Nutrient Digestibility and Blood Composition of Broiler Chickens Fed Diets containing Biodegraded Sweet Orange (*Citrus sinensis*) Fruit Peel

ABSTRACT

Aims: To determine the nutrient digestibility and blood composition of broiler chickens fed biodegraded sweet orange fruit peel-based diets.
Study design: Completely randomized design.
Place and Duration of Study: Livestock Research Farm, Federal University of Agriculture Makurdi, Nigeria; fifty-six days
Methodology: Sweet orange peels and cattle rumen content were collected. Rumen content was mixed with water at a ratio 1 kg : 1 litre and sieved to get rumen filtrate (RF). RF was mixed with Sweet orange peels at a ratio of 1 litre : 2.5 kg and fermented for 48 hours, sun-dried, milled, and added to broiler diets at different levels: 0% (T1), 5% (T2), 10% (T3), 15% (T4), and 20% (T5). A total of 150 day-old chicks, randomly assigned to five dietary treatments and three replicates per treatment were raised in a 56-day feeding trial. Three chickens of average live body weight similar to the treatment group average were selected and used to determine nutrient digestibility and blood composition.
Results: The results showed that the coefficient of digestibility of dry matter, crude protein, crude fibre, ether extract and nitrogen-free-extract did not differ significantly ($P > .05$) among the treatment groups. Haematological indices; haemoglobin, red blood cell (RBC), packed cell volume, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH), did not differ significantly ($P > .05$) across the dietary groups, while white blood cell (WBC) and mean corpuscular haemoglobin concentration (MCHC) varied significantly ($P < .05$). Serum indices; total protein (TP), globulin, glucose, cholesterol, and alkaline phosphatase were significantly affected ($P < .05$) by the experimental diets, while albumin, aspartate transaminase and alanine transaminase did not differ significantly ($P < .05$) across the dietary groups.
Conclusion: Biodegraded sweet orange peel-based diets can replace maize grains up to 20% without affecting nutrient digestibility or having any adverse effect on blood constituents of broiler chickens.

Keywords: Orange peel, Biodegradation, Nutrient digestibility, Blood, Growth, Chickens

1. INTRODUCTION

Livestock plays an indispensable role in Nigeria's agriculture sector, making a significant contribution of about 20% of the gross domestic product [1]. Agriculture is the backbone of Nigeria's economy, accounting for 24% of the national GDP, providing livelihoods for about 70% of the country's active labor force, and the livestock sub-sector growing at a faster rate of 12.7% than the agricultural growth rate of 6.8% being driven by the rising consumer demand [2]. However according to FAO, the average protein consumption of 45.4g in Nigeria is lower than the recommended 64g, therefore there is a need to improve the efficient supply of affordable meat for the generality of the populace.

Nutrition represents the foremost concern within the poultry industry, because the survival of the sector is dependent upon the accessibility of feed resources [3]. The primary sources of these feedstuffs are essentially human food components, such as maize, sorghum, millet, wheat, oat, soybean, groundnut, and sunflower. Feed costs are high, accounting for over 70% of the overall production cost [4] and for this reason, it is necessary to develop a feeding programme that will guarantee minimum cost to achieve optimal production. Sweet orange (*Citrus sinensis*) fruit peel is an abundant, unutilised agro-industrial by-product among several others in Nigeria and it is available at no cost and contains high energy content [5]. This agro-industrial by-product has limited nutritional value, primarily due to its low nutrient concentration, high fibre content, unappealing taste, and the presence of substances that interfere with nutrient absorption. The feed value of this by-product can however be improved by the application of some simple processes such as soaking, moist or dry heating, and fermentation. Rumen content is one valuable and available animal by-product in the Nigerian abattoirs, which can be transformed into a useful waste product by taking advantage of its microbial population. By utilizing rumen content and its microbial population, sweet orange fruit peel can be processed, leading to value addition and making it a more suitable dietary energy source for livestock production [6].

In animal nutrition, nutrient digestibility and blood composition are critical factors in evaluating feed quality and determining the feed's nutritional value. This evaluation is necessary to ensure that the animal receives sufficient nutrients to achieve optimal health, growth, and production. Thus, the present study aimed to evaluate the impact of incorporating biodegraded sweet orange peels into broiler diets as a partial replacement for maize grains on the nutrient digestibility and blood constituents of broiler chickens.

