DOSE-DEPENDENT EFFECTS OF BIOCHAR INCLUSION ON GROWTH, HEMATOLOGICAL, MANURE AND CARCASS CHARACTERISTICS OF RABBITS

***Ansah, K.O.,[1] Osafo E.L.K., [2]Osman, A.,[2] Opoku, G.C.,[2]Ansah, A.S.[2]**
[1]*Council for Scientific and Industrial Research –*
Animal Research Institute, P. O. Box AH 20, Achimota, Accra, Ghana.
[2]*Department of Animal Science,*
Kwame Nkrumah University of Science and Technology. Kumasi.
**Corresponding Author's Email: ojake6@yahoo.com*
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ABSTRACT
A 10-week trial was conducted to evaluate effects of graded levels of rice husk biochar (RHB) on growth, digestibility, hematological and carcass characteristics of twenty-four (24) weaned cross-bred rabbits averaging 840 g in weight. Animals were allotted to six dietary treatments in a 3 x 2 factorial arranged in Completely Randomized Design: Brachiaria mulato II (BM) + concentrate without RHB; BM + concentrate with 15 g/kg RHB; BM + concentrate with 30 g/kg RHB (BMB30); Brachiaria decumbens (BD) + concentrate without RHB; BD + concentrate with 15 g/kg RHB and BD + concentrate with 30 g/kg RHB. All data were analyzed statistically with GLM procedure of Minitab®18.1 and means were separated at 5% probability level using Tukey's test. BM contained 35.25% fibre and 8.11% crude protein (CP) and BD contained 32.76% fiber and 6.90% CP. Concentrate contained 22.49% CP, and 11.75% fiber. As RHB was included, CP and fiber contents of concentrates declined. Dry matter (DM), CP and neutral detergent fiber (NDF) intake differed among treatments and ranged from 98.26-124.44 g/day, 8.75-10.92 g/day and 88.19-98.39 g/day with BM based diets having highest DM and CP intakes. Average daily gain (ADG) ranged from 12.74-18.30 g/day and was highest for BD without RHB. Diets had no significant effect (p>0.05) on digestibility of DM and metabolizable energy (ME) but significantly affected (p<0.05) that of CP (61.03-68.34%), organic matter (OM) (51.87-54.31%) and NDF (48.07-57.66%). For hematology, only platelet count (184-216.67 x 10^{*3} / uL) differed significantly (p<0.05) among treatments. RHB inclusion significantly affected (p<0.05) kidneys (11.00-13.33 g) and liver (38.67-52.98 g). N/P/K, C/N (18.37-28.24) and lignin/nitrogen (6.33-8.84) ratios as well as other mineral characteristics assessed for manure indicated RHB improved manure characteristics. Diets with or without RHB inclusion had no detrimental effect on rabbits hence up to 30 g/kg of biochar produced from rice husk can be recommended as a feed additive for rabbits.

Keywords: *rice husk, Brachiaria, additive, concentrate*

INTRODUCTION
Meat production in most parts of Sub-Saharan Africa (SSA), of which Ghana is part, has been below what is demanded by natives hence the over reliance on imports (Frimpong, 2009). Rabbit (*Oryctolagus cuniculus*) production provides a sustainable means for the achievement of food security, poverty alleviation and meeting protein

needs in most SSA households (Idahor *et al*., 2018; Schönfeldt and Hall, 2012). The special attributes of the rabbit, such as low-cost management requirements (Gono *et al*., 2013), short gestation periods (Akinmutimi *et al*., 2006), high birth and growth rates, ability to utilize forage and agricultural by-products and adaptability to a wide range of ecological environments (Abu *et al*., 2008) renders the rabbit ideal for meat production in SSA (Osei *et al*., 2012).

Anti-microbial resistance (Huyghebaert *et al*., 2011; European Union, 2003) and health problems in humans (Hajati and Hazaei, 2010; Saleha *et al*., 2009), arising from the use of antibiotics (non-therapeutic) has led to their ban in Europe and most parts of the world. It is thus imperative to identify and use alternatives that can reduce health implications on humans and pathogen load without compromising growth and productivity of livestock. Biochar, a carbon-rich product obtained through pyrolysis (breaking down by heating at 350-700 °C) or gasification of biomass waste materials in the absence (or under low levels) of oxygen (International Biochar initiative, 2018; Toth and Dou, 2016; Tang *et al*., 2013), can serve this purpose.

Biochar as a livestock feed additive has been reported to improve digestibility, feed efficiency, weight gain and dietary energy absorption (Gerlach and Schmidt, 2012). The appeal for use as an input in animal feeding systems to enhance animal performance and reduce environmental impact stems from the ability of biochar to bind to different chemicals (Thies and Rilling, 2012) and to adsorb microorganisms (Leng, 2014) and toxins (Toth and Dou, 2016). Inclusion of biochar in livestock diets have been reported to detoxify feed, enhance feed intake and digestion, promote animal live weight gain as well as improve the quantity and quality of products such as milk, eggs and meat (Toth and Dou, 2016). Biochar incorporation into livestock feed has however been limited to cattle (Winders *et al*., 2019), poultry (Goiri *et al.,* 2021), pigs (Kim *et al*., 2017) and rarely on sheep (McAvoy *et al*., 2020), goats (Silivong and Preston, 2016) and other domesticated livestock. Limitations on the use of biochar as a feed additive stems from the inadequate and contradicting research findings, potential contamination, knowledge gap of end-users (livestock producers) and affordability as discussed in a review by Lao and Mbega (2020). Schmidt *et al.* (2019) also asserted that the long-term feeding of biochar can potentially shift gastrointestinal tract (GIT) microbiome and adsorb feed nutrients. There is thus the need to estimate safe and appropriate inclusion levels of biochar in the diet of livestock without deleterious effects on growth and other indices of livestock. This study thus investigates the dose-dependent effects of varying inclusion levels of biochar produced from rice husk on growth, hematology, manure and carcass characteristics of rabbits.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at the Micro-livestock section of the Department of Animal Science, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi in the Ashanti Region of Ghana. Day and night time temperatures ranged between 24-30 °C and 20-25 °C respectively whiles the relative humidity was 70% (minimum) and 92% (maximum) during the experimental period (November, 2022 to February, 2023).

