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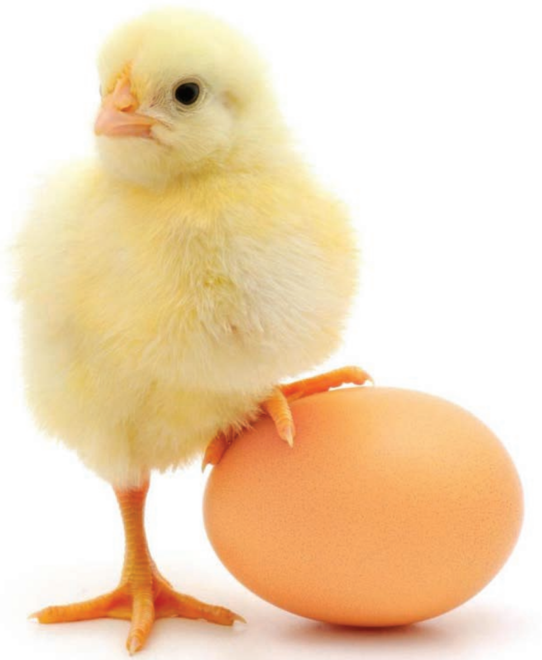

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Research Article
Apparent Digestibility and Gut Integrity of Chicken Fed a Diet Supplemented with African Safou (*Dacryodes edulis*)

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Abstract
Background and Objective: In the present study, which is the second in a series of three publications on a project aimed at evaluating the efficacy and safety of utilizing African Safou (*Dacryodes edulis*) in poultry feeds as an additive, the goal was to determine the apparent digestibility and gut integrity of chicken fed a diet supplemented with *Dacryodes edulis* parts. The first paper explored the impact of the plant parts on growth traits, ceca microbiota and blood parameters in dual-purpose indigenous chicken. **Materials and Methods:** In the current study, a total of 288 male dual-purpose indigenous chicken were fed six experimental diets. The diets differed in terms of the *D. edulis* part that was included plus its inclusion level. Data on the growth performance of the chicken fed the experimental diets were compared to chicken fed a positive (T^+) and a negative (T^-) control diet. The chickens were fed the diets for a period of 14 weeks. The apparent digestibility variability of various nutrients and gut morphometric variables were measured using standard methods. Data were collected and analyzed. **Results:** All the experimental diets improved the apparent digestibility of metabolizable energy (AME), dry matter (DM), fat, crude protein (CP), crude fiber (CF) and calcium . Conversely, the values for zinc, magnesium and phosphorus were negative. The AME, DM content and fat digestibility differed significantly between and among treatment diets. The gut morphometric variables were not significantly different across the treatment groups. **Conclusion:** The findings obtained in this study showed that parts of *D. edulis* plant positively impacted the apparent digestibility of the feed.

Key words: *Dacryodes edulis*, African Safou, digestibility, gut health, blood parameters, minerals, chicken

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sustainable poultry meat and egg production are important for providing quality protein in human nutrition[1]. Numerous constraints limit increases in poultry production in developing countries. The main constraint is the high cost of feed, which accounts for up to 70% of the poultry production cost[2]. Feed additives, such as growth promoters, are a major contributor to the high feed cost[2]. The use of locally available resources, such as plant-based growth promoters, would result in a reduction in the cost of feeds. Information on the safety and efficacy of locally available plant-based alternatives is lacking. Studies have shown the need to maintain chicken gut health and its integrity because it influences the efficiency, absorption and conversion of feedstuff into valuable nutrient needed for metabolism and production[3-5]. In intensive poultry production systems, chicken commonly receive in-feed antimicrobials aimed at improving feed efficiency and preventing infections[4]. However, the uncontrolled use of antibiotics in animal production has led to the emergence of antibiotic-resistant bacterial strains. This effect has negative implications for the management of bacterial diseases in humans and livestock. Furthermore, there is a potential risk of public health concerns due to antibiotic residues in poultry products[5,6].

There is a need to develop safe substitutes to in-feed antibiotic growth promoters. Novel strategies, such as using plant-based additives, are desirable. These strategies have been shown to have nutraceutical properties, which have been shown to aid the maintenance of a healthy gut and simultaneously mitigate intestinal disorders[7]. Oso *et al.*[5] showed that phytogenic feed additives are a novel alternative to the use of antibiotics. Alternative medicine has existed long before the advent of conventional medicine[8]. Various efficacy tests of different phytogenic feed additives, including *Moringa oleifera*[9], thyme[10], garlic[11] and ginger[12], have been confirmed to enhance nutrient digestibility and gut health in chicken. Upadhaya and Kim[13] proposed that the benefits resulted from improved nutrient utilization possibly by stimulating the secretion of several digestive enzymes, such as lipase, amylase and protease, while Sobolewska *et al.*[14] attributed it to enhanced gut health.

African Safou (*Dacryodes edulis*) is a tree native to Africa and its parts have traditionally been used in human and livestock nutritional and medical interventions[15]. This tree is widely cultivated in various African countries, including Cameroon. Proximate and phytochemical analyses of the *D. edulis* leaves, stem, bark, fruit and seed have qualified and quantified rich essential nutrients, as detailed in Ebana *et al.*[16,17]. Moreover, *D. edulis* plant/part extracts have antioxidant, antifungal, antiviral, bactericidal, hypoglycemic, hypolipidemic and hepatoprotective properties[8,16,18]. These properties are good indicators of the plant potential for use as prebiotic and growth promoters for substitution of antibiotics in poultry feeds. Tangomo *et al.*[19] explored the impact of *D. edulis* plant parts on chicken growth traits, ceca microbiota and blood parameters of dual-purpose chicken. The study findings did not identify any negative impacts on all the parameters considered. However, information on the impact of supplementing chicken diets with *D. edulis* plant parts on digestibility and gut integrity is lacking. This study aimed to determine the apparent digestibility and gut integrity as part of efficacy and safety tests needed to determine the potential of using *D. edulis* in poultry feeds without negatively impacting human or animal health and welfare.

MATERIALS AND METHODS

Animal use ethical compliance: At the start of this study, ethical approval for the use of animals was sought from the Animal Ethics Review Committee of Jomo Kenyatta University of Agriculture and Technology. Animal care and use procedures were shared with the committee, which performed an evaluation, after which a compliance certificate (reference number JKU/2/4/896C) was granted. The committee constantly monitored adherence to the care and procedures.

Experimental procedure: A total of 288 male dual-purpose day-old chicks of the breeding stock described by Gikunju *et al.*[20] were sourced from Kenya Agricultural and Livestock Research Organization (KALRO), Naivasha Centre, Kenya. The chickens were transported to Jomo Kenyatta University of Agriculture and Technology (JKUAT), where the feeding trials and laboratory analysis were carried out. The chicks were provided a glucose solution upon arrival at JKUAT from the Kenya Agricultural and Livestock Research Organization[21], Naivasha to reduce transportation stress. At JKUAT, the chicks were intensively brooded from 1-21 days of age in deep litter pens measuring 3×3.4 ft. under the same experimental conditions. During this period, the brooder temperature was set to meet the heat requirements of the chicks at different growing stages[22]. Moreover, the chicks were subjected to a 24 h light regime during the brooding period. The chicks were fed a starter diet devoid of the *D. edulis* plant part powder during the first 7 day of brooding. The starter and finisher diet ingredients and nutrient composition are presented in Table 1, as previously

