Contents lists available at ScienceDirect

Heliyon



journal homepage: www.cell.com/heliyon

Research article

5²CelPress

Incorporation of yeast treated Dialium bark meal on growth performance, blood indices and intestinal physiology of broilers

I.P. Ogbuewu^{a,b,*}, C.A. Mbajiorgu^b

^a Department of Animal Science and Technology, Federal University of Technology, P.M.B. 1526, Owerri, Imo State, Nigeria
 ^b Department of Agriculture and Animal Health, University of South Africa, Florida Science Campus, Private Bag X6, Florida, 1710, South Africa

ARTICLE INFO

Keywords: Dialium bark Yeast Broilers Growth Gut histomorphology Blood parameters

ABSTRACT

Incorporation of beneficial microbes to enhance the nutritive value of non-traditional feed additives is presently being promoted as a cost-effective way of increasing animal performance. Therefore, this trial investigated the impact of the incorporation of Dialium bark meal treated with yeast (TS) into broiler diets on intestinal histology, growth, and blood characteristics. One hundred and eighty days old Ross 308 broiler chickens were assigned to three treatments with five replications of 12 birds in a complete randomized design. The treatments were designed as (TS0: control) which is the (starter and finisher) diets without TS additive; whereas the TS0.5 and TS1.0 diets have TS additive at 0.5 and 1.5 g kg⁻¹, respectively. The feeding trial lasted for 56 days comprising (28 days each for starter and finisher phases). Feed intake, body weight gains (BWG), and feed conversion ratio (FCR) were determined at the end of the starter and finisher phases. Blood and intestinal histomorphological indices were determined at the end of the finisher phase. Results indicate that TS contains 8.15 % crude protein, 29.84 % crude fibre, and 11.61 % ash. In addition, starter broilers in the TS0.5 and TS1.0 groups gained less weight and had a higher feed intake than those in the TSO group. Broilers (29-56 d) in the TS0.5 and TS1.0 groups recorded better (p < 0.05) final live weight (FLW), BWG, and FCR than those in the TS0 group. Feed intake responded linearly to increasing TS levels in the diet during the finisher production phase. Broilers in group TS1.0 tended (p < 0.05) to have higher haemoglobin concentration, packed cell volume, red blood cell count, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration compared to those in group TSO. Conversely, there were no treatment effects (p > 0.05) on mean cell volume, white blood cells, lymphocytes, heterophils, and serum biochemical variables. Broilers on TS diets had significantly improved histoarchitecture of the jejunum in broilers. In conclusion, results suggest that Dialium bark meal treated (TS) with dried active yeast is high in fibre and ash, and its addition at 0.5 and 1.0 g kg⁻¹ feed improved body weight gain, feed conversion ratio, aspects of the blood characteristics and intestinal histomorphology of the jejunum of broilers at the finisher production phase. It is recommended that TS can be added to broiler finisher diet up to 1.0 g kg⁻¹ feed for best growth performance, intestinal histomorphological, and blood characteristics.

https://doi.org/10.1016/j.heliyon.2024.e31725

Received 4 November 2023; Received in revised form 20 May 2024; Accepted 21 May 2024

Available online 22 May 2024

^{*} Corresponding author. Department of Animal Science and Technology, Federal University of Technology, P.M.B. 1526, Owerri, Imo State, Nigeria.

E-mail addresses: dr.ogbuewu@gmail.com, ifeanyi.ogbuewu@futo.edu.ng (I.P. Ogbuewu).

^{2405-8440/© 2024} The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC license (http://creativecommons.org/licenses/by-nc/4.0/).

1. Introduction

In the last decade, global poultry production has been adversely affected by the high costs of conventional feedstuffs and additives [1]. This escalating cost of poultry feedstuffs and additives has been and will continue to pose a major problem to the poultry industry, if not addressed. One likely approach to maximising poultry productivity at a reduced cost is to utilise the barks of tropical plants such as *Dialium guineense* Willd (Black velvet tamarind) bark. The plant is indigenous to Africa and other tropical countries [2]. Detailed information on the taxonomy and medicinal values of the plant has been documented in the literature [3,4]. Chemical profiling of Dialium bark meal indicates that it is rich in ash, fibre (i.e., lignin, cellulose, and hemicellulose), flavonoids, and total phenols [2,4,5]. In addition, Dialium bark meal contains anti-nutritional factors (ANFs) like tannins, cyanogenic glycosides, and trypsin inhibitors [5]. A recent feeding experiment in our station [5] found that addition of Dialium bark meal to broiler diet at 0.5 g kg⁻¹ feed improved BWG and FCR. However, broilers offered Dialium bark meal at 1.0–1.5 g kg⁻¹ feed gained less weight. The reasons behind the poor productivity of broilers fed 1.0–1.5 g kg⁻¹ feed Dialium bark meal are not clear but could possibly be linked to its high fibre content and the presence of ANFs, among other factors.

