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Haematological and Serum Biochemical Indices of Broiler Chickens Fed Diets Containing Graded Levels of Biodegraded Sweet Orange (*Citrus sinensis*) Peel

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Abstract: *A 56 day feeding trial was conducted to determine the effect of rumen content biodegraded sweet orange peel on haematological and serum biochemical parameters of broiler chickens. Fresh rumen content (RC) was collected daily from eviscerated cattle and fresh sweet orange peels (SOP) were collected daily from orange retailers. The SOP was mixed thoroughly with one kilogram of RC in a ratio of 1SOP: 0.3 RC. The mixture was put in polythene bags, tied and left to ferment for 48 hours and then sun-dried. The dried mixture was separated from the RC, milled and used to replace dietary maize in the control diet D1 at 2%, 4%, 6%, 8% and 10% to obtain diets D2, D3, D4, D5 and D6 respectively. A total of 216, day-old Marshal MY broiler chicks were randomly assigned to six dietary treatments in a Completely Randomized Design and replicated thrice. The values of globulin, cholesterol and serum glutamic oxalaacetic transaminase (SGOT) were influenced significantly ($P < 0.05$) by the dietary treatments. Globulin values ranged from 0.83% in 2% SOP diet to 2.33% in 8% SOP diet. Globulin increased as the inclusion level of SOP in the diet increased. Cholesterol values ranged from 127.43% in 10% SOP diet to 154.90% in 2% SOP diet. Cholesterol decreased as the level of SOP inclusion increased in the diet. SGOT values ranged from 20.74% in 0% SOP diet to 59.90% in 8% SOP diet. SGOT had no consistent variation with increase in SOP inclusion in the diet. The utilization of biodegraded SOP had no deleterious effect on haematological and serum biochemical parameters of broiler chickens.*

Key Words*: Sweet orange peel, rumen content, biodegradation, haematological and serum biochemical indices.*

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I. Introduction

A major challenge facing the development of poultry production in Nigeria is the availability and high cost of feed stuffs. Feed cost accounts for about 70% of the total cost of production and is thereby considered a major determinant of the profitability, sustainability and development of the poultry enterprise (Adejinmi *et al.*, 2011). Research on non-conventional feed ingredient (NCF) for poultry has been on-going. The use of industrial by-products represents a valuable resource capable of replacing conventional feed stuffs such as maize. These by-products are low-cost, readily available and have no direct nutritional significance for humans. Sweet orange peel ingredients are interesting from a nutritional point of view since they are a source of vitamin C, phenolic compounds, coumarin, volatile oils, nobiletin, flavonoids, pectin, bioflavonoids, naringin and hesperetin (Shafeghat, 2010; Parhiz *et al.*, 2014). Dried *Citrus sinensis* peel can significantly improve the immune system activities, and this action has been attributed to their antioxidant properties (Chen *et al.*, 2010). Oluremi et al. (2007) had earlier reported that sweet orange peel could serve mainly as an energy source because of its high energy value (2990 kcal/kg). The direct disposal of large quantities of SOP waste on the environment represents an important loss of natural resources, which could be bioconverted into poultry feed with a higher commercial value. Rivas *et al.* (2008) reported that orange peel is in fact constituted by soluable sugar 16.90% weight (Wt); starch, 3.75% wt; fiber (cellulose, 9.21% wt; hemicelluloses, 10.50% wt; lignin, 0.84% wt; and pectin, 42.50%), ashes 3.5% wt; fats 1.95% wt; and protein, 6.50%. Citrus peels are rich in nutrients and contain many phytochemicals with strong potential for use in drug production and as food supplement (Chede, 2013). The rumen content (RC) is a substantial waste generated daily at abattoirs. It is plant material at various stage of digestion, rich in protein and other micro-flora such as fungi, protozoa and bacteria, (Esonu *et al.*, 2006). The RC is an important source of energy and vitamins especially vitamin B complex. Despite the nutritional potential of SOP, its use has been limited by the high concentration of anti-nutritional factors like limonene, tannin, saponin, phytate and oxalate which interfere with digestive processes, prevent effective absorption and utilization of both micro and macro nutrients in the body (Oluremi *et al.*, 2007). In order to fully understand the possible impact of the use of biodegraded sweet orange peel, there is need for studies on health assessment impact of this feedstuff incorporated into broiler chicken diets. This study is therefore aimed at investigating the effect of biodegraded sweet orange peel on haematological and biochemical indices of broiler chickens.

