

EFFECTS OF DIETARY INCLUSION OF *ZYMOMONAS MOBILIS* TREATED SAWDUST ON HAEMATOLOGY, SERUM BIOCHEMISTRY, CARCASS CHARACTERISTICS AND SENSORY EVALUATION OF MEAT OF BROILER CHICKENS

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ABSTRACT

This study was conducted to investigate the effects of including *Zymomonas mobilis*-treated sawdust (ZTSD) as unconventional feed in broiler chicken diets for 56 days by observing the haematology, serum metabolites, carcass characteristics and sensory evaluation. A total of 375, 1-day old broiler chicks were randomly assigned into 5 groups, each with 5 replicates of 15 birds each in a Completely Randomized Design for the study. Five diets containing untreated and treated sawdust were formulated to replace wheat offal at 0, 50 and 100 % at starter and finisher phases. At the starting phase, the broiler chickens, fed 100 % untreated sawdust-based diet (UTSD), had highest (p < 0.05) values for packed cell volume (PCV), red blood cell (RBC) and lymphocytes. Whereas the birds, fed 100 % *Zymomonas mobilis*-treated based diet (ZTSD), had the highest value for white blood cell (WBC). The finishing broiler chickens, fed 50 % UTSD, had the highest values for total protein (TP), albumin, globulin and uric acid, while the birds on 50 % ZTSD had the least values for TP and albumin. The birds, fed 50 % ZTSD, had highest (p < 0.05) value for liver, while the highest value of the whole gastrointestinal tract was observed at the group with 100 % ZTSD. The control group had the highest value for the overall acceptability of the meat. In conclusion, the inclusion of ZTSD improved PCV, Hb, RBC, TP and blood glucose. ZTSD, given at 50 %, increased the breast, thigh, drumstick and back of the broiler chickens. The application 100 % UTSD and 100 % ZTSD increased the sensory attributes of the meat.

Key words: haematology; serum biochemistry; carcass characteristics; sensory evaluation; sawdust; Zymomonas mobilis

INTRODUCTION

The utilization of wheat offal as a major dietary fibre source in most parts of poultry producing areas in many countries has escalated its price, thereby necessitating a search for a cheaper and locally available alternative (Lamidi *et al.*, 2008). Therefore, animal nutritionists are currently focusing on cheap but suitable alternative feedstuffs especially crop residues and industrial by-products, to sustain livestock industry (Alhassan, 1985). The evaluation of these unconventional feed resources besides other strategies would reduce pressure on the demand for conventional feed resources thereby ensuring attainment of feed security for poultry (Fajimi *et al.*, 1993).

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Sawdust is a lignocellulosic material that is burnt away annually in industrial sites resulting in pollution thereby aggravating the existing environmental hazards. Millions of tons of these lignocellulosic materials, which are wasted every year are found around industrial sites such as sugar mills and sawmills (Pigden and Bender, 1975). Although, there is scarce information concerning the utilization of sawdust by chickens (Oke and Oke, 2001). Ibrahim (2003) attempted a study on exploring the potentials of sawdust as a livestock feed and reported encouraging results. Moreover, Anigbogu et al. (2008) revealed that sawdust, ammoniated sawdust and biodegraded sawdust have been used in animal feeding. Gohl (1981) reported the use of sawdust in livestock feed in an attempt to solve the problem of disposing of the by-product. The major limitation in the use of sawdust as feed (which constitute about 62.10 % of crude fibre-lignocellulosic plant material) is the crystalline nature of the cellulose and recalcitrance of lignin (Chesson et al., 1983).

Efforts to improve the bioconversion of cellulosic material as feed have been made during the last decades. The chemical and physical methods of treatment have improved to some extent the availability of nutrients in feeds, but are not yet acceptable to the farmers (Igba-Shan and Miller, 1983).

Moreover, microbial technology using efficient microorganisms and Innovation Solid-State Fermentation (SSF) technology (Anigbogu et al., 2009) may be appropriate for the biological conversion of sawdust (lignocellulosic waste) to valuable feed, making enzymatic hydrolysis more accessible in the rumen (Lewis et al., 1996). Oke and Oke (2007) reported that up to 80 g.kg⁻¹ sawdust from Daniella ogea tree can be included in broiler chicken diets without any detrimental effect on their weight gain and reduced cost of production. The authors suggested biological and chemical treatment of this lignocellulose waste in order to improve its digestibility. Zymomonas mobilis is a bacterium belonging to the Zymomonas genus that is known for its bio-ethanol production efficiency (Seo et al., 2005, Gunasekaran and Chandra, 1999), with activities that surpass yeast in some aspects. It is generally found in African palm wine and Mexican pulque. It is a rod-shaped gram-negative bacterium. It is 2-6 µm long and $1-1.4 \mu m$ wide but can vary significantly

(Yanase *et al.*, 2005; Cazetta *et al.*, 2007). Its ability to efficiently ferment carbohydrates using the Entner-Doudoroff pathway makes it an attractive option for life-enzyme for animal feed (Onyejekwe, 2010). *Zymomonas mobilis* has shorter fermentation time (300-400%) than yeast with higher ethanol yield (92-94% versus 88-90% for yeast). It can convert sugar mixtures to ethanol with 90-95%efficiency as reported in the University of Energy Efficiency and Renewable Energy report, United States of America (Ichita, 2006).

However, blood functions in the transportation of nutrients, gases and metabolic waste products around the body of livestock (Zhou et al., 1999), it can be used to assess the clinical and nutritional health status of the animals (Olorode and Longe, 2000). The effects of feedstuffs on the haematological factors of the poultry birds can be used in deciding whether such feedstuffs should be included or not in poultry nutrition (Mmereole, 2008). Mitruka and Rawnsley (1977) reported that high packed cell volume and haemoglobin contents are linked with high feed conversion efficiency while a high percentage of lymphocytes is associated with the ability of the poultry birds to perform well under very stressful conditions (Mmereole, 2008). The chemistry of serum or analysis of serum metabolites such as cholesterol, urea, etc., in the blood system, is for the purposes of detecting organ diseases in domestic animals and the amount of available proteins in the diets (Iyayi and Tewe, 1998). The serum biochemical constituents are positively correlated with the quality of the diet (Brown and Clime, 1972; Adeyemi et al., 2000). Kaneko (1997) reported that the serum protein profile and the absolute values of individual fractions are an excellent basis for a tentative diagnosis.

