

## RESPONSE OF BROILER CHICKENS TO GRADED LEVELS OF DECORTICATED RAW SANDBOX SEEDS (*HURA CREPITANS*) AS SOURCE OF METHIONINE

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### ABSTRACT

A 56-day feeding trial was performed to investigate the use of decorticated sandbox seeds (*Hura crepitans*) as a source of methionine in broiler production. One hundred and twenty (120) Ross 308 day-old broiler chicks were used in a completely randomized design study with four dietary treatments each replicated thrice. The four diets for each of starter and finisher phase were formulated to be isocaloric and isonitrogenous with 0 %, 0.1 %, 0.2 % and 0.3 % inclusion of sandbox seeds (SBS) and designated as D1, D2, D3 and D4, respectively. Data were collected on growth performance, nutrients retention, haematology and serum biochemistry. All data collected were subjected to one-way ANOVA and treatment means were separated.

The D2 treatment (0.1 % DL-Methionine + 0.1 % SBS) was significantly better in terms of total feed intake and weight gain, while the D4 treatment (0.3 % SBS) was significantly worst (2.52) in its effect on FCR. There were no significant differences among the treatments in their effects on nutrient retention. There were no significant differences ( $P > 0.05$ ) among the treatments in their effects on haematological and serum biochemical parameters except the D4 treatment, which was significantly lowest ( $P < 0.05$ ) in its effect on red blood cell counts and albumin value.

It was concluded that the use of decorticated SBS, as a source of methionine in broiler production, has no detrimental effect on nutrient utilization and blood profile.

**Key words:** broiler; performance; decorticated; Sandbox; methionine

### INTRODUCTION

The importance of amino acids in poultry nutrition cannot be overemphasized. Amino acids are the building blocks of protein (Hadinia *et al.*, 2014) and the quality of protein feedstuff is determined by its amino acids profile. While requirements for some amino acids are easily met by regular feedstuffs, requirements for others cannot be met except by additional feed supplementation. This has necessitated synthetic amino acid use especially for those highly deficient in common feedstuffs. Methionine has a vital role in the metabolic functioning of animal and humans, which is why it is also known

as functional amino acid (Maggawa *et al.*, 2022). High demand for protein synthesis and feather development has caused for methionine to be considered the first limiting amino acid in poultry and, therefore, supplementation of synthetic methionine in poultry diets to balance the dietary amino acids is a common practice (Kim *et al.*, 2019). Furthermore, methionine and lysine are the first two limiting amino acids that play important role in the growth and development of broilers. They are in short supply relative to need (Rehman *et al.*, 2019). Their presence in enough quantity or otherwise determines the utilization of other amino acids that are present at enough quantity. Methionine is responsible

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for initiation of protein synthesis (McDonald *et al.*, 2010). Supplementing broiler feeds with both methionine and lysine improves feed efficiency, body weight gain, carcass yield and meat quality (Bregendahl *et al.*, 2006). This has made methionine supplementation a constant practice among feed millers and poultry farmers eventually leading to increased feed cost. Maggawa *et al.* (2022) reported that cost of synthetic methionine has been on the increase with resultant surge in price of finished feeds.

Furthermore, the need for precision formulation, based on amino acids' requirement rather than based on crude protein in livestock feeding, has been emphasized. Actually, there is no crude protein requirement by poultry birds instead amino acids' requirements (NRC, 1994). Livestock production with minimal negative impact on the environment is essential to mitigate the effects of livestock production on climate change. This can only be achieved through optimal amounts and proportion of proteinogenic amino acids (Bailey, 2020). Deamination of amino acids, according to Ribeiro *et al.* (2007), lead to formation of ammonia, which is mostly excreted as uric acid in poultry birds. Diets, that are high in poor quality protein, result in more deamination and more nitrogen loss in uric acid than diets high in good quality protein with appropriate amino acids profile. Methionine affects nutrients digestibility, feed conversion ratio and carcass characteristics of broilers (Ahmed and Abbas, 2011). Since alternative, that can completely eliminate the use of synthetic methionine especially as it relates to the production of organic poultry, is currently lacking (Burley *et al.*, 2016), there is a need for the industry to start exploring this avenue and investigate natural sources of these amino acids, especially when several countries plan to ban synthetic amino acids in broiler production.