Table 1: Gut morphometric of chicken fed with the different experimental diets

Variables	Dietary treatment groups[1]								
	TL_{0Ed}	TL_{1Ed}	TB_{0Ed}	TB_{1Ed}	TS_{0Ed}	TS_{1Ed}	T^{+}	T^{-}	p-value
TMT (µm)									
8th week	675.44±63.8	656.88±109.0	665.52±35.9	701.41±39.9	543.01±12.2	572.68±22.0	707.03±91.3	876.55±74.5	0.068
14th week	670.48±71.4	718.77±82.5	576.36±72.5	837.43±97.5	596.01±53.2	715.03±51.4	556.73±63.1	617.36±41.8	0.190
LPT (µm)									
8th week	125.76±6.8	131.70±10.0	150.54±10.9	175.83±31.2	128.08±9.5	160.94±6.3	140.09±28.8	118.54±23.4	0.400
14th week	176.97±20.3	141.14±29.1	131.50±11.2	185.13±24.7	164.63±16.3	174.51±14.1	110.79±10.8	149.17±4.8	0.120
MMT (µm)									
8th week	19.72±2.9	18.96±2.3	20.79±1.7	25.20±5.5	21.23±3.8	26.34±6.5	17.73±3.1	15.91±1.1	0.550
14th week	25.48±4.6	29.42±12.3	19.61±0.5	18.65±2.5	26.12±1.9	39.35±9.5	22.06±3.4	22.84±2.1	0.350
SBT (µm)									
8th week	77.45±10.6	49.44±7.2	46.30±5.1	65.79±16.1	44.01±6.4	67.76±18.6	50.61±10.8	58.30±2.6	0.370
14th week	72.28±16.0	73.66±13.3	61.82±9.9	56.39±4.3	52.20±2.6	75.85±15.4	41.30±2.4	89.81±12.6	0.120
MET (µm)									
8th week	33.00±4.0	17.83±1.2	22.79±1.8	22.08±1.2	18.56±3.7	22.56±2.2	20.67±0.4	22.32±0.5	0.010*
14th week	21.45±3.1	16.90±1.1	24.91±2.4	19.38±3.2	21.94±2.5	27.15±6.2	20.16±2.9	28.34±4.5	0.350
VH (µm)									
8th week	543.51±59.9	541.54±113.0	514.76±15.8	575.91±56.9	404.32±28.1	394.17±39.4	557.71±66.3	741.84±61.6	0.029*
14th week	507.53±70.6	539.15±70.6	478.02±91.0	660.85±56.7	458.46±49.1	526.14±65.8	506.13±65.0	482.55±30.3	0.500
VWT (µm)									
8th week	56.35±16.9	53.91±2.7	92.15±2.3	78.20±11.3	67.66±16.6	78.05±7.7	72.92±6.0	53.13±13.2	0.210
14th week	82.22±7.7	84.12±14.2	107.63±5.3	95.75±4.0	70.78±4.3	79.43±18.0	85.33±15.9	79.09±3.7	0.400
VWJ (µm)									
8th week	96.67±10.4	106.85±5.9	125.02±3.5	131.13±15.7	102.55±7.2	122.70±7.1	111.08±8.2	104.23±2.3	0.110
14th week	135.66±13.9	123.31±6.1	122.40±6.9	135.77±9.4	111.36±4.2	141.55±10.5	122.96±27.1	115.89±1.0	0.660
CD (µm)									
8th week	113.13±9.5	111.32±4.9	149.11±9.6	170.26±24.9	118.14±19.1	156.21±11.5	114.21±27.8	127.28±32.7	0.310
14th week	147.48±11.9	142.75±37.5	160.82±26.4	171.44±28.3	166.78±14.2	190.32±9.0	98.39±9.9	148.83±31.2	0.300
VH/CD									
8th week	4.96±0.9	4.84±0.9	3.47±0.1	3.60±0.7	3.73±0.9	2.58±0.4	5.41±1.3	6.78±1.8	0.200
14th week	3.44±0.4	4.15±0.9	3.09±0.5	4.06±0.6	2.76±0.2	2.80±0.4	5.18±0.6	3.57±0.7	0.170
VH/VWT									
8th week	11.50±3.5	10.25±2.5	5.59±0.2	7.49±0.6	6.85±1.8	5.25±1.0	7.87±1.5	15.72±3.9	0.076
14th week	6.14±0.4	6.75±1.3	4.55±1.1	6.93±0.6	6.57±1.0	7.58±2.1	6.06±0.4	6.09±0.1	0.700
VH/VWJ									
8th week	5.68±0.5	5.07±1.0	4.13±0.1	4.53±0.7	3.95±0.1	3.22±0.3	5.17±1.0	7.11±0.5	0.021*
14th week	3.71±0.1	4.34±0.3	3.86±0.5	4.91±0.4	4.11±0.3	3.78±0.6	4.28±0.3	4.17±0.3	0.550
VST (µm²)									
8th week	94059.92±26.1	90272.01±15.5	148933.78±56.7	144556.58±31.1	88121.98±26.1	94945.42±60.0	126121.06±17.0	122323.87±28.7	0.280
14th week	134091.40±29.5	142030.53±27.0	158592.00±22.4	198449.16±17.8	101345.19±99.0	123809.21±99.2	141792.92±43.6	120534.46±12.4	0.260
VSJ (µm²)									
8th week	166939.69±29.1	182422.75±40.80	201977.42±74.4	237583.51±40.2	131210.98±17.9	152745.29±21.01	191253.51±10.8	243258.84±23.6	0.095
14th week	222325.66±49.3	211481.71±38.3	187246.29±45.4	281896.88±31.8	161136.39±20.8	233086.24±33.5	206292.41±69.1	175424.25±96.5	0.570

TMT: Total mucosa thickness, LPT: Lamina propria thickness, MMT: Muscularis mucosa thickness, SBT: Submucosa thickness, MET: Muscularis externa, VH: Villus height, VWT: Villus width at the top, VWJ: Villus width at the junction, CD: Crypt depth, VH/CD: Villus height/crypt depth ratio, VH/VWT: Villus. height/villus width at the top, VH/VWT villus height/villus width at the junction (), VST: Villus surface area at the top () and VSJ: Villus surface area at the junction described by Tangomo *et al.*[19]. Both diets were provided *ad libitum* during the two growth phases. Water was freely accessible at all times. The chicks were vaccinated following the guidelines by Farrell[2].

D. edulis plant parts were used to formulate the six experimental diets as described by Tangomo *et al.*[19]. The diets differed in the *D. edulis* part (leaves, stem and bark combination and seed) that was incorporated, as well as the level (0.5 or 1%) of inclusion. The diets were TL_{0Ed} (0.5% leaves powder), TL_{1Ed} (1.0% leaves powder), TB_{0Ed} (0.5% stembark powder), TB_{1Ed} (1.0% stembark powder), TS_{0Ed} (0.5% seeds powder) and TS_{1Ed} (1.0% seeds powder). Moreover, a positive (T^+ positive control; 0.5 g oxytetracycline as recommended by the manufacturer) and a negative (T^- negative control; having no commercial antibiotic and no plant supplement) diet were formulated and fed to the chickens at starter and finisher phases.

The negative control diet was fed to all chicks for 1 week before introduction to the experimental starter diet for a further 7 weeks. From week 8, the growing chicks were fed the finisher diet until the end of the experimental period, which was 14 weeks. At week 4, each of the chicks was weighed and allocated to a cage. A total of 72 cages were used and each was assigned 4 chicks of similar weight. The experimental units (cages) were randomly assigned to the six experimental diets plus the negative and positive control diets. Thus, each treatment group had 9 replicates with a total of 36 birds. No data or samples were collected in the first week after the chicks were allocated to their respective cages. This measure was taken to allow the chicks to acclimatize to the experimental conditions. Thus, the chicks were subjected to the experimental diets from week 5, which was also the time at which sample collection commenced. Feed and water were freely accessible throughout the experimental period. Vitamins and trace elements were provided in the drinking water.

