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Supplementation of biochar: Impact on growth performance, haematology and serum metabolites of weaned rabbit bucks

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Abstract

Biochar is a feed additive with potent growth and immune benefits for various livestock. The present study determined the effect of biochar supplementation on performance, haematology and serum metabolites of 36 weaned male rabbits. The 56-day experiment was a completely randomized design, consisting of three dietary treatments, replicated three times, and fed from point of placement at 35 days of age till end of study at 91 days of age. Treatments included 1: Control diet + 0% biochar; 2: Control diet + 15 g biochar/kg diet; 3: Control diet + 20 g biochar/kg diet. At 15 g/kg, biochar improved final body weight, average daily weight gain, average daily feed intake, feed conversion ratio, protein intake and protein efficiency ratio, compared to other treatments. Rabbits fed biochar supplemented diets at the various inclusion levels (15 g/kg and 20 g/kg) had higher survivability compared to rabbits in the control group. Changes in body weight were higher in the 15 g/kg biochar group, from week 6 until week 8, compared to rabbits in the other groups. The digestibility of the various nutrients was higher in the biochar supplemented groups, except for ash, which was higher in the control group than in rabbits that received biochar at 20 g/kg. Rabbits fed control diet had higher haematology parameters than those fed biochar, except for the leukocytes, red cell volume and urea levels. The levels of alanine aminotransferase, alkaline phosphatase and aspartate aminotransferase were elevated in biochar-fed rabbits compared to the control rabbits. The results of this study showed that biochar supplementation at 15 g/kg has potential to enhance the growth, nutrient digestibility, and survivability of weaned rabbit bucks.

Keywords: Growth rate, metabolic health, micro-livestock, sustainability, vegetal carbon

1. Introduction

Rabbits have high growth rate, and feed utilisation efficiency, short breeding cycle and large litter size [1, 2]. Rabbit production is profitable for smallholder farming households and communities that make up the dominant farming population in developing countries. Rabbit production is a low cost and odor-free venture, and a valuable source of raw materials for Luxury curtains, hats, pelts, cushions, and other agro-allied industrial applications [3, 4]. Intensified rabbit production at the intersection of safe dietary additives, animal welfare, and ethically compliant husbandry techniques, can therefore be a significant contributor to food security, poverty alleviation and protein supply in developing countries.

Rabbit meat is high in protein (about 20.8%), with low concentrations of fat, sodium and cholesterol [5, 6]. Due to its high nutritive value, rabbit meat can help curb protein deficiency at all levels. Despite the capacity for high feed conversion ratio and protein synthesis, limited research still exists on the use of safe and locally sourced feed additives such as biochar in rabbit production. Nutritional trials have shown that biochar has demonstrable growth promoting and immune-modulatory benefits. Biochar is a carbon-rich and non-toxic feed additive, with toxin-binding and electron mediatory effects, resulting from the low-temperature (350 and 1000°C) pyrolysis of biomass [7, 8]. Biochar was approved for animal feed supplementation by the European Union (EU) Regulation, No. 68/2013 that listed "vegetal carbon; (charcoal)" as a feed item [8, 9, 10]. The organic resources used for biochar production

Could be trunk, rootstocks, harvest leftovers, cotton fibers, or biogas plant fermentation remnants [9, 11]. The porous structure and large surface area of biochar enables it to absorb gases, toxins, microorganisms, inorganic nutrients, and soluble organic wastes [12, 13]. Dietary supplementation of biochar in livestock enhances animal performance and health, increases weight gain, feed intake, digestive efficiency, improves the animal's ability to absorb dietary energy, and increases the quantity and quality of the resulting animal products [8, 14, 15]. The capacity of biochar to enhance performance and immune function in animals has been attributed to the detoxifying and microbial adsorption capacities of the feed additive [10, 16]. Biochar supplementation has been investigated in goats [17], sheep [18], cattle [16, 19], pigs [12, 20], layers [21], turkey poults [22], broilers [23], quails [24], and aquaculture [25].

The potential of biochar to exert a positive impact on animal performance and health parameters has been established. Currently, only a few studies exist on the dietary effects of biochar in rabbits [26, 27]. Given the inconsistent results reported in some studies, and the paucity of literature on biochar supplementation in rabbits, the present study was conducted to determine the dietary supplementation effect of biochar on weaned male rabbits.

1. Materials and methods

2.1. Ethical and animal welfare considerations

The study was conducted in accordance with the guidelines approved by the Institutional Committee on Animal Care and Use of the Faculty of Veterinary Medicine, University of Nigeria (reference number UNN/FVM/A024/0051). High level of care was provided for the animals to ensure that clinical problems were detected early and disease spread minimised.

