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Research Article

Effects of varying inclusion levels of Fermented Hunteria umbellata Seed Oil on the growth performance, carcass characteristics, caecal microbial evalaution and liver histology of growing rabbits

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Abstract

The rising demand for organic livestock products has necessitated the exploration of phytogenic feed additives as alternatives to synthetic growth promoters. Hunteria umbellata is a medicinal plant rich in bioactive secondary metabolites, yet its fermented oil's efficacy in rabbit nutrition remains largely unexplored. This study evaluated the effects of varying inclusion levels of Fermented Hunteria umbellata Seed Oil (FHUSO) on the growth performance, carcass characteristics, caecal microbial evaluation and liver histology of growing rabbits. Fifty growing male rabbits (initial body weight 656.3 g) of about 8 weeks of age were randomly assigned to five treatments (n=10) in a 90-day trial using a Completely Randomized Design (CRD). Following a two-week quarantine and deworming period, rabbits were housed individually and fed a basal diet supplemented with FHUSO at 0 (T1), 5 (T2), 10 (T3), 15 (T4), and 20 (T5) ml/kg. Result on the chemical composition of FHUSO reveals the presence of 14 major bioactive with therapeutic properties. Growth indices, including average daily weight gain and feed intake, were significantly ($P<0.05$) highest in T5, followed by the intermediate groups (T2–T4), and lowest in T1. The feed conversion ratio (FCR) was most efficient in T5. Carcass weight and dressing percentage mirrored the trend of feed intake, peaking in the T5 group. While the relative weights of the liver, spleen, heart, lungs, and kidneys showed no significant differences ($P>0.05$), the intestinal length was significantly altered, increasing with higher oil inclusion. Histological examination of the liver revealed no pathological changes across all treatments, indicating a high safety margin for the oil. Population of Clostridium spp, Escherichia coli and Coliform decreased ($P<0.05$) as the level of FHUSO increased across the treatment. Treatment 1 shows a higher pH corresponding to a lower total volatile fatty acid [TVFA]. Conversely, animals feed diet containing FHUSO had a lower pH with higher TVFA. Supplementation with FHUSO at 20 ml/kg significantly enhances growth performance and carcass yield without inducing systemic toxicity. The findings suggest that the bioactive synergy of flavonoids and terpenoids in FHUSO optimizes gut morphology and nutrient utilization, making it a potent natural growth promoter in rabbit production.

Keywords: *Hunteria umbellata,* Rabbits, Growth Performance, Carcass Yield, Liver Histology, Phytogenics..

Introduction

The global livestock industry is currently undergoing a paradigm shift, transitioning away from synthetic growth promoters toward sustainable, "green" feed additives [1]. Rabbit production (Oryctolagus cuniculus) stands at the forefront of this transition due to the species' high reproductive rate, rapid growth, and ability to convert forage into high-quality protein [2]. However, intensive rabbit farming is often hampered by the "weaning crisis," characterized by digestive disturbances and oxidative stress, which leads to poor growth performance and significant economic losses [3]. Traditionally, antibiotic growth promoters (AGPs) were utilized to mitigate these issues; however, their subsequent ban—driven by concerns over antimicrobial resistance (AMR)—has necessitated the exploration of phytogenic feed additives (PFAs) [3]. Hunteria umbellata, a significant medicinal plant traditionally used in West African ethnomedicine, has emerged as a candidate of high pharmacological interest [4]. The seeds are recognized for their dense concentration of bioactive secondary metabolites, including flavonoids (17.33%), phenols (12.66%), and terpenoids (8.77%) [5]. While raw seeds are potent, the application of fermentation to the seed oil represents a strategic biotechnological intervention. Fermentation acts as a bio-enhancer, breaking down complex phytochemicals into smaller, more bioavailable molecules and reducing potential anti-nutritional factors [6-7]. Despite the potential of Hunteria umbellata, its application in rabbit nutrition is not yet standardized. Rabbit producers frequently grapple with intestinal dysbiosis—the disruption of the delicate cecal microflora—which results in poor feed conversion ratios (FCR) and stunted growth [7]. Furthermore, the metabolic demands of rapid growth in rabbits often trigger oxidative stress, which compromises the immune system and reduces muscle deposition [8]. Currently, there is a dearth of comprehensive data regarding the optimal inclusion levels of fermented Hunteria umbellata seed oil (FHUSO) and how these varying concentrations influence the physiological growth curve of growing rabbits [8].