Proximate analysis of Dacryodes edulis powder parts and experimental feed: Proximate analysis of the *D. edulis* plant parts and experimental diets were performed for moisture, crude protein, ash and ether extract according to the procedure described by Tangomo *et al.*[19]. The dry matter contents were calculated as the weight lost after drying the samples at 105°C for a period of 24 h. The ash content was calculated from the weight loss following incineration of the sample in a muffle furnace at 550°C for 6 h. The crude protein was determined using the Kjeldahl technique and the calculated N was multiplied by 6.25. The crude fiber (CF) and ether extract (EE) were measured using methods described by the Association of Official Analytical Chemists[23], whereas the Nitrogen Free Extract (NFE) was determined using the difference method:

$$100\text{-}(CP+EE+CF+Ash)$$

Apparent digestibility: The feed intake (FI) was measured as the difference between the amount of feed provided to the birds and the remnant after a period of 24 h. All the excreta from each cage was collected in intervals of 3 day. The samples were weighed, freeze-dried and stored at -20°C according to the procedure described by Zarei *et al.*[24]. The samples were later reweighed, homogenized, dried at 90°C for 16 h overnight and ground to pass through a 0.5 mm sieve for analysis of dry matter, carbohydrate, lipids, crude protein, ash, crude fiber, apparent metabolize energy (AME) and minerals (Ca, P, Zinc, Mg and Iron). The proximate analyses were performed according to the official method recommended by the AOAC[25]. The nutrient digestibility was estimated according to the methods previously used by Nkukwana[26] and Zarei *et al.*[24]. Proximate analysis of excreta and experimental diet were performed to calculate the apparent digestibility:

$$\text{Apparent digestibility} = \frac{\text{Concentration of nutrient in diet} - \text{concentration of nutrient in excreta}}{\text{concentration of nutrient in diet}}$$

Gut morphometric and histology: Gut integrity was determined at the end of the 8th and 14th week periods. Two birds were selected from each of the treatment replicate groups at the end of the two sampling periods. The birds were randomly selected before they were fasted for 24 h, after which the live weight was measured. The birds were then stunned using concentrated CO_2 gas before they were slaughtered by severing the carotid arteries and jugular veins using a sharp knife. The chicks were completely bled and scalded using hot water; the feathers were plucked and the carcass was washed before evisceration. A 2 cm portion of the midpoint of the jejunum segment was obtained from each selected bird and dissected for morpho-histology analysis. Each sample was preserved in 10% buffered formalin solution until further processing as described by Hashemi *et al.*[27]. Each sample was later embedded in paraffin. A 5 µm section of each sample was placed onto a glass slide and stained with hematoxylin and eosin for the histological analysis.

The method described by Bäcker[28] was used to measure the total mucosa thickness (TMT), lamina propria thickness (LPT), muscularis mucosa thickness (MMT), submucosa thickness (SBT), muscularis externa (MET), villus height (VH),

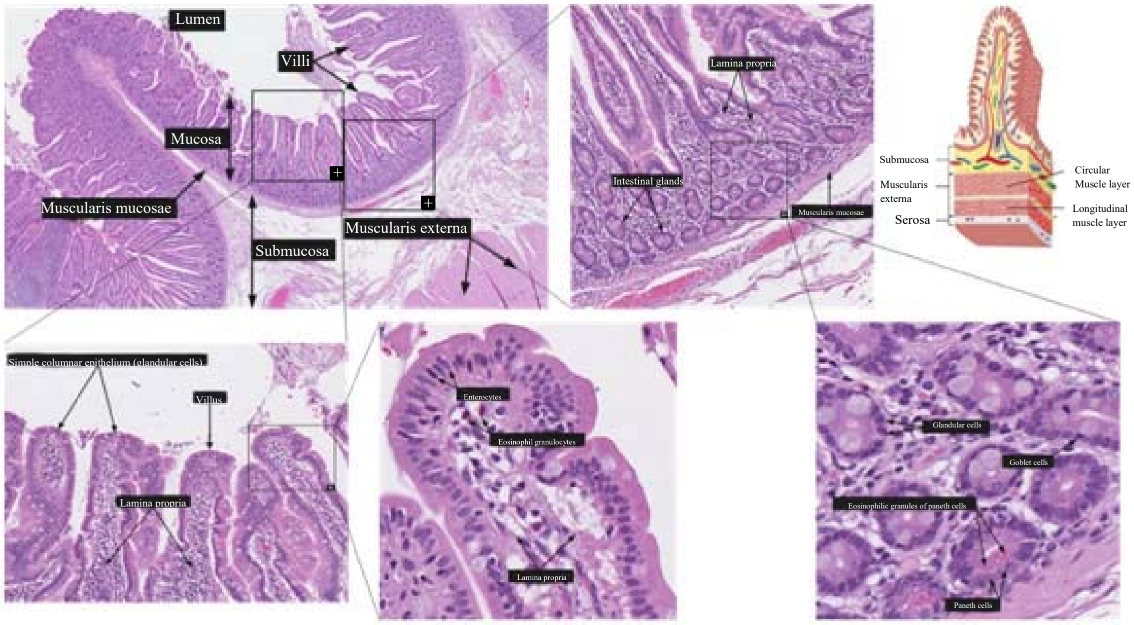

Fig. 1: The description of intestinal histology of layer[31]

villus width at the top (VWT), villus width at the junction (VWJ), crypt depth (CD), villus height/crypt depth ratio (VH/CD), villus height/villus width at the top (VH/VWT), villus height/villus width at the junction (VH/VWT), villus surface area at the top (VST) and villus surface area at the junction (VSJ). The surface area of the villus was calculated using the formula:

$$\text{Surface area} = 2\pi \times \frac{\text{VW}}{2} \times \text{VL}$$

where, the VW is the villus width at the top (VWT) or at the junction (VWJ) and the VH is the villus height. Crypt depth was measured from the base upward to the region of transition between the crypt and villus[12,27,29,30]. The Human Protein Atlas[31] (Fig. 1) was used for the description of intestinal histology and the interpretation referred to Das *et al.*[32] as a guide.

Statistical analysis: The statistical analysis were performed using RStudio software version 3.6.1[33]. The normality was tested using the Shapiro-Wilk test. The effect of treatment on the apparent digestibility and gut integrity in the chickens was analyzed using one-way ANOVA. Values of $p<0.05$ were considered statistically significant.

RESULTS

Proximate composition in *D. edulis* leaves, stembark and seed powder: Proximate composition analyses of *D. edulis* are presented in Fig. 2. The results demonstrated that the *D. edulis* powder significantly ($p\leq 0.05$) differed in terms of DM, ash, crude fiber, crude protein and energy between and among treatment groups. The stembark had the highest DM, ash and crude fiber contents, while the leaves were highest in crude protein. The experimental diet that included seed powder at a 0.5% inclusion level resulted in the highest apparent digestibility.

Seeds of *D. edulis* plant had significantly more zinc than the leaves and stembark, while they were lowest in magnesium, calcium and iron. The stembark and leaves were high in magnesium, calcium and iron in all cases. These results are presented in Fig. 3.

Proximate composition in the droppings of local chicken: The proximate composition analyses of droppings of chicken fed various experimental diets are shown in Fig. 4. There were significant differences in the DM, ash, fat, crude fiber and crude protein. The nutrient digestibility differed between and among the treatment diets, as well.