Ethical Approval

The study was carried out following approved procedures by the Animal Research and Ethics Committee (AREC, 2022) of the Quality Assurance and Planning Unit of the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana with reference **KNUST/AREC/C.1** and ethical clearance number, **ETHICAL CLEARANCE (KNUST 0030).**

Management of Animals

A total of twenty-four (24) rabbits weaned at 6 weeks' old were divided into 6 groups of 4 rabbits each. At the beginning of the experiment, the average live body weight of these rabbits of mixed breed was 843 ± 3 g. Each rabbit was housed individually. The housing structure was subdivided into individual cubicles measuring 60 cm long, 45 cm wide and 40 cm high (60 x 45 x 40 cm^3). Housing structure was constructed with wood with wire meshed floors and partitions. It was then raised about 45 cm from the ground and about 6 m from the roof of the building within which it was placed. Each cage had two earthenware (clay) pots; one as a drinker and the other as a feeder. Feed and water were offered at 08:30 hours every morning *ad libitum* throughout the study period.

Experimental Feeds and Design

Dietary treatments comprised concentrates with varying inclusions of biochar produced from rice husk and *Brachiaria sp.* (*B. decumbens or B. mulato*). Concentrates were formulated to be isonitrogenous. Biochar was obtained from Lucky Star company at Bamang in the Ejisu Juabeng Municipality in the Ashanti Region of Ghana under the supervision of the CSIR-Forestry Research Institute at Fumesua in Kumasi. Other ingredients for the concentrates were obtained from the open market and *Brachiaria* grass harvested daily from cultivated stands at the Department of Animal Science, KNUST.

Rabbits were fed for 70 days including two weeks' adaptation to experimental diets. *Brachiaria sp.* (*B. mulato* and *B. decumbens*) were the basal feed and was supplemented with concentrates containing 0, 15 and 30 g/kg rice husk Biochar (RHB) in a 3x2 factorial arranged in a completely randomized design (CRD) with four rabbits per treatment. Six (6) dietary treatments used were: *Brachiaria mulato II* (BM) + concentrate without RHB (BMB0); BM + concentrate with 15 g/kg RHB (BMB15); BM + concentrate with 30 g/kg RHB (BMB30); *Brachiaria decumbens* (BD) + concentrate without RHB (BDB0); BD + concentrate with 15 g/kg RHB (BDB15) and BD + concentrate with 30 g/kg RHB (BDB30). Grass and concentrates were fed together every morning. Grasses were cut daily from cultivated stands and fed together with formulated concentrates every morning at 100 g grass and 5% body weight of rabbits for concentrates.

Table 1 shows the gross and calculated composition of concentrates mixed with RHB.

Representative samples of concentrate and *B. mulato* and *B. decumbens* were collected and

Table 1: Gross and calculated composition of concentrate

	Calculated Nutrient Compositions of concentrates, %							Gross Composition, %
	CP	**ASH**	**EE**	**CF**	**DM**	**NFE**	**ME (Kcal/kg)**	
Millet mash	5.96	1.18	2.01	1.42	56.64	48.43	2104.16	58.5
Soya Bean Meal	3.04	1.503	0.32	0.92	16.83	13.22	607.91	19.2
Wheat Bran	9.6	0.92	0.39	2.21	18.09	13.28	858.73	20.0
DCP	0	0	0	0	0	0	0	1.0
Vitamin Premix	0	0	0	0	0	0	0	0.5
Salt	0	0	0	0	0	0	0	0.5
Toxin binder	0	0	0	0	0	0	0	0.5
Total	18.60	3.60	2.72	4.54	91.564	74.94	3570.79	100

** Premix composition per kg diet: Vitamin A – 3,200,000iu, Vitamin D3 1,200iu, Vitamin E 3,200iu Vitamin K3 800mg, Vitamin B1 – 400mcg, Selenium (Se) 40mg, Manganese (Mn) 32,000mg, Pantothenic acid 2000mg, Folic acid 200mg, Choline chloride 60000mg, Iron (Fe) 8,000mg, (Cu) 3,200mg, Zinc (Zn) 2,00mg, Cobalt (Co) 90mg, Iodine (I) 800mg.*
CP-Crude protein; EE-Ether extract; CF-Crude Fiber; DM-Dry matter; NFE-Nitrogen free extract; Metabolizable energy; DCP-Di Calcium Phosphate

dried in an oven at 60°C for 48 hours. Dried feed samples were ground to pass a 1.0 mm sieve and sent to ascertain their mineral and proximate compositions as described by AOAC (2000) at the Nutrition and Soil Science laboratories at the Department of Animal Science and Department of Crops and Soil Science at KNUST, Kumasi-Ghana. Proximate compositions analyzed for included Dry matter (DM), organic matter (OM), Ash, Ether Extract (EE), Crude protein (CP), Crude Fiber (CF) and Nitrogen free extract (NFE). Metabolizable energy (ME) was calculated using the formula, ME = 37 x %CP + 81.8 x %EE + 35 x %NFE as outlined by Pauzenga (1985). The neutral detergent fibre (NDF) and the acid detergent fibre (ADF) fractions were found based on the methods described by Goering and Van Soest (1970). Following the methodology developed by Van Soest *et al.* (1991), acid detergent lignin (ADL) was also determined.

Feed Intake and Growth parameters

Feed (*BM, BD* and concentrates with 0, 15 and 30 g/kg RHB) was weighed every morning and offered to animals in their individual cubicles. Feed left was collected and weighed every morning and deducted from feed offered to estimated voluntary intake of feed offered. Animals were weighed weekly and at the end of the 10th week. Initial weight was deducted from the final weight to ascertain the weight gain. Weight gain was divided by the number of days (56 days) to derive the average daily gain.