Previous investigations into Hunteria umbellata have primarily been clinical, with [9] highlighting the plant's significant anti-inflammatory and antipyretic properties. In the realm of animal science, studies on similar phytogenics (such as black cumin and ginger oils) have shown that phenolic compounds can stimulate the secretion of endogenous digestive enzymes, thereby improving nutrient digestibility [10-12]. Fermentation specifically has been shown to increase the antioxidant capacity of plant extracts by liberating bound phenolics [13-15].

This study is justified by the urgent need to establish an empirical baseline for FHUSO as a natural growth promoter. By evaluating a range of inclusion levels (5 to 20 ml/kg), this research will help to optimize feed efficiency, harness bioactive synergy and support sustainable agriculture.

Materials and Methods

Experimental Location and Ethical Approval

The experiment was conducted at the Rabbitry Unit of the Gandhi College of Agriculture, Rajasthan. The region is characterized by a semi-arid climate, and the environmental temperature and humidity were monitored to ensure they remained within the comfort zone for growing rabbits. All experimental protocols were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Gandhi College of Agriculture. The handling and management of the animals were performed in strict accordance with the guidelines for the care and use of laboratory animals to minimize stress and ensure animal welfare throughout the 90-day trial.

Collection of Hunteria umbellata seeds and processing of oil Following the acquisition of healthy Hunteria umbellata seeds from a Rajasthan market and their formal authentication at Gandhi College of Agriculture (Voucher No. MB20071), the samples were processed through manual cracking and a controlled 7-day aqueous fermentation. Post-fermentation, the seeds were subjected to hydrodistillation using a Clevenger-type apparatus to isolate the essential oil fraction. The chemical constituents of the oil were characterized using a Shimadzu GCMS-QP2020 NX (Japan), equipped with a high-performance SH-Rxi-5Sil MS capillary column (30 m × 0.25 mm ID × 0.25 μm). The analysis was managed via GC MS solution software, utilizing an ultra-fast scanning speed of 20,000 u/sec and a high-sensitivity ion source. This setup, featuring a dual-stage filament and a high-capacity differential vacuum system, ensured precise fragmentation patterns which were cross-referenced against the Wiley and NIST mass spectral databases for definitive compound identification.

Experimental Animals and Management

A total of 50 growing male rabbits of about 7 weeks old (Chinchilla × New Zealand White) with an initial average body weight of 656.3 g were used for this experiment. The rabbits were purchased from a reputable breeding farm in Rajasthan. Two weeks prior to the arrival of the animals, the experimental pens and equipment (feeding and watering troughs) were thoroughly washed and disinfected using a broad-spectrum disinfectant. Upon arrival, the rabbits underwent a two-week quarantine and acclimatization period. During this phase, they were fed the basal diet and dewormed using a combination of anti-helmintics to treat endo- and ectoparasites. Following quarantine, the rabbits were stratified by weight and housed individually in galvanized battery cages (60 cm×45 cm×40 cm) equipped with separate feeders and nipple drinkers.

Experimental Design and Treatments

The experiment followed a Completely Randomized Design (CRD). The 50 rabbits were randomly assigned to five treatment groups (n=10). The treatments consisted of a basal diet formulated to meet the nutrient requirements of growing rabbits [16], supplemented with varying levels of fermented Hunteria umbellata seed oil (FHUSO):

- **T1: Basal diet only (Control)**

Ingredients	**g/kg DM**
Maize	405
Wheat bran	100
Palm kernel meal	200
Soybean meal	200
Bone meal	20.0
Methionine	10.0
Lysine	10.0
Growers Mineral-Vitamin Premix	25.0
Salt	30.0
Total	**1000**
Chemical composition	
Dry matter	879
Crude protein	168
Crude fibre	131
Ether extract	21.0
Ash	80.0
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 1: Ingredient and chemical composition of the basal diet (g/kg of DM)

Bioactive compounds	**Reaction time (min)**	**Concentration (mg/g)**
Limonene	13.24	753.5
β-Caryophyellene	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 2: Major bioactive compounds in fermented Hunteria umbellata seed oil