The potential of beneficial microbes to break down the complex carbohydrates in tree bark to their monomers has been highlighted in the literature [6–8]. Yeast (*Saccharomyces cerevisiae*) is one type of microorganism capable of producing lignocellulolytic enzymes such as xylanase, laccases, cellulases, and pectinases that biodegrade cell wall component into their different monomer units [6,7]. It was hypothesised that yeast will provide the enzymes that will biodegrade cell wall component in Dialium bark meal into their different monomer units thereby releasing the trapped nutrients and bioactive compounds. In addition treating Dialium bark meal with yeast would result in a value-added end-product, with prebiotic and probiotic properties leading to an enhancement in production indices and the physiological well-being of chickens. Thus, this study was undertaken based on the hypothesis that Dialium bark meal treated with dried active yeast (TS) is rich in important nutrients and bioactive compounds, and that its incorporation into broiler diets would improve growth performance, intestinal histomorphology, and blood components.

2. Materials and methods

Table 1

2.1. Ethics statement, collection, and processing of test materials

This investigation was done at the Poultry Unit, Federal University of Technology Owerri ($4^\circ 4'$ and $6^\circ 3'$ N and $6^\circ 15'$ and $8^\circ 15'$ E) Nigeria. Experimental procedures were approved by the institution's Animal Ethics Committee (Ref#: 2023/AST/134). Dialium stem bark was obtained from the institution's Botanical Garden in March 2023 and processed into meals as described by Ogbuewu and Mbajiorgu [5]. The dried active baker's yeast (STK Royal®) used in this experiment was procured from a reputable pharmaceutical store in Owerri, Imo State. Nigeria. Dialium bark meal used in the present feeding experiment was fortified with dried active yeast (*S. cerevisiae*) at a ratio of 10.0 g–1.0 g yeast and designated TS.

Ingredients	Starter (1–28 d)	Finisher (29–56 d)	
Corn	540	600	
SBM	300	250	
Spent grain	20	10	
Wheat offal	40	30	
PKC	50	50	
Bone meal	30	35	
Oyster shell	10	15	
Salt	2.5	2.5	
Vitamin/mineral premix	2.5	2.5	
Lysine	2.5	2.5	
Methionine	2.5	0.25	
Total	1000	1000	
Determined composition (g/kg)			
CP	239.0	203.9	
ME (Kcal/kg)	3000	3200	
CF	40.8	42.8	
EE	41.2	44.4	
Ash	16.5	16.6	
Calcium	16.3	16.5	
Phosphorus	11.3	11.4	

Tuble I	
Ingredient and nutrient composition of broile	er diets (g kg $^{-1}$).

To provide the following per kg of feed: vitamin A - 2, 000,000 i.u; vitamin D3 - 100 i.u; vitamin E - 8 g; vitamin K - 0.4 g; vitamin B1 - 0.3 g; vitamin B2, 1.0 g; vitamin B6 - 0.6 g; vitamin C - 2.40 g, vitamin B12 - 40 g; manganese - 160 g; iron, 8.0 g; zinc - 7.2 g; copper - 0.3 g; iodine, 0.25 g; cobalt - 36.0 mg; selenium - 16.0 mg; SBM - soybean meal; PKC - palm kernel cake; CP -crude protein; ME - metabolisable energy; CF - crude fibre; EE - ether extract.

2.2. Experimental birds and management

In the feeding trial, 180 day-old chicks (Ross 308 broiler) were randomly allocated to 3 treatment groups (TS0, TS0.5, and TS1.0.) of 60 chicks each. Each group was replicated five times, with twelve birds per replicate. Broiler chicks in TS0 group received basal starter and finisher diets (Table 1) without TS feed additive and served as the control treatment. Broiler chicks in TS0.5 and TS1.0 groups received basal starter and finisher diets with TS additive at 0.5 and 1.0 g kg⁻¹ feed, respectively. The feeding programme consisted of broiler starter (1–28 days) and finisher (29–56 days) mash diets. Birds were housed in a dwarf-walled naturally ventilated building partitioned into floor pens measuring 4 m² and fitted with drinkers and feeders. The floor of the pens was scrubbed with a hard brush and thereafter disinfected with ISO®. Wood shavings were used as litter, covering the concrete pen floor to a depth of 5 cm. All the birds had *ad libitum* access to clean water and feed throughout the feeding period. Basal starter diets (Table 1) were prepared to meet the nutritional requirements of Ross 308 broilers [9].

2.3. Data collection and experimental procedure

2.3.1. Proximate analyses and growth performance

The proximate content of TS and nutrient composition of basal diets were determined as described by AOAC [10]. Analyses were done in triplicate and results were presented in percentages. Crude protein was determined using the Micro-kjeldah method [11]. The initial live weights of the chicks were determined at the beginning of the study using a digital weighing balance (Model: HESA 3303) and weekly thereafter to calculate BWG. Feed intake was computed by subtracting the quantity of feed leftover from the quantity given the previous morning. Feed conversion ratio was computed as feed consumed divided by weight gain at the end of the starter, finisher, overall production phases.