2. MATERIALS AND METHODS

2.1 Experimental site

The study was conducted in the Teaching and Research Farm, Federal University of Agriculture Makurdi, Nigeria. Makurdi is located at a latitude of 7^{o}.43´N and a longitude of 8^{o}.53´N.

2.2 Collection and Preparation of Test Ingredients

Sweet orange fruit peels were obtained free from orange retailers in Makurdi and likewise, the fresh rumen content were obtained from cattle slaughtered at a government abattoir in the city. Rumen filtrate (RF) was prepared by adding water to rumen content in a ratio of 1 litre : 1 kg, mixing the contents, and then sieving the mixture. The filtrate thus obtained was mixed with sweet orange peels in the ratio of 1 litre : 2.5 kg, poured into polythene bags tied at the open end and kept under shade to ferment for 48 hours. The fermented sweet orange peels were then spread on a concrete platform, sun-dried to below 10 % moisture and then milled. Homogenous milled sample was analyzed for proximate constituents [7] and was used to formulate experimental broiler diets at the starter (Table 1) and finisher (Table 2) phases. Its proximate composition has earlier been reported [8]. The fermented sweet orange peels was used as a replacement for maize grains at levels of 0% (T1), 5% (T2), 10% (T3), 15% (T4) and 20% (T5).

2.3 Experimental Animal, Design and Procedure

The study used 150-day-old unsexed broiler chicks, weighed and grouped into five groups, each group having 30 chicks with similar live weight. Each group was randomly assigned to one of the five diets with three replicates (10 birds per replicate), and all treatment replicates were randomly allotted to fifteen experimental pens. The experiment was a completely randomized design. The birds were raised in a deep litter of wood shavings and served with feed and drinking water *ad libitum for 56 days*. The birds were vaccinated at day old against Newcastle disease (i/o), infectious bursal disease at 14 days, Newcastle disease (lasota) at 21 days and infectious bursal disease at 28 days. Anti-stress supplements were administered 24 hrs prior to and post-vaccination and weighing, coccidiostat at alternate weeks, and antibiotics when necessary.

Table 1: Ingredients Composition of Experimental Diets (kg/100 kg)

Ingredients	Broiler Starter Diets	Broiler Finisher Diets

	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
Maize grains	49.22	46.76	44.30	41.84	39.38	54.31	51.59	48.88	46.16	43.45
SOPM	0	2.46	4.92	7.38	9.84	0	2.72	5.43	8.15	10.86
SBM	37.08	37.08	37.08	37.08	37.08	30.00	30.00	30.00	30.00	30.00
Maize meal	4.30	4.30	4.30	4.30	4.30	5.29	5.29	5.29	5.29	5.29
BDG	4.00	4.00	4.00	4.00	4.00	4.50	4.50	4.50	4.50	4.50
Bone ash	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Fish meal	0.70	0.70	0.70	0.70	0.70	1.20	1.20	1.20	1.20	1.20
Limestone	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20
Palm oil	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Premix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Table salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Methionine	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Analysis (%)										
CP	22.73	22.70	22.66	22.63	22.60	20.63	20.60	20.56	20.53	20.49
EE	4.21	4.18	4.14	4.11	4.08	4.25	4.21	4.19	4.15	4.11
Crude fibre	4.73	4.94	5.16	5.36	5.56	4.60	4.82	5.05	5.29	5.51
Ca	1.60	1.60	1.60	1.60	1.60	1.31	1.31	1.31	1.31	1.31
Available P	0.72	0.72	0.72	0.72	0.72	0.71	0.70	0.69	0.68	0.68
Lysine	1.10	1.09	1.09	1.08	1.07	0.97	0.96	0.96	0.95	0.94
Methionine	0.38	0.37	0.36	0.35	0.35	0.35	0.35	0.34	0.34	0.33
ME (MJ/kg)	11.95	11.89	11.83	11.77	11.72	12.18	12.11	12.05	11.98	11.81