2.2. Screening, selection and management considerations prior to the experiment

Prior to the experiment, the rabbits were clinically examined by a veterinarian to confirm the absence of clinical signs of respiratory infection, gastroenteritis, ectoparasites, or other overt health abnormalities. The animals were selected for the trial based on the following selection criteria; (a) uniform age (35 ± 2 days), (b) absence of visible morphological deformities, (c) normal faecal consistency, and (d) responsive behavior to handling. Baseline faecal samples (approximately 5 g per rabbit) were collected directly from the rectal ampulla using sterile swabs and submitted to the Veterinary Parasitology Laboratory of the University of Nigeria, for microscopic examination of parasitic infections. Only rabbits cleared of gastrointestinal helminths and protozoan parasites were used for the study. Body condition scoring was also performed by a licensed veterinarian using a five-point scale (1 = emaciated, 5 = obese), with only animals scoring 3-3.50 (moderate condition) chosen, to ensure uniform metabolic status at the commencement of the trial.

Before placement in experimental pens, the rabbits were exposed to an acclimatisation period of 14 days, during which they were fed a standard commercial diet. On days 3 and 10 of acclimatisation, all rabbits received subcutaneous injections of ivermectin (Vetrimox injection 1%, 200 µg/kg body weight, administered at 0.1 ml per 100 g body weight (Vetamed Limited, Lagos, Nigeria) to eliminate potential endoparasites. On day 5 of acclimatisation, all the animals received oral probiotic supplementation (Protexin Avian, containing *Lactobacillus acidophilus*, *L. casei*, *L. rhamnosus*, *Bifidobacterium bifidum*, *B. longum*, and *Enterococcus*

faecium at 1×10^9 CFU/g; Protexin Limited, Devon, United Kingdom). The probiotic was administered in the drinking water according to the manufacturer's recommendation to achieve beneficial gut microbiota in the experimental animals. The rabbits were monitored daily, and sick animals separated into isolation pens, equipped with separate feeders and watering troughs. Study location, experimental design and management of animals The study was conducted at the Rabbit Production Unit of the Department of Animal Science Teaching and Research Farm, University of Nigeria (UNN), Nsukka (latitude 6.86°N, longitude 7.49°E; elevation 447 m above sea level). Nsukka experiences a tropical guinea savanna climate, characterised by a mean annual rainfall of approximately 1,600 mm, distributed between May and October [28]. The study was carried out during the dry season (January to March), when ambient air temperature ranged between 22 and 26°C, with fluctuations in relative humidity between 45 and 65%, and a consistent photo period consisting of natural daylight variations of approximately 12 hours light and 12 hours darkness. A total of 36, healthy, weaned male rabbits, of Hyla strain, with an average initial weight of 500 g, were sourced from the Rabbit Production Unit of the UNN Experimental Farm, with an established, genetically documented rabbit breeding colony. The rabbits were randomly assigned at 35 days of age to three dietary treatments consisting of 3 replications of rabbits in a replicate. Experimental trial lasted for 56 days during which time the rabbits were fed *ad libitum* from the point of placement at 35 days of age, until slaughter age at 91 days of age. Each rabbit was housed individually in standardized galvanised steel wire mesh cages (50 × 40 × 40 cm; internal volume, 80 liters). All cages were positioned at uniform height (1.2 m above floor level) on a three-tier metal stand (materials: 40 × 40 × 3 mm steel square pipe; dimensions: 180 × 120 × 150 cm) fabricated locally to specification. Enrichment materials included: (a) wooden gnawing sticks (*Acacia* sp., diameter 12 mm, length 150 mm), replaced every 7 days; (b) newspaper bedding materials (approximately 50 g per cage) placed on the floor below the mesh; and (c) cardboard box shelters (15 × 15 × 10 cm) fitted under each cage to provide refuge and simulate burrow-like structures. Animals were handled gently and minimally, particularly during non-data collection periods, to minimise stress-induced physiological perturbation.