Sandbox tree belongs to the *Euphorbiaceae* family. The fruits are large and garden egg-shaped capsules about 3–5 cm long and 5–8 cm in diameter and are brown when ripe and matured. The matured fruit explodes and splits into segments and catapults the seeds as far as 100 m (Feldkamp, 2006). Seeds of the sandbox tree (*Hura crepitans*) have crude protein value of 24.63 % and 25.75 %, as reported by Jimoh *et al.* (2023) and Fowamola and Akindahunsi (2017), respectively, while amino acid profile reveals higher methionine level than conventional soya bean meal (2.30 % vs. 0.6 %, resp.; Jimoh *et al.*, 2023; Ogundipe *et al.*, 2022). This study investigated utilization of diets containing graded levels of decorticated raw sandbox seeds (*Hura crepitans*) as a source of methionine

in broiler production. The study focused on growth performance, nutrient retention and blood profile of the birds fed the diets.

## MATERIALS AND METHODS

The study was carried out at the Livestock Teaching and Research Farm, Joseph Sarwuan Tarka University (formerly Federal University of Agriculture), Makurdi, Benue State, Nigeria. Makurdi is located within latitude 7.450N and longitude 8.320N (GPS, 2012). It is characterized by tropical dry and wet climate with five months of dry season and seven months of rain from April to October.

Matured and brown sandbox fruits were plucked from the sandbox trees located within the campus of Joseph Sarwuan Tarka University, Makurdi, Nigeria and its environs. The fruit capsules were broken and seeds removed. Afterwards, the flat seeds were decorticated and sundried for three days, then milled and included in the formulation of experimental diets. Synthetic DL-methionine was purchased from a reputable distributor of feed additives.

Four experimental diets were formulated for each of broiler starter and finisher phases to meet broiler chicken nutrient requirements according to Ogundipe *et al.*, (2022) and NRC (1994). Decorticated, dried and milled sandbox seeds were included into each of the formulated diets at 0.0 %, 0.1 %, 0.2 % and 0.3 % levels. The experimental diets were: D1, which served as control diet with DL-methionine 0.2 % + sandbox seed 0 %; D2 diet with DL-methionine 0.1 % + sandbox seed 0.1 %; D3 diet with DL-methionine 0 % + sandbox seed 0.2 % and D4 diet with DL-methionine 0 % + sandbox seed 0.3 %. All the diets were formulated to be isonitrogenous and isocaloric. Table 1 shows the composition of experimental diets used in this study.

A total of one hundred and twenty (120) Ross 308 day-old broiler chicks were obtained from a commercial hatchery, South West Nigeria. They were randomly allotted to four dietary treatments with three replicates per treatment and ten birds per replicate. The birds were raised on a deep litter system. The pen was cleaned and disinfected prior to the arrival of birds. Starter feed was fed to the birds from one-day old to four weeks, while finisher feed was given from fifth week to the end of eighth week. Experimental feeds and water were supplied to the birds *ad libitum*.

**Table 1. Composition of experimental diets**

Feedstuffs	Broiler Starter Diets (%)				Broiler Finisher Diets (%)			
	D1	D2	D3	D4	D1	D2	D3	D4
Maize	58	58	58	58	65.2	65.2	65.2	65.1
Maize offal	3	3	3	3	2	2	2	2
SBM	25.15	25.15	25.15	25.05	22.23	22.23	22.23	22.23
Blood Meal	10	10	10	10	6.67	6.67	6.67	6.67
Bone	3	3	3	3	3	3	3	3
Lysine	0.1	0.1	0.1	0.1	0.15	0.15	0.15	0.15
Methionine	0.2	0.1	0	0	0.2	0.1	0	0
SBS	0	0.1	0.2	0.3	0	0.1	0.2	0.3
Salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Broiler Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100	100	100
Proximate composition								
CP, %	23.98	24	24.03	24.01	20.51	20.54	20.56	20.58
ME, Kcal/Kg	2888.75	2888.75	2888.75	2886.25	2924.85	2924.85	2924.85	2921.6
EE, %	3.63	3.66	3.69	3.71	3.73	3.73	3.73	3.72
CF, %	3.83	3.84	3.86	3.83	3.71	3.71	3.73	3.74
Lysine, %	1.62	1.62	1.62	1.62	1.37	1.37	1.37	1.37
Methionine, %	0.53	0.44	0.34	0.34	0.48	0.39	0.3	0.3
Calcium, %	1.14	1.14	1.14	1.14	1.12	1.12	1.12	1.12
Av. Pho., %	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78