*SOPM = Sweet orange peel meal, BDG = Brewers dried grain, SBM = Soybean meal, CP = Crude protein, EE = Ether extract, ME = Metabolizable energy, *Premix containing: Vitamin premix per kg; vit. A 12000 IU, vit D3 3000 IU, vit.E 30 mg, vit. K3 2.5 mg, Folic Acid 1 mg, Niacin 40 mg, Calpan 10 mg, vit. B2 5 mg, vit.B12 0.2 mg, vit. B1 2 mg, vit B6 3.5 mg, Biotin 0.08 mg, Antioxidant 125 mg, Mineral premix per kg; Cobalt 0.25 mg, Selenium 0.25 mg, Iodine 1.2 mg, Iron 40 mg, Manganese 70 mg, Copper 8 mg, Zinc 60 mg, Choline chloride 200 mg. T1 = 0% maize replacement with SOPM (Control diet); T2 = 5% maize replacement with SOPM; T3 = 10% maize replacement with SOPM; T4 = 15% maize replacement with SOPM; T5 = 20% maize replacement with SOPM*

2.4 Nutrient Digestibility

Evaluation of nutrient digestibility was done in the last week of the feeding trial to access nutrient utilization by the broiler chickens. One bird per replicate of average live weight similar to the group average was selected and transferred into metabolic cages. They were allowed the first 3 days to adapt to the cage environment. The birds were deprived of feed for twelve hours on the third day to empty the gut of the previously consumed diets prior to faecal collection and also on the seventh day at the termination of the trial. The birds were served daily weighed ration during the digestibility trial, the leftover was collected and weighed to determine the feed intake by difference. Wet faecal droppings were collected on a daily basis from each replicate, weighed and oven-dried at 105 ^{o}C for 24 hours. The oven-dried faecal samples were bulked per replicate, milled and analyzed to determine proximate nutrients using [7]. The quantity of nutrients in diets and faeces was determined by multiplying nutrient percentage in diets and in faeces by their corresponding dry matter values in diets and faeces, respectively. Nutrients retained were determined by the difference between the nutrient intake (Ni) and that voided in the faeces (Nf).

$$Apparent\ coefficient\ of\ digestibility\ of\ nutrient = \{Ni - Nf\ /\ Ni\}\ x\ 100.$$

2.5 Blood Constituent Evaluation

Two ml out of about 5 ml blood taken from each bird per replicate was dispensed into labelled sterile bottles containing EDTA. Haematological indices; red blood cell count (RBC), white blood cell count

(WBC), haemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV) and mean haemoglobin concentration (MHC) were determined [9]. The rest blood was dispensed into separate bottles without anticoagulants for serum biochemical analysis. constituents determined were total protein, albumin, globulin, creatinine, glucose, cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphate (ALP) using [10].

2.6 Statistical analysis

Data collected were subjected to one-way analysis of variance (ANOVA) using SPSS [11], and the means of significantly different ($P < .05$) parameters were separated using Duncan's Multiple Range Test.

3. RESULTS AND DISCUSSION

3.1 Nutrient Digestibility

The result of the apparent coefficient of nutrient digestibility of the diets is presented in Table 2. The broiler chickens receiving the different diets had comparable digestibility of all the nutrients with the exception of ash retention which decreased significantly from 72.91% to 60.28% as the maize grains replacement with sweet orange peel meal (SOPM) increased from 0% to 20%. For the other nutrients, coefficients of digestibility were high across the treatments ranging from 83.36% - 84.52% for CP, 80% - 83.41% for CF, 80.00% - 83.04% for EE, 74.88% - 79.27% for NFE and 76.25% - 80.47% for dry matter. This showed that the replacement of maize with SOPM had no negative effect on the digestibility of these nutrients.

Table 2: Effect of Biodegraded Sweet Orange Peel Meal on the Nutrient Digestibility of Finisher Broiler Chicken

	Experimental diets						
Nutrients (%)	**T1**	**T2**	**T3**	**T4**	**T5**	**SEM**	**P-value**
Dry matter	80.47	77.67	78.77	77.71	76.25	0.73^{ns}	0.92
Crude protein	84.52	83.73	84.40	83.66	83.36	0.82^{ns}	0.97
Crude fibre	80.18	81.73	81.83	83.41	81.45	0.90^{ns}	0.90
Ether extract	83.04	82.37	81.81	80.00	82.05	1.21^{ns}	0.74
NFE	79.27	76.18	77.37	76.41	74.88	0.78^{ns}	0.94
Ash	72.91^{a}	71.92^{a}	69.24^{ab}	66.13^{ab}	60.28^{b}	1.70^{*}	0.03