The mineral composition results obtained from the chicken excreta are presented in Fig. 5. The calcium, iron, zinc, magnesium and phosphorous significantly differed between and among the experimental diets.

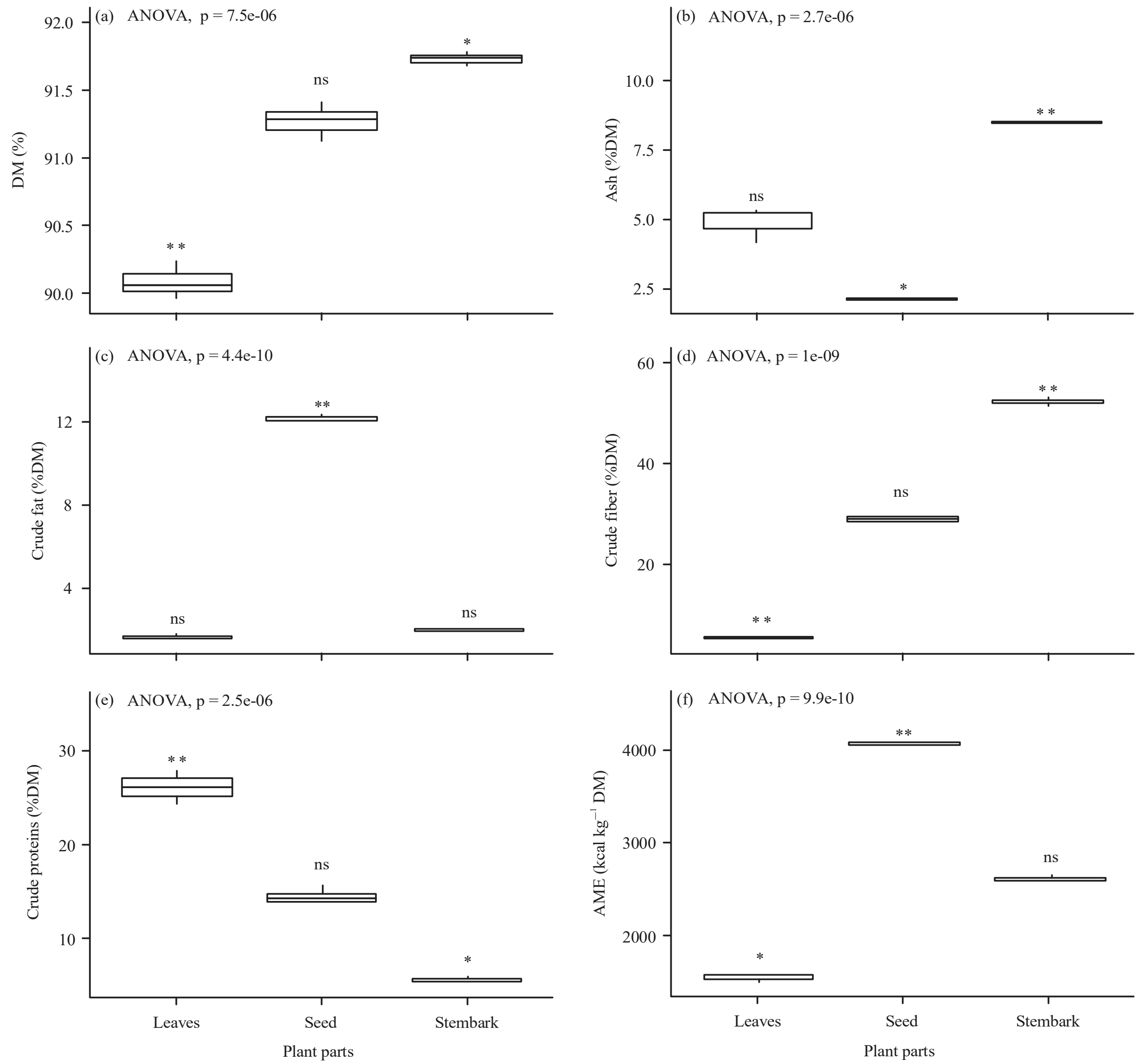

Fig. 2(a-f): Proximate composition analyses of the *D. edulis* results- a: DM (%), b: Ash (%), c: Fat (%DM), d: Crude Fiber (%DM), e: Crude Proteins (%DM) and f: apparent metabolizable energy (AME; kcal kg^{-1} DM)

Data are median with 25 and 75% quartile (box) and non? outlier range (whisker), ns: Non significant difference

The mineral content in the negative control and TS_{0Ed} diet were significantly different from the other test diets in terms of calcium. There were significant differences between and among the experimental diets for zinc and iron. The results further revealed that TB_{1Ed}, TS_{0Ed} and TS_{1Ed} were significantly different from the other test diets in terms of magnesium.

Apparent digestibility: The apparent digestibility of different proximate contents in the experimental diets is presented in Fig. 6. A statistical difference was observed between and among the different experimental diets for DM, AME, ash, crude fiber, fat and crude protein.

The results for the apparent digestibility of different mineral constituents in the experimental diets are shown in Fig. 7. There was a significant difference between and among the experimental diets regarding calcium, iron, zinc, magnesium and phosphorous.

The positive values for different nutrients and minerals indicate that digestion took place. Moreover, they differed between and among the experimental diets. The TS_{0Ed}

