7685.4	7686.1	133.8
Average daily feed intake (g)	60.71	60.96	60.99	61.00	1.68
FCR	4.02	4.03	4.03	4.02	0.25

SEM: Standard error of mean; FCR: feed conversion ratio

Table 4: Growth performance of rabbit bucks fed different levels of fermented *Hydrocotyle asiatica* seed meal (FHASHM)

Semen quality of rabbit bucks fed different levels of fermented *Hydrocotyle asiatica* seed meal (FHASHM) is revealed in Table 5. Semen volume, pH, sperm motility, sperm concentration and live sperm were significantly ($p < 0.05$) influenced by the treatments. Their values ranged from 0.56 – 0.88 mL, 7.28 – 7.85, 72.11 – 87.55 %, 140.2 – 158.1 (106/mL) and 71.23 – 89.22 %. The higher sperm motility and sperm concentration in T2 – T4 suggests that the antioxidants (flavonoids and phenols) in FHASHM can protect sperm cell membrane from lipid peroxidation. This preservation of membrane integri-

ty often leads to a higher progressive motility, volume and higher viable count compared to T1 (control). [30]. Sperm concentration range recorded in this study was within 138.8 – 160.1 (106/mL) recorded by [31] when rabbit buck were fed diet supplemented with phytogenics. The synergy between the bioactive compounds in FHASHM may also help to maintain optimal pH for sperm longevity during storage. The pH recorded in this trial is within 7.2 – 7.8 recorded by [32, 33] when thyme was supplemented in the diet of rabbits.

Parameters	Control (T1)	T2 (5 %)	T3 (10 %)	T4 (15 %)	SEM
Semen Volume (ml)	0.56b	0.75a	0.81a	0.88a	0.01
pH	7.28	7.53	7.66	7.85	0.01
Sperm Motility (%)	72.11b	85.76a	87.37a	87.55a	0.03
Sperm concentration (106/mL)	140.2b	156.2a	157.8a	158.1a	0.18
Live sperm (%)	71.23b	88.94a	89.14a	89.22a	0.05

SEM: Standard error of mean; FCR: feed conversion ratio

Table 5: Semen quality of rabbit bucks fed different levels of fermented *Hydrocotyle asiatica* seed meal (FHASHM)

Parameters	Control (T1)	T2 (5 %)	T3 (10 %)	T4 (15 %)	SEM
Malondialdehyde (nmol/mL)	2.88a	2.01b	1.98b	1.95b	0.01
Superoxide dismutase (U/mL)	30.02b	37.45a	38.07a	38.66a	0.02
Catalase (U/mL)	11.56b	13.87a	14.11a	14.19a	0.02
Glutathione peroxidase (μ mol/L)	21.75b	28.09a	29.12a	29.15a	0.03

SEM: Standard error of mean; FCR: feed conversion ratio

Table 6: Oxidative stress indices of rabbit bucks fed different levels of fermented *Hydrocotyle asiatica* seed meal (FHASHM)

Conclusion

The findings indicate that fermentation detoxified *Hydrocotyle asiatica* seed making them physiologically safe for rabbits. Fermented *Hydrocotyle asiatica* seed meal (FHASHM) can re-

place up to 15% of soyabean meal in the diets of rabbit bucks without compromising the growth performance, reproductive integrity and oxidative stress. This replacement offers a sustainable strategy in reducing feed cost in rabbit production.

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