D1: Contains 0.1 % of methionine and 0 % of SBS; D2: Contains 0.1 % of methionine and 0.1 % of SBS; D3: Contains 0 % of methionine and 0.2 % of SBS; D4: Contains 0 % of methionine and 0.3 % of SBS; SBM: Soya bean meal; SBS: Sandbox Seeds; CP: Crude protein; ME: Metabolizable Energy; EE: Ether extract; CF: Crude fiber; Av. Pho.: Available phosphorus

Data on feed intake, weight gain and feed conversion ratio were collected. Daily feed intake was determined as difference between feed offered to birds in the morning and feed left over the following morning. Weight of birds was determined weekly. Weight gain was determined as the difference between initial and final weights of birds. Feed conversion ratio was calculated by dividing total feed intake by weight gain.

Total Feed Intake (g/bird) = Feed offered – Feed left over

Weight gain (g/bird) = Final weight of bird – Initial weight of bird

FCR = Total feed intake /Weight gain

At the end of sixth week of the study, nutrient retention trial was carried out using total faecal collection method. One bird per replicate was selected and housed in a previously disinfected two-tier wire floor metabolic cage with dimension of 0.7 x 0.6 m floor spacing per cell and a dropping tray for easy collection of faecal droppings. The birds were allowed five days acclimatization period

and fed *ad libitum* with experimental diets. Thereafter, a weighed amount of dietary treatment was offered to birds in each replicate for five days. The quantity of feed consumed per bird was determined as difference between feed offered and feed left over. Droppings were collected, weighed and oven-dried on daily basis for five days. The collections for each day were pooled together for each replicate and subjected to proximate analysis. Nutrient intake, nutrient output and nutrient retention were calculated using the formulas below:

Nutrient Intake = Nutrient coefficient in feed × weight of feed consumed (g)

Nutrient Output = Nutrient coefficient in droppings × weight of droppings (g)

Apparent Nutrient Retention (%) =  

$$\frac{\text{Nutrient Intake (g)} - \text{Nutrient Output (g)}}{\text{Nutrient Intake (g)}} \times 100$$

Serum biochemical and haematological analyses were carried out at the eighth week of the study. For serum biochemical analysis, two millilitres (2 ml) of blood were obtained from a bird per replicate through wing vein with the aid of needle and syringe. The blood was drained into a bottle without anticoagulant and was allowed to clot to obtain the serum. It was later centrifuged using high speed Wintrobe Microhaematocrit for 10 min at 2000 revolutions per minute to separate cells from serum. The serum was analysed for total protein, albumin, globulin, glucose, cholesterol, creatinine and liver enzymes namely alkaline phosphatase, aspartate transaminase (AST) and alanine transaminase (ALT). For haematological analysis, further two millilitres (2ml) of blood were collected from the wing vein of selected birds and drained into a labelled sterile bottle containing EDTA (ethylenediamine tetra acetic acid) to prevent coagulation. Haematological parameters analysed include packed cell volume (PCV) using the procedure of Dacie and Lewis (1991). The procedure of Jain (1986) was used to determine white blood cell (WBC), red blood cells (RBC), haemoglobin concentration (Hb), mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, neutrophils, lymphocytes, monocytes, eosinophils and basophils.

All data collected for growth performance, nutrient retention and blood profile were subjected to a one-way Analysis of Variance suitable for a completely randomized design using Statistical Package for Social Science (SPSS, 2011). Treatment means were separated using Duncan

Multiple Range Test (Duncan, 1955) at 5 % significance level.