2.3. Biochar preparation and production

The experimental biochar was produced through the low-temperature pyrolysis of mixed hardwood biomass, using a locally fabricated cylindrical shaped metal kiln. The biomass consisted principally of *Acacia* and *Eucalyptus* sp. collected locally from forestry pruning operations. The biomass was sun-dried for 14 days, during which time the moisture content was reduced to 12%. Thereafter, the dried biomass was loosely stacked on the grill to a height of 1.2 m to permit air circulation during pyrolysis. The thermochemical heating was initiated by igniting kindling wood underneath the locally made kiln, designed to have a large heating chamber that extends to the tapered funnel-top with an airtight cover for controlling low-oxygen conditions during pyrolysis (Figure 1). Pyrolytic temperature was monitored using a calibrated thermocouple (Cole-Palmer, USA) inserted through the funnel-top into the heating chamber, with readings recorded every 30 minutes. The pyrolysis process continued for four hours once temperature in the heating chamber reached 500-600°C, maintained by periodic open-and-close adjustments of top-cover to restrict oxygen inflow while preventing complete kiln starvation. The resultant char was

unloaded from the kiln and spread on dry concrete floor to cool passively for 48 hours under ambient conditions. Following kiln discharge and subsequent cooling, the biochar (yield, approximately 25-30% initial dry weight of biomass) was passed through a single-stage hammer mill (FAM Model MH 400, motor power 7.5 kW; FAM Maschinenbau GmbH, Halberstadt, Germany), equipped with a 2 mm screen to achieve uniform particle size distribution. The sieved biochar was stored in sealed polyethylene-lined canvas bags maintained in a dry storage room (22-25°C; <70% relative humidity) until needed for dietary treatment inclusion. Proximate composition of the biochar was determined according to official methods of the Association of Official Analytical Chemists; AOAC [29], and the results are shown in Table 1.

2.4. Experimental diets

Three treatment diets were fed to the growing rabbit bucks from point of placement at 35 days of age until the end of the trial at 91 days of age. A standard, commercial formulated basal concentrate feed (Top brand, Premier Feeds Limited), was procured from a reputable feed company. Percentage composition of the basal concentrate feed is shown in Table 2. Biochar was supplemented into the basal diet at 15 g/kg and 20 g/kg. Biochar supplementation was mixed daily into the feed, and any leftover feed was discarded to prevent rancidity or nutritional degradation. Clean water was provided for the rabbits throughout the period of the experiment.

2.5. Data collection

2.5.1. Growth performance

The live body weight of rabbits was measured at the point of placement (day 0) and once a week until the end of the trial, using a calibrated digital electronic balance (Camry Model EK-7601, readability ± 1 g; Camry Electronics, Hangzhou, China). The data obtained was used to determine the average daily weight gain (ADWG) for each buck. The daily feed intake (DFI) was determined as the difference between feed supplied the previous day and the leftover feed remaining in the feeding trough the next day. Average daily feed intake (ADFI) was calculated using the values obtained from the DFI. Feed conversion ratio (FCR) was obtained by dividing the feed intake by the weight increase of the bucks. Protein intake (PI) was calculated as the product of average daily dry matter intake and the analysed crude protein concentration of each diet. Protein efficiency ratio (PER) was calculated by dividing the body weight gain (g) of rabbits by their PI (g) values. Mortality records were kept throughout the trial, and survivability was calculated as follows: $\text{Survivability} = \text{Number of rabbits alive at end of trial} / \text{initial number of rabbit's} \times 100$.

2.5.2. Digestibility trial

At the end of the trial (day 56), two rabbits per replicate were transferred into metabolism cages for a 5-day digestibility trial. The cages were designed to allow for a separate collection of feces and urine. A 2-day pre-collection period was allowed to enable the rabbits to adapt to metabolic cage confinement. The animals were given *ad libitum* access to their respective treatment diets, and faecal samples were collected during days 3-5 of the trial. Total faecal output was weighed daily, oven-dried, ground through a 2 mm sieve using a hammer mill and used for proximate analysis. Apparent nutrient digestibility coefficients were calculated using the standard method of Noblet and Perez [30].

2.5.3. Haematology and serum biochemistry

At the end of the trial (day 56), two rabbits per replicate were randomly selected for blood collection. 2.5 ml of blood was

collected into ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes, via puncture of the marginal ear vein using 25-gauge sterile needles. The rabbits were gently restrained in cloth bags, while blood collection was done between 0900-1000 hours to minimize circadian variation in haematological parameters. The blood collected into the EDTA tubes were analysed using an automated haematology analyser. Blood parameters such as haematocrit, red blood cell count, leukocyte count, haemoglobin concentration, mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration were assayed.