$^{a,\ b,}$Means with different superscripts in the same rows are significantly different (p<0.05), *(p < .05), ^{ns}Not significantly different (p > .05), SEM = Standard error of mean, NFE = Nitrogen free extract, SOPM = Sweet orange peel meal, P = Probability level
T1 = 0% maize replacement with SOPM (Control diet); T2 = 5% maize replacement with SOPM; T3 = 10% maize replacement with SOPM; T4 = 15% maize replacement with SOPM; T5 = 20% maize replacement with SOPM

3.2 Blood Composition

Table 3 shows the impact of the experimental diets on haematological and serum biochemical indices. The partial replacement of maize grains with biodegraded SOPM did not significantly ($P > .05$) affect Hb, RBC, PCV, MCV and MCH, but WBC and MCHC values showed significant ($P < .05$) differences across the dietary groups. The variation of WBC values decreased as the percentage of dietary SOPM increased from 0% to 20% but remained within the normal range of 3.98×10^{9} L to 10.82×10^{9} L [12] for broiler chickens. WBC is important for fighting off infections and diseases, and high values indicate an immune response, while low values increase the risk of infection. The MCHC values though differed significantly, were similar to the normal range of 27 to 35 g/dl reported by [13]. It is concluded that the level of haemoglobin in each cell was sufficient and that the consumption of the biodegraded SOPM-based diets did not make the chickens more vulnerable to infection. The Hb, RBC, PCV, MCV, and MCH values did not differ significantly and were within the normal range of 7.50 g/dl to 16.04 g/dl Hb, 1.97 x 10^{12} L to 3.75 x 10^{12} L RBC, 27.43 % to 37.30 % PCV, and 24.4 pg to 57.2 pg MCH [12, 14], reported for healthy broiler chickens. The MCV was higher than the reference range 111 fL to 144 fL MCV reported by [7], but comparable to the values of 97.07 fL to 166.45 fL reported by [15]. Nonetheless, it was not significantly different within the dietary treatments, indicating that the SOPM did not alter the MCV level. The normal levels of MCV and MCH indicated that SOPM did not negatively affect nutrient absorbability for blood formation and function in broiler chickens, nor did it present any toxic effects. Additionally, the MCHC levels indicated the absence of normocytic anaemia among the chickens. This showed that processing the sweet orange peel meal may have reduced its anti-nutritional factors and improved its energy and protein content, which could have enhance broiler chicken performance.

Table 3: Effect of Biodegraded Sweet Orange Peel Meal on Haematological and Serum Biochemical Indices of Finisher Broiler Chicken

	Experimental Diets						
Blood constituents	**T1**	**T2**	**T3**	**T4**	**T5**	**SEM**	**P-Value**
Haemoglobin (g/dL)	11.87	12.10	11.67	11.67	11.77	0.12^{ns}	0.82
Red blood cell (× 10^{12}/L)	2.10	2.30	2.17	2.10	2.23	0.04^{ns}	0.60
Packed cell volume (%)	35.67	36.33	35.00	34.67	35.33	0.35^{ns}	0.67
White blood cell (× 10^{9}/L)	6.73^{a}	6.13^{ab}	6.07^{ab}	5.53^{b}	5.47^{b}	0.17*	0.04
MCV (fL)	169.77	159.03	161.87	165.23	158.40	1.90^{ns}	0.31
MCH (pg)	56.50	52.77	53.90	55.13	52.77	0.62^{ns}	0.25
MCHC (g/dl)	33.23^{a}	33.27^{a}	33.23^{a}	33.20^{a}	25.87^{b}	1.00*	0.03
Total protein (mg/dl)	4.03^{a}	4.23^{a}	3.70^{ab}	2.97^{b}	2.93^{b}	0.17*	0.01
Globulin (g/dl)	2.27^{a}	2.10^{ab}	2.17^{a}	1.23^{bc}	0.83^{c}	0.19*	0.02
Albumin (g/dl)	1.77	2.13	1.60	2.07	2.10	0.11^{ns}	0.50
Albumin/globulin ratio	0.78^{d}	1.01^{c}	0.74^{d}	1.68^{b}	2.5^{a}	0.18*	0.00
Glucose (mg/dl)	121.57^{ab}	137.03^{a}	92.30^{b}	77.40^{c}	73.83^{c}	8.49*	0.04
Cholesterol (mg/dl)	92.93^{a}	90.90^{a}	83.37^{b}	84.63^{b}	80.73^{c}	1.29*	0.00
AST (µ/L)	55.67	46.60	41.87	37.87	36.27	3.23^{ns}	0.35
ALT (µ/L)	28.70	27.10	28.07	25.80	27.60	0.77^{ns}	0.85
ALP (µ/L)	93.13^{a}	89.20^{a}	74.00^{b}	81.73^{ab}	74.13^{b}	2.50*	0.02