## RESULTS

The growth performance of broiler chickens fed diets containing graded levels of decorticated sandbox seeds (*Hura crepitans*) as a source of methionine is shown in Table 2. All growth performance parameters in broilers were significantly affected by the experimental diets. Birds fed D2 (DL-methionine 0.1 % + sandbox seed 0.1 %) showed significantly increased ( $p < 0.05$ ) total feed intake, weekly feed intake and daily feed intake compared to other treatment groups, which were similar including the control group (D1). Effects of D2 on final weight, total weight gain, weekly weight gain and daily weight gain were significantly higher ( $p < 0.05$ ) compared to the effect of D4 (DL-methionine 0 % + sandbox seed 0.3 %). However, the effect of D2 was comparable to effects of D1 control diet (DL-methionine 0.2 % + sandbox seed 0 %) and D3 (DL-methionine 0 % + sandbox seed 0.2 %) on the same weight parameters. For feed conversion ratio, the D2 treatment was similar ( $p < 0.05$ ) to D1 and D3 treatments but was significantly improved ( $p < 0.05$ ) compared to the D4 treatment.

Table 3 shows the apparent nutrient retention of broiler chickens fed diets containing graded levels of decorticated sandbox seed as a source of methionine.

**Table 2. Growth performance of broiler chickens fed diets containing graded levels of decorticated sandbox seeds (*Hura crepitans*) as a source of methionine**

Parameters	Treatments				P-value
	D1	D2	D3	D4	
Initial weight (g)	37.27	37.07	36.70	36.15	0.79
Total feed intake (g)	3794.79 <sup>b</sup>	4554.77 <sup>a</sup>	3834.50 <sup>b</sup>	3721.90 <sup>b</sup>	0.04
Weekly feed intake (g)	474.35 <sup>b</sup>	569.35 <sup>a</sup>	479.31 <sup>b</sup>	465.24 <sup>b</sup>	0.04
Daily feed intake (g)	67.76 <sup>b</sup>	81.34 <sup>a</sup>	68.47 <sup>b</sup>	66.46 <sup>b</sup>	0.04
Final weight (g)	2231.33 <sup>a,b</sup>	2775.33 <sup>a</sup>	2218.00 <sup>a,b</sup>	1513.00 <sup>b</sup>	0.02
Total weight gain (g)	2194.07 <sup>a,b</sup>	2738.27 <sup>a</sup>	2181.30 <sup>a,b</sup>	1476.85 <sup>b</sup>	0.02
Weekly weight gain (g)	274.26 <sup>a,b</sup>	342.28 <sup>a</sup>	272.66 <sup>a,b</sup>	184.61 <sup>b</sup>	0.02
Daily weight gain (g)	39.18 <sup>a,b</sup>	48.90 <sup>a</sup>	38.95 <sup>a,b</sup>	26.37 <sup>b</sup>	0.02
Feed conversion ratio	1.81 <sup>a</sup>	1.67 <sup>a</sup>	1.77 <sup>a</sup>	2.52 <sup>b</sup>	0.01

<sup>a,b</sup>Means within the same row with different superscripts are significantly different ( $p < 0.05$ ); D1: Control with methionine; D2: Contained 0.1 % methionine + 0.1 % sandbox seeds; D3: Contained 0.2 % sandbox seeds without methionine; D4: Contained 0.3 % sandbox seeds without methionine

**Table 3. Nutrients retention of broiler chickens fed diets containing graded levels of decorticated sandbox seeds (*Hura crepitans*) as a source of methionine**

Nutrients	Treatments				P-value
	D1	D2	D3	D4	
Dry matter	82.90	84.37	78.55	75.82	0.28
Crude protein	79.33	82.28	75.01	70.88	0.18
Ether extract	64.20	63.85	47.76	45.31	0.39
Crude fibre	83.25	86.49	81.00	76.26	0.28
Total ash	64.26	64.68	47.31	49.67	0.16
Nitrogen-free extract	89.00	89.68	87.23	84.44	0.55

D1: Control with methionine; D2: Contained 0.1 % methionine + 0.1 % sandbox seeds; D3: Contained 0.2 % sandbox seeds without methionine; D4: Contained 0.3 % sandbox seeds without methionine

There were no significant differences ( $p > 0.05$ ) in the effect of experimental treatments on retention of dry matter, crude protein, ether extract, crude fibre, total ash and nitrogen-free extract.