A second set of blood (2.5 ml) was collected into plain serum separator tubes (10 $\times$ 75 mm, clot activator beads, barrier gel; Beckton Dickinson, Franklin Lakes, NJ, USA), for serum analysis. The blood was allowed to clot for 20-30 minutes at room temperature (22-25°C), centrifuged at 3,500 rpm for 15 minutes using a laboratory centrifuge (Sunline Model 5424R, rotor 24-place swinging bucket; Eppendorf AG, Hamburg, Germany) to separate serum, and stored at -20°C until when required for analysis. Serum samples were thawed at room temperature and analysed in duplicates using a semi-automated biochemistry analyser (Randox Daytona plus Analyzer, software version 2.1; Randox Laboratories Limited, Antrim, United Kingdom) with species-specific reagent kits. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed using the enzymatic colorimetric method [31], and the results expressed in international units per liter (IU/L). Alkaline phosphatase (ALP) was determined by reference measurement [32] and expressed in IU/L. Serum urea and creatinine activities were analysed using Raman spectroscopy method [33], and the results expressed in milligrams per deciliter (mg/dL). Uric acid was assayed by enzymatic colorimetric method [34] and expressed in mg/dL.

2.5.4. Proximate analysis of diets and faecal samples

The experimental diets and faecal samples from experimental rabbits were analysed at the Central Analytical Laboratory of the Department of Animal Science, University of Nigeria, Nsukka. The proximate contents (Table 3) of dry matter, crude protein, crude fiber, ether extract, ash, nitrogen-free extract, and metabolisable energy, were determined following the standard procedures of the AOAC [29].

2.6. Statistical analysis

Data were analysed using the statistical analysis software IBM SPSS Statistics Version 22.0 (IBM Corporation, Armonk, NY, USA). The study employed a completely randomised design (CRD) with three dietary treatments, replicated three times, with four rabbits per replicate. Data normality was assessed using the Duncan Multiple Range test ($\alpha = 0.05$) to determine appropriateness of parametric statistical testing. One-way analysis of variance (ANOVA), with the General Linear Model procedure, was used for the dependent variables, with dietary treatment as a fixed factor. The equation below describes the linear model used.

$$Y_{ijk} = \mu + T_i + e_{ijk}$$

Where Y_{ij} = response variable; μ = population mean; T_i = effect of the i th treatment ($i = 1, 2, 3$), and e_{ij} = random error associated with each Y . When F-ratios from ANOVA were significant at the predetermined α level ($p < 0.05$), pairwise comparisons among treatment means were done using Duncan's New Multiple Range Test, and significance of difference declared between if $p < 0.05$.

3. Results

3.1. Growth performance

Table 4 shows the results of feeding varying dietary levels of biochar on the growth performance of weaned rabbits.

Rabbits fed 15 g biochar/kg feed (T2) had higher ($p < 0.05$) final body weight, ADWG, ADFI and FCR compared to rabbits fed 0 g/kg (T1) and 20 g/kg (T3) of dietary biochar. Protein intake and protein efficiency ratios were higher ($p < 0.05$) in T2 group compared to the other treatment groups, except that, rabbits in the 15 g/kg biochar group had similar ($p > 0.05$) protein intake ratio with those in the 20 g/kg group. Survivability was higher ($p < 0.05$) in the T3 group compared to that of rabbits in the other treatment groups. Regardless of the level of inclusion, all biochar-supplemented dietary groups had higher ($p < 0.05$) survivability compared to the control group.

3.2. Weekly body weight changes

Results on the weekly body weight changes of rabbits that received different dietary inclusion levels of biochar are presented in Table 5. From week 1-week 5, rabbits in the T2 group had comparable ($p > 0.05$) body weight changes with rabbits in the control and T3 groups. From week 6, changes in body weight became pronounced, ($p < 0.05$) in the T2 rabbit group, and was sustained until week 8, compared to rabbits in other treatment groups.

3.3. Digestibility parameters

As shown in Table 6, supplementation of biochar at 15 g/kg resulted in higher ($p < 0.05$) digestibility of crude protein, crude fibre, ash and nitrogen-free extract, while ether extract digestibility increased ($p < 0.05$) at 20 g/kg inclusion of biochar. Evidently, the digestibility of the various nutrients was higher ($p < 0.05$) in the biochar supplemented groups, compared to the control, except for ash digestibility where rabbits in the control group had higher ($p < 0.05$) ash digestibility than rabbits in the 20 g/kg dietary biochar group.

3.4. Haematology and serological metabolites

Rabbits fed the control diet (T1) had higher ($p < 0.05$) haematocrit, erythrocytes and haemoglobin concentrations, compared to rabbits fed biochar, regardless of the levels of inclusion in the diet. The reverse was, however, the case for leucocytes, red cell volume, ALT, ALP, and AST levels, with the biochar rabbit groups having higher concentrations (Table 7). Creatinine levels did not differ ($p > 0.05$) among treatment groups, whereas all biochar supplemented groups had higher ($p < 0.05$) urea levels than the control group.