Therefore, this study was carried out to determine the effects of untreated or treated sawdust on haematology, serum metabolites, carcass characteristics and sensory evaluation of the meat of broiler chickens.

MATERIAL AND METHODS

Research Station and tested ingredients

The study was carried out at the poultry unit, Directorate of University Farms, Federal University of Agriculture, Abeokuta, Nigeria. The sawdust was collected from different sawmills in Abeokuta, Ogun State, Nigeria, bagged and stored in pallets. The sawdust was biologically treated by the traditional setting according to Anigbogu *et al.* (2009) using 50 kg sawdust placed into fermentation vat (Volume = 200 litres) with 100 litres of water added to 5 kg of previously fermented dough containing *Zymomonas mobilis,* which acted as starter inoculums. The sample was homogeneously mixed and kept to be fermented for a period of 20 days at room temperature in the range of 23.1 °C to 24.6 °C, after which the fermented product was sun-dried, analysed and stored as life-enzyme (sawdust degraded *Z. mobilis* microbes) for the experimental study.

Management of birds and experimental diets

Three hundred and seventy-five (375) 1-day old unsexed marshal broiler chicks were obtained from Obasanjo Farms Nigeria Limited, Nigeria. They

Ingredients	Starter diets				Finisher diets					
		-Z. mobilis -Z. mobilis +Z. mobilis Z. mobilis				-Z. mobilis -Z. mobilis +Z. mobilis +Z. mobilis				
	0%	50 %	100 %	50 %	100 %	0 %	50 %	100 %	50 %	100 %
Maize	53.60	53.60	53.60	53.60	53.60	54.60	54.60	54.60	54.60	54.60
Soybean meal	29.50	29.50	29.50	29.50	29.50	23.50	23.50	23.50	23.50	23.50
Fish meal	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Groundnut cake	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Wheat offal	5.00	2.50	0.00	2.50	0.00	10.00	5.00	0.00	5.00	0.00
Sawdust	0.00	2.50	5.00	2.50	5.00	0.00	5.00	10.00	5.00	10.00
Bone meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Limestone	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Broiler premix ^{ab}	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Methionine ^c	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Toxin binder	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis	<u>:</u>									
Metabolizable										
energy (MJ/Kg)	12.00	11.88	11.76	11.95	11.91	11.92	11.68	11.44	11.83	11.74
Crude Protein (%)	23.38	23.01	22.64	23.14	22.89	21.68	20.94	20.20	21.19	20.70
Crude Fibre (%)	3.62	4.97	6.32	4.69	5.77	3.67	6.37	9.07	5.82	7.97
Ether Extract (%)	3.83	3.75	3.68	3.86	3.90	3.83	3.69	3.54	3.91	3.98
Ash (%) Nitrogen	2.60	2.82	3.03	2.65	2.70	2.26	2.68	3.10	2.36	2.46
Free Extract (%)	50.57	49.45	48.33	49.65	49.33	52.56	50.32	48.09	50.72	48.89
Calcium (%)	1.48	1.48	1.48	1.48	1.48	1.47	1.47	1.46	1.47	1.46
Phosphorus (%)	0.57	0.59	0.61	0.59	0.61	0.54	0.57	0.61	0.57	0.61

Table 1. Percentage composition of experimental broiler chicken diets (DM- Basis)

^aStarter Vitamin-Mineral Premix: (Rotinol) based on 2.5 kg/ton (Thiamine, 2000 mg, riboflavin, 7000 mg, pyridoxine, 5000 mg, cyanocobalamine, 1700 mg, niacin, 30,000 mg, D-panthotenate, 10,000 mg, folic acid, 800 mg, biotin, 2000 mg, Retinyl acetate, 12,000 iu., cholecalciferol, 2,400,000 iu., tocopherol acetate, 35,000 iu., menadione, 4,000 mg, ascorbic acid, 60,000 mg, manganese, nill, iron, 70,200 mg, zinc, nill, copper, nill, cobalt, 200 mg, iodine, 400 mg, selenium, 80 mg, choline chloride, 500,000 mg.

^bFinisher Vitamin-Mineral Premix: (Rotinol) based on 2.5 kg/ton (Thiamine, 1000 mg, riboflavin, 6000 mg, pyridoxine, 5000 mg, cyanocobalamine, 25 mg, niacin, 60,000 mg., D-panthotenate, 20,000 mg, folic acid, 200 mg, D-biotin, 8 mg, Retinyl acetate, 40 mg, cholecalciferol, 500mg, tocopherol acetate, 40,000 mg., menadione, 800 mg, ascorbic acid, 60,000 mg, manganese, nill, iron, 80,000 mg, zinc, nill, copper, nill, cobalt, 80 mg, iodine, 400 mg, selenium, 40 mg, choline chloride, 80,000 mg. ^cMethionine Hydroxyl Analog (MHA): (Novus International Inc.St. Charles, MO), feed supplement providing 84% Methionine activity.

were weighed and randomly allotted to five dietary treatments. A total of 75 birds were used per treatment and the treatments were replicated 5 times with 15 birds each. The chicks were brooded for 2 weeks. All routine vaccinations and necessary medication were administered to the birds. Feed and water were supplied to the broiler chickens *ad libitum*. The birds were raised for eight weeks (0–4 weeks for the starter phase and 4–8 weeks for the finisher phase). The test diets were actually formulated to include untreated and *Z. mobilis* - treated sawdust at varying levels of 0, 50 and 100 % replacing wheat offal weight for weight basis.

The composition of the experimental broiler chicken diet is shown in Table 1.

Chemical analysis

The proximate composition of crude protein, crude fibre, ether extract and ash of the samples of untreated and treated sawdust was determined according to the standard procedures of AOAC (2015). The fibre fraction such as neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) was determined by the methods of Van Soest *et al.* (1991). Calcium and phosphorus of the test ingredients were determined by the methods of Grueling (1966). Gross energy of the samples was determined using a Gallenkamp Ballistic bomb calorimeter (Cam Metric Ltd., Cambridge, UK).