Parameters	Diet 1 (0 ml/kg FHUSO)	Diet 2 (5 ml/kg FHUSO)	Diet 3 (10 ml/kg FHUSO)	Diet 4 (15 ml/kg FHUSO)	Diet 5 (20 ml/kg FHUSO)	SEM
Number of Rabbits	10.00	10.00	10.00	10.00	10.00	-
Duration of experiment	12.00	12.00	12.00	12.00	12.00	-
Average Initial body weight (g)	681.9	682.1	682.3	682.4	680.7	0.08
Average final body weight (g)	2095.5c	2381.2b	2385.3b	2387.6b	2670.1a	2.69
Body weight gain (g)	1413.6c	1699.1b	1703.0b	1705.2b	1989.4a	1.65
Average daily weight gain (g/day)	15.71c	18.88b	18.92b	18.93b	22.10a	0.02
Total feed intake (g)	8311.2b	8633.1a	8636.2a	8638.1a	8639.2a	15.85
Average daily feed intake (g/day)	92.35b	95.94a	95.96a	95.97a	95.99a	0.96
Feed conversion ratio	5.88a	5.08b	5.07b	5.07b	4.34c	0.01

a,b,c Means in the same row with different superscript are significantly (P< 0.05) different; SEM: standard error of mean

Table 3: Effect of dietary supplementation of fermented Hunteria umbellata seed oil (FHUSO) on growth performance of growing rabbits

Parameters	Diet 1 (0 ml/kg FHUSO)	Diet 2 (5 ml/kg FHUSO)	Diet 3 (10 ml/kg FHUSO)	Diet 4 (15 ml/kg FHUSO)	Diet 5 (20 ml/kg FHUSO)	SEM
Average live weight (g)	1917.2c	2315.2b	2313.8b	2351.2b	2800.2a	3.05
Dressed weight (g)	1211.4c	1610.3b	1609.2b	1602.4b	2000.3a	2.44
Dressing percentage	63.19c	69.55b	69.55b	68.15b	71.43a	0.02
Spleen (%)	0.071	0.073	0.072	0.075	0.077	0.001
Liver (%)	4.22	4.27	4.32	4.41	4.46	0.02
Kidney (%)	0.53	0.55	0.54	0.51	0.57	0.01
Lungs (%)	2.33	2.46	2.65	2.87	2.81	0.01
Abdominal fat (%)	1.75	1.08	1.05	1.01	0.96	0.11
Intestinal length (cm)	271.2b	304.6a	311.4a	315.7a	320.8a	1.61

a,b,c Means in the same row with different superscript are significantly (P< 0.05) different; SEM: standard error of mean

Table 4: Effect of dietary supplementation of fermented Hunteria umbellata seed oil (FHUSO) on carcass characteristics of growing rabbits

Microbial group (Log10 CFU/g)	Diet 1 (0 ml/kg FHUSO)	Diet 2 (5 ml/kg FHUSO)	Diet 3 (10 ml/kg FHUSO)	Diet 4 (15 ml/kg FHUSO)	Diet 5 (20 ml/kg FHUSO)	SEM
pH	6.72a	5.75b	5.78b	5.77b	5.80b	0.02
Total Coliforms	5.97a	4.29b	4.25b	4.18b	4.12b	0.03
Escherichia coli	6.11a	3.93b	3.81b	3.21b	3.18b	0.01

Clostridium perfringens	3.88a	2.21b	2.16b	2.08b	2.01b	0.02
Lactobacillus spp	4.16b	5.92a	5.98a	6.07a	6.10a	0.15
TVFA (mmol/l)	60.07b	89.07a	89.12a	90.20a	90.25a	2.64

TVFA: Total volatile fatty acid; a,b Means in the same row with different superscript are significantly ($P < 0.05$) different; SEM: standard error of mean

Table 5: Caecal count and microbial activity of growing rabbits fed varying levels of FHUSO

Growth performance evaluation

All the rabbits were weighed at the beginning of the experiment before they were allocated to the treatments. The experimental diets were offered to the rabbits thrice daily (7:00 H, 12:00 and 16:00 H). Daily feed intake was estimated as the difference between feed served and the left over. Fresh clean water was also provided at all times throughout the experiment. Records of average daily feed intake and daily body weight gain were recorded. The daily weight gain was calculated as the weekly weight gain divided by 7. Mortality rate was monitored and Feed Conversion Ratio (FCR) was calculated as the ratio of feed intake to body weight gain.