4. Discussion

Emerging research has shown that biochar is a beneficial feed additive with the potential to promote growth and enhance immune functions in livestock, through improved gastrointestinal pH modulation, selective microbial colonisation, toxin adsorption, and enhanced nutrient bioavailability. Biochar tends to exhibit superior effects, at high inclusion levels, reflecting a dose-dependent biphasic response pattern [27, 35, 36].

In the present study, 15 g/kg biochar supplementation increased the final body weight, body weight gain, feed intake, and feed conversion efficiency of growing weaned rabbits. This superior performance at 15 g biochar is likely due to the physicochemical profile of biochar, which plausibly triggered metabolic remodeling that translated into enhanced digestive efficiency and nutrient utilisation in the experimental rabbits [25, 37, 38]. Reports from previous studies showed that biochar supplementation can modulate the gastrointestinal environment, leading to increased bioavailability of nutrients for utilisation, particularly when fed at higher dietary inclusion levels [20, 36, 40]. Evidently, the porous nature and large surface area of biochar confer the high adsorption capacity on the feed additive, allowing it to effectually bind with harmful compounds, toxins, and gases that often interfere with nutrient absorption in the gut. This

stern adsorptive capacity of biochar also extends to proteins, amino acids, and digestive enzymes, thereby creating a more favorable environment for nutrient retention and utilisation [8, 25, 40].

The dose-dependent response observed in the experimental rabbits fed 15 g/kg inclusion, agrees with earlier reports that biochar supplementation follows a quadratic response pattern, wherein low-to-intermediate doses provide optimal benefits [10, 24, 41]. This response pattern likely relates to the balance between beneficial adsorption of harmful compounds, and potential interference with nutrient absorption at increased levels of supplementation in the diet. The superior weekly weight gains observed at a 15 g/kg biochar dosage indicate enhanced benefits that occur with adjustments in the gastrointestinal microbiome. This adaptation phase likely represents the period needed for beneficial microbes to colonise the biochar surfaces and for digestive processes to optimise [42, 43].

Improved digestibility of crude protein, crude fiber, ash, and nitrogen-free extract at the 15 g/kg supplementation of biochar corresponds with prior research demonstrating positive impact of biochar supplementation on nutrient utilisation in farm animals [35, 38]. The observed increase in crude protein digestibility carries significant economic weight, considering the high cost of protein in rabbit diets [44], with improved utilisation directly translating to better growth rates and improved cost-efficiency. Due to the microporous design of biochar, the supplement offers an ecological haven for beneficial microbes to thrive, resulting in better fermentation and increased microbial protein production [36, 37, 45]. The reports of Mirheidari *et al.* [37] showed that biochar supplementation acts as a booster for rumen microbes, directly increasing microbial protein synthesis. Because rabbit ceca are major sites of microbial fermentation (like rumen), it is plausible that feeding biochar to rabbits can result in comparable improvements in nutrient digestion.

The improved fiber digestibility in the biochar-fed rabbits may be attributed to the ability of biochar to modulate pH and provide surface area for fibrolytic bacteria attachment [46, 47]. The highly porous structure of biochar provides microhabitats for beneficial, fiber-degrading microbes [46, 48, 49], while its ability to adsorb inhibitory compounds removes barriers to efficient fiber digestion in the gut [8, 35, 50]. Ether extract digestibility was enhanced at higher biochar inclusion rates, suggesting biochar's positive influence on lipid metabolism and absorption. Possible mechanisms include biochar-mediated bile acid adsorption and recycling [51, 52], or modulation of the gut microbiome to support improved lipid utilisation [36, 53].

The haematological profile offers significant insights into the physiological impact of biochar. The elevated erythrocyte, haemoglobin, and haematocrit levels found in the control subjects is contrary to initial expectations, suggesting that various adaptive mechanisms may be at play. Rabbits in both the control and treatment groups had hemoglobin concentration levels that were within the standard 8-17 g/dL range [54]. Biochar-fed rabbits showed slightly lower red blood cell counts, and this likely suggests enhanced metabolic efficiency rather than anemia, as improved nutrition can lead to better oxygen utilisation and reduced need for compensatory red blood cell production [55, 56].

The elevated leucocyte counts could be a pointer to an enhanced immune function in the biochar supplemented rabbits, aligning with previous reports of the known antimicrobial properties of biochar [56, 57]. This improved immune response translated into superior survival rates for the biochar-treated groups, notably in the rabbits that received 20 g biochar per kg feed.