2.3.2. Histomorphological examination

Histomorphology of the jejunum was performed at 56 days of age. Five chickens (one per replicate) were selected and humanely slaughtered as described by Liza et al. [8]. The slaughtered bird was dissected by cutting open the abdomen via a midline incision to remove the jejunum (from the pancreatic loop to Meckel's diverticulum). Thereafter, the proximal section of the jejunum was excised following standard procedures [12,13]. The excised samples were flushed with saline, fixed in 10 % neutral buffered formalin for 24 h, and thereafter embedded in paraffin wax. The embedded samples were sectioned and stained with haematoxylin and eosin (H&E) following the procedure described by Bancroft et al. [14]. Tissue sections (5 µm thick) were cut using a microtome and captured via a microscope (Leica Microscope GmbH, Wetzlar, Germany) fitted to a digital camera (Sony DXC-930P, Sony Corporation, Tokyo, Japan). Image analysis was done via Leica Biosystems Image scope software with Leica application suite version 3.4. Villus height (VH) and crypt depth (CD) were determined as described by Rady et al. [13], whereas, the VH/CD ratio was determined by dividing the VH by the CD. The presence of intact lamina propria was the criterion for the selection of villus.

2.3.3. Haematological and serum biochemical assay

On the 56th day of the feeding experiment, five broilers (one per replicate) were weighed and euthanized through the brachial vein using the methods described by Ogbuewu and Mbajiorgu [5]. Blood samples (7.0 mL) were collected from each of the selected birds using a 10 mL syringe and thereafter transferred into ethylenediaminetetraacetate, EDTA (3 mL), and non-EDTA (4 mL) Vacutainer tubes. Blood samples were analysed at the Clinical Chemistry and Haematology Diagnostic Laboratory of the Federal Medical Centre, Owerri, Nigeria. Haematological parameters, viz. packed cell volume (PCV), red blood cell (RBC), haemoglobin (Hb) concentration, white blood cell (WBC), lymphocytes (L), heterophils (H), and H/L ratio were determined as described by Ogbuewu and Mbajiorgu [5]. Mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular haemoglobin concentration (MCHC) were computed using standard methods [15]: MCV = $10 \times PCV/RBC$, MCH = Hb/RBC and MCHC = Hb $\times 100/PCV$. AGGAPE assay kits (AGAPPE Diagnostics Switzerland GmbH) were used to determine the following serum biochemical parameters: total protein (Biuret method), albumin (Bromocresol green method), globulin (determined by subtracting albumin value from total protein), glucose (GOD-PAP method), urea (Urease/GLDH method), creatinine (Jaffe's method), cholesterol (CHOD-PAP method), aspartate aminotransferase (International Federation of Clinical Chemistry recommended procedure), and alanine aminotransferase (International Federation of Clinical Chemistry recommended procedure), and alanine aminotransferase (International Federation of Clinical Chemistry recommended procedure), and alanine aminotransferase (International Semi-auto chemistry analyser (BA-88A model, Mindray, Nansha, Shenzhen, China).

2.4. Statistical analysis

Statistical analyses were performed using Statistical software (SPSS for Windows Version 10; SPSS GmbH, Munich, Germany) to assess if means differed across treatment groups. Results were presented as means, standard deviation (SD), and standard of error of the mean (SEM). Differences between means were determined at a 5 % probability level using the Least Significant Difference (LSD) test. The model used was: $Y_{ij} = \mu + B_i + E_{ij}$; where $Y_{ij} =$ an observation (growth performance, intestinal histomorphology, and blood variables), μ is the general mean, Bi = the fixed effect of the TS additive levels (i = 3; 0, 0.5, and 1.0 g kg⁻¹ feed), and E_{ij} = random error linked with each observation.

3. Results

3.1. Proximate composition of TS and growth performance

The results of the proximate composition of TS as displayed in Table 2 show that TS is low in crude protein (8.15 %) and high in crude fibre (29.84 %) and ash (11.61 %). Growth parameters of broilers aged 1–28 d (starter phase), 29–56 d (finisher phase), and 1–56 d (overall phase) were summarised in Table 3. In the starter phase (1–28 d), there were statistical differences (p > 0.05) in feed intake among broilers. Broiler chicks in the TS0 (control) group had heavier (p < 0.05) FLW and BWG than those in the TS1.0 group, but similar to those in the TS0.5 group. However, broiler chickens in TS0.5 and TS1.5 had similar (p > 0.05) body weight gain. Broilers in the control group had a better (p < 0.05) FCR than broilers in the TS1.0 group. However, the TS0 and TS0.5 groups had similar FCR. Finisher broilers (29–56 d) in the TS0.5 and TS1.0 groups had significantly improved (p < 0.05) FLW, BWG, and FCR compared to broilers in the TS0 group. There was a statistical difference (p < 0.05) in feed intake among broilers in the finisher phase. In the overall phase (1–56 d), feed intake (p < 0.05) increased as the levels of TS were increased in the diet. However, BWG was increased by TS additive levels. Broilers in the TS1.0 group had better FCR than those in the other two dietary groups.