Carcass and Organ weight assessment

Five animals were randomly selected from each treatment group at the end of the 90 days feeding trial, weighed, slaughtered and skinned. The animals were eviscerated, all the internal organs and gastro-intestinal tract removed before the dressed weights were taken using sensitive digital scale. Weight of organs such as heart, liver, kidney, spleen and lungs were taken using the weighing balance. Paired organs were weighed separately and recorded, then both were added together to obtain the paired weight for the organs.

Percentage relative weights of organs were calculated using the formula below:

$$\text{Relative weight of organ} = \frac{\text{Absolute weight of the organ}}{\text{Live weight of the rabbit}} \times 100$$

Caecum count and microbial evaluation

At the end of the experiment, caecal content was aseptically collected into sterile containers (rabbits used for carcass characteristics) into sterile containers and the pH is recorded; for microbial quantification, 1 g of the fresh digesta is homogenized in 9 ml of sterile anaerobic diluent to create a 10-1 stock suspension. A 10-fold serial dilution is then performed up to 10-7 under strict aseptic conditions. Aliquots of 0.1 ml from the targeted dilutions is spread plated onto selective media. The counted colonies are expressed as Log 10 CFU/g caecal contents. Total volatile fatty acids was determined using Markham apparatus as described by Markham [17].

Histopathology

Liver samples (from animals used for carcass evaluation) were fixed in 10% neutral buffered formalin, processed, and stained with Hematoxylin and Eosin (H&E) for microscopic examination.

Statistical Analysis

Data obtained were subjected to a One-way Analysis of Variance (ANOVA) using the General Linear Model (GLM) procedure. Significant differences between means were separated using Duncan's Multiple Range Test at a 5% ($P<0.05$) level of significance.

Results and discussion

Effect of dietary supplementation of fermented Hunteria umbellata seed oil (FHUSO) on growth performance of growing rabbits is revealed in Table 3. Average daily weight gain was lower ($p<0.05$) among rabbits fed treatment 1 (15.71 g/day) than treatment 2 (18.88 g/day), treatment 3 (18.92 g/day), treatment 4 (18.93 g/day) and treatment 5 (22.10 g/day). Average daily feed intake was higher ($p<0.05$) in FHUSO group compared to the control. The results of this study indicate that dietary supplementation with fermented Hunteria umbellata seed oil (FHUSO) significantly improved the growth performance of rabbits, with the highest efficacy observed at the 20 ml/kg level (T5). The higher average daily weight gain and feed intake observed in T5, followed by intermediate results in T2-T4, suggest that FHUSO acts as a potent natural growth promoter [18]. This enhancement can be attributed to the high concentration of Limonene (753.5 mg/g) and α-pinene (206.2 mg/g). These polyphenolic compounds are known to improve the palatability of feed and stimulate the secretion of digestive enzymes, which enhances nutrient digestion and absorption [19, 20]. Furthermore, the antioxidant properties of these compounds help mitigate oxidative stress, allowing the animals to redirect energy from cellular repair toward tissue growth [21]. The significant reduction in FCR (improved efficiency) in the FHUSO groups, particularly T5, compared to the control (T1) highlights the biological efficiency of the oil's bioactive components. The synergy between all the bioactive compounds in FHUSO helps to modulate the gut microbiota by suppressing pathogenic bacteria while favoring beneficial microbes [22]. The presence of terpinene-4-ol (97.03 mg/g), β-caryophyellene (86.22 mg/g), α-terpineol (45.11 mg/g), 1,8-cineole (20..96 mg/g) and β-citronella (11.42 mg/g) likely improved the integrity of the intestinal mucosa, increasing villi height and thus the surface area available for nutrient uptake [23]. The fermentation process likely increased the bioaccessibility of these phyto-compounds or bioactive compounds. The small concentrations of cubenol (1.77 mg/g), α-terpinolene (2.33 mg/g) and trans-linalool oxide (2.54 mg/g) may have provided additional benefits without reaching anti-nutritional thresholds. At the levels provided, these compounds likely acted synergistically to improve metabolic health and immune status, which is reflected in the superior growth metrics of the supplemented groups compared to the lower-performing control group [24]. The outcome obtained is in agreement with the reports of [25].