The antimicrobial properties of biochar have been well-documented, particularly, its ability to bind and neutralise pathogenic bacteria such as *Campylobacter*, *Salmonella*, and *Escherichia* [16, 42, 58]. The reduced disease pressure in the biochar-supplemented rabbits and improved survival rates may thus be linked to the selective adsorption of gram-negative bacteria and their endotoxins by biochar. Overall, rabbits fed biochar supplemented diets had enhanced nutrient digestibility and feed conversion efficiency of the biochar fed rabbits, and improved health status and disease resistance. Well-nourished animals typically show greater resilience to environmental stresses and pathogenic challenges [27, 58], which may explain the higher survivability observed in the present study for the biochar treatment groups.

The higher red cell volume resulting from all levels of biochar inclusion is indicative of improved red blood cell maturation and function, and possible enhancement in mineral absorption. Vijayaraghavan [59] had earlier reported that biochar contains significant amounts of minerals, including magnesium, potassium, and calcium, which are essential for proper haematopoiesis and cellular function.

There was a significant increase in the levels of ALT, ALP, and AST, among rabbits fed diets containing biochar. While elevated liver enzymes typically indicate hepatocellular damage, normal physiological variations in liver enzyme levels can also occur due to increased metabolic activity, enhanced protein synthesis, or adaptive responses to dietary changes [10, 35, 60]. Hence, the observed increases in the levels of these liver enzymes in our study may not be due to hepatotoxicity, and future studies can be done to confirm these claims.

The absence of significant differences in creatinine levels between the control and biochar groups indicates that renal function remained unaffected by the dietary treatments, suggesting that the observed metabolic changes were specific to hepatic processes rather than systemic toxicity. Elevated urea levels in the biochar-treated rabbits suggested improved

protein utilisation and higher protein intake. High protein intakes/utilisation naturally increases urea production as a byproduct of amino acid breakdown [60]. Our study observed increased protein consumption in groups fed biochar, consistent with poultry literature suggesting that initial biochar-induced elevations in liver enzymes are temporary and often normalise as health and performance improve over time [10, 22]. Nutrition studies have shown that adapting to new diets can initially cause an increase in metabolic enzyme activity associated with enhanced overall performance.

Table 1: Proximate composition of experimental biochar

Proximate nutrients	kg
Dry matter	5.50
Crude protein	8.60
Crude fiber	2.50
Ether extract	0.01
Ash	8.50
Nitrogen-free extract	77.4

Table 2: Ingredient composition of basal concentrate feed

Ingredients	%
Ground maize	42.50
Soybean meal	18.00
Wheat bran	15.00
Fish meal	6.50
Bone meal	4.00
Vitamin-mineral premix	2.50
Salt (NaCl)	1.50

All values are on a as-offered basis except where specified. Vitamin-Mineral Premix composition: Vitamin A 10,000 IU/kg, Vitamin D₃ 2,000 IU/kg, Vitamin E 50 mg/kg, Vitamin K₃ 2 mg/kg, Vitamin B₁ 2 mg/kg, Vitamin B₂ 4 mg/kg, Niacin 20 mg/kg, Pantothenic acid 10 mg/kg, Vitamin B₁₂ 0.02 mg/kg, Manganese 80 mg/kg, Zinc 100 mg/kg, Iron 80 mg/kg, Copper 10 mg/kg, Iodine 1 mg/kg, Cobalt 0.2 mg/kg.

Table 3: Proximate compositions of experimental diets and faecal matter

	T1	T2	T3
Dietary nutrients			
Moisture	10.05	11.44	11.99
Crude protein	21.70	20.60	20.06
Crude fiber	7.80	8.90	9.01
Ether extract	3.70	3.08	2.99
Ash	2.50	3.62	3.98
NFE	54.25	52.36	51.97
Faecal nutrients			
Moisture	10.15	9.91	9.89
Crude protein	18.05	17.60	17.20
Crude fiber	5.50	5.60	5.91
Ether extract	3.50	1.50	1.00
Ash	1.00	2.80	3.13
NFE	61.80	62.59	62.87

NFE: Nitrogen-free extract; T1; Control (0% biochar kg⁻¹ feed) T2 (control diet + 15 g biochar kg⁻¹ feed); T3 (control diet + 20 g biochar kg⁻¹ feed)

Table 4: Growth indices of weaned rabbits fed with biochar for 56 days (g)