Data collection

Blood collection and Analysis

Blood samples were collected individually from 75 broiler chickens (3 birds per replicate) via the wing veins using sterilized syringe at the end of the starting and finishing phases of the feeding trials. About 2.5 ml of the blood sample were collected from each bird into vials containing ethylene diamine tetra-acetic acid (EDTA) as an anticoagulant for the determination of haematological parameters. However, another set was collected into heparinised tubes for serum biochemistry measurement. Haemoglobin concentration was estimated using the cyanmethaemoglobin method (Cannan, 1958), packed cell volume (PCV), red blood cell (RBC) and white blood cell (WBC) count of the blood samples were determined in Wintrobe haematocrit tube according to the method of Schalm et al. (1975). Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular

Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated as described by Jain (1986).

Carcass and organ weight determination

At the end of 8 weeks, seventy-five broiler chickens (3 birds per replicate) were selected at random and starved for about 18 hours to empty their crops for each experiment. They were slaughtered by cervical dislocation, allowed to bleed, scalded in warm water and defeathered. They were thereafter taken to the laboratory where other measurements like the dressed weight, the weight of the cut parts and organs were taken with a sensitive electronic scale within 1 hour after slaughtering. The weight of the cut-up parts and organs was expressed as a percentage of live weight according to "Modified Kosher" method as described by Abe *et al.*, (1996), while the dressing percentage was calculated as follows:

Dressing % =
$$\frac{\text{Eviscerated weight}}{\text{Liveweight}} \times \frac{100}{1}$$

Sensory evaluation

The sensory evaluation of cooked samples of broiler chicken breast minced meat from three birds per replicate was carried out by twenty panellists. Parameters that were evaluated by the panellists include colour, juiciness, flavour, tenderness and overall acceptability. Each meat sample was coded and presented one after the other to each member of the panel. Each member rinsed his or her mouth with water after assessing each meat sample to avoid carry-over effect. The panellists awarded scores using a nine (9) point hedonic scale of (i) Dislike extremely (ii) Dislike very much (iii) Dislike moderately (iv) Dislike slightly (v) Intermediate (vi) Like slightly (vii) Like moderately (viii) Like very much (ix) and Like extremely (Ogunwole *et al.*, 2013).

Experimental design and Statistical analysis

The experimental design used for this study was a completely randomized design (CRD). All data collected were subjected to one-way analysis of variance (ANOVA) as outlined by Daniel (1995) with the aid of SAS (2001) and the significant means separated by Duncan's multiple range test at 5 % level of significance (Steel and Torrie, 1980).

RESULTS

The result of the proximate composition of the untreated and treated sawdust is shown in Table 2. The biodegradation of the sawdust with *Zymomonas mobilis* led to increased (p < 0.05) values of crude protein, ether extract, nitrogen free extract, gross energy and metabolizable energy. There was a significant reduction (p < 0.05) in the values of crude fibre, fibre fraction and ash of the sawdust. The crude fibre, neutral detergent fibre, acid detergent fibre and acid detergent lignin had 21.37 %, 35.32 %, 58.92 % and 189.36 % reduction, respectively after degradation.

Table 2. Proximate analysis of untreated and treated sawdust^{*} (SD) (DM-basis)

Components (%)	Untreated SD	Treated SD	t-test (P-value)
Dry matter	89.51ª	86.50 ^b	21.69 (0.0001)
Moisture	10.49 ^b	13.50 ^a	-21.69 (0.0001)
Crude protein	2.14 ^b	7.13ª	-35.95 (0.0001)
Crude fibre	62.48°	51.50 ^b	79.10 (0.0001)
Ether extract	0.60 ^b	5.00 ^a	-31.70 (0.0001)
Nitrogen free extract	15.82 ^b	20.87ª	-36.38 (0.0001)
Ash	8.47 ^a	2.00 ^b	46.61 (0.0001)
Neutral detergent fibre	89.31°	66.00 ^b	167.93 (0.0001)
Acid detergent fibre	76.76 ^a	48.00 ^b	207.20 (0.0001)
Acid detergent lignin	40.51°	14.00 ^b	191.00 (0.0001)
Calcium (g.kg ⁻¹ DM)	0.05	0.04	1.23 (0.2879)
Phosphorus (g.kg⁻¹ DM)	0.85	0.83	0.144 (0.8924)
**Metabolizable energy (MJ.kg ⁻¹)	2.90 ^b	5.92°	-21.76 (0.0001)

Means on the same row having different superscripts are significantly different (P < 0.05).

*Average of three determinations.

**Metabolizable energy values were calculated using the method.

37 x % CP + 81 x % EE + 35.5 x % NFE for poultry birds (Fisher and Boorman, 1986).

The haematological and serum metabolites of starting broiler chickens fed diets containing untreated and Zymomonas mobilis-treated sawdust are shown in Table 3. The dietary treatments influenced (p < 0.05) the packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC), heterophil, lymphocytes, eosinophils, monocytes and basophils. The broiler chickens fed 100 % untreated sawdust-based diet (UTSD) had the highest (p < 0.05) values for packed cell volume, red blood cell and lymphocytes while the birds fed 100 % Zymomonas mobilis-treated sawdust-based diet (ZTSD) had the highest value for white blood cell and birds on 50 % ZTSD had the highest value for heterophil. However, the birds fed 100 % ZTSD had the lowest values for PCV, RBC, WBC and lymphocytes while the least value for heterophil was recorded in 100 % UTSD. The dietary treatments significantly (p < 0.05) influenced the total protein,

albumin, globulin, glucose, cholesterol, uric acid, creatinine, AST and ALT. The birds fed 50 % ZTSD had highest (p < 0.05) values for total protein, albumin and globulin but the lowest values for these serum metabolites were obtained in the control group and 100 % ZTSD.

