BIO-EVALUATION OF HAEMATOLOGICAL AND BIOCHEMICAL INDICES OF BROILER CHICKENS FED MUNICIPAL ORGANIC WASTE DEGRADED WITH DIASTIC MICROBES FROM SNAIL (*ACHATINA ACHATINA*)

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ABSTRACT

A forty-two-day feeding trial sought to bio-evaluate the haematological and serum biochemical responses of broiler birds fed municipal organic wastes degraded with diastic microbes of Achatina achatina as part of formulated diets, using one-hundred-and-twenty-day-old broiler chicks. The day-old chicks (DOC) were assigned to four treatments diets of 30 birds per group; in three replications of 10 birds per replicate, in a completely randomized design (CRD). T_A (control), T_B (5%), T_C (10%), and T_D (15%) of degraded municipal organic wastes (DMOW) inclusion levels were assigned. There were no significant ($P<0.05$) differences in the haematological and biochemical indices. Reported significant differences in the serum biochemistry only occurred in protein profile, total cholesterol and high-density lipoprotein. Serum protein values were significantly ($P<0.05$) higher at 15%DMOW. Birds fed 5%DMOW had better ($P<0.05$) cholesterolic values. The results of the overall experiments indicated that DMOW can be fed to broilers without causing harm to the birds and humans when consumed.

Keywords: *Achatina achatina*, Broiler, Diastic microbes, MOW, Haematology, Serum Biochemistry

INTRODUCTION

Broilers' greatest potentials as food had been reported by Eze *et al.* (2024). These include short production interval, efficiency in feed utilization, high carcass to feed ratio and overall fast return on investment, which is challenged by high cost of feed amidst other impending factors. There is "demand traffic" on various feedstuffs used in diet formulations. This calls for diversification of feed alternatives. Biodegradable municipal organic wastes (DMOW) come handy in the novel attempt, since the degraded forms are loaded with induced favorable microflora for modification of lignified carbohydrate and fibre (Anigbogu, 2014). The municipal organic waste (MOW) cause pollution and the emission of greenhouse gases from the biogenic carbon greatly contributes to the overall ozone layer depletion (Eze *et al*., 2024). Using MOW as broiler diet not only serve as emission mitigation strategy, waste management option but also in broiler feeding. However, some identified constraints limit the use of MOW in poultry diet. Nutritional aspects of these constraints was reported by Mandey *et al*. (2017) to include "variability (or lack of consistency) in nutrient quality, limited information on the availability of nutrients, high fibre content, presence of anti-nutritional factor(s) and need for nutrient supplementation (added cost)". Technical aspects include "seasonal and unreliable supply, bulkiness, physical characteristics, need for de-hulling and/or processing (drying, detoxification), limited research and development facilities for determining nutrient, and composition and inclusion levels in poultry diets" (Eze *et al.* (2024). Nevertheless, the gut system of Snail (*Achatina achatina*) is designed to degrade high fibrous materials during feeding Eze *et al.* (2024). This is made possible by diverse species of microorganism such as bacteria, fungi, virus, protozoa, enzymes, etc. These are collectively called Diastic microbes (Dm) (Anigbogu and Chukwurah, 2014). During the feeding of snail, the feed materials which could have fermented to release biogases are entrapped by these indigenous microbes, during which some would die while others would survive and adapt for degradation process. The adapted microbes multiply and are referred as Adapted Diastic microbes (ADm) (Anigbogu and Chukwurah, 2014). The present study aimed at testing this novel feedstuff of the wholesomeness in broilers' health and when consumed, through bio-evaluation of the haematological and biochemical indices.

MATERIALS AND METHODS

Study period and location

The study was conducted in Abakaliki Municipality. Abakaliki is the capital city of Ebonyi State, South-Eastern Nigeria, West Africa. It is located 64 kilometres (40 mi) South-Eastern of Enugu. It lies on Latitudes $7^030^{\cdot}$ E and $8^030^{\cdot}$ E and Longitudes $5^0$40'N and $6^0$45'N (Wikipedia, 2024). It is an agrarian state of the nation, whose municipal organic waste constitute is agro-based. The waste collection lasted twenty-eight (28) day; degradation of waste ten (10) days while feeding trial lasted forty-two (42) days, making a total of 80 days.