Apparent Digestibility study and assessment of Manure characteristics

Faecal samples were collected every morning into zip lock bags for the last seven (7) days of the rabbit feeding trial. Daily fecal samples were stored in a freezer after collection. Fecal samples were thawed and mixed together by dietary treatments. Five hundred (500) grams of each sample was taken and oven dried at 60°C to a constant weight and cooled in desiccators to prevent atmospheric moisture absorption. The dried samples were subdivided into two samples of 250 g each per treatment and one half sent to the Nutrition laboratory at the Department of Animal Science at KNUST for determination of their proximate compositions using AOAC (2000) procedures. Digestibility of nutrients was calculated by inserting determined proximate compositions of faeces and feed into the formula:

Apparent digestibility

$$= \frac{\%\,\text{nutrientin feed} - \%\,\text{nutrientin faces}}{\%\,\text{nutrientin feed}} \times 100$$

The other halves of 250 g per treatment were sent to the Department of Crops and Soil Science at KNUST for laboratory assessment of manure characteristics such as Dry matter (DM), Ash, Organic matter (OM), pH, Total Nitrogen (N), Organic Carbon (OC), Available Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), Sodium (Na) and lignin. Carbon to Nitrogen ratio (C/N) and Lignin to Nitrogen ratios were also calculated from the analyzed results.

Hematological Analysis

Three rabbits were randomly selected from each treatment, bled by puncturing the ear vein with sterile syringes and free flowing blood collected into labeled test tubes with the anti-coagulant, Ethylene Diamine Tetra Acetic Acid (EDTA) for hematological analysis. Samples collected in EDTA reinforced tubes were sent to the Ashanti Regional Veterinary Office of the Ministry of Food and Agriculture, Kumasi-Ghana. An *SK8800 Veterinary Full-auto Hematology Analyzer®* was used to ascertain the effect of dietary treatments on blood parameters such as White Blood Cell (WBC) count, Red Blood Cell (RBC) count, Hemoglobin (HGB), Packed cell volume (PCV), Mean Cell Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and Platelet (PLT) count.

Carcass Evaluation

The rabbits were prepared for carcass evaluation when they were finished, that is at the end of the 10th week, where they were about 16 weeks old. A total of eighteen rabbits (three animals per treatment) were selected, starved for 12 hours and slaughtered for carcass study. The slaughter weight, warm and chilled carcass weight, dressing percentage, full and empty gastro intestinal tract (GIT) weight and organs like liver, lungs (and trachea), spleen, kidney and heart were taken.

Statistical Analysis

Data on growth and other performance indices were compared using four rabbits in each treatment as replications. All data were analyzed using the general linear model (GLM) of Minitab® version 18.1 (Minitab, 2017) as a 3x2 factorial arranged in a completely randomized design (CRD) with four replications. Test for significant differences among treatment means was conducted using Tukey's test at 5% significance. Model applied for analysis of all rabbit feeding trial parameters was;

$$Y_{ijk} = \mu + B_i + G_j + (BG)_{ij} + e_{ijk},$$

Where Y_{ijk} was the observation; μ was the overall mean for each parameter; B_i was the fixed effect of the $_i th$ level of Biochar; G_j was the fixed effect of the $_j th$ grass (*Brachiaria sp.)*; BG_{ij} is the fixed interaction effect between jth grass type and the $_i th$ level of Biochar and e_{ijk} is random residual error.

RESULTS AND DISCUSSION

Chemical composition of feed

Table 2 shows the proximate and fibre fraction composition of grasses and concentrate fed. The CP of BM was higher (8.11%) than that of BD (6.90%). Although concentrates were formulated to be isonitrogenous and isocaloric, the addition of RHB of 15-30g/kg led to a reduction in the DM, CP and ME contents. Decrease in DM as biochar inclusion was increased indicates the hygroscopic (water absorption) nature of biochar (Tan *et al*., 2021). Crude Protein and ME for basal diets (BM and BD) and concentrates varied numerically from 6.9- 8.11% CP and 2531.86-2539.86 Kcal/kg ME for the basal diets and 20.19-22.49% CP and 3104.89-3177.07 Kcal/kg ME for concentrates.

Table 2: Chemical composition of Grass and Concentrates

	Analysed chemical composition of forages and concentrate				
	Forages		Concentrates		
	Brachiaria mulato (BM)	***Brachiaria decumbens (BD)***	**0 g/kg RHB**	**15 g/kg RHB**	**30 g/kg RHB**
MOISTURE, %	7.61	8.30	6.03	8.66	9.91
DM, %	92.40	91.71	93.98	91.35	90.10
ASH, %	4.26	7.55	7.19	9.20	12.89
CP, %	8.11	6.90	22.49	21.61	20.19
EE, %	8.21	8.69	5.74	6.61	5.72
NFE, %	44.18	44.11	51.23	51.25	53.25
NDF, %	71.22	74.20	53.05	59.05	69.05
ADF, %	39.15	46.40	14.63	22.20	28.97
ADL, %	4.99	5.86	11.20	11.95	12.51
ME (Kcal/kg)	2539.86	2531.86	3177.07	3159.643	3104.892

CP-Crude protein; EE-Ether extract; CF-Crude Fiber; DM-Dry matter; NFE-Nitrogen free extract; Metabolizable energy; NDF- Neutral detergent fiber; ADF-Acid detergent fiber; ADL-Acid detergent lignin

Ash, NDF, ADF and ADL contents also increased as RHB inclusion level was increased. Metabolizable Energy, CP and DM contents were nominally highest for concentrates without RHB. Ash, ADL, NDF and ADF were higher in concentrates with RHB inclusion with the highest values recorded for concentrates with 30 g/kg RHB inclusion. Concentrates showed higher values for CP, ADL, Ash, NFE and ME than the respective values for BM and BD, whereas BD and BM showed higher values of CF, DM, NDF, ADF and EE.