Growth indices (g)	T1 (control)	T2 (15 g/kg)	T3 (20 g/kg)	SEM	p-value
Initial body weight	540 ± 18.2	545 ± 98.2	557 ± 71.60	23.8	0.756
Final body weight	1089 ± 35.8 ^b	1530 ± 60.8 ^a	1232 ± 151 ^b	60.5	0.001
Average daily weight gain	549 ± 50.5 ^b	984 ± 140 ^a	675 ± 112 ^b	54.7	0.003
Average daily feed intake	1098 ± 101 ^b	1527 ± 204 ^a	1252 ± 141 ^b	60.6	0.026
Feed conversion ratio (%)	49.9 ± 0.14 ^c	64.4 ± 2.10 ^a	53.7 ± 3.20 ^b	1.68	0.000
Protein intake	238 ± 21.9 ^b	311 ± 39.3 ^a	288 ± 31.9 ^{ab}	11.7	0.041
Protein efficiency ratio	2.30 ± 0.00 ^b	3.13 ± 0.10 ^a	2.19 ± 0.29 ^b	0.12	0.000
Survivability (%)	75.9 ± 0.60 ^c	85.9 ± 0.40 ^b	90.1 ± 0.01 ^a	1.61	0.000

Mean values having different superscripts in a row are statistically significant at 5% probability level; SEM: standard error of mean; T1: control, 0% biochar kg⁻¹ feed; T2: control diet + 15 g biochar kg⁻¹ feed; T3: control diet + 20 g biochar kg⁻¹ feed.

Table 5: Body weight changes of weaned rabbits fed with biochar for 56 days (g)

Body weights (g)	T1	T2	T3	SEM	p-value
Initial body weight	540 ± 18.2	545 ± 98.2	557 ± 71.9	23.8	0.756
Week 1	567 ± 29.3	598 ± 96.3	584 ± 72.8	21.6	0.138
Week 2	678 ± 42.3	629 ± 78.6	692 ± 106	28.0	0.061
Week 3	745 ± 51.8	721 ± 42.0 ^a	765 ± 103	29.1	0.150
Week 4	788 ± 84.6	906 ± 38.2	841 ± 108	33.3	0.140
Week 5	879 ± 25.5 ^b	1022 ± 87.3 ^a	926 ± 144 ^{ab}	33.8	0.047
Week 6	938 ± 22.2 ^b	1183 ± 76.4 ^a	1003 ± 132 ^b	42.3	0.004
Week 7	1001 ± 41.9 ^b	1316 ± 120 ^a	1114 ± 155 ^b	48.9	0.010
Week 8	1089 ± 35.8 ^b	1530 ± 60.8 ^a	1232 ± 151 ^b	60.5	0.001
Average body weight	803 ± 15.0 ^b	931 ± 13.9 ^a	857 ± 111 ^{ab}	29.9	0.005

Mean values having different superscripts in a row are statistically significant at 5% probability level; SEM: standard error of mean; T1: control, 0% biochar kg⁻¹ feed; T2: control diet + 15 g biochar kg⁻¹ feed; T3: control diet + 20 g biochar kg⁻¹ feed.

Table 6: Effect of biochar supplemented diets on apparent digestibility (%) of rabbits

Parameters	T1	T2	T3	SEM	p-value
Crude protein	35.2 ± 0.01 ^c	69.5 ± 0.02 ^a	63.2 ± 0.01 ^b	3.89	0.000
Crude fiber	45.1 ± 0.01 ^c	77.5 ± 0.02 ^a	71.9 ± 0.01 ^b	3.78	0.000
Ether extract	26.3 ± 0.00 ^c	82.6 ± 0.02 ^b	85.7 ± 0.01 ^a	7.15	0.000
Ash	68.8 ± 0.01 ^b	72.4 ± 0.00 ^a	65.5 ± 0.01 ^c	0.81	0.000
Nitrogen-free extract	11.3 ± 0.00 ^c	57.3 ± 0.02 ^a	48.2 ± 0.01 ^b	5.23	0.000

Mean values having different superscripts in a row are statistically significant at 5% probability level; SEM – standard error of mean; T1; Control (0 g biochar kg⁻¹ feed); T2 (control diet + 15 g biochar kg⁻¹ feed); T3 (control diet + 20 g biochar kg⁻¹ feed).