Haematological and serum metabolites of finishing broiler chickens fed sawdust-based diets are shown in Table 4. The PCV, haemoglobin, RBC and WBC were influenced (p < 0.05) by the experimental diets. The birds fed 50 % UTSD had the highest value for PCV while the least value was recorded in 100 % ZTSD. The highest values recorded in birds fed 50 % UTSD for haemoglobin and RBC were similar (p > 0.05) to those of the birds fed the control diet. The MCH, MCHC and MCV were affected (p < 0.05) by the dietary treatments. The highest value for MCV recorded in birds fed 50 % ZTSD was similar (p > 0.05) to the values obtained for birds on the

	Dietary treatments					
Parameters	1	2	3	4	5	
	Control diet	50 % UTSD	100 % UTSD	50 % ZTSD	100 % ZTSD	SEM
Haematological parameters:						
Packed cell volume (%)	27.50 ^c	30.50 ^b	32.00 ^a	26.00 ^d	26.50 ^{cd}	0.65
Haemoglobin (g.dl ⁻¹)	8.90	10.00	10.50	8.50	8.70	0.31
Red blood cell (x 10 ¹² .L ⁻¹)	2.25 ^{ab}	2.70 ^{ab}	2.88ª	2.15 ^b	2.50 ^{ab}	0.11
Mean Corpuscular Haemoglobin (pg)	40.19	37.06	37.35	39.56	35.88	1.61
Mean Corpuscular Haemoglobin						
in Concentration (g.dl ⁻¹)	32.41	32.86	32.80	32.77	32.85	0.86
Mean Corpuscular Volume (fl)	123.07	112.96	113.66	121.54	108.67	3.66
White blood cell (x 10 ⁹ .L ⁻¹)	19.50 ^b	13.90 ^d	12.05 ^e	16.20 ^c	21.15°	0.91
Heterophil (%)	31.00 ^{cd}	33.50 ^{bc}	29.00 ^d	37.00 ^a	36.00 ^{ab}	0.86
Lymphocytes (%)	65.50 ^b	63.50 ^b	70.00 ^a	61.50 ^b	62.50 ^b	0.95
Eosinophil (%)	0.50 ^b	1.00 ^a	0.00 ^b	0.00 ^b	0.50 ^b	0.12
Monocytes (%)	1.50 ^a	2.00 ^a	0.50 ^b	0.50 ^b	0.50 ^b	0.18
Basophils (%)	1.50ª	0.00 ^c	0.50 ^{bc}	1.00 ^{ab}	0.50 ^{bc}	0.17
Serum metabolites:						
Total protein (g.dl ⁻¹)	3.50 ^b	4.20 ^a	3.40 ^b	4.50 ^a	2.55 ^c	0.19
Albumin (g.dl ⁻¹)	1.30 ^b	2.10 ^a	2.00 ^a	2.20ª	1.35 ^b	0.11
Globulin (g.dl-1)	2.20ª	2.10 ^a	1.40 ^b	2.30ª	1.20 ^b	0.13
Glucose (mg.dl ⁻¹)	118.50 ^d	112.00 ^e	127.50 ^a	125.50 ^b	123.50 ^c	1.49
Cholesterol (mg.dl ⁻¹)	90.00 ^c	88.50°	97.00 ^b	103.00ª	90.50 ^c	1.48
Uric Acid (mg.dl ⁻¹)	4.20 ^c	5.75ª	4. 20 ^c	5.35 ^b	3.95 ^d	0.19
Creatinine (mg.dl ⁻¹)	0.65 ^b	0.50 ^c	0.75 ^b	0.70 ^b	0.95ª	0.04
Aspartate Amino-Transferase (µKat.L ⁻¹) 1.11	0.80	0.92	0.87	0.80	0.05
Alanine Amino-Transferase (μ Kat.L ⁻¹)	0.36	0.35	0.45	0.42	0.37	0.04

Table 3. Haematological parameters and serum metabolites of starting broiler chickens (0 – 4 weeks) fed diets containing untreated and treated sawdust

Means on the same row having different superscripts are significantly different (P < 0.05); SEM: Standard Error of Mean; n = 5.

control diet and 100 % ZTSD while the least value was recorded in broilers fed 50 % UTSD. The birds fed the control diet had the highest value for WBC, however, the least value was observed in birds fed 50 % ZTSD. The highest value for heterophil was recorded in 50 % UTSD with a similar value in 100 % UTSD while the lowest value obtained in 50 % ZTSD was similar (p > 0.05) to the value in control group and 100 % ZTSD. The finishing broiler chickens fed 50 % UTSD had the highest values (p < 0.05) for total protein, albumin, globulin and uric acid while birds on 50 % ZTSD had the least values for total protein and albumin. The AST and ALT were significantly (p < 0.05) affected by the dietary treatments. The highest value of AST was obtained in 100 % ZTSD while the lowest value was recorded in the control diet. Moreover, the birds fed 50 %

UTSD had the highest (p < 0.05) value for ALT while the lowest value was recorded in 100 % UTSD.

The carcass characteristics of broiler chickens fed sawdust-based diets are shown in Table 5.

The dietary treatments influenced (p < 0.05) the dressed weight and eviscerated weight. The broiler chickens fed 50 % ZTSD had the highest value while the least value was observed in birds fed 100 % UTSD. The birds on other diets had similar (p > 0.05) values with the control group. The birds fed 50 % UTSD had highest (p < 0.05) value of eviscerated weight which had similar (p > 0.05) values with birds on the control diet and 100 % ZTSD. The highest value (p < 0.05) of breast recorded in the birds fed control diet was similar (p > 0.05) to birds fed 100 % UTSD while the lowest value was observed in birds fed 50 % UTSD. The highest value (p < 0.05) of breast recorded in the birds fed control diet was similar (p > 0.05) to birds fed 100 % UTSD while the lowest value was observed in birds fed 50 % UTSD. The highest