Source, treatment, sampling and storage

Municipal solid wastes ready to be dumped in landfills were collected and source separated into municipal organic wastes (without papers) and impurities. Other metals were removed by magnetic separation. A fermentation vat of volume, 3.5 litres was used to prepare the starter inoculum of municipal organic waste substrate (500g) with the addition of 100 ml of Diastic microbes (of *Achatina achatina*) suspension as a homogenous mixture, kept at room temperature for 10 days. After which, the fermented product was used as starter inoculums (fermented dough) for the study, while each 2kg of the dough was used to relatively degrade each 20kg of the MOW using 4 liters of water. After which, the fermentation product was sun-dried, analysed and stored for feeding study. The dried wastes were milled to a maximum particle size of 13mm, and were collected in 1 kg portions packed in polythene plastic bags and cartons. Samples for chemical analysis were taken from every carton. The wastes were frozen and stored at a temperature of -24^{0}C. The remaining was prepared as degraded products for the feeding experiment for the broiler birds, as described by Anigbogu and Uchealor (2014).

Experimental Broilers, management and design

A total of one hundred and twenty day-old-chicks were assigned randomly to 4 experimental diets in three replications of 10 birds per replicate in a completely randomized design. Each replicate group was housed in a cleaned and disinfected experimental pen. Routine vaccination program was followed as required. Feed and water were provided *ad libitum.* The experiment lasted for 42 days.

Experimental Diets

The municipal organic waste-based broiler ration consisted of three differently prepared diets where the municipal organic waste formed part of each diet. Diet A= Control diet, diet B = 5% municipal organic waste, diet C= 10% municipal organic waste and diet D = 15% municipal organic waste. All other feed materials were used to balance the ration as in table 1.

Table 1. Percentage Composition of MOW based Broiler diets.

INGREDIENTS (kg)	T_A	T_B	T_C	T_D
Maize grain	60.00	55.00	50.00	45.00
Municipal organic wastes	-	5.00	10.00	15.00
Wheat offal	2.00	2.00	2.00	2.00
Soybean meal	30.00	30.00	30.00	30.00
Palm Kernel Cake	2.00	2.00	2.00	2.00
Fish meal	4.00	4.00	4.00	4.00
Bone meal	1.00	1.00	1.00	1.00
Lysine	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Total(kg)	**100**	**100**	**100**	**100**
Calculated composition				
Crude protein (%)	21.16	20.91	20.66	20.41
Energy (Kcal/kg ME)	3164.5	3099.71	3034.91	2970.12
Crude fibre (%)	4.04	4.64	5.24	5.87

TA=0%DMOW; TB=5%DMOW; TC=10%DMOW; TD=15%DMOW. DMOW=Degraded Municipal Organic Waste.

Data collection and analysis

Haematological and serum biochemical indices

At the end of the experiment, blood samples were taken from webal subclavicle vein of each of 12 birds randomly selected from each replicate. The selected birds from respective research groups were bled and 7 ml of blood was collected into a bijou bottle treated with ethylene diamine tetra acetic acid (EDTA). The blood collected was used for haematological assay for red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), haemoglobin concentration (Hb), blood serum protein (BSP), mean corpuscular haemoglobin (MCH) and mean corpuscular concentration as described by Schalm *et al.* (1975) and Ramnik (1999). While the blood samples for biochemical analysis were collected into EDTA-free test tubes and allowed to clot for serum separation. Serum was separated from the blood by centrifugation of blood at 4000 rpm for 15 minutes and thereafter stored at -20^{0}C,

and was later used for blood chemistry analysis. The blood chemistry analysis was conducted based o standard procedure.