3.2. Haematological values

Table 4 shows the haematological values of broilers fed TS diets. Broilers in the TS1.0 group had statistically increased (p < 0.05) Hb and PCV relative to those in the TS0 group. Conversely, birds in TS0 and TS0.5 groups recorded comparable Hb and PCV values. Dietary TS additive affected RBC, with the birds in the TS1.0 group having a higher RBC (p < 0.05) than the birds in the TS0 group. Conversely, broilers in groups TS0 and TS0.5 had comparable RBC values. MCV ranged from 97.53 to 103.89 fl with broilers in the TS0.5 group returning the lowest MCV, while those in the TS1.0 group had the highest value. However, the identified difference was not significant (p > 0.05). Likewise, the impacts of TS diets on WBC, lymphocytes (L), and heterophils (H) were not significant (p > 0.05). MCH and MCHC ranged between 30.16 and 41.63 pg and 29.67–40.10 g/dl, respectively; however, the identified differences were significant (p < 0.05). Broilers fed TS diets had similar (p > 0.05) H/L ratios.

3.3. Serum biochemical parameters

The serum biochemical values of broilers are summarised in Table 5. Total protein and albumin values ranged from 49.72 to 50.63 g/dl and 19.34–20.72 g/dl, respectively. However, the recorded difference was not significant (p > 0.05). Likewise, serum globulin and albumin/globulin ratios were not affected by TS additive. Similar glucose, urea, and creatinine levels were found among broilers fed TS diets. There were no significant differences (p > 0.05) in the serum cholesterol values among the groups. Likewise, the effect of TS diets on aspartate aminotransferase (AST), alanine aminotransferase (ALT), and the AST/ALT ratio was not statistically significant (p > 0.05).

3.4. Intestinal histomorphology

Table 6 displays the results of jejunal villus height (VH), crypt depth (CD), and VH/CD ratio in broilers on TS additive. The mean values of VH, CD, and VH/CD ratio of broilers offered TS diets were 1714.43 μ m, 135.43 μ m, and 12.78, respectively. Villus height and VH/CD ratio were significantly higher (p < 0.05) in broilers in groups TS0.5 and TS1.0 compared to birds in the TS0 group. Broilers in the TS0 group had significantly higher CD (p < 0.05) than those in TS0.5 and TS1.0 groups.

4. Discussion

4.1. Nutrient content and growth performance

There are growing numbers of trials on the utilisation of non-traditional feedstuffs and additives to promote broiler productivity, and the focus here was dietary TS. This study determined the proximate composition of TS and the impact of its addition at 0, 0.5, and 1.0 g kg⁻¹ feed on health and productivity of broilers. The results of proximate composition show that TS is rich in ash (minerals) and fibre, which is similar to the values recorded in the literature [5]. During the starter phase, broilers that received 1.0 g TS kg⁻¹ feed

Table 2

Proximate composition of TS feed additive (%).					
Parameters	TS				
Dry matter	85.99				
Crude protein	8.15				
Ether extract	1.83				
Carbohydrates	35.56				
Crude fibre	29.84				
Total ash	11.61				

Table 3

Growth performance of broilers offered TS feed additive.

Parameters 1–28 d	Additive levels of TS			Mean	SD	SEM	<i>p</i> -value
	TS0	TS0.5	TS1.0				
ILW (g/bird)	47.30	48.02	47.54	47.62	0.50	0.17	0.212
FLW (g/bird)	606.40 ^a	586.80 ^{ab}	531.47 ^b	574.89	42.81	14.27	0.024
BWG (g/bird)	21.66 ^a	20.96 ^{ab}	18.98^{b}	25.11	2.04	0.68	0.023
FI (g/bird)	54.42^{b}	64.21 ^a	60.49 ^a	59.71	6.07	0.02	0.128
FCR	2.51^{b}	3.06 ^{ab}	3.19^{a}	2.40	0.33	0.11	0.027
29–56 d							
FLW (g/bird)	2200.00°	2420.00^{b}	2700.00^{a}	2440.00	219.06	73.02	< 0.001
BWG (g/bird)	56.91 ^c	67.45 ^b	75.47 ^a	66.611	8.14	2.71	< 0.001
FI (g/bird)	118.91 ^b	129.10^{b}	142.98 ^a	130.33	10.67	3.56	< 0.001
FCR	2.09^{a}	1.91 ^b	1.89 ^b	1.97	0.10	0.03	0.007
1–56 d							
BWG (g/bird)	38.44 ^c	42.37 ^b	47.36 ^a	50.90	1.05	1.55	< 0.001
FI (g/bird)	86.67 ^c	94.79 ^b	103.59 ^a	100.06	7.92	2.64	< 0.001
FCR	2.25	2.24	2.18	1.97	0.44	0.15	0.313

^{a,b,c} Means not having the same superscript are significant (p < 0.05; LSD test). LSD least significant difference; *ILW* initial live weight; *FLW* final live weight; *BWG* body weight gain; *FI* feed intake; *FCR* feed conversion ratio; *SD* standard deviation; *SEM* standard error of the mean; *P* probability.