	Dietary treatments						
Parameters	1	2	3	4	5		
	Control diet	50 % UTSD	100 % UTSD	50 % ZTSD	100 % ZTSD	SEM	
Haematological parameters:							
Packed cell volume (%)	39.00 ^b	53.00 ^a	37.00 ^b	34.00 ^c	28.00 ^d	2.23	
Haemoglobin (g.dl ⁻¹)	12.50 ^a	11.90 ^a	12.40 ^a	11.00 ^b	9.50°	0.31	
Red blood cell (x 10 ¹² .L ⁻¹)	3.30 ^{ab}	3.50 ^a	3.00 ^{bc}	2.60 ^{cd}	2.30 ^d	0.13	
Mean Corpuscular Haemoglobin (pg)	37.91 ^{ab}	34.01 ^b	42.14ª	42.30 ^a	41.29 ^a	1.12	
Mean Corpuscular Haemoglobin							
in Concentration (g.dl ⁻¹)	32.09°	22.47 ^b	33.57ª	32.40 ^a	33.96 ^a	1.22	
Mean Corpuscular Volume (fl)	31.25 ^b	44.53 ^a	29.83 ^b	30.98 ^b	29.55 ^b	1.58	
White blood cell (x 10 ⁹ .L ⁻¹)	23.10 ^a	17.00 ^c	15.60 ^d	12.90 ^e	18.20 ^b	0.91	
Heterophil (%)	31.00 ^{bc}	35.00 ^a	32.00 ^{ab}	28.00 ^c	31.00 ^{bc}	0.70	
Lymphocytes (%)	65.00 ^b	60.00 ^c	67.00 ^{ab}	70.00 ^a	68.00 ^{ab}	1.05	
Eosinophil (%)	0.00 ^b	1.00 ^a	0.00 ^b	0.00 ^b	0.00 ^b	0.12	
Monocytes (%)	3.00 ^a	2.00 ^{ab}	1.00 ^{bc}	1.00 ^{bc}	0.00 ^c	0.30	
Basophils (%)	1.00 ^{ab}	2.00 ^a	0.00 ^b	1.00 ^{ab}	1.00 ^{ab}	0.21	
Serum metabolites:							
Total protein (g.dl ⁻¹)	3.10 ^c	6.30 ^a	4.60 ^b	2.10 ^e	2.70 ^d	0.41	
Albumin (g.dl ⁻¹)	1.80 ^b	2.90 ^a	1.50 ^{bc}	1.00 ^c	1.80 ^b	0.18	
Globulin (g.dl ⁻¹)	1.30 ^c	3.40ª	3.10 ^b	1.10 ^d	0.90 ^e	0.29	
Glucose (mg.dl ⁻¹)	120.00 ^c	122.00 ^b	112.00 ^e	115.00 ^d	133.00 ^a	1.94	
Cholesterol (mg.dl ⁻¹)	87.00 ^b	76.00 ^e	84.00 ^c	82.00 ^d	90.00 ^a	1.29	
Uric Acid (mg.dl ⁻¹)	3.20 ^b	5.00 ^a	4.80 ^a	3.00 ^b	2.80 ^b	0.28	
Creatinine (mg.dl ⁻¹)	0.10 ^c	0.40 ^{ab}	0.40 ^{ab}	0.30 ^b	0.50 ^a	0.04	
Aspartate Amino-Transferase (µKat.L ⁻¹) 0.77	0.85	0.83	0.82	1.07	0.05	
Alanine Amino-Transferase (µKat.L-1)	0.41	0.48	0.24	0.32	0.39	0.04	

Table 4. Haematological parameters and serum metabolites of finishing broiler chickens (4 – 8 weeks) fed diets containing untreated and treated sawdust

Means on the same row having different superscripts are significantly different (P < 0.05); SEM: Standard Error of Mean; n = 5.

value of thigh recorded in the birds fed control diet were similar (p > 0.05) to the values of birds fed 50 % UTSD, 100 % UTSD and 50 % ZTSD except in birds fed 100 % ZTSD which had the least value. The back had similar (p > 0.05) values across the dietary treatments except in 100 % ZTSD which had the least value. The organ weight was affected (p < 0.05) by the dietary treatments. The highest value of the whole gastrointestinal tract was observed in 100 % ZTSD while the lowest value was recorded in 50 % UTSD.

The sensory evaluation of meats from broiler chickens fed sawdust-based diets is shown in Table 6. The dietary treatments significantly (p < 0.05) influenced the colour, juiciness, flavour, tenderness and overall acceptability. The highest value recorded

in the control diet for colour was statistically (p > 0.05) similar to the values obtained in 100 % UTSD and 50 % ZTSD but the lowest value was recorded in 50 % UTSD. The control diet had the highest value for overall acceptability which was similar (p > 0.05) to the values obtained in 100 % UTSD and 50 % ZTSD but the lowest value was recorded in 50 % UTSD.

DISCUSSION

The proximate composition of the experimental diets met the nutrient requirements of the starting and finishing broiler chickens in the tropics as stated by Olomu (1995).

	Dietary treatments					
Parameters	1	2	3	4	5	
	Control diet	50 % UTSD	100 % UTSD	50 % ZTSD	100 % ZTSD	SEM
Live weight (g)	1850.00	1900.00	1900.00	1900.00	1850.00	17.75
Dressed weight (g)	1720.00 ^b	1740.00 ^b	1500.00 ^c	1850.00ª	1680.00 ^b	33.07
Eviscerated weight (g)	1320.00 ^{ab}	1360.00ª	1280.00 ^b	1250.00 ^b	1300.00 ^{ab}	12.73
Dressing percentage (%)	71.35	71.79	67.47	65.77	70.33	0.97
Cut parts (% of LW)						
Head (%)	3.24 ^b	3.16 ^b	3.16 ^b	3.16 ^b	4.32ª	0.13
Breast (%)	21.62ª	18.95 ^c	21.05 ^{ab}	20.00 ^b	19.46°	0.30
Thigh (%)	10.81ª	10.53ª	10.53ª	10.53ª	9.73 ^b	0.10
Drumstick (%)	10.81	10.53	10.53	10.53	11.89	0.24
Wing (%)	8.65°	9.47 ^b	8.42 ^d	9.47 ^b	16.22ª	0.78
Back (%)	16.22ª	16.84ª	16.84ª	15.79ª	9.73 ^b	0.74
Neck (%)	3.24 ^b	3.16 ^b	3.16 ^b	4.21 ^a	4.32ª	0.17
Shank (%)	5.41ª	5.26ª	4.21 ^b	5.26ª	5.41ª	0.15
Organ weight (% of LW)						
Heart (%)	0.39°	0.42 ^b	0.59ª	0.53ab	0.59ª	0.03
Spleen (%)	0.13 ^b	0.16 ^b	0.19 ^b	0.29ª	0.19 ^b	0.02
Liver (%)	1.89 ^d	2.11 ^c	2.37 ^b	2.63ª	2.16 ^c	0.07
Kidneys (%)	0.22 ^b	0.21 ^b	0.21 ^b	0.21 ^b	0.32ª	0.01
Gizzard (%)	2.16 ^c	2.11 ^c	4.21ª	3.16 ^b	2.16 ^c	0.22
Whole GIT (%)	12.97°	11.71 ^e	13.68 ^b	12.63 ^d	14.05ª	0.22

Table 5. Carcass characteristics of broiler chickens fed diets containing untreated and treated sawdust

Means on the same row having different superscripts are significantly different (P < 0.05); SEM: Standard Error of Mean; LW: Live weight; GIT: Gastro-intestinal tract; n = 5.