The presence of bioactive compounds likely improved nutrient digestibility and absorption. When rabbits consume more high-quality nutrients (as seen in T2-T5), there is a corresponding increase in muscle deposition and bone development, which directly elevates the carcass weights. The dressing percentage is a key metric of profit in rabbit production. The improvement in this parameter suggests that FHUSO helped partition nutrients toward edible carcass components rather than non-carcass fat or waste, a result of the synergistic action of bioactive compounds in optimizing protein synthesis [26]. Phytochemicals, are known to influence the morphology of the gastrointestinal tract. An increase in intestinal length (or weight) often correlates with increased villi height and crypt depth. This expansion increases the total surface area available for nutrient absorption. The fermented oil likely acted as a mild “trophic” agent, stimulating the growth of the intestinal mucosa, which explains why the T5 group could process feed more efficiently (lower FCR) than the control group [27]. The fact that the relative weights of the liver, spleen, heart, lungs, and kidneys were not significantly different across treatments is a vital safety indicator [28]. In toxicology, an increase in the relative weight of the liver or kidneys often signals a “hypertrophic” response to toxins, as these organs work harder to detoxify the body [28]. The stability of these weights across all FHUSO levels indicates that the oil, even at 20 ml/kg, does not contain anti-nutritional factors at levels high enough to cause organ stress [29]. The stability of the spleen weight suggests that the FHUSO did not trigger an inflammatory or overactive immune response, which can sometimes occur with high-dose plant extracts.