II. Materials And Methods

Sweet orange (*Citrus sinensis*) peels were collected from sweet orange fruit retailers in Gboko metropolis in Benue State who normally peel the orange fruit before selling to consumers. The rumen content was collected from freshly slaughtered white Fulani cattle at the slaughter slab at Rice Mill also in Gboko. One kilogram (1.0 kg) of RC was thoroughly mixed with the SOP in a ratio of 1SOP:0.3 RC packed, tired in polythene bags and left to ferment for 48 hours. The fermented materials were sun-dried, separated from the rumen content and the peels were milled. Samples of the processed meals were analyzed for proximate composition according to AOAC (2002). Phytate was determined using spectrophotometric method (Kirk *et al.*, 1991), tannin and saponin using the procedure of Harbone (1998), oxalate using the procedure by Onwuka (2005) and flavonoid using the technique of Ozyurt (2014).

Six experimental diets were formulated for each of starter and finisher phases such that the control diet contained no sweet orange peel (0%) and no rumen content (0%). The biodegraded SOP was used to replace dietary maize in the control diet (D1) at 2%, 4%, 6%, 8% and 10% to obtain diets D2, D3, D4, D5, and D6, respectively.

Two hundred and sixteen day-old Marshel MY broiler chicks were balanced for weight and randomly assigned to the six dietary treatments in a complete randomized design (CRD). Each treatment group of 36 birds was further sub-divided into three replicates of 12 chicks each. Feed and water were provided *ad libitum* and the birds were subjected to standard management procedure. Feed intake and body weights were recorded weekly.

At the end of the 56 days feeding trial, one bird per treatment replicates of live weight similar to the group average was bled to obtain samples for haematological and serum biochemical analysis. The blood for haematology was collected in test bottles containing ethylene diamine tetra acetic acid (EDTA) while blood for serum biochemical analysis was collected in test bottles and allowed to set. The blood samples were stored in an ice pack and transported immediately to Department of Veterinary Physiology, Pharmacology and Biochemistry, College of Veterinary Medicine University of Agriculture, Makurdi for analysis.

Data collected were subjected to one way analysis of variance (ANOVA) using Minitab (2014), and where significant differences occurred the means were separated using the least significant difference (LSD) as contained in Minitab (2014).

III. Results And Discussion

The proximate composition of biodegraded sweet orange peel is presented in Table 1. The result showed that it contained 7.18% crude protein, 12.76% crude fibre, 2.70% ether extract, 7.50% ash, 61.78% NFE and 2648.82 kcal ME/kg. The nutrient quality of feed ingredient is one of the major prerequisite for production of good quality feeds. The basic nutrients that cannot be compromised in the choice of ingredients for feed formulation are protein and energy. The crude protein value obtained in this study is comparable to 7.40% reported by Ojabo *et al*. (2014). The disparity in crude protein composition could be attributed to the type of pasture consumed by the cattle, the stage of digesta degradation in the rumen and the proportion of the constituent mixtures. The energy value obtained in this study is comparable to 2.89 Mcal/kg reported by Ani *et al.* (2015). The high energy value of biodegraded SOP in this study is in agreement with the findings of Iyayi and Aderolu (2004) who stated that biodegraded agro-by-products increase energy value. The high energy value of biodegraded SOP in this study, suggests its utilization as an energy source in diets for broiler chickens. The crude fibre value obtained in this study was lower than 13.50% and 14.60% reported by Ojabo *et al*. (2014) and Ani *et al.* (2015) respectively. This reduction could be attributed to advanced fermentation. The ash value obtained in this study was higher than 4.47% reported by Ani *et al.* (2015) but comparable to 8.19% reported by Ojabo *et al.* (2014). The high ash content could be attributed to the addition of some nutrients into the material with advanced fermentation period.

Quantitative composition of the anti-nutritional factors in the biodegraded SOP showed that it contained 0.008% tannin, 0.70% saponin, 2.50% alkaloid, 0.45% phytate and 3.02% oxalate. Tannin had a lower concentration than the other anti-nutrients in the biodegraded SOP. The low tannin concentration in this study was influenced by biodegradation. This observation is in agreement with the findings of Osman (2007) who reported that solubilization of tannins occurred during fermentation resulting to a more significant decrease in tannin content. The anti-nutritional factor values obtained in this study were lower than those reported by Oluremi *et al.* (2007) in sun-dried citrus fruit peel. The low anti-nutritional factor values obtained in this study were influenced by biodegradation. Oluremi *et al.* (2010) reported a decrease in the concentration of oxalate, flavonoid, tannin, saponin and phytate detected in SOP as duration of fermentation increased from 0 to 48 hours.