Table 6. Sensory evaluation of meat from broiler chickens fed diets containing untreated and treated sawdust

	Dietary treatments						
Parameters	1	2	3	4	5		
	Control diet	50 % UTSD	100 % UTSD	50 % ZTSD	100 % ZTSD	SEM	
Colour	6.65ª	5.75°	6.60ª	6.60ª	6.25 ^b	0.09	
Juiciness	6.05 ^b	5.35°	6.35ª	5.95 ^b	6.12 ^{ab}	0.10	
Flavour	5.75°	5.15 ^b	6.10ª	5.70 ^a	5.85°	0.10	
Tenderness	6.20 ^c	5.95 ^d	6.45 ^b	6.65ª	6.40 ^b	0.07	
Overall acceptability	6.50°	6.00 ^b	6.40ª	6.40 ^a	6.15 ^b	0.05	

 abc Means on the same row having different superscripts are significantly different (P < 0.05); SEM: Standard Error of Mean; n = 5.

Proximate composition of *Zymomonas mobilis*-fermented sawdust

The crude protein of *Zymomonas mobilis*fermented sawdust of 7.13 % was greater than the value of 5.56 % reported by Anigbogu and Anosike (2010) for *Zymomonas mobilis*-degraded sawdust but lower than the value reported for wheat offal by Aduku (1993). The crude fibre of 51.50 % obtained in the study was lower than the value (58.85 %) reported by Anigbogu and Anosike (2010). However, the value of ether extract of 5.00 % was greater than 0.16 % reported by the same authors. The difference might

be due to the species of trees, length of storage of the timber, sawdust at the sawmills and the milling pattern (Oke and Oke, 2007). There was an improvement in the gross energy of the fermented sawdust. This might be due to the hydrolysis of the crude fibre into disaccharides and monosaccharide which resulted in the availability and utilization of liberated energy (Faniyi, 2006; Adedire *et al.*, 2012).

Haematological and serum metabolites of starting broiler chickens

At the starter phase, the dietary treatment influenced the haematological parameters with the exception of haemoglobin, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC). The blood variables most often influenced by dietary treatments were identified as red blood cell (RBC), packed cell volume (PCV), plasma protein, glucose and clotting time (Aletor, 1989; Aletor and Egberongbe, 1992). The PCV, haemoglobin (Hb) and RBC values were within the normal ranges (22.00-35.00%), (7.00-13.00 g.dl⁻¹) and (1.58-3.28 x 10¹²L) reported by Jain (1986) and Bounous et al. (2000). High PCV, Hb and RBC improved oxygen-carrying capacity of the cells, which result in better availability of nutrients (Oleforuh-Okoleh et al., 2015). Although there were no differences among dietary treatments for MCH, MCV and MCHC, their values were within the normal ranges (33.00 – 47.00 pg), (26.00 – 35.00 g.dl⁻¹) and (90.00-140.00 fl) cited by Jain (1993) and Benerjee (2004). This may indicate similar haemoglobin content. This observation agreed with the findings of Fasuyi and Aletor (2005). They reported no significant differences in MCH, MCV and Hb when cassava leaf protein concentrate replaced fish meal in broiler diets. The white blood cell values were within the normal values for broiler chickens (1.20-3.00 x 104 μl) reported by Jain (1993). The WBC, heterophil, eosinophil, basophils, monocytes and lymphocyte indicate the immunity potential of the chickens. The sawdust-based diets influenced the serum metabolites of the broiler chickens. The values of total protein were within the normal range for Gallus gallus species that is 2.5 to 4.5 g.dl⁻¹ as cited by Thrall (2007). The values of albumin obtained in the control group and 100 % ZTSD are similar but are lower than the normal range

 $(2.00-3.50 \text{ g.dl}^{-1})$ reported by Anon (1980) and Jain (1986). The birds on 100 % UTSD and 50 % ZTSD had values of globulin which were lower than the normal range (2.00-3.50 g.dl⁻¹) reported by Marieb and Hoehn (2007). However, the values were within the normal range (0.5 to 1.8 g.dl⁻¹) as cited by Thrall (2007). The total protein, albumin and globulin values obtained in the study attest to the nutritional adequacy of untreated and treated sawdust in replacing wheat offal in meeting the protein needs of the broiler chickens.

Moreover, the values of glucose (112.00– $-127.50 \text{ mg.dl}^{-1}$) obtained in this study were lower than the normal range (200–500 mg.dl $^{-1}$) reported by Café *et al.* (2012). The values of cholesterol (88.50–103.00 mg.dl $^{-1}$) observed in the study were within the values (58.00–128.00 mg.dl $^{-1}$) reported by Zomrawi *et al.* (2012) for broiler chickens fed ginger root powder at levels 0.5, 1 and 1.5% respectively. The birds fed 100% ZTSD had the lowest value of uric acid compared to other diets. Babatunde and Pond (1987) observed that blood urea concentration is inversely related to protein quality, therefore, the lowest value of total protein observed in 100% ZTSD may be due to the inferior protein quality and/or nutrition of the sawdust.