RESULTS AND DISCUSSION

Haematology of Broiler birds fed DMOW

The results of the effects of MOW degraded with diastic microbes of *Achatina achatina* on the haematology of broiler birds is as presented in Table 2. The PCV and MCV of broiler birds fed control diet were significantly higher than others but fell within the range reported for poultry. Birds on the control diet had the highest PCV and MCV (35.00% and 78.30pg) respectively while those on 5% DMOW had the least (27.33% and 61.01pg) respectively, though similar (P>0.05) to those fed 10% DMOW and 15% DMOW. The haemoglobin (Hb), white blood cells (WBC), red blood cells (RBC), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) were not significantly (P>0.05). The packed cell volume or haematocrit is a measure of the relative mass of red cells present in a sample of whole blood while the mean corpuscular volume (MCV) expresses the relative volume of individual red blood cell (Khanam *et al*., 2013). The significant higher (P<0.05) values of these parameters as observed in the control experiment dissociates anaemic condition among birds fed other treatment diets. This is because the Hb, MCHC, MCH as well as RBC were all similar (P>0.05) in all the experimental animals. The values obtained for other haematological parameters which were similar (P>0.05) all fell within the values reported for broilers under tropical condition (Makeri *et al*., 2017). These haematological indices are indicators of the haemoglobin concentration in the PCV which defines the differential quality of the oxygen carrying capacity of the red blood cells.

Table 2. Haematology of broiler birds fed DMOW

Dietary Treatments	A	B	C	D
Parameters	**0% DMOW**	**5% DMOW**	**10% DMOW**	**15% DMOW**
PCV (%)	35.00±2.52[a]	27.33±1.45[b]	29.00±1.73[b]	28.33±1.20[b]
HGB (g/dL)	9.27±0.27	9.00±0.44	8.43±0.62	8.77±0.30
WBC (x 10^9/L)	12.13±0.19	12.35±0.52	12.66±0.67	13.16±0.30
RBC (x 10^{12}/L)	4.46±0.23	4.51±0.28	4.55±0.20	4.59±0.05
MCHC (%)	26.89±2.90	32.94±0.15	29.51±3.69	31.09±2.02
MCH (fl)	20.96±1.77	20.12±1.64	18.50±0.59	19.09±0.51
MCV (pg)	78.30±1.65[a]	61.01±5.15[b]	64.38±6.79[ab]	61.79±3.24[b]

[ab] Row means with different superscript are significantly different (P<0.05). Keys: DMOW=degraded municipal organic wastes

Biochemical indices of the experimental birds.

The results of the effects of treatments on biochemical indices of the experimental birds are as presented in Table 3. Statistical analysis revealed that the effects of the various levels of DMOW on serum protein profile value (total protein, albumin and globulin) were significantly different (P<0.05). Birds that are fed diet D (15% DMOW) had the highest total protein which differs significantly (P<0.05) from the values obtained for other treatments. The highest was reported against birds on diet D (53.33g/L) followed by those on the control diet (42.67g/L) whereas the least was observed in serum of birds placed on diet C (39.00g/L). The serum concentration of the albumin of birds placed on diets B (5% DMOW) and D (15% DMOW) were similar (P>0.05) but differ significantly (P<0.05) from albumin concentration of birds fed diet C (10% DMOW). Serum album concentration of diets A and B were not significantly different (P>0.05). Also, the concentrations of diets A, B and D were similar (P>0.05). Birds on diet D (15%) had the highest albumin concentration (24.33g/L) while birds on diet C (10% DMOW) had the least (16.33g/L). The treatments affected the serum concentration of the globulin significantly (P<0.05). Globulin level of birds fed diet D (15% DMOW) was significantly higher (P<0.05) than those of the birds fed treatments A (control diet) and B (5% DMOW). Those of diets A (control), B (5% DMOW) and C (15% DMOW) were not significantly different (P>0.05) while those of birds fed diets C and D were also similar (P>0.05). The urea levels were not significantly (P>0.05) affected by the various levels of DMOW. The highest value (9.43 mg/L) was seen in birds fed diet D (15% DMOW) while birds fed control diet had the least (5.88 mg/L). The effects of treatments on liver enzymes: Aspartate Aminotransferase (AST), Alaninine Aminotransferase (ALT), Alaninine Phosphate (ALP) and bilirubin concentration were not significantly (P>0.05) different. The bilirubin concentration followed the same pattern. Results show significant differences (P<0.05) in total cholesterol (TC) and high-density lipoprotein (HDL). Birds fed 5% DMOW had significantly higher (P<0.05) cholesterolic and HDL values (4.23mmol/L, 1.16 mmol/L) respectively than (3.60 mmol/L, 0.72 mmol/L), (3.20 mmol/L, 0.36 mmol/L) and (3.47 mmol/L, 0.69 mmol/L) recorded for birds fed 0% DMOW (control diet), 10% DMOW diet and 15% DMOW diet respectively. Total cholesterolic and HDL values of birds fed control diet (3.60 mmol/L, 0.72 mmol/L) and