The CP content of concentrates with RHB was higher than the reported values of about 16-18% reported by Owoleke *et al*. (2016) for growing rabbits. Crude protein, EE and CF of BD and BM-RHB based diets used in this study was also higher than the 12% of CP, 14% of crude fibre, and 2% of EE daily requirements for rabbits suggested by Cheeke (1987). Comparatively, higher CP (8.1%), ash (7.8%) and OM (92.2%) as well as lower DM (20.9 %), EE (2.8%) and NDF (63.1%) values have been reported for *Brachiaria decumbens* (Rahman *et al*., 2020). Higher values of CP and ash for BD have also been reported by Binuomote *et al*. (2019) for *B. decumbens.* Crude protein, EE and CF obtained for *B. mulato* was higher than those reported by Daning and Riyanto (2017). Crude protein (8.5%) reported by Imran *et al*. (2016) for BM was, however, similar to this study. Variation in CP, NDF, Ash and other nutrient contents of forages in comparison to other works could be attributed to factors such as age, stage of maturity differences in climate and location of study (Suhaimi *et al*., 2017; Silva *et al*., 2016).

Feed intake

Table 3 shows the effects of RHB on nutrient intake and growth performance of rabbits. Feed intake is generally directly proportional to nutrient intake as the more feed an animal consumes,

Table 3: Nutrient Intake and Growth performance of Rabbits fed diets containing graded levels of Rice Husk biochar (RHB)

GRASS	*Brachiaria mulato*			*Brachiaria decumbens*			SEM
BIOCHAR	0 g/kg	15 g/kg	30 g/kg	0 g/kg	15 g/kg	30 g/kg	
Nutrient Intake, gday^{-1}							
DM	116.71^{ab}	124.44^{a}	114.44^{b}	102.78^{c}	101.45^{c}	98.26^{c}	3.56
ASH	5.38^{a}	5.73^{a}	5.27^{a}	10.01^{b}	9.89^{b}	9.57^{b}	0.26
CP	10.24^{ab}	10.92^{a}	10.04^{ab}	9.15^{c}	9.04^{c}	8.75^{c}	0.32
EE	10.37^{bc}	11.06^{abc}	10.17^{c}	11.52^{a}	11.38^{ab}	11.01^{abc}	0.36
NDF	89.96^{ab}	95.92^{ab}	88.19^{b}	98.39^{a}	97.17^{a}	94.06^{ab}	3.06
ADF	49.45^{a}	52.72^{a}	48.48^{a}	61.52^{b}	60.76^{b}	58.82^{b}	1.82
ADL	6.30^{a}	6.72^{a}	6.18^{a}	7.77^{b}	7.68^{b}	7.43^{b}	0.23
OM	111.33^{a}	118.71^{a}	109.15^{a}	92.77^{b}	91.62^{b}	88.69^{b}	3.32
NFE	55.80^{a}	59.50^{a}	54.71^{a}	58.49^{a}	57.77^{a}	55.91^{a}	1.86
Growth							
Initial weight, g	842.00	843.00	844.00	843.00	842.00	840.00	119.00
Final Weight, g	1933.00^{a}	2015.00^{a}	1950.00^{a}	2125.00^{a}	1927.00^{a}	1764.00^{a}	140.00
Weight Gain, g	972.00	1172.00	1106.00	1282.00	1074.00	924.00^{a}	168.00
ADG, g/day	17.36^{a}	20.93^{a}	19.74^{a}	22.90^{a}	19.17^{a}	16.50^{a}	3.00
FCR	7.54^{ab}	5.71^{b}	6.71^{ab}	5.84^{b}	6.96^{ab}	7.86^{b}	0.75
BWC, %	6.55^{a}	6.80^{a}	6.46^{a}	6.24^{a}	6.88^{a}	7.32^{a}	0.62

Means in the same row with different superscripts are significantly different (p<0.05); SEM- Standard Error of Mean; ADG-Average daily gain; FCR-Feed conversion ratio; BWC- Percent Body weight consumed; CP-Crude protein; EE-Ether extract; CF-Crude Fiber; DM-Dry matter; NFE-Nitrogen free extract; Metabolizable energy;NDF- Neutral detergent fiber; ADF-Acid detergent fiber; ADL-Acid detergent lignin the more nutrients it consumes. Nutrient intakes were generally higher for DM, CP and OM for BM based diets than for BD based diets.

The reverse was observed for Ash, NDF, ADF, ADL and EE intakes. Significant differences ($p<0.05$) were observed for all except NFE intake ($p>0.05$). Nutrient intakes ranged from 98.26-102.78 g/day DM, 88.69-92.77 g/day OM, 8.75-9.15 g/day CP, 94.06-98.39 g/day NDF, 11.01-11.52 g/day EE, 9.57-10.01 g/day Ash, 58.82-61.52 g/day ADF, 7.77-7.43 g/day ADL and 55.91-58.49 g/day NFE for BD grass-based diets. For BM grass-based diets, nutrient intake ranged from 114.44-124.44 g/day DM, 109.15-118.71 g/day OM, 10.04-10.92 g/day CP, 88.19-95.92 g/day NDF, 10.17-11.06 g/day EE, 5.27-5.73 g/day Ash, 49.45-52.72 g/day ADF, 6.1-6.72 g/day ADL and 54.71–59.50 g/day NFE.

Rabbits fed with BM and concentrates with 15 g/kg RHB diet showed significantly ($p<0.05$) higher (10.92 g/day) CP intake followed by rabbits fed with BM and concentrates without RHB diet (10.24 g/day). Similarly, higher DM (124.44 g/day), NFE (59.50 g/day) and OM (118.71 g/day) intakes were observed for rabbits on BM and concentrates with 15 g/kg diet. This indicates that optimum DM, CP, NFE and OM intakes were observed at the BM-15g/kg RHB inclusion level. Rabbits fed with *Brachiaria decumbens* and concentrate diets without RHB showed the highest EE (11.52 g/day), Ash (10.01 g/day), NDF (98.39 g/day), ADF (61.52 g/day) and ADL (7.77 g/day) intakes followed by *Brachiaria decumbens* and concentrate diets with 15 g/kg RHB with 9.89 g/day Ash, 11.38 g/day EE, 97.17 g/day NDF, 60.76 g/day ADF and 7.68 d/day ADL intakes. Hence optimum EE, Ash, NDF, ADF, and ADL intakes were observed at the BD-concentrate diets without RHB inclusion (0 g/kg). Crude protein intakes for BM based diets increased at 15 g/kg RHB inclusion level but decreased as RHB inclusion was increased to 30 g/kg RHB. A similar trend was observed for DM, OM and NFE intakes for all BM based diets. For BD based diets, DM, CP, Ash, EE, NDF, ADF, ADL, OM and NFE intakes decreased as RHB was incorporated in the diets. Differences in intake trends can be attributed to the level of feed intake since it was observed that the higher the total feed intake for *Brachiaria-concentrate-RHB diets,* the higher the nutrient consumption by the rabbits. Also, higher NDF, ADF, ADL, EE and Ash content in BD coupled an increase in these nutrients as RHB was incorporated could have contributed to the higher intakes for these nutrients for the BD-based diets.