Table 4

Haematological characteristics of broilers fed TS feed additive.

Parameters	Additive levels of TS			Mean	SD	SEM	P-value
	TS0	TS0.5	TS1.0				
Hb (g/dl)	7.67 ^b	8.77 ^{bc}	11.87 ^a	9.43	2.04	0.68	0.003
PCV (%)	25.83 ^b	25.70 ^b	29.60 ^a	27.04	2.14	0.71	0.008
RBC ($\times 10^6 \mu$ l)	2.56^{b}	2.64 ^{ab}	2.85 ^a	2.69	0.18	0.06	0.041
MCV (fl)	101.36	97.53	103.89	100.93	8.33	2.78	0.703
MCH (pg)	30.16^{b}	33.31 ^{ab}	41.63 ^a	35.04	6.61	2.20	0.027
MCHC (g/dl)	29.67 ^{bc}	34.18 ^{ab}	40.10 ^a	34.65	5.43	1.81	0.029
WBC ($\times 10^3 \mu l$)	8.80	8.23	8.80	8.611	0.94	0.31	0.750
Lymphocytes (%)	75.33	76.00	75.00	75.44	3.24	1.08	0.946
Heterophils (%)	21.66	22.65	21.33	21.89	1.90	0.63	0.729
H/L ratio	0.29	0.30	0.29	0.29	0.033	0.01	0.912

Means not having the same letters are significant (p < 0.05; LSD test). LSD least significant difference; Hb haemoglobin; PCV packed cell volume; RBC red blood cell; MCV mean corpuscular volume; MCH mean corpuscular haemoglobin; MCHC mean corpuscular haemoglobin concentration; WBC white blood cell; H heterophils; L lymphocytes; fl femtolitre; pg picogram; SD standard deviation; SEM standard error of the mean; P probability.

Table 5

Serum biochemistry of broilers fed TS feed additive.

Parameters	Additive levels of TS			Mean	SD	SEM	P-value
	TS0	TS0.5	TS1.0				
Total protein (g/dl)	49.93	50.63	49.72	50.10	3.87	1.29	0.966
Albumin (g/dl)	19.98	20.72	19.34	20.01	1.58	0.53	0.628
Globulin (g/dl)	29.95	29.92	30.38	30.08	3.16	1.05	0.985
ALB/GLO ratio	0.67	0.71	0.64	0.67	0.08	0.03	0.635
Glucose (mg/dl)	159.67	162.85	160.67	161.06	5.29	1.76	0.801
Urea (mg/dl)	5.60	5.74	5.42	5.59	0.54	0.18	0.807
Creatinine (mg/dl)	0.40	0.50	0.43	0.44	0.09	0.03	0.422
Cholesterol (mg/dl)	83.67	84.00	85.67	84.44	1.94	0.65	0.460
AST (IU/L)	12.51	12.54	11.73	12.26	0.90	0.30	0.518
ALT (IU/L)	14.33	15.00	13.33	14.22	1.20	0.40	0.256
AST/ALT ratio	0.88	0.84	0.89	0.87	0.11	0.04	0.879

Means having the same letters are not significant (p < 0.05; LSD test). LSD least significant difference; ALB albumin; Glob globulin; AST aspartate aminotransferase; ALT alanine aminotransferase; SD standard deviation; SEM standard error of the mean; P probability.

recorded a higher FCR (poor), gained less weight, and had a higher feed intake than those that received a diet without TS additive. This implies that inclusion of TS at 1.0 g kg⁻¹ feed in a chicken diet does not support growth performance at the early stage of life. The reason behind this poor performance is not clear; however, it could be attributed in part to poor digestion and utilisation of the experimental diets by birds, as their gastrointestinal tracts were not fully developed to utilise TS diets. On the other hand, broilers (1–28 d) that received TS diet at 0.5 g kg⁻¹ feed had similar BWG with the control broilers, suggesting that 0.5 g kg⁻¹ feed could be the

Table 6

VH, CD and VH/CD ratio of the jejunum of broilers fed TS feed additive.

Parameters d 56	Additive levels of TS			Mean	SD	SEM	p-value
	TS0	TS0.5	TS1.0				
VH (μm)	1623.62 ^b	1754.38 ^a	1765.30 ^a	1714.43	12.68	17.67	0.001
CD (µm)	149.29 ^a	130.16^{b}	126.84 ^b	135.43	70.80	6.71	0.031
VH/CD ratio	10.90 ^b	13.49 ^a	13.96 ^a	12.78	1.53	0.53	0.002

 a,b,c Means not having the same superscript are significant (p < 0.05; LSD test). LSD least significant difference; VH villus height; CD crypt depth; SD standard deviation; SEM standard error of the mean; P probability.

tolerance limit of the chicks. It would be interesting to further investigate the histomorphological characteristics of the small intestine of broiler chicks offered diets with and without TS additive.