Haematological and serum metabolites of finishing broiler chickens

The dietary treatments influenced the haematological parameters of the finishing broiler chickens. The birds fed the control diets, 50 % UTSD and 100 % UTSD had higher values of PCV compared with the normal range (22.00-35.00 %) by Bounous et al. (2000) while the values of PCV of birds on 50 % ZTSD and 100 % ZTSD were within the normal range. This is an indication that the fibrous feedstuffs ensure the good health status of the birds because low PCV values indicate anaemia. The haemoglobin values were within the normal range (7.00-13.00 g.dl⁻¹) reported by Jain (1993). This might indicate that the replacement of wheat offal with untreated and treated sawdust in the broiler chicken diets was nutritionally adequate in providing a sound plane of nutrition. Lindsay (1977) reported that haemoglobin concentration decreased in livestock on low protein intake, parasite infection or liver damage. However, the PCV and Hb are correlated with the nutritional

status of the livestock which directly relates to the nutritional balance of the diet fed to the livestock (Church et al. 1984, Babatunde et al. 1987). This further indicated that all the broiler chickens had higher tendency to resist respiratory stress because Hb which is carried by the RBC is the oxygen-carrying pigment as earlier reported by Muhammad and Oloyede (2009). The red blood cell values obtained in the study were within the normal range (2.54-3.30 x 10⁶ mm⁻³) reported by Aletor and Egberongbe (1992). It had been reported by Ugwuene (2011) that reduced RBC indicates a reduction in the level of oxygen that would be carried to the tissues as well as the level of carbon dioxide returned to the lungs. The values of MCH observed in the study were within the normal range (33.00-47.00 pg) reported by Jain (1993). The values of MCHC in birds fed 50 % UTSD were lower than the normal range (26.00-35.00 g.dl⁻¹) for broiler chickens (Jain 1993). However, all the values obtained for MCV were lower than the normal range (90.00-140.00 fl) reported by Jain (1993). MCV is an important trait which is responsible for the cell size of erythrocytes and it is an essential factor in determining the ability of poultry birds to withstand prolonged oxygen starvation (Mitruka and Rawnsley, 1977). The values of WBC were within the normal range of 9.20-31.00 x 10⁶ mm⁻³ reported in the literature (Riddell, 2011; Mitruka and Rawnsley, 1977; Banks, 1974) for healthy Nigerian local chickens. The white blood cells play an essential role in disease resistance, especially in the production of antibodies and the process of phagocytosis. The lymphocytes were the most numerous and frequent white blood cell type followed by heterophils, eosinophils and the monocytes (Afolabi et al., 2011). The same trend was observed by Bounous et al. (2000) and described the lymphocytes as the most numerous WBC in chickens and turkeys. However, the result of the study did not agree with their reports because the values obtained for eosinophil were lower than the values of monocytes. The lymphocytes and monocytes, which are agranulocytes of WBC, were within the normal range from 47.2 to 85.0 % and 0.06 to 5.0 % respectively for a healthy chicken (Riddell, 2011; Mitruka and Rawnsley, 1977). However, the birds on 100 % ZTSD had zero value for monocytes which was lower than the value reported

in the literature. Banks (1974) reported 6 % monocytes for domestic chickens and Islam et al. (2004) reported 3.42 +/- 0.50 %) monocytes for local chicken of Bangladesh. Moreover, lymphocytes are involved in antibody production, as they are reactive cells in inflammation and delayed hypersensitivity (Banks, 1974). Small lymphocytes may be responsible for the development of clones of plasma cells while monocytes are phagocytic cells. The high lymphocytes and heterophil count in this study are consistent with the findings of Afolabi et al. (2010) who also observed high lymphocytes and heterophils in chickens. This is in contrary to the reports of Oyewale (1987) who observed higher white blood cell count and lower lymphocyte counts in Nigerian fowls. The heterophils and the eosinophils that are granulocytes of WBC were within normal range from 10 to 53.6 % and 0.00 to 15 % respectively for a healthy chicken (Riddell, 2011; Pampori and Igbal, 2007; Mitruka and Rawnsley, 1977). The eosinophils function in phagocytosis while the basophils are responsible for the elaboration of histamines and heparin in circulating blood (Afolabi, et al., 2011). Moreover, the dietary treatments influenced the serum metabolites of the finishing broiler chickens. The values of total protein obtained in birds fed 50 % ZTSD (2.10 g.dl⁻¹) and 100% ZTSD (2.70 g.dl⁻¹) were lower than the normal range $(3.00 - 5.00 \text{ g.dl}^{-1})$ reported by Obikaonu et al. (2012) but the higher value (6.30 g.dl⁻¹) was recorded in 50 % UTSD was within the normal range (5.00 – 8.00 g.dl⁻¹) reported by Anon (1980). Reddy and Salunkhe (1984) reported decreased total protein which was attributed to inhibition of protein utilization in broiler chickens. The value of albumin (1.00 g.dl⁻¹) recorded in 50 % ZTSD was lower than the normal range (2.10-3.45 g.dl⁻¹) reported by American Metabolic Testing Laboratories (2001). However, the values of globulin obtained in the control group, 50 % ZTSD and 100 % ZTSD (0.90 - 1.30 g.dl⁻¹) were lower than the normal range (2.00-3.50 g.dl⁻¹) reported by Marieb and Hoehn (2007). Globulin carries essential metals through the bloodstream to the various parts of the body of farm animals. It helps to fight infections in the body of animals. Therefore, high globulin levels are often pronounced in birds with serious infections because of abnormally increased production of antibodies.

The values of globulin observed in the study revealed that the inclusion of treated sawdust in the broiler diet did not precipitate any severe effects on the health status of the birds. Serum urea can be used as a test of protein break down, renal function, hydration status and liver failure (Agboola et al., 2013). The concentration of uric acid also depends on a diet especially those with high protein content. However, the values of uric acid in this study were lower than the normal range (7.00-21.00 mg.dl⁻¹) reported by American Metabolic Testing Laboratories, (2001). The values of uric acid obtained in 50 % ZTSD and 100 % ZTSD were similar to the value in the control group. This probably suggested that there were better digestion, utilization and absorption of protein from the treated sawdust used which invariably improved protein utilization. However, a high concentration of urea may be toxic to both the liver and kidney while low levels could be due to low protein intake or severe liver failure (Oyebimpe, 2012). It had been reported by Baron (1973) that increased concentration of creatinine is associated with renal impairment. The values of glucose in this study were within the normal range (65.00 – 140.00 mg.dl⁻¹) reported by American Metabolic Testing Laboratories (2001). The cholesterol values were within the values (76.30–115.57 mg.dl⁻¹) reported by Onyimonyi *et al.* (2012) who fed dried garlic powder to broiler chickens. However, the values were lower than (100.30–108.21 mg.dl⁻¹) reported by Aderemi (2004), (93.33-116.67 mg.dl⁻¹) by Nworgu (2004) and (143.10-163.00 mg.dl⁻¹) reported by Nworgu et al. (2007). This will restore the confidence of consumers who earlier had reduced or stopped their consumption of chicken due to cholesterol scare. Also, this will protect the consumers from the negative effect of cholesterol which includes obesity, heart attack and stroke (Onyimonyi et al., 2012). Ekpenyong and Biobaku (1986) reported that the levels of SAST and SALT are normally low in blood but they become high when the plane of nutrition is low or when there is an occurrence of liver damage by toxic substances. The values of aspartate aminotransferase (AST) were comparable to the values reported by Sobayo et al. (2013) when they fed graded levels of Garcinia kola (Bitter kola used as phytobiotic in broiler chicken diets. Moreover, the values of alanine aminotransferase