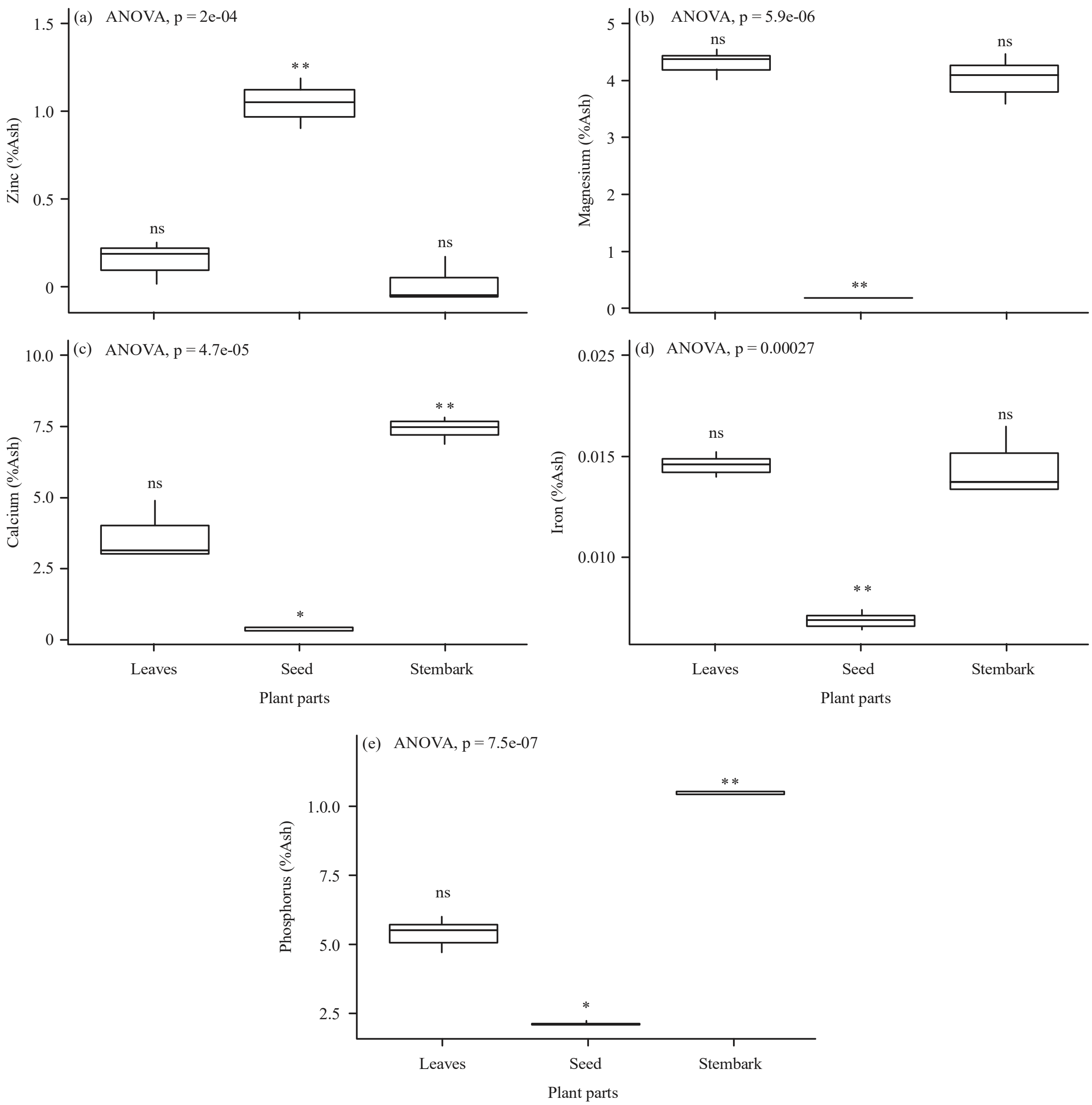

Fig. 3(a-e): The *D. edulis* plant parts mineral content analysis results- a: Zinc (%Ash), b: Magnesium (%Ash), c: Calcium (%Ash), d: Iron (%Ash), e: Phosphorus (%Ash)

Data are median with 25 and 75% quartile (box) and non-outlier range (whisker). ns: Non significant difference

recorded the highest DM and AME digestibility. The crude fiber, fat and crude protein digestibility were significantly different between and among dietary treatment groups. The lowest crude fiber digestibility was recorded in the control diets, while it was the highest in TL_{1Ed} and TS_{0Ed}.

Gut morphometric and comparative histology: The results of the gut morphometric and histology examination for the local chickens fed the tests diets are presented in Table 1 and Fig. 8.

We obtained the values for the total mucosa thickness (TMT), lamina propria thickness (LPT), muscularis mucosa thickness (MMT), submucosa thickness (SBT), muscularis externa (MET), villus height (VH), villus width at the top (VWT), villus width at the junction (VWJ), crypt depth (CD), villus height/crypt depth ratio (VH/CD), villus height/villus width at the top (VH/VWT), villus height/villus width at the junction (VH/VWT), villus surface area at the top (VST) and villus surface area at the junction (VSJ). In all cases, there were no significant

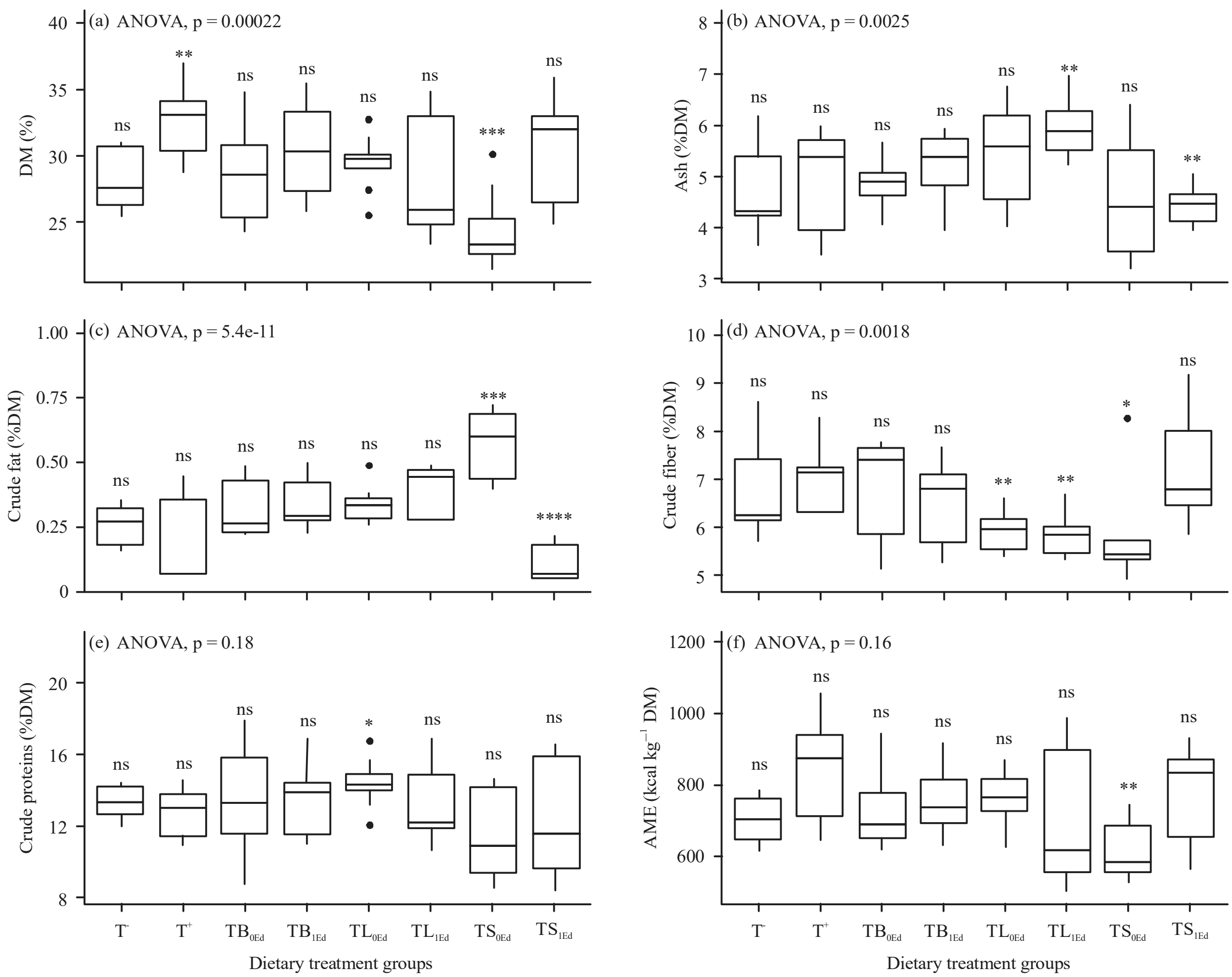

Fig. 4(a-f): The proximate composition analyses of chicken droppings from the experimental diets- a: DM (%), b: Ash (%), c: Fat (%DM), d: Crude fiber (%DM), e: Crude proteins (%DM) and f: Apparent metabolizable energy (AME; kcal kg^{-1} DM)
Data are median with 25 and 75% quartile (box) and non-outlier range (whisker); See text for the description of the treatment diets, ns: Non significant difference

differences within respective parameters between and among the different experimental diets except in the MET and VH at the end of the 8 week feeding trial. The difference in the VH contributed to the significant difference obtained in VH/VWJ by the end of the same period.

The mucosa, muscularis mucosa, submucosa, muscularis externa and serosa were distinctly recognizable for all dietary treatments according to the gut histology. There was a notable reduction in the villi height for samples collected from the chicken fed the positive control diet at weeks 8 and 14. A similar observation was made for samples collected from chicken fed TB$_{0Ed}$ at 8 weeks, TL$_{1Ed}$ at 14 weeks and TS$_{0Ed}$ and TS$_{1Ed}$ at 8 and 14 weeks, respectively.