Table 7: Haematology and serology parameters of rabbits fed biochar supplemented diets

Parameters	T1	T2	T3	SEM	p-value
Haematocrit (%)	40.0 ± 0.00 ^a	35.7 ± 0.57 ^b	33.3 ± 0.57 ^c	0.76	0.000
Erythrocytes (×10 ⁶ mm ³)	10.9 ± 0.08 ^a	8.48 ± 0.14 ^b	8.21 ± 0.09 ^c	0.32	0.000
Leukocytes (×10 ⁹ mm ³)	9500 ± 100 ^b	12000 ± 400 ^a	1266 ± 1059 ^a	384	0.001
Haemoglobin conc. (g/dL)	10.3 ± 0.01 ^a	8.50 ± 0.30 ^b	8.10 ± 0.10 ^b	0.26	0.000
Red cell volume (fL)	36.8 ± 0.26 ^c	42.1 ± 1.30 ^a	40.6 ± 0.68 ^b	0.80	0.000
Red cell haemoglobin (pg)	9.53 ± 0.03	10.0 ± 0.52	9.86 ± 0.03	0.12	0.162
Red cell haem. conc. (g/dL)	25.8 ± 0.03 ^a	23.8 ± 0.54 ^b	24.3 ± 0.49 ^{ab}	0.35	0.018
Alanine aminotrans. (IU/L)	34.7 ± 0.57 ^b	65.3 ± 1.15 ^a	65.7 ± 3.05 ^a	3.86	0.000
Alkaline phosphatase (IU/L)	53.0 ± 2.64 ^c	81.7 ± 2.51 ^b	90.7 ± 3.05 ^a	4.28	0.000
Aspartate aminotrans. (IU/L)	24.3 ± 0.57 ^c	45.7 ± 2.08 ^b	55.3 ± 3.05 ^a	3.43	0.000
Creatinine (mg/dL)	4.62 ± 0.30	6.77 ± 0.34	6.87 ± 0.09	0.43	0.102
Urea (mg/dL)	39.0 ± 1.00 ^b	66.7 ± 1.15 ^a	69.0 ± 5.56 ^a	3.67	0.000

Mean values having different superscripts in a row are statistically significant at 5% probability level: Haem. – Haemoglobin; conc. – concentration; aminotrans. Aminotransferase; SEM – standard error of mean; T1; Control (0% biochar kg⁻¹ feed) T2 (control diet + 15 g biochar kg⁻¹ feed); T3 (control diet + 20 g biochar kg⁻¹ feed)

5. Conclusion

This study demonstrated that supplementing weaned rabbit bucks with biochar improved their growth, nutrient digestibility, and survival rates. Higher biochar levels slightly increased liver enzymes, likely reflecting an adaptive metabolic response rather than liver damage. Furthermore, creatinine levels remained consistent across dietary groups, highlighting the improved performance of rabbits in the biochar groups, without any sign of kidney damage or dysfunction. The increased leucocyte counts and improved survivability, contributes to the growing body of evidence supporting biochar as a multifunctional feed additive that improves animal performance and health status, and ensures sustainability. Overall, the findings of the present study suggest that up to 15 g/kg of biochar has the potential to enhance growth, nutrient digestion, immune response, and survival rates of weaned rabbit bucks. The observed temporal and dose-dependent effects observed with biochar supplementation, establish critical protocols for biochar application in commercial rabbit farming, and the resulting performance improvements address significant bottlenecks in the rabbit industry.

5.1. Practical implications and applications

This study offers significant, practical, and economical insights for industrial rabbit farming, suggesting that at 15

g/kg inclusion rate, biochar can be readily utilized as a dietary supplement for rabbit production. By improving growth rates and feed conversion, biochar supplementation can increase profitability and reduce input costs. Furthermore, biochar can boost the survival rate of rabbits, making it an essential feed additive for mitigating the high mortality in livestock caused by environmental stress and diseases in tropical climates. For optimal results in tropical regions, combining this inclusion level of biochar (15 g/kg) with an 18% crude protein diet is recommended to maximise both nutritional efficiency and overall health of rabbits. Beyond animal performance, biochar provides environmental advantages by improving nutrient use and lowering nitrogen excretion. Due to the highly stable nature of biochar, the resulting manure provides lasting soil fertility benefits when used on cropland.

5.2. Limitations and future perspectives

The relatively short duration of this experiment may not be sufficient to fully capture the long-term effects of biochar supplementation on rabbit health and performance. Longer-term studies would be valuable to assess whether the beneficial effects of biochar are sustained over complete production cycles. Also, the single biochar source and production method used in this study limits the generalisability of findings linked to other biochar types. Future research should investigate biochar from different

substrates and production conditions, to optimise the beneficial properties of biochar for rabbit nutrition applications. The elevation of liver enzymes observed at higher biochar inclusion levels requires further investigation through histopathological examination and longer-term monitoring. While the current evidence suggests that these changes may be adaptive rather than pathological, comprehensive toxicological studies may be required to establish biochar as a safe additive for rabbits. Metagenomics and metabolomics approaches could also illuminate the complex interactions between biochar, gut microorganisms, and host metabolism, thus showing how biochar extensively exert its beneficial effects in rabbits.

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