The haematological parameters of broiler chickens fed diets containing graded levels of decorticated sandbox seeds (*Hura crepitans*) as a source of methionine are shown in Table 4. The results showed no significant ( $p > 0.05$ ) differences among the treatments in their effects on haematological parameters considered except for red blood cells and monocytes. Birds on the D4 diet

showed significantly lower red blood cell value ( $p < 0.05$ ) compared to broiler chickens on other experimental treatments, where the red blood cell values were similar ( $p < 0.05$ ). The value of monocytes was significantly higher for birds fed D2 diet ( $p < 0.05$ ) compared to birds on other treatments. Significantly increased total protein and albumin values ( $p < 0.05$ ) were observed in broilers on the D1 diet, while birds on the D4 diet had significantly lower albumin value ( $p < 0.05$ ) compared to birds in other treatments.

**Table 4. Haematological parameters of broiler chickens fed diets containing graded levels of decorticated sandbox seeds (*Hura crepitans*) as a source of methionine**

Parameters	Treatments				P-value
	D1	D2	D3	D4	
Packed cell volume (%)	35.00	34.67	35.50	34.50	0.99
Red blood cells ( $\times 10^{12}/l$ )	2.40 <sup>a</sup>	2.40 <sup>a</sup>	2.45 <sup>a</sup>	2.15 <sup>b</sup>	0.05
Haemoglobin (g/dl)	11.67	11.56	11.84	11.50	0.99
MCV (fl)	145.13	145.02	145.00	160.50	0.44
MCH (pg)	48.38	48.25	48.27	53.27	0.10
MCHC (g/dl)	33.20	33.33	33.35	33.30	0.44
White blood cells ( $\times 10^2/l$ )	6.46	6.07	6.00	5.90	0.66
Lymphocytes (%)	46.67	46.33	47.50	48.50	0.36
Neutrophil (%)	48.00	46.67	48.50	46.50	0.28
Eosinophil (%)	1.67	1.67	1.50	1.50	0.98
Basophil (%)	0.00	0.00	0.00	0.00	
Monocytes (%)	3.67 <sup>a,b</sup>	5.33 <sup>a</sup>	2.50 <sup>b</sup>	3.50 <sup>b</sup>	0.03

<sup>a,b</sup>Means within the same row with different superscripts are significantly different ( $p < 0.05$ ); D1: Control with methionine; D2: Contained 0.1 % methionine + 0.1 % SBS; D3: Contained 0.2 % SBS without methionine; D4: Contained 0.3 % SBS without methionine; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration

**Table 5. Serum biochemical parameters of broiler chickens fed diets containing graded levels of decorticated sandbox seeds (*Hura crepitans*) as a source of methionine**

Parameters	Treatments				P-value
	D1	D2	D3	D4	
Total protein(g/dl)	6.16 <sup>a</sup>	4.86 <sup>b</sup>	5.02 <sup>b</sup>	5.22 <sup>b</sup>	0.04
Albumin (g/dl)	2.27 <sup>a</sup>	2.15 <sup>a</sup>	2.16 <sup>a</sup>	1.85 <sup>b</sup>	0.03
Cholesterol (mg/dl)	89.23	70.88	71.13	70.20	0.28
Glucose (mg/dl)	84.23	64.88	71.13	62.20	0.22
AST (u/l)	169.18	143.54	144.31	132.39	0.31
ALT (u/l)	44.22	41.94	44.51	45.83	0.81
ALP (u/l)	97.07	83.87	85.20	84.40	0.28
Creatinine (mg/dl)	0.82	0.65	0.63	0.75	0.18
Uric acid	8.93	8.37	8.27	8.34	0.47

<sup>a,b</sup>Means within the same row with different superscripts are significantly different ( $p < 0.05$ ); D1: Control with methionine; D2: Contained 0.1 % methionine + 0.1 % SBS; D2: Contained 0.2 % SBS without methionine; D3: Contained 0.3 % SBS without methionine; AST: Aspartic amino transferase; ALT: Alanine amino transferase; ALP: Alkaline phosphatase

Total protein values ranged from 4.86 g/dl to 6.16 g/dl (Table 5). Albumin values, however, were similar among D1, D2 and D3 treatments. All other serum biochemistry parameters considered were not significantly influenced.