DISCUSSION

Different approaches have been used to evaluate the efficacy and safety of utilizing *D. edulis* in animal feeds. The key aspect for the evaluation is the availability of nutrients/chemicals in the material being tested or its effect on the set limits of animal health and welfare. Feeding trials have traditionally been used to determine the suitability (efficacy and safety) of using specific resources in animal feeding. In this study, 288 dual-purpose male chickens were fed diets supplemented with different parts (leaves, stem and bark combination and seeds) of *D. edulis*[19] as part of a project aimed at evaluating the suitability of using the plant/parts as an additive to poultry diets to reduce/eradicate the use of

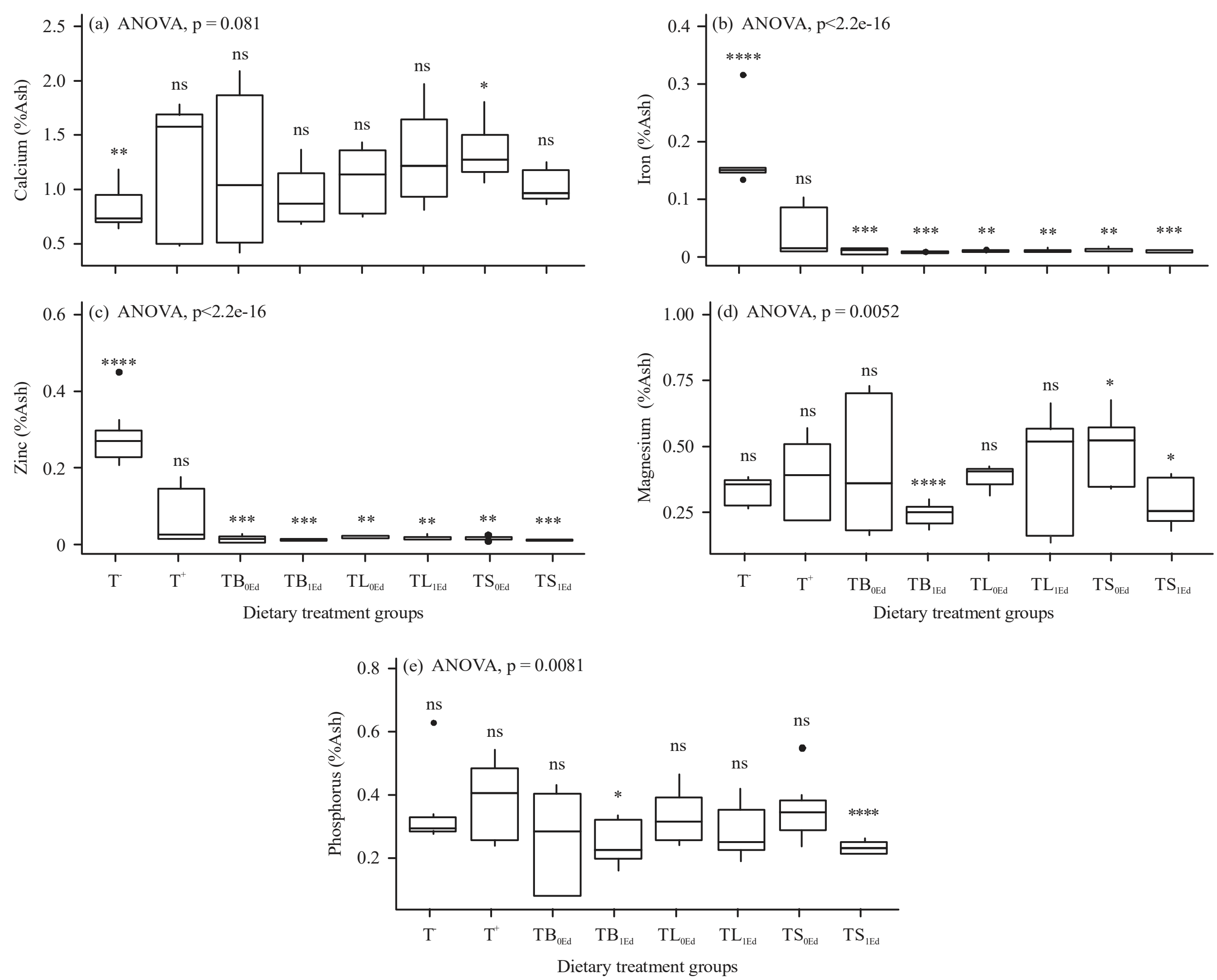

Fig. 5(a-e): Excreta mineral contents of the chicken fed different experimental diets- a: Zinc (%Ash), b: Magnesium (%Ash), c: Calcium (%Ash), d: Iron (%Ash), e: Phosphorus (%Ash)

Data are median with 25 and 75% quartile (box) and non-outlier range (whisker). ns: Non significant difference

antibiotics whose uncontrolled use as a growth promoter in animal feeds has had a negative impact on human and animal health and welfare[5,7-11]. This effort is undertaken to reduce the increasing cost of poultry feeds that are partially due to the high price of the conventionally/commercially available feed additives[5]. The first paper in this project reported the impact of plant parts on dual-purpose chicken growth traits, ceca microbiota and blood parameters[19].

The choice of *D. edulis* was due to its extensive use in African traditional human and veterinary medicine[8,16,18], as well as its use in the provision of limiting nutrients and minerals in human diets[16,17]. Moreover, the plant is abundant in the African Continent because it is found in Sierra Leone, Nigeria, Cameroon (West), Uganda (East), Angola, Zimbabwe (South) and other African countries[15]. In Africa, where this plant (*D. edulis*) is abundant and poultry production is limited due to the high price of feed additives, no study has been carried out to determine the efficacy and safety of using plant/parts as feed additives in poultry diets.

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We hypothesized that the use of poultry diets supplemented with *D. edulis* leaves, a combination of stem and bark and seed powder would improve the apparent digestibility, gut integrity and utilization of the available nutrients. This is through maintenance of a healthy gut and by reducing/eradicating intestinal disorders[7,14] as well as enhancing nutrient digestibility[13] possibly by stimulating secretion of several digestive enzymes such as lipase, amylase and protease.

It is evident from the results obtained in this study that the *D. edulis* plant is characterized by components that have nutritional (proximate and digestibility) and medicinal qualities (gut integrity). The findings indicated that the *D. edulis* stembark is high in DM, ash, fiber and Ca, while the seed powder is superior in fat and Zinc and is more digestible. This concurs with the results presented by Ogboru *et al.*[15]. Bratte[34] reported that the *D. edulis* seed powder has the potential to be used as an energy source due to the higher fat content. Furthermore, evidence of the potential use of *D. edulis* was indicated by the higher digestibility of AME, DM, fat, crude protein, crude fiber and