Dry Matter intake was higher than the 76-99.99 g/day reported by Dong *et al*. (2008) who fed para grass (*Brachiaria mutica*) water spinach or sweet potato vines with or without fiber supplement. The DM intakes recorded in this study was in agreement with the assertion by Ensminger (1991) that young rabbits with a body weight of 1.8 kg consume feed around 112 g DM/head/day. Aderinola *et al*. (2018) reported lower range of DM (60.81-75.22 g/day), Ash (5.39-6.13g/day) and EE (2.55-2.88 g/day) and higher range of CP (11.31-12.77 g/day) intakes when they fed crossbred weaner rabbits with mixtures of legumes and grass. Rahman *et al*. (2020) also reported higher DM (121.2 ± 4.9 g/day), OM (115 ± 4.7 g/day), similar CP (9.6 g/day), lower EE (3.9 g/day) and NDF (55.8 ± 2.6) intakes when rabbits were fed *Brachiaria decumbens* supplemented with commercial pellets on *ad libitum* basis. These differences in feed intakes could be attributed to the varying nutrient composition, palatability of diets fed as well as the nutrient content of the feed offered. Differences in nutrient intake may be attributed to differences in nutrient composition, level of feed intake and palatability as RHB was incorporated in the diets.

Averagely, total weight gain and the daily body weight gain were similar ($p>0.05$) across all dietary treatments indicating that the final body weight gains were not significantly different between rabbits fed BM and BD – RHB based diets. Average daily gain (ADG) ranged from 12.74 – 18.30 g/day for BD – RHB based diets and 13.33- 16.55 g/day for BM – RHB based diets. Daily gain was least for rabbits on BD and

30 g/kg RHB inclusion levels and highest for rabbits on BD and 30 g/kg RHB inclusion levels. The ADG of 12.74-18.30 g/day reported in this study was higher than the findings of Ogunsipe *et al.* (2014) who fed Napier grass with concentrates but similar to reports by Onyimonyi and Ene (2003) who fed *Panicum maximum.* Similar ADG range (10.56-20.20 g/day) have also been reported by Al-Amin *et al*. (2019) who fed New Zealand White rabbits with varying levels of leucaena leaf meal in pelleted complete diets. Lower daily weight gains of 3.3-11 g/day have also been reported by Rahman *et al*. (2020) who fed *B. decumbens* and commercial pellets to rabbit. Iyeghe-Erakpotobor *et al*. (2006) reported daily gains of -2.08-6.7 g/day when rabbits were fed diets containing *B. decumbens and* concentrates containing soybean cheese waste/maize offal.

Apparent Digestibility

Table 4 shows apparent digestibility indices of rabbits fed diets containing biochar produced from rice husk. Dry matter, NDF and CF digestibility generally decreased as RHB inclusion increased for both BD and BM-based diets but the reverse was observed for OM and CP digestibility. Differences ($p>0.05$) in CP digestibility of BM-RHB based diets and diets without RHB existed. Rabbits on BM supplemented with 15 g/kg RHB recorded the highest CP (68.34%), ADF (57.81 %) and ME (64.57 %) digestibility. The values gotten for crude protein digestibility (61.03-68.34%) was lower than 69.50-75.55% reported by Azi and Alu (2022) who fed weaner and grower rabbits with graded levels of acha offal supplemented with enzymes. Crude protein digestibility was, however, similar to the range of 60.40-68.20% obtained by Ukim *et al.* (2012) who fed sorghum, millet and maize offals to weaner rabbits.

Differences in digestibility especially for CP could be attributed to the differences in CP content of feed resulting from the inclusion of RHB. Differences existed in digestibility of crude fibre and fiber fractions among treatment diets. Animals on BM and BD supplemented with 30 g/kg RHB had significantly ($p<0.05$) lower CF digestibility. Digestibility values obtained from this study indicates that biochar might positively influence enzymatic digestion in the small intestines and microbial digestion in the large intestines as suggested by Kim *et al.* (2017) and Saleem *et al*. (2018).

Hematology

Table 5 shows the effects of biochar on hematology of rabbits. With the exception of platelet

Table 4: Effects of Rice Husk biochar on Apparent Digestibility indices of rabbits

GRASS	*Brachiaria mulato*			*Brachiaria decumbens*			SEM
BIOCHAR	0 g/kg	15 g/kg	30 g/kg	0 g/kg	15 g/kg	30 g/kg	
Parameters, %							
DM	64.17^{a}	60.62^{a}	58.89^{a}	59.55^{a}	59.42^{a}	55.33^{a}	2.24
CF	25.23^{a}	21.22^{ab}	19.67^{bc}	19.90^{bc}	24.32^{ab}	14.81^{c}	1.03
CP	64.21^{b}	68.34^{a}	67.91^{a}	64.32^{b}	64.48^{b}	61.03^{c}	0.50
EE	62.38	58.55	61.25	60.01	65.24	59.77	0.67
N	65.35	59.53	68.01	74.10	75.41	73.08	0.18
NDF	57.06^{a}	48.07^{d}	56.74^{ab}	57.66^{a}	54.15^{c}	55.79^{b}	0.22
OM	51.87^{c}	53.14^{b}	54.31^{a}	53.99^{a}	53.01^{b}	54.20^{a}	0.09
ME	53.79	64.57	65.36	55.10	61.67	63.55	3.14

Means in the same row with different superscripts are significantly different ($p<0.05$); SEM- Standard Error of Mean. CP-Crude protein; EE-Ether extract; CF-Crude Fiber; DM-Dry matter;OM-Organic matter; NFE-Nitrogen free extract; Metabolizable energy; NDF- Neutral detergent fiber; CF-Crude fiber; N- Nitrogen.