## DISCUSSION

The diets containing graded levels of decorticated raw sandbox seeds (*Hura crepitans*) as a source of methionine showed significant influence on the growth performance parameters in broilers fed the diets from day-old to 8 weeks. This implies that using decorticated raw sandbox seeds as a source of methionine to replace synthetic methionine (DL-methionine) at graded levels in diets of broilers resulted in marked differences in their growth performance parameters. Earlier reports (Chattopadhyay *et al.*, 2006; Narayanswamy and Bhagwat, 2010; Kumari *et al.*, 2012) also show response of broilers in their growth performance to DL-methionine and herbal methionine supplemented diets.

The inclusion of decorticated raw sandbox seeds at 0.1 % to the diet containing 0.1 % DL-methionine (D2) resulted in significantly better total feed intake. Further increase in inclusion levels (0.2 %, 0.3 %) of decorticated raw sandbox seeds without DL-methionine in the diet did not result in higher feed intake and the same trend was observed for weekly feed intake and daily feed intake. This agrees with Maggawa *et al.*

(2022) that at starter and finisher phase, alternative sources of methionine (moringa seeds, jatropha seeds, hibiscus sabdariffa seeds and cashew nut seeds) significantly influenced feed intake of birds. However, feed intake was not significantly affected by replacement of sesame seeds for synthetic methionine in broilers at starter phase (Agbulu *et al.*, 2010).

Birds on treatments D2 and D3 demonstrated significantly better final weight, total weight gain, weekly weight gain and daily weight gain. Values of final weight observed in this study are similar to values obtained by Archibong *et al.* (2022), where sandbox seed meal replaced soybean meal in the diets of finisher broilers. Kumari *et al.* (2012) reported that replacement of 0.1 % synthetic DL methionine by 0.1 % herbal methionine supplement (Methiorep) in broiler diets significantly improved their live weight gain. Similarly, Narayanswamy and Bhagwat (2010) found that chicks on herbal methionine had increased body weight compared to chicks in the control group and numerically higher weight gain compared to chicks in the synthetic methionine group. Addition of DL-methionine and herbal methionine resulted in heavier body weight and body weight gain of broilers fed 15 g herbal methionine/kg diet compared to other treatments (Chattopadhyay *et al.*, 2006). Furthermore, the similarity between birds on treatments D2 and D3 in weight parameters may be attributed to a slight increase in the inclusion of sandbox seeds, which caused an increase in the methionine content of the diet. The significantly

reduced performance of birds on the D4 treatment (DL-methionine 0 % + sandbox seed 0.3 %) with no synthetic methionine could be an indication that more inclusion of sandbox seeds in the absence of DL-methionine will be needed to meet the methionine requirements of the birds.

Feed conversion ratio obtained in birds on the D2 diet was significantly better than values for birds on other treatments. Kumari *et al.* (2012) reported that replacement of 0.1 % synthetic DL methionine by 0.1 % herbal methionine supplement (Methiorep) in broiler diets significantly improved feed conversion ratio. The FCR values, obtained in this study, are better than the range of values obtained by Archibong *et al.* (2022) in a study, where sandbox seed meal was included at 32.5 % to replace soya bean meal. Feed conversion ratio of broiler fed 15 g herbal methionine/kg diet was significantly better than that of broilers fed 10 g herbal methionine or DL-methionine/kg diet (Chattopadhyay *et al.*, 2006). Daily weight gain and feed conversion ratio of broilers were significantly affected at starter phase as sesame seed replaced synthetic methionine at different levels (Agbulu *et al.*, 2010).