The consumption of fibre-rich diets by chickens is governed by the bird's energy requirements [17]. There is compensatory feeding behaviour in birds when the energy level of the feed is diluted [17]. This may explain the significantly higher feed intake for broilers aged 29–56 d fed 0.5 and 1.0 g TS kg⁻¹ feed in this experiment. This agrees with the results of Ogbuewu and Mbajiorgu [5], who found elevated feed intake in broilers fed Dialium bark meal at 1.5 g kg⁻¹ feed for 47 days.

Feed conversion ratio was significantly influenced by dietary TS additive at the finisher production phase, with a lower FCR (improved) that compared favourably with the target FCR of 1.5 for Ross 308 broilers [9]. Earlier feeding experiments indicated that broilers given Dialium bark meal at 1.0 and 1.5 g kg⁻¹ feed had poor FCR and BWG [5] which differed from the results presented here. The observed disparity could be associated with differences in the quantity of Dialium bark meal added to the ration and the processing method used. This study hypothesised that TS additive would enhance BWG because of its likely ability to modulate intestinal microbiota composition via antagonistic behaviour and competitive exclusion, leading to reduced pathogenic bacteria competition with the host for available nutrients. The higher BWG in broilers fed TS0.5 and TS1.0 diets indicates the ability of yeast present in the test feed additive to modulate gut microbiota composition [18] and absorptive capacity of the intestine, leading to an improvement in gut health and nutrient uptake. This observation corroborates with Hetland et al. [19] who discovered that inclusion of moderate levels of fibrous materials in the chicken feed had no adverse consequence on growth performance even though the nutrient content of the feed was diluted. The fact that broilers fed 0.5 and 1.0 g TS kg⁻¹ during the finisher production phase had a lower FCR than broilers at the starter production phase suggests that broilers' ability to utilise a fibrous diet increases as they grow older. The observed difference in BWG between starter and finisher broilers in this study could be ascribed to digestive tract adaptability to utilise TS additive. The overall growth phase results indicate that TS additive increased BWG at a similar FCR to the control diet. These results suggest that, outside of improving FCR, TS additive can enhance BWG in broilers via other modes of action, as evidenced by intestinal histomorphological results. These findings are consistent with those of [18], who reported that yeast, a component of TS, enhanced BWG in broilers by preserving normal intestinal microbiota via competitive exclusion and antagonism, resulting in decreased bacterial competition with the host for available nutrients.

4.2. Haematological characteristics

Blood variables were employed as diagnostic tools for screening the feeding quality of non-traditional feedstuffs and additives [20]. In this study, dietary TS additive increased Hb, PCV, and RBC values in broilers; however, values fall within the range of 7–13 g/dl (Hb), 22–35 % (PCV), and 2.5–3.5 × $10^6 \mu l$ (RBC) reported for healthy broilers [21]. The improved Hb, PCV, RBC, and haematological indices (MCV, MCH, and MCHC) at 1.0 g TS kg⁻¹ feed can be attributed to enhanced utilisation by the broilers, resulting in improved BWG. In addition, MCV which determines the size of RBC was higher in broilers fed 1.0 g TS kg⁻¹ feed than the control, although values fell within the range (90–140 fl) reported for healthy chickens [21]. Similarly, MCH and MCHC values recorded for broilers fed diets containing TS at 0 and 0.5 g kg⁻¹ feed were within the reference values of 33–47 pg (MCH) and 26–35 g/dl (MCHC) reported for healthy chickens [21], indicating macrocytic normochromic red cells. The improved MCH and MCHC values in broilers fed 1.0 g TS kg⁻¹ feed could be attributed to enhanced nutrient utilisation.

The WBC helps to protect the body from disease-causing organisms. No differences in WBC, lymphocytes, or heterophils were observed in this study, implying that TS diets had no stimulatory effect on the immune system of broilers. However, WBC, lymphocytes, and heterophils values were within the reference range of $4.78-9.73 \times 10^3/\mu$ l (WBC), 51.17-75.33 % (lymphocytes), and 21.46–36.17 % (heterophils) reported for healthy Ross 308 broilers by Kongpechr et al. [22]. The present study is consistent with Ogbuewu and Mbajiorgu [5], who demonstrated that addition of Dialium bark meal at 0, 0.5, 1.0, and 1.5 g/kg⁻¹ feed to broiler diets did not affect WBC, lymphocytes, or heterophils. The H/L ratio reflects the immune response of poultry and is used in medical and nutritional assessments to investigate chicken stress responses [23,24]. In this study, the similarity in H/L ratios in broilers among the treatment groups ruled out the possibility of nutritional and environmental stress.

4.3. Serum biochemical values

Ogbuewu et al. [20] found a positive relationship between serum proteins and dietary protein intake. Results indicate that the serum proteins were not affected by TS additive, indicating the quality of protein in the diets was not compromised. Similar results were observed by Ogbuewu and Mbajiorgu [5] in broilers fed Dialium bark meal diets. Elevated serum levels of liver enzymes (ALT and

AST) are excellent indicators of hepatic damage in farm animals [25]. The AST/ALT ratio is usually greater than one in the case of hepatic damage [26]. The fact that serum AST/ALT ratios in this trial were <1.0 indicates that the test feed additive is not toxic to broilers. There was no effect of dietary TS additive on serum cholesterol concentrations. This result agrees with Ogbuewu and Mbajiorgu [5] who reported similar serum cholesterol levels in broilers fed Dialium bark meal. However, these values were within the normal range (87–192 mg/dl) reported for healthy broilers by Meluzzi et al. [27].