Table 5: Effects of biochar on hematology of rabbits

GRASS	*Brachiaria mulato*			*Brachiaria decumbens*			Ref. Ranges**	SEM
BIOCHAR	0 g/kg	15 g/kg	30 g/kg	0 g/kg	15 g/kg	30 g/kg		
WBC, 10^{*3} / Ul	4.80	4.27	4.70	3.73	3.90	5.53	5.2-13.5	0.62
GRAN#, 10^{*3} / uL	1.94	1.71	1.93	1.33	1.51	2.78	2.0-7.5	0.40
RBC, 10^{*6}/Ul	5.67	5.08	5.26	4.90	5.82	5.48	5.00-7.60	0.31
HGB, g/Dl	13.70	13.35	12.30	11.47	13.17	12.83	10.5-17.0	0.54
PCV, %	41.87	37.47	38.57	36.50	43.50	39.83	31.0-46.0	2.38
MCV, fL	73.87	73.73	73.37	74.53	74.73	72.90	56.8-66.5	0.72
MCH, pg	24.07	22.73	23.30	23.33	22.67	23.37	20.1-25.1	0.71
MCHC, g/dL	32.67	30.90	31.83	31.33	32.10	32.13	32-37	0.86
PLT, 10^{*3} / Ul	186.67^{bc}	199.33^{abc}	207.00^{abc}	184.00^{c}	216.67^{ab}	224.00^{a}	100-712	9.79
MPV, fL	8.07	6.93	7.90	8.20	8.10	6.90	3.8-6.8	0.94

***Reference ranges as displayed on the SK8800 Veterinary Full-auto Hematology Analyzer. Means in the same row with different superscripts are significantly different (p<0.05); SEM- Standard Error of Mean. WBC- White Blood Cell; RBC-Red Blood Cell; HGB-Hemoglobin; PCV-Packed cell volume; MCV-Mean cell volume; MCH- Mean Corpuscular Hemoglobin (MCH); MCHC-Mean Corpuscular Hemoglobin Concentration; PLT-Platelet;*

counts between BM without RHB (186.60 x 10^{*3}/Ul), BD without RHB (184.00 x 10^{*3}/Ul) and that of BD with 30 g/kg RHB (224.00 x 10^{*3}/Ul) where differences ($p<0.05$) were observed, all other blood indices were similar ($p>0.05$) indicating that diets had generally minimal adverse effects on hematological indices of rabbits.

Hemoglobin (HGB) which gives an indication of the animal's ability to withstand some level of respiratory stress (Sainsbury, 1983) was similar ($p>0.05$) with values (11.4-15.7 g/dl) obtained in this study within the normal reported ranges of 10.1-15.1 g/dl (Gallego, 2017) and 10-15 g/dl (Wesche, 2014) for rabbits. Yeh *et al*. (2019) also reported similar range of HGB (12.8-13.9 g/dl) for rabbits. Lower HGB levels indicates dietary deficiency of iron, copper, vitamin and amino acids (Frandson, 1981) hence results obtained for HGB shows diets had adequate nutrients to support growth. Values for RBC were within the normal ranges of 5.1-7.9 $x10^{*6}$/Ul and 4.6-7.4 $x10^{*6}$/Ul reported by Varga (2014) and Gallego (2017) respectively for rabbits. Normal to higher ranges of RBC is an indication of adequacy of protein in the diets for optimal growth and development of rabbits.

With the exception of WBC (5.53 10^{*3} / uL) for BD with 30 g/kg RHB inclusion which was within range, the remaining diets were lower than the range of 5.4 – 9.9 x 10^{*3} / uL reported by Archetti *et al*. (2008), Moore (2006) and Bortolotti *et al*. (1989). White blood cell values for BM - based diets (4.27- 4.8 10^{*3} / uL) were all within the normal physiological ranges reported by Gallego (2017) and Graham and Mader (2012) for rabbits. According to Odesanmi *et al*. (2010), a reduction in WBC count is indicative of leucocyte suppression and production from the bone marrow which may impact disease resistance of the animals. This should have been the case for BD-based diets but similarities in WBC amongst treatments did not suggest susceptibility to diseases. Also, since there were no elevated WBC counts, which is indicative of initiation of disease resistant cells, it can be concluded that no harmful pathogen attacked rabbits fed diets containing rice husk biochar.

The range of PCV (36.5-43.50%) obtained in this study were within the ranges of 33-46% by Gallego (2017), 33-48% by Varga (2014) and the 33-55% by Graham and Mader (2012). Lower PCV is indicative of blood dilution (Wilson and Brigstoke, 1981) and if less than 30% with low

RBC and HGB indicates anemic condition (Melillo, 2007). Normal PCV range obtained in this study shows rabbits were in a good physiological state. The values of MCH, MCV and MCHC are important hematological parameters that serve as indicators of anaemic condition (Campbell and Ellis, 2007). From the study, RHB inclusion had no health implications on rabbits due to the similar (p>0.05) MCV, MCHC and MCH values obtained for diets with/without RHB inclusion.

According to Akinmutimi (2004), the quantity and quality of feed consumed by animals greatly affect their hematological indices. From the results obtained, indices such as HGB, PCV, RBC and WBC concentrations which showed no significant differences (p>0.05) indicate that rabbits tolerated the BM- and BD-concentrate (with varying RHB levels or without RHB) diets and that the diets had no toxicological or disease impact on the animals. Differences in hematological indices with other works could be attributed to climate, breed, age and physiological state of the rabbits. Values obtained for blood hematological indices in this study indicate RHB can be incorporated into rabbit diets to support normal growth of rabbits.