Methionine is an essential amino acid that cannot be synthesized by poultry species and is classified as the first limiting amino acid (Carvalho *et al.*, 2018). As a limiting amino acid, the initiation of tissue formation responsible for growth and development depends on its quantity and availability. Therefore, it could be inferred that the significantly improved performance of birds on the D2 treatment (DL-methionine 0.1 % + sandbox seed 0.1 %) may probably be because quantity of methionine supplied by sandbox seeds was complemented by DL-methionine. Nonetheless, this finding differs from reports that methionine at different levels did not alter performance indices for broilers (Khajali *et al.*, 2006; Kluge *et al.*, 2015; Rehman *et al.*, 2019), which, according to Rehman *et al.* (2019), might be attributed to the satisfaction of methionine requirements at the lower standard level. It differs also from reports of Dilger and Baker (2008) and Powell *et al.* (2015), who did not observe significant differences in feed intake and FCR due to supplementation of L-methionine and DL-methionine.

All nutrient retention values in this study did not differ among treatments but are higher than values obtained by Archibong *et al.* (2022). This could be attributed to the relatively low crude fibre content of the seeds and the finished diets. According to Montagne *et al.* (2003), there is an inverse relationship

between dietary fibre and bioavailability of nutrients. Results of this study, therefore, indicate that fibre content of the seeds had little effect on digestibility and retention of nutrients in the feed.

Blood is an important index of physiological, pathological and nutritional status in the organism (Olorode and Ajayi, 2005). The values of all measured haematological indices in this study are within the normal range, as reported by Mitruka and Rawnsley (1977) and Ross *et al.* (1978) for healthy birds. The values of red blood cells, obtained in this study, were within the normal range of  $1.97-3.75 \times 10^{12}$  L reported by Talebi *et al.* (2005). Monocyte values (2.50–5.33 %) were within range of reference values (0.06–5.00 %), as reported by Riddell (2011). In sites of infection, direct pathogen destruction and clean-up of cellular debris are affected by monocytes (Britannica, 2024). Non-significant differences were observed in haematological parameters determined for birds fed experimental diets, except RBC and monocytes, thus implying that decorticated sandbox seeds had no negative effect on immune system of experimental birds even up to 0.3 % inclusion level. The non-significant difference in the values obtained for most of the haematological indices suggests the safety and adequacy of the test ingredient (Alu *et al.*, 2009), which is decorticated raw sandbox seeds (*Hura crepitans*), as a source of methionine in this study. Rekhateh *et al.* (2010) reported that herbal methionine had no significant effect on haematological profiles of broiler chickens, thus, suggesting that dietary herbal methionine has no detrimental effect on chicken survivability. Feeding diets containing herbal methionine did not alter haematological parameters in broilers (Igbasan and Olugosi, 2013) and in domestic layer birds (Igbasan *et al.*, 2012).

The serum biochemical parameters were within normal range reported for chicken (Meluzzi *et al.*, 1992). Total protein values (4.86–6.16 g/l) obtained in this study are within the range of 5.00–8.00 g/l for broiler chicken, as reported by Muhammad *et al.* (2015). The values for total protein were higher in birds on control compared to other treatments. However, no deleterious effect of the SBS on serum biochemical parameters was observed in this study.

Adeniran *et al.* (2017) reported high total protein in birds fed 0 and 50 g/kg fermented castor oil seed meal with either DL-methionine or herbal methionine supplementation. Plasma protein indicates a body with protein reserve, thus, reflecting chicken's ability to store reserve protein even when maximum capacity

for depositing tissue is attained (Azis *et al.*, 2012). This implies the ability of birds to store protein for tissue development due to good protein reserve. The trend for total protein and albumin in this study was similar to Obikaonu *et al.* (2011); they both depend on protein availability in animal diet.

## CONCLUSION

Conclusively, the use of sandbox seeds as a source of methionine has no detrimental effects on performance, nutrients digestibility and blood profile of broiler chickens. Sandbox seeds have the potential to serve as an alternative source of methionine in the diets of broiler chickens.

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## AUTHORS CONTRIBUTIONS

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All authors have read and agreed to the manuscript.

## DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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