4.4. Intestinal histomorphology

The digestive tract is an intricate and constantly changing organ that plays an important part in feed digestion, nutrient uptake, and immune function [28]. The use of gut histomorphological indices in pathophysiology and nutritional studies has been documented [29,30]. Dietary TS additive increased jejunal VH, suggesting a higher absorptive capacity. Reduced jejunal CD and increased VH/CD ratio as observed in broilers fed TS at 0.5 and 1.0 g kg⁻¹ feed suggest enhanced ability for nutrient absorption. The enhanced histomorphology of the jejunum in broilers fed TS diets may be ascribed to the stimulatory action of the product. Yeast present in TS diets may have improved the histoarchitecture of the intestine, which supports the findings of others [18,31]. This could be the main factor that explains the impact of dietary TS additive on growth indices during the finisher production phase, thereby enhancing the uptake of nutrients needed for body growth and development.

5. Conclusion

This study found that dietary TS is rich in ash (minerals) and fibre, and its addition at 0.5 and 1.0 g kg⁻¹ feed have the potential to improve growth performance, the histoarchitecture of the jejunum, and aspects of the blood characteristics of Ross 308 broilers at the finisher phase. In contrast, during the starter phase, results indicate that broilers on TS diets at 0.5 and 1.0 g kg⁻¹ feed gained less weight and had a higher feed intake than those fed a control diet, indicating the inability of TS diets to support early life growth in Ross 308 broilers. Although, there were observed differences in the serum chemistry of broilers that were offered control diets compared to those that received the test diets. Further experiments are required to determine the optimal level of TS that supports growth performance, blood characteristics, and intestinal histomorphology of broilers.

Funding statement

This study did attract any grant from any funding agency.

Additional information

No additional information is available for this study.

Data availability

Data will be made available on request.

CRediT authorship contribution statement

I.P. Ogbuewu: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Conceptualization. **C.A. Mbajiorgu:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- G. Thirumalaisamy, J. Muralidharan, S. Senthilkumar, R.H. Sayee, M. Priyadharsini, Cost-effective feeding of poultry, Int. J. Sci. Environ. Technol. 5 (2016) 3997–4005.
- [2] A.D. Akinpelu, T.O. Awotorebo, O.M. Agunbiade, A.O. Aiyegoro, I.A. Okoh, Anti-vibrio and preliminary phytochemical characteristics of crude methanolic extracts of the leaves of *Dialium guineense* (Willd), J. Med. Plants Res. 5 (2011) 2398–2404.
- [3] E.E. Besong, M.E. Balogun, S.F.A. Djobissie, D.C. Obu, J.N. Obimma, Medicinal and economic value of *Dialium guineense*, Afr. J. Biomed. Res. 19 (2016) 163–170.
- [4] I.P. Ogbuewu, M.M.C. Modisaojang-Mojanaja, B.G. Mokolopi, C.A. Mbajiorgu, Nutritional and chemical composition of Black velvet tamarind (*Dialium guineense* Wild) and its influence in animal production: a review, Open Agric 8 (2023) 20220174.
- [5] I.P. Ogbuewu, C.A. Mbajiorgu, Dietary Dialium guineense stem-bark supplementation improves growth performance and haemato-biochemical characteristics of broiler chickens, Heliyon 9 (2023) e17341.