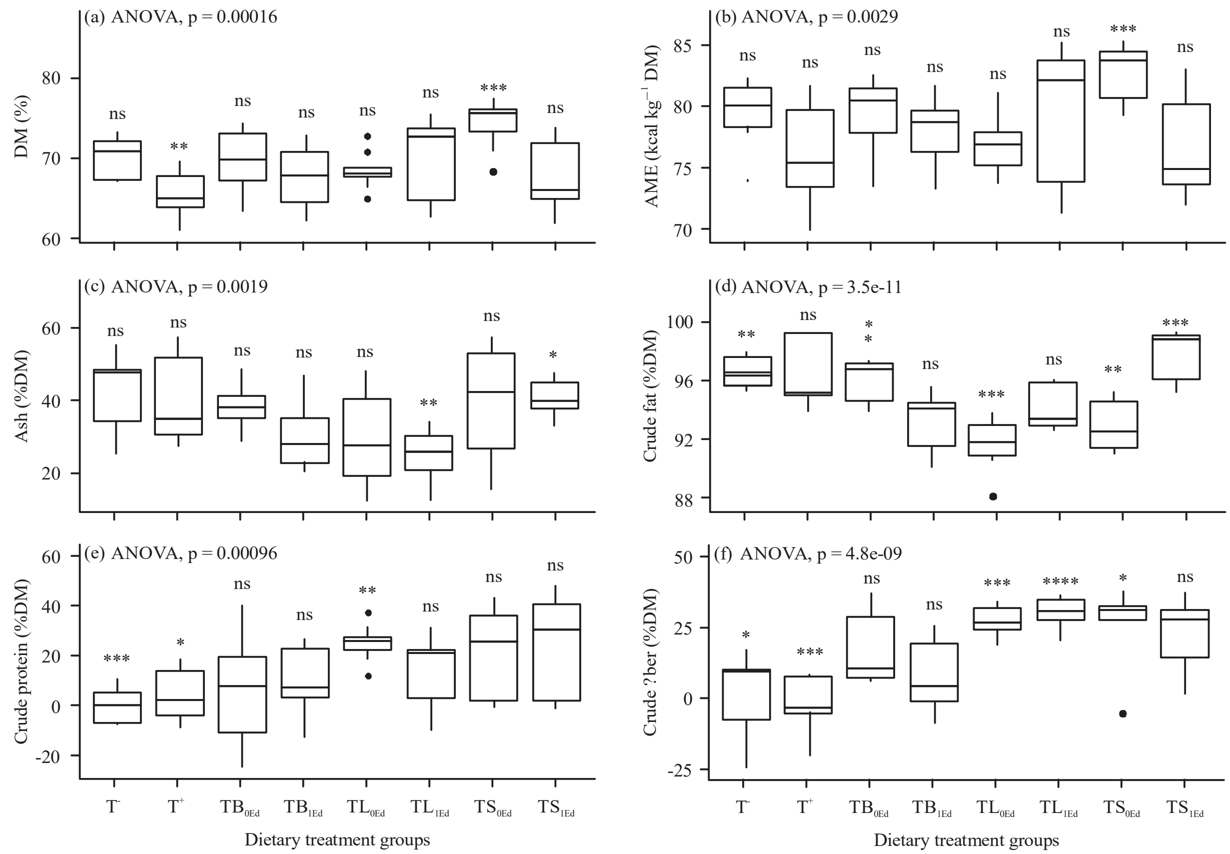

Fig. 6(a-e): Apparent digestibility analysis of different experimental diets - a: DM (%), b: apparent metabolizable energy (AME; kcal kg^{-1} DM), c: Ash (%), d: Fat (%DM), e: Crude proteins (%DM), f: Crude fiber (%DM)

Data are median with 25 and 75% quartile (box) and non-outlier range (whisker); See text for the description of the treatment diets; ns: Non significant difference

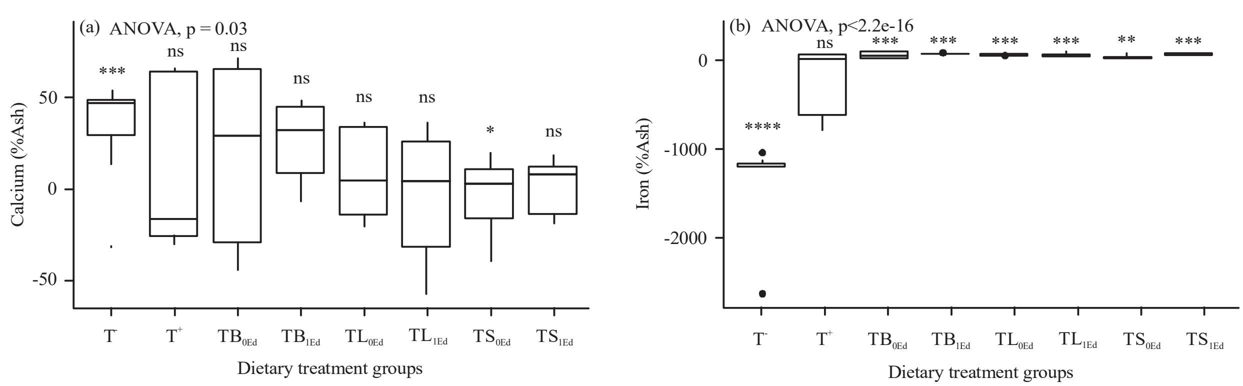

Fig. 7(a-e): Continue

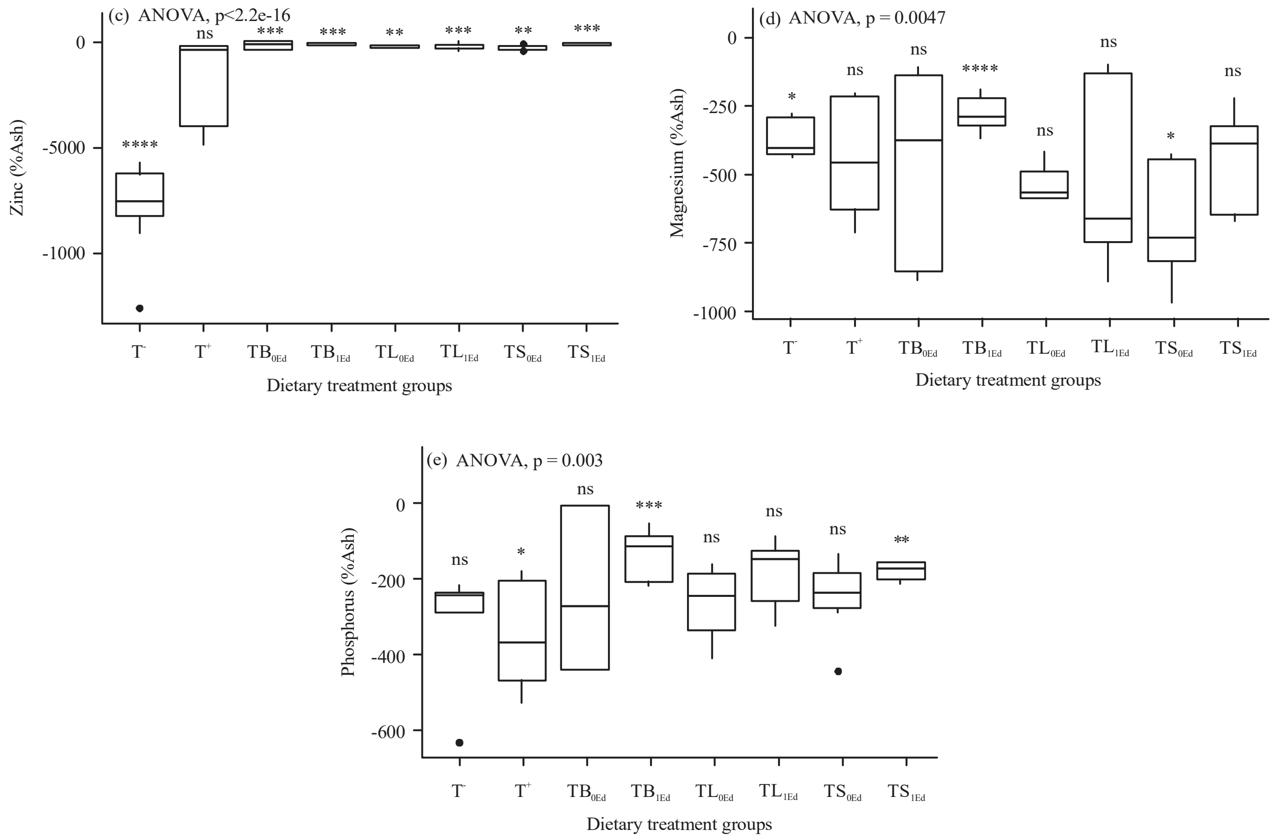

Fig. 7(a-e): Apparent digestibility of different mineral constituents of the experimental diets- a: Calcium, b: Iron, c: Zinc, d: Magnesium and Phosphorus