those fed 15% DMOW (3.47 mmol/L, 0.69 mmol/L) were similar (P>0.05) respectively. Whereas Total cholesterol of birds fed control diet and 15% DMOW differs significantly (P<0.05) from those fed 10% DMOW (3.20mmol/L) which was the least. Their HDL values were similar (P>0.05). Low density lipoprotein (LDL) was similar (P>0.05) in all the experimental birds. Very low-density lipoprotein (VLDL) and serum triglyceric values followed the same pattern. There were no significant differences (P>0.05) among the birds fed the respective diets.

Table 3. Biochemical indices of the experimental birds.

Dietary Treatments	**A**	**B**	**C**	**D**
Parameters	**0% DMOW**	**5% DMOW**	**10% DMOW**	**15% DMOW**
Total protein (g/L)	42.67±2.84^{b}	40.00±0.58^{b}	39.00±2.89^{b}	53.33±3.18^{a}
Globulin (g/L)	21.00±2.87^{b}	16.33±0.33^{b}	22.67±1.16^{ab}	29.00±2.91^{a}
Albumin (g/L)	21.67±1.20^{ab}	23.67±0.33^{a}	16.33±0.33^{b}	24.33±6.03^{a}
Urea (mg/L)	5.88±1.13	6.53±0.44	7.68±0.83	9.43±1.45
Creatinine	0.15± 0.02	0.15±0.02	1.10±0.01	0.09±0.012
Aspartate Aminotransferase (µ/l)	29.00±2.52	27.67±0.67	28.00±0.58	28.00±1.15
Alaninine Aminotransferase (µ/l)	14±2.52	13±1.67	14±2.52	12±0.57
Alaninine Phosphate (µ/l)	40.00±0.58	43.67±0.88	44.33±2.19	37.33±3.39
AST/ALT ratio	2.15±0.26	2.09±0.25	2.11±0.30	2.35±0.37
Bilirubin (mg/l)	9.83±0.44	10.00±0.58	9.13±0.13	9.70±0.17
Total cholesterol (mmol/L)	3.60± 0.06^{b}	4.23± 0.03^{a}	3.20 ±0.10^{c}	3.47± 0.07^{b}
High density lipoprotein (mmol/L)	0.72±0.01^{b}	1.16±0.17^{a}	0.36±0.14^{b}	0.69± 0.01^{b}
Low density lipoprotein (mmol/L)	2.52± 0.18	2.63± 0.06	2.26± 0.10	2.32± 0.10
Very Low-density lipoprotein (mmol/L)	0.36 ± 0.14	0.44± 0.12	0.58 ± 0.10	0.45± 0.07
Triglycerides (mmol/L)	0.80± 0.31	0.97± 0.27	1.27± 0.23	1.00± 0.15

^{ab} Row means with different superscript are significantly different (P<0.05). Keys: DMOW=degraded municipal organic wastes

CONCLUSION

The adoption of DMOW has become imperative having proven through the current research, its wholesomeness to the in-fed broiler chickens and humans when consumed. The haematological as well as serum biochemical indices were closely related to those fed conventional broiler diets. The haematological parameters, serum proteins, liver enzymes and serum cholesterol were not negatively impacted at any level of the DMOW included. Hence, we recommend its adoption as alternative feedstuff and for waste management option as well as reduction in the biogenic carbon values that could cause greenhouse gases emission when dumped in landfills.

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