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(ALT) were within the values reported by the same authors.

Carcass characteristics of broiler chickens fed diets containing untreated and treated sawdust

There was no difference in the live weight of the broiler chickens fed the experimental diets. This was in concert with the observation of Odeh et al. (2016) who reported no differences between treatments for the live weight. However, there were differences in dressed weight and eviscerated weight. This was contrary to the findings of Abdulraheem et al. (2006) who observed no statistical differences between treatment groups when rice bran was used to replace maize in broiler chicken diets. Birds on 50 % ZTSD had superior higher value of dressed weight compared with a control diet. The higher dressed carcass weight (1850.00 g) of broiler chickens fed 50 % ZTSD may be considered to be a direct consequence of the better body weight and FCR of the broiler chickens in this treatment. Although birds fed the control diet with a dressed weight of 1720.00 g and 100 % ZTSD with a value of 1680.00g did not have the highest live weight per finisher broiler chicken, they manifested remarkable dressed weight as a percentage of live weight indicating that all diets supported a proportional cumulative weight gain. However, it implies that the dressed weight of broiler chickens was not directly proportional to the weight gain or performance traits. Also, high weight gain value may not imply a concomitant increase in the dressed weight value expressed as a percentage of live weight (Fasuyi and Aletor, 2005). The dressing percentage was not influenced by dietary treatments. This may suggest that untreated and treated sawdust can be utilized to replace wheat offal in broiler diets. The values of dressing percentage in the study were lower than the values (74.15-86.29%) reported by Odeh et al. (2016). The dietary treatments influenced the cut-up parts of the broiler chicken except for the drumstick. The birds fed 100 % ZTSD had the lowest value of thigh and back compared with birds fed other diets. It may imply that the replacement of wheat offal by treated sawdust may not fully support the growth of some body parts of the broiler chickens. This observation agrees with the reports of Fasuyi and Aletor (2005).

The birds fed 100 % UTSD had the highest value of gizzard which might be due to the extra muscular activity required to process high fibre content of the feed. The increased weight of liver of the birds fed 50 % ZTSD and 100 % UTSD might be due to the role of this organ in eliminating metabolic waste and toxin from farm animals. Also, Onyeyilli et al. (1998) reported that the liver was a primary organ of biotransformation in farm animals. The values of the whole gastrointestinal tract did not follow any trend, but birds fed 100 % UTSD and 100 % ZTSD had highest values while the least value was obtained in birds on 50 % UTSD. Abdelsamie et al. (1983) reported that higher fibre contents at similar feed intakes enhanced relative weight and length of the gastrointestinal tracts of broiler chickens. Longe and Ogedengbe (1989) reported that the gravity of feeding dietary fibre on growth response is a function of the source and concentration of the fibre source.

Sensory evaluation of meat from broiler chickens fed untreated or treated sawdust

The replacement of wheat offal with untreated and treated sawdust had an influence on the sensory attributes of the broiler chicken meat. The values obtained in 100 % UTSD, 50 % ZTSD and 100 % ZTSD were similar to the control group. This implied that the taste of panellists could not differentiate the meat samples. The sawdustbased diets improved the juiciness and tenderness of the meat. Therefore, Z. mobilis-treated sawdust promoted overall acceptability of meat from broiler chickens without any deleterious influence on the meat quality. Breidenstein and Carpenter (1983) reported that colour, flavour, juiciness and tenderness are the essential parameters of the eating quality of meat. Also, Pippen et al. (1969) reported that the components responsible for flavour are from the lean portion and dissolved in the fat during cooking. However, Awosanya et al. (1990) observed that the only factor which was responsible for consumers' overall acceptability of rabbit meat is the age at which the animal is slaughtered. Therefore, the younger the age of the livestock, the more acceptable is its meat. Moreover, juiciness is important in the tenderness of meat because it provides lubrication to the consumers, and enhances mouthfeel (Owens et al., 2004). Tenderness had been reported as a major

quality determinant and probably the most essential sensory characteristic of meat (Deatherage, 1963). Tenderness score followed a similar trend as juiciness and flavour. Ouali (1990) and Smulders et al. (1991) reported that meat tenderization is a multifactorial process which depends on a number of biological and environmental factors. The utilization of treated sawdust increased the degree of tenderness, as assessed by the taste of panellists. This agreed with the findings of Omojola and Adesehinwa (2007) who reported that exogenous enzyme increased the degree of tenderness of breast meat from broiler chickens. However, the result for texture, colour and overall acceptability did not agree with the reports of the same authors who observed that these parameters were not affected by the inclusion of enzyme in the broiler chicken diets.

The dietary treatments influenced the sensory parameters.

CONCLUSION

Dietary inclusion of 100 % Zymomonas mobilistreated sawdust replacing wheat offal for broiler chicks is not recommended at the starter phase since it potentiates increased white blood cell counts of the chicks. This may be reflective of the imbalance defence mechanism of the starting chicks to the introduced bacteria. As a result of the established gut ecology of the older birds, Zymomonas mobilis treatment of the sawdust posed no negative effects on the health status of the finishing broilers. Dietary inclusion of 50 % Zymomonas mobilis-treated sawdust significantly improved carcass yield in terms of breast, thigh, drumstick and back weights. Hence, dietary inclusion of Zymomonas mobilis-treated sawdust is only recommended at the finisher phase.

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