Data are median with 25 and 75% quartile (box) and non-outlier range (whisker); See text for the description of the treatment diets, ns: Non significant difference

calcium in the experimental diets compared to the control diets. The differences in the digestibility of different parts of *D. edulis* can explain the differences observed in the proximate composition of chicken droppings. For instance, González-Alvarado *et al*.[35] and Nkukwana *et al*.[36] revealed that the fiber content in feed significantly increases the coefficient of total tract nutrient absorption. Emami *et al*.[37] reported that a diet supplemented with 200 mg kg^{-1} essential peppermint oil increased the apparent crude protein digestibility of broilers. Furthermore, Nkukwana *et al*.[36] demonstrated that the speed at which various diets pass through the digestive tract is a function of exogenous (feed proximate contents) and endogenous factors such as intestinal integrity and enzymatic gland function. The increased activity of digestive enzymes improves gut function[13]. An improvement in nutrient digestibility consequently enhances the health status of animals.

The studied gut morphometric parameters indicated that there was a notable inconsistency in the mode of action of *D. edulis* compared to other phytogenic feed additives[27]. There were no significant changes in the tested gastrointestinal morphometry in chicken fed the experimental diets except in the muscularis externa and villus height, which have previously been reported by Gadde *et al*.[38] in weanling pigs fed diets containing plant extracts of carvacrol, cinnamaldehyde and *capsicum oleoresin*. Similarly, Oetting *et al*.[39] did not report any significant change in gut intestinal morphometry of birds fed a mixture of essential oils of thyme, clove, oregano eugenol and carvacrol. Significant changes in the gut morphology and histology are not desirable, as they would indicate interference with the genetically mediated cell growth. Reduction in gut villi number, structure and height would lead to a decline in the absorption capacity of the gut, hampering nutrient absorption[13] and a consequent delay in animal growth. Upadhaya and Kim[13] further noted that phytogenic feed additive may also negatively hamper the functioning of the intestinal mucosa, thus affecting nutrient absorption.

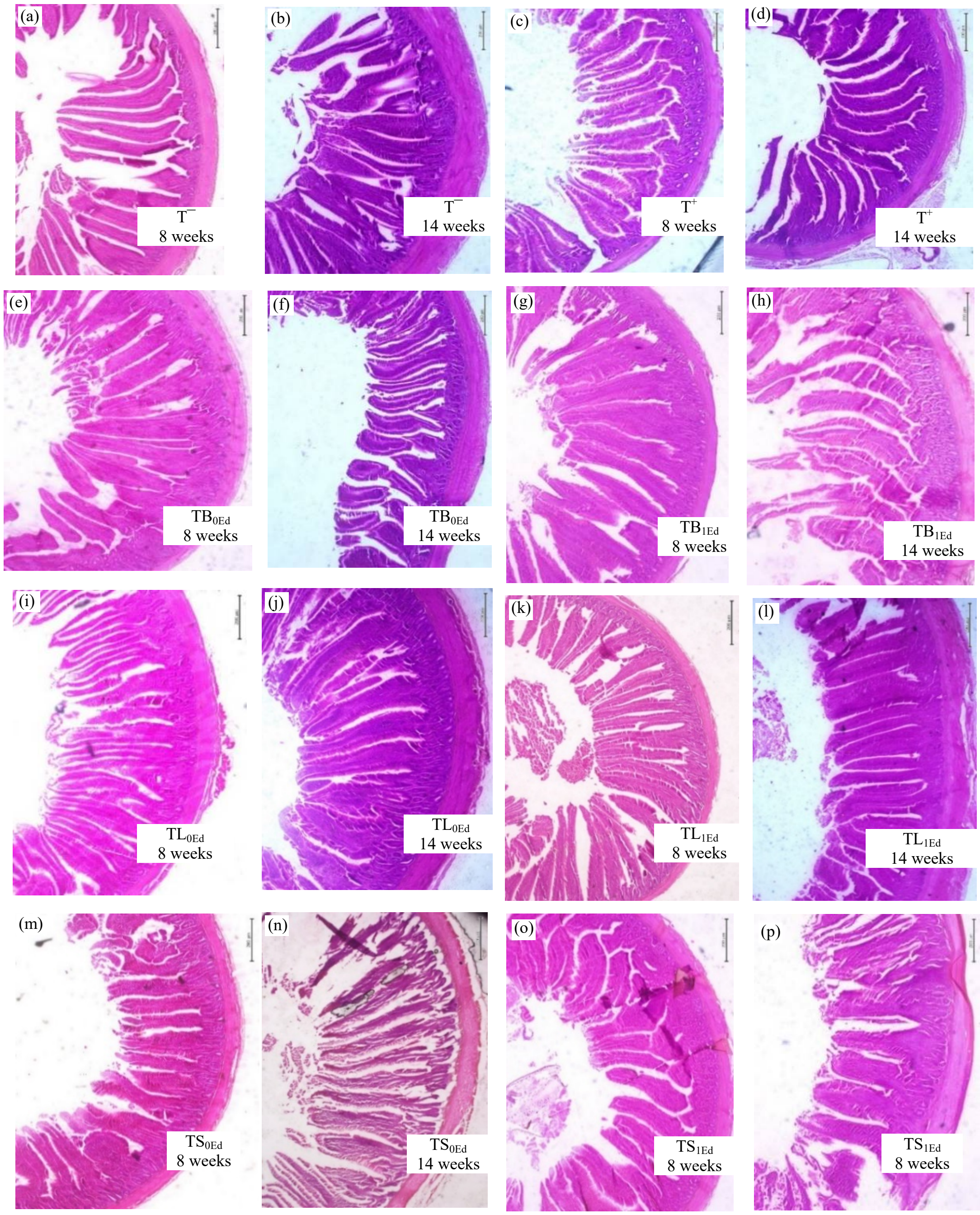

Fig. 8(a-p): Gut histology of the local chickens fed with 8 weeks and 14 weeks different experimental diets

TL_{0Ed}: 0.5% leaves powder, TL_{1Ed}: 1.0% leaves powder, TB_{0Ed}: 0.5% stem and bark powder, TB_{1Ed}: 1.0% stem and bark powder, TB_{0Ed}: 0.5% stem and bark powder, TB_{1Ed}: 1.0% stem and bark powder, TS_{0Ed}: 0.5% seeds powder), TS_{1Ed}: 1.0% seeds powder, TPos: Positive control, 0.5 g oxytetracycline powder per liter of drinking water, and TNeg: Negative control

CONCLUSION

The proximate and mineral composition of the *D. edulis* plant differed between and among different parts, implying that the parts can be used to satisfy different requirements of poultry feeds. This is further highlighted by the significant differences between the proximate analysis of the droppings and mineral contents of the chicken excreta. The findings of this study further showed that *D. edulis* plant parts positively impact the apparent digestibility of the feed but may have an adverse effect on the intestinal histology of local chicken in the long-term.

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