Carcass Evaluation

Table 6 shows the effect of biochar on carcass characteristics of rabbits. Rabbits slaughtered were of similar (p>0.05) weights. Differences (p<0.05) existed for liver, kidney and full GIT weights. For the relative weight of organs, the only difference (p<0.05) existed in the lungs for BM without biochar and BM with 30 g/kg biochar.

Dressing percentage reported in this study (53.48 -56.75%) was higher than that reported (45.3 – 50.2%) by Njidda and Isidahomen (2011) when rabbits were fed with diets containing sesame seed meal but was lower when compared to the range of 61.98-69.74% by Teye *et al.* (2020) who fed rabbits with graded levels of palm kernel oil residue. Differences in dressing percentage may be attributed to the parts considered as edible as well as the age and weight at slaughter.

Similarities in relative weights of organs could be attributed to the similarities in final weight and body weight gains of rabbits fed biochar-based diets. Similar lung (13.39-14.78g), heart (4.15-4.67g), kidney (10.11-11.15 g) and higher liver weight (55.34-57.77 g) have been reported by Teye *et al*. (2020) who fed rabbits with graded levels of palm kernel oil residue. Oloruntola *et al*. (2015) also reported lower weights for

Table 6: Effects of biochar inclusion levels on carcass characteristics of rabbits

GRASS	*Brachiaria mulato*			*Brachiaria decumbens*			
BIOCHAR	0 g/kg	15 g/kg	30 g/kg	0 g/kg	15 g/kg	30 g/kg	SEM
Absolute weights, g							
Slaughter weight, g	1720.00	1743.00	1589.00	1842.00	1770.00	1502.00	141.00
Warm weight, g	940.00	975.00	865.33	1046.00	972.00	830.00	105.00
Chilled weight, g	922.00	953.00	848.00	1024.00	953.00	811.00	97.13
Full GIT, g	312.33^{ab}	364.33^{a}	310.70^{ab}	355.70^{ab}	372.30^{a}	276.30^{b}	26.80
Empty GIT, g	123.70	120.00	103.00	138.70	138.70	127.30	13.80
Heart, g	5.67	5.67	5.33	6.00	5.67	5.33	0.51
Lung, g	11.33	12.33	13.33	13.33	13.11	11.33	2.09
Liver, g	47.67^{ab}	48.33^{ab}	38.67^{b}	53.00^{a}	46.33^{ab}	40.67^{ab}	4.55
Kidney, g	12.00^{ab}	11.00^{b}	12.00^{ab}	13.33^{a}	12.33^{ab}	11.33^{ab}	0.67
Spleen, g	0.93	0.83	1.00	1.00	1.12	0.85	0.18
Head, g	153.30	160.70	148.70	163.70	166.00	153.00	12.15
Limbs, g	35.05	43.76	35.19	48.96	41.48	33.21	5.22
Drip loss	1.97	2.30	1.99	2.05	2.24	2.41	0.35
Dressing	54.66	56.15	53.83	56.71	54.41	54.83	2.49

Means in the same row with different superscripts are significantly different (p<0.05); SEM- Standard Error of Mean lungs (6.3 g), liver (37.4 g), heart (3.5 g) and kidneys (7.6 g) when rabbits were fed with Napier grass-based diets.

Changes in organ size, shape, weight and colour indicates a disease condition (Teye *et al*., 2015) or effects of anti-nutritional factors on rabbits (Akinmutimi, 2004). Also, according to Ahamefule *et al.,*2006), internal organ weights during and after nutritional studies give an indication of negative effects the nutritional interventions have on animals. The similarities in the weights of organs thus indicates biochar inclusion did not affect the physiological and anatomical functions the organs.

Manure characteristics

Table 7 shows the effects of biochar on characteristics of manure generated from rabbits fed diets containing biochar produced from rice husk. Weights of daily manure generated by rabbits fed RHB included diets did not differ significantly ($p>0.05$) and ranged from 28.24-39.01 g. This range was in agreement with the findings of Sholihah and Wahyunigrum (2016) that adult rabbits weighing 1 kg produces 28 g of faeces per day.

Dry matter (DM) contents of the manure from rabbits ranged from 66.77% for rabbits fed BM based diets without RHB to 74.89% for rabbits fed BD based diets with 30 g/kg RHB. Higher DM (79.6%) contents of rabbit manure have been reported by Li *et al.* (2022). Organic carbon (OC) content (40.83-50.73%) in the manures generated significantly increased ($p<0.05$) as biochar levels in the feed increased. Organic carbon contents of rabbit manure obtained in this study were higher than the 30.1%, 16.7%, and 10% reported by Adekiya *et al.* (2020), Hassan (2012) and Tumimbang *et al*. (2016), respectively. Ash contents of manure was significantly different ($p<0.05$) among treatments with diets having 30 g/kg RHB inclusion levels recording the highest ash contents. Ash ranged from 10.43 % for BM without RHB to 17.63 % for BM with 30 g/ kg RHB. The ash recorded for this study