- [6] M.Y. Abduh, R. Yulianto, D. Avima, A.K. Widianto, R. Alfianny, Solid-state fermentation of cinnamon bark using Aspergillus awamori to increase cinnamon oil yield extracted using hydrodistillation, maceration, and soxhlet extraction, Nat. Volatiles and Essent. Oils 8 (2021) 1575–1588.
- [7] M. Azrinnahar, N. Islam, A.A.S. Shuvo, A.K.M.A. Kabir, K.M.S. Islam, Effect of feeding fermented (*Saccharomyces cerevisiae*) de-oiled rice bran in broiler growth and bone mineralization, J. Saudi Soc. Agric. Sci. 20 (2021) 476–481.
- [8] R.I. Liza, J. Ismita, K.M.S. Islam, R. Chowdhury, M.R. Debi, N.R. Joy, Effect of feeding yeast (Saccharomyces cerevisiae) fermented rice bran with urea on the performance of broilers, J. Bangladesh Agric. Univ. 20 (2022) 57–63.
- [9] Ross Aviagen, 308 Broiler: Performance Objectives, Aviagen Limited Newbridge, Midlothian, 2019. http://en.aviagen.com/assets/Tech_Center/Ross_Broiler/ Ross-308-Broiler-PO-2019-EN (accessed September, 2021).
- [10] AOAC, Official Methods of Analyses, eighteenth ed., Association of Official Analytical Chemists, Washington DC, 2005.
- [11] R.A. Koln, M.S. Allen, Enrichment of proteolysis activity relative to nitrogen in preparations from the rumen for in vitro studies, Anim. Feed Sci. Technol. 52 (1995) 1–4.
- [12] M. Samanya, K. Yamauchi, Histological alterations of intestinal villi in chickens fed dried *Bacillus subtilis* var. natto, Comp. Biochem. Physiol. Mol. Integr. Physiol. 133 (2002) 95–104.
- [13] W.F. Rady, A.N. Sayed, H.A. Abdel-Raheem, Effect of dietary supplementation of echinacea and nucleotides on productive performance, intestinal histomorphology and gene expression of broiler chickens, Assiut Vet. Med. J. 69 (2023) 141–155.
- [14] J.D. Bancroft, C. Layton, S.K. Suvarna, Theory and Practice of Histological Techniques, seventh ed., Churchill Livingstone, New York, 2013.
- [15] N.C. Jain, Veterinary Haematology, Fourth ed., Lea and Febiger, Philadelphia, PA, 1986, p. 297, 1221.
- [16] Y. Akiba, L.S. Jensen, C.R. Bart, R.R. Kraeling, Plasma estradiol, thyroid hormones and liver lipids determination in birds, J. Nutr. 112 (1982) 299–308.
- [17] M.M. Van Krimpen, R.P. Kwakkel, C.M.C. Van der Peet-Schwerin, L.A. en Hartog, M.W.A. Verstegen, Effect of nutrient dilution and non-starch polysaccharides concentration in rearing and laying diets on eating behaviour and feather damage of rearing and laying hens, Poultry Sci. 88 (2009) 759–773.
- [18] I.P. Ogbuewu, V.M. Okoro, E.F. Mbajiorgu, C.A. Mbajiorgu, Yeast (Saccharomyces cerevisiae) and its effect on production indices of livestock and poultry a review, Comp. Clin. Pathol. 51 (2019) 669–677.
- [19] H. Hetland, B. Svihus, V. Olaisen, Effect of feeding whole cereals on performance starch digestibility and duodenal particle size distribution in broiler chickens, Br. Poultry Sci. 43 (2002) 416–423.
- [20] I.P. Ogbuewu, O.O. Emenalom, I.C. Okoli, Alternative feedstuffs and their effects on blood chemistry and haematology of rabbits & chickens: a review, Comp. Clin. Pathol. 26 (2015) 277–286.
- [21] D. Bounous, N. Stedman, Normal avian hematology: chicken and Turkey, in: B.F. Feldman, J.G. Zinkl, N.C. Jain (Eds.), Schalm's Veterinary Haematology, Wiley, New York, 2000, pp. p1147–p1154.
- [22] S. Kongpechr, P. Sukon, D. Sohsuebngarm, The study of haematology in commercial broiler chickens of Cobb 500, Ross 308, and Arbor Acres Plus, J. Mahanakorn Vet. Med. 15 (2020) 209–221.
- [23] S. Azis, Performance and heterophil to lymphocyte (H/L) ratio profile of broiler chickens subjected to feeding time restriction, Int. J. Poultry Sci. 11 (2012) 153–157.
- [24] P. Minias, Evolution of heterophil/Lymphocyte ratios in response to ecological and life-history traits: a comparative analysis across the avian tree of life, J. Anim. Ecol. 88 (2019) 554–565.
- [25] V.H.A. Enemor, J.C. Anosike, B.E.B. Nwoke, P.C. Chikezie, Serum aminotransferases and bilirubin levels in malaria patients, Int. J. Nat. Appl. Sci. 1 (2005) 85–89.
- [26] M. Cheesbrough, District Laboratory Practice in Tropical Countries. Tropical Health Laboratory, second ed., Cambridge University Press, United Kingdom, 2000. Part 2.
- [27] A. Meluzzi, G. Primiceri, R. Giordani, G. Fabris, Determination of blood constituent's reference values in broilers, Poultry Sci. 71 (1992) 337–345.
- [28] Y. Lan, M. Verstegen, S. Tamminga, B. Williams, The role of the commensal gut microbial community in broiler chickens, World's Poult. Sci. J. 1 (2005) 95–104.
 [29] W.A. Awad, K. Gharee, S. Abdel-Raheem, J. Böhm, Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens, Poultry Sci. 88 (2009) 49–55.
- [30] S.S.M. Beski, P.A. Iji, Effect of a processed soy protein product on growth and gut physiology of broiler chickens, J. Appld. Anim. Nut. 3 (2015) 1–10.
- [31] S. Seifi, R. Sayrafi, R. Khoshbakht, A. Gilani, B. Goudarzi, Evaluation of yeast culture and direct-fed microbial on gut histology and serum components of broilers challenged with suboptimal diets under heat stress, Acta Sci. Anim. Sci. 42 (2020) e46828.