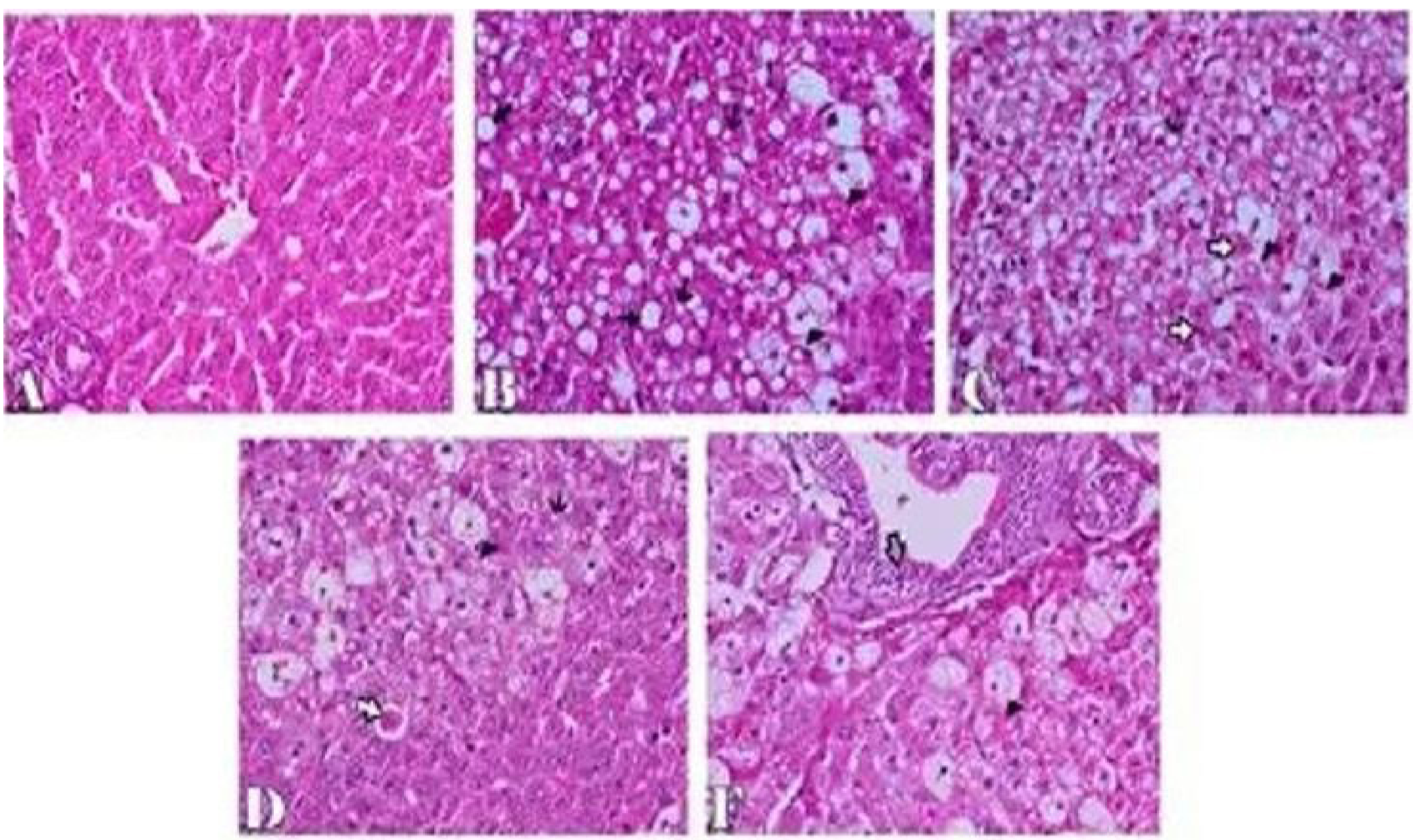

Plate 1-5 Liver Histology of growing rabbits fed varying levels of FHUSO

Caecal count and microbial activity of growing rabbits fed varying levels of FHUSO is presented in Table 5. Total coliforms, Escherichia coli and Clostridium perfringens count which varied from 4.12 – 5.97 (Log10 CFU/g), 3.18 – 6.11 (Log10 CFU/g) and 2.01 – 3.88 (Log10 CFU/g) were higher ($p<0.05$) in T1 compared to the other groups. Conversely, Lactobacillus spp in the caecum was higher in rabbits fed FHUSO compared to the control. Rabbits are hindgut fermenters, relying heavily on a healthy cecal microbial population. The bioactive compounds in FHUSO act as natural ionophores. In this experiment, it was observed that high concentration of this phyto-compounds lead to a significant reduction in pathogens (total coliform, E.coli and Clostridium spp). According to [30], essential oil are highly hydrophobic in nature with the capacity to penetrate the lipid bilayer of cell membranes of Gram negative bacteria like E.coli. This typically results in a significant drop in Coliform counts giving more space and nutrient to become available for beneficial bacteria such as Lactobacillus spp in the gut. Treatment 1 shows a higher pH (6.72) corresponding to a lower total volatile fatty acid [TVFA] (60.07 mmol/L). Conversely, animals feed diet containing FHUSO had a lower pH with higher TVFA. This inverse relationship suggests that FHUSO facilitates fermentation in the gut providing the rabbits with an additional energy source that contributed to the higher Average Daily Weight Gain (ADWG). The result obtained in this study aligns with the report of [31-32].

The most definitive evidence of the safety of FHUSO is the unaffected liver histology. The liver is the primary site for the metabolism of phyto-compounds. If these compounds were toxic, histology would reveal fatty changes, necrosis, or cellular infiltration [33]. On the contrary, the high content of limonene likely provided a hepatoprotective effect. Limonene and α-pinene scavenge free radicals and stabilize hepatic cell membranes, protecting the hepatocytes from lipid peroxidation. The normal histological architecture across treatments confirms that FHUSO is a “safe” additive that promotes growth without compromising the internal health of the rabbit [34]. The result obtained is in agreement with the reports of [35].

Conclusion

In conclusion, FHUSO supplementation at 20 ml/kg had significant impact on the growth and carcass characteristics of rabbits. Aside proving itself as a natural growth promoter, it was also discovered that it has the ability to inhibit the activities of pathogenic organisms in the gut and improve the proliferation of beneficial bacteria in order to maintain balance and improve nutrient absorption and energy during fermentation. Studies on the liver histology showed that all cells had a normal architecture suggesting that it is nontoxic to animals. FHUSO can further help to promote livestock sustainability and reduce antimicrobial resistance.

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