Table 7: Effects of biochar on manure characteristics of rabbits

GRASS	*Brachiaria mulato*			*Brachiaria decumbens*			SE
BIOCHAR	0 g/kg	15 g/kg	30 g/kg	0 g/kg	15 g/kg	30 g/kg	
WT, g	39.01 ^{a}	32.48 ^{a}	28.24 ^{a}	32.86 ^{a}	36.60 ^{a}	27.74 ^{a}	6.71
DM, g	66.77 ^{a}	72.36 ^{a}	73.19 ^{a}	69.35 ^{a}	68.54 ^{a}	74.89 ^{a}	3.90
ASH, %	10.43 ^{a}	14.83 ^{b}	17.63 ^{d}	12.37 ^{c}	12.53 ^{c}	15.43 ^{b}	0.17
pH	6.23^{c}	6.45 ^{bc}	6.84 ^{a}	6.26 ^{c}	6.61 ^{ab}	6.72 ^{ab}	0.06
N, %	2.28 ^{a}	2.41 ^{b}	1.86 ^{c}	1.99 ^{d}	1.74 ^{e}	1.86 ^{c}	0.01
P, mg/kg	0.70 ^{a}	0.63 ^{b}	0.48 ^{c}	0.61 ^{d}	0.80 ^{e}	0.52 ^{f}	0.005
K, mg/kg	1.27 ^{a}	1.11 ^{b}	1.09^{b}	1.21 ^{c}	1.44 ^{d}	0.85 ^{e}	0.01
Ca, mg/kg	0.49 ^{a}	0.52 ^{b}	0.66 ^{c}	0.54 ^{d}	0.69 ^{e}	0.61 ^{f}	0.002
Mg, mg/kg	0.26 ^{a}	0.29 ^{b}	0.30 ^{c}	0.28 ^{d}	0.38 ^{e}	0.26 ^{a}	0.002
Na, mg/kg	0.11 ^{a}	0.06 ^{b}	0.06 ^{b}	0.06 ^{b}	0.08 ^{c}	0.09 ^{c}	0.001
OC, %	41.95 ^{a}	47.78 ^{b}	49.40 ^{c}	40.83 ^{d}	49.05 ^{c}	50.73 ^{e}	0.10
OM, %	89.57 ^{a}	85.17 ^{b}	82.37 ^{c}	87.63 ^{d}	87.47 ^{d}	84.57 ^{b}	0.17
L, %	14.68 ^{a}	15.26 ^{b}	16.44 ^{c}	13.31 ^{d}	13.86 ^{d}	13.88 ^{d}	0.14
C/N	18.37 ^{a}	19.83 ^{b}	26.59 ^{c}	20.49 ^{d}	28.24 ^{e}	27.23 ^{f}	0.12
L/N	6.44 ^{ab}	6.33 ^{b}	7.46 ^{c}	6.69 ^{ab}	7.97 ^{cd}	8.84 ^{d}	0.23

Means in the same row with different superscripts are significantly different ($p<0.05$); SEM- Standard Error of Mean. WT- Weight; DM-Dry matter; OM-Organic matter; pH-Acidity or alkalinity; N-Total Nitrogen' OC-Organic Carbon; P-Available Phosphorus; K-Potassium; Ca-Calcium; Mg-Magnesium; Na-Sodium; L-lignin; C/N-Carbon to Nitrogen ratio; L/N- Lignin to Nitrogen was higher than the 10.37% reported for rabbits by Peiretti *et al.* (2014). As biochar inclusion increased, DM, OC and Ash contents of manure increased with a reduction in OM content (82.37-89.57%). Moula *et al*. (2018) reported lower OM (77.29) for rabbit manure. Increases observed in this study and differences in Ash, DM and OC contents compared to other studies can be attributed to the carbonaceous, dry and charred nature of biochar incorporated in the diets.

Total nitrogen (N), phosphorus (P) and potassium (K) contents of manure differed ($p<0.05$) between treatments and ranged from 1.74-2.41% N, 0.48-0.80% P and 0.85-1.44% K. Moula *et al.* (2018) reported N (1.79 mg/kg) and P (0.59 mg/kg) within the range of this study but lower K (0.67) contents for rabbit manure. Adeniyan *et al*. (2011) also reported lower N (1.04%) but higher P (0.99%) and K (2.05%) contents for rabbit manure. Tumimbang *et al.* (2016) reported similar total N value (2%) and K (<1%) higher P (1.5%) for rabbit manure. The range of values obtained in this study for N, P and K in rabbit manure were within the range reported by Rajendran *et al*. (2019) to increase uptake and availability of nutrients when applied on soils.

Other manure parameters assessed were the pH (6.23-6.84), lignin (13.31-16.44%), C/N ratio (18.37-28.24) and lignin/N ratio (6.33-8.84). These parameters differed significantly ($p<0.005$) between dietary treatments. The pH, C/N ratio and lignin levels in manure increased as RHB was included in the diet. Lignin contents of 14.8% and 21.39% have been reported by Adekiya *et al.* (2020) and Li *et al* (2022) respectively for rabbit manure. High lignin and C/N ratio of diets with RHB is an indication that those manures when used as soil amendment will result in nutrient immobilization, organic matter accumulation and humus formation (Adekiya *et al*., 2018). The ratio of lignin to N content of rabbit manure was lower than the ratio obtained by Adekiya *et al*. (2020) for rabbit manure indicating a slower rate of decomposition than manure in our study. Biochar has been found to be alkaline in nature due to the abundant carbonyls, phosphates, carbonates and other alkaline substances in them (Dai *et al.,* 2017) hence the ability to neutralize acidic soils. The increase in manure pH can thus be attributed to the inclusion of biochar in the diets. Increase in lignin can also be attributed to the biochar feedstock, rice husk, which is known to contain as high as 25% lignin (Ramesh *et al*., 2022). Increase in C/N ratio of manure of rabbits as biochar level increased can also be attributed to the high carbon content of biochar. A C/N ratio of 20-30 similar to our study has been reported to result in a state of equilibrium between N mineralization and immobilization (Brust, 2019) and this indicates that the use of manure from rabbits fed diets containing RHB as a soil amendment can either result in N release for crop growth or N unavailability for crops.

CONCLUSIONS

Generally, nutrient intakes were higher for *Brachiaria mulato* based diets supplemented with concentrates containing 15 g/kg biochar. Daily gains were similar for all dietary treatments indicating the potential for the use of biochar as a feed additive up to levels in this study.

Normal hematological levels indicate diets were well tolerated by experimental animals and provided adequate nutrients for optimal physiological development and resistance to pathogens. The similarities of weights of organs such as the heart, kidney, liver and lungs show that the inclusion of biochar in the diets did not affect the physiological and anatomical functions of the organs. Manure generated from rabbits after feeding biochar-based diets showed appreciable levels of N, P, K and other essential soil minerals which can potentially serve as soil amendment. Results of this study suggests the incorporation of up to 30 g/kg biochar produced from rice husk in the diet of weaner rabbits will not have any negative effect on feed intake, growth, nutrient digestibility, hematology, carcass and manure characteristics. This study thus serves as a basis for further trials with biochar from different feedstock (biomass), breeds of rabbits and livestock to ascertain the true effects of biochar on animal performance.

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