*$^{a, b, c}$ Means with different superscripts in the same row are significantly different (p < .05), *(p < .05), ^{ns} Not significantly different (p>0.05), SEM = Standard error of mean, SOPM = Sweet orange peel meal, MCV= Mean corpuscular volume, MCH= Mean corpuscular haemoglobin, MCHC= Mean corpuscular haemoglobin count, AST= Aspartate transaminase, ALT= Alanine transaminase, ALP = Alkaline phosphatase, P = Probability level. T1 = 0% maize replacement with SOPM (Control diet); T2 = 5% maize replacement with SOPM; T3 = 10% maize replacement with SOPM; T4 = 15% maize replacement with SOPM; T5 = 20% maize replacement with SOPM*

The experimental diets had significant (*P < .05*) effects on serum indices; including TP, globulin, albumin : globulin ratio, glucose and cholesterol. With increase in dietary maize replacement with SOPM from 0% to 20%, serum indices decreased. However, albumin, AST, and ALT were not significantly (*P > .05*) affected. The levels of TP and globulin were found to be within the normal ranges of 2.2 g/dl to 5.5 g/dl and 0.8 % to 5.5 %, respectively [16], indicating normal functioning of the liver and kidney. The glucose concentrations were lower than the normal range of 200 - 400 mg/dl reported by [16]. The blood glucose reflects the nutritional and endocrinal condition of the birds. When serum glucose levels are too high (hyperglycemia), it causes nausea, and shortness of breath, and may cause damage to the heart, kidney, and nerve, and when blood glucose levels are low (hypoglycemia) it may give rise to hunger, irritability, shaking, twitching and weakness [16, 17]. However, these symptoms were not observed in the experimental birds. The serum cholesterol values obtained in this research were also within the normal reference of 52.00 mg/dl to 148 mg/dl [18], even though it tended to decrease with an increase in the dietary SOPM. This showed decreased absorption and /or synthesis of cholesterol with increasing SOPM levels.

The albumin levels were also within the normal range of 1.1 g/dl to 2.1 g/dl [19], indicating the birds were healthy and adequately utilized protein in their respective diets. Liver enzyme aspartate transaminase (AST) tended to decrease as the level of dietary maize replacement by SOPM increased but not significantly. The levels of serum ALT in the different dietary treatments were lower than 32 to 62 µ/l [20] and 37.80 to 65.36 µ/l [21] for broiler chickens. Alkaline phosphatase (ALP) values differed significantly among the treatment groups, ranging between 74.00 to 93.13 µ/l, but were within the normal range of 30.00 ± 1.54 µ/dL [22]. This revealed that SOPM did not damage the liver of the experimental broiler chickens. The non-significant variations in ALT and ALP concentrations among the treatment groups in relation to the control treatment in this study implied that the replacement of dietary maize with SOPM did not impair the liver and kidney function.

The blood parameters are major indices of the physiological, pathological and nutritional status of an organism, and changes in the constituent compounds of blood when compared to normal values could be used to interpret the metabolic state of an animal as well as the quality of feed [23]. Blood constituents' evaluation in this study has shown clearly that rumen filtrate biodegraded sweet orange peel meal can be a partial substitute for maize up to 20% without affecting the livability of broiler chickens.

4. CONCLUSION

The findings of this study revealed the possibility of transforming sweet orange fruit peel as an agricultural waste into a valuable feed resource, and that biodegraded sweet orange peel meal obtained from rumen filtrate can be used as a viable alternative to maize grains in broiler chicken feed up to a replacement level of 20%, without any adverse effects on nutrient digestibility or blood composition.

ETHICAL APPROVAL

All authors hereby declare that the Principles of laboratory animal care were followed and approved by the appropriate College ethics committee.

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