Lawal *et al*. (2011) stated that success has been recorded in the use of microbial technology to improve the value of low-quality agro-industrial by-products. Fermentation technology modifies biological materials into useful products, reduces anti nutrients and fiber content in feeds.

The result of the haematological and serum biochemical parameters of the broiler chickens fed diets containing graded levels of biodegraded SOP is presented in Table 4 and Table 5 respectively. The result showed that PCV, RBC, WBC, Hb, MCV, MCH and MCHC did not differ significantly ($P > 0.05$) among the dietary groups. The level of biodegraded SOP inclusion in the diet did not appear to have a definite influence on the PCV, RBC, WBC, Hb, MCV, MCH and MCHC values of the broiler chickens. The values obtained for PCV, RBC, WBC, Hb, MCV, MCH and MCHC fell within the values in literature (Mitruka and Rawnsley 1977; Oyewole *et al.,* 2013; Olajide, 2017). The MCV value of 136.93fl for the chickens in D1 (control group) was highest though not significantly different, while D5 had the least (84.13fl). The slight variation in MCV value measured tends to confirm that the diets affected the blood profile of the broiler chickens. The MCV values obtained in this study were higher than the range of 68.59fl to 104.40fl reported by Lawal *et al* (2011) but comparable to MCV value range of 97.00m^3

Table 1: Proximate Analysis, Energy and Ant-nutritional Factors in Biodegraded sweet orange peel

Parameters (%)	Biodegraded sweet orange peel
Dry matter	91.92
Crude protein	7.18
Crude fibre	12.76
Ether extract	2.70
Ash	7.50
Nitrogen free extract	61.78
Energy (kcal ME/kg)	2648.82
Tannin	0.008
Saponin	0.70
Alkaloid	2.50
Phytate	0.45
Oxalate	3.02

Table 2: Ingredient and Dietary Composition of Broiler Starter Diets containing Biodegraded SOP

	Experimental Diets					
Ingredients	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**
Maize	53.30	52.23	51.17	50.10	49.04	47.97
SOPM	0.00	1.07	2.13	3.20	4.26	5.33
SBM	35.60	35.60	35.60	35.60	35.60	35.60
BDG	5.00	5.00	5.00	5.00	5.00	5.00
Bone ash	2.05	2.05	2.05	2.05	2.05	2.05
Limestone	1.50	1.50	1.50	1.50	1.50	1.50
Blood meal	1.50	1.50	1.50	1.50	1.50	1.50
Methionine	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.30	0.30	0.30	0.30	0.30	0.30
Common salt	0.25	0.25	0.25	0.30	0.30	0.30
Vit./Min. Premix*	0.25	0.25	0.25	0.30	0.30	0.30
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Nutrients						
Crude Protein %	22.91	22.90	22.89	22.87	22.85	22.83
Crude fibre %	4.36	4.34	4.56	4.68	4.80	4.91
Fat %	3.76	3.75	3.75	3.74	3.73	3.71
ME (Kcal/Kg)	1932.24	1929.32	1926.67	1923.76	2815.59	2825.72
Methionine (%)	0.61	0.62	0.62	0.62	0.61	0.61
Lysine (%)	1.53	1.53	1.57	1.52	1.52	1.51
Calcium (%)	1.39	1.39	1.38	1.38	1.38	1.38
Phosphorus (%)	0.61	0.61	0.61	0.61	0.61	0.61

Vitamin – mineral premix* (animal care ®) supplied the following nutrients per kg of feed. Vitamin: A (1,200,000 I.U) D_3 (300,000 I.U), E (3,000mg), K_3 (250mg), folic acid (10mg), Niacin (4,000mg), Calpan (1,000mg), B_2 (500mg), B_{12} (2mg), B1 (200mg), B_6 (350mg), Biotin (8mg), Antioxidant (12,500mg), Minerals; Cobalt (25mg), Selenium (25mg), Iodine (120mg), Iron (4,000mg), Manganese (700mg), Copper (800mg), Zinc (6,000mg), and Chlorine chloride (20,000mg).

To 108.00 m^3 reported by Albokhadaim (2012). The MCH values obtained in this study had a similar trend to the MCV values. MCH value of 45.63pg for chickens in D1 (control group) was highest though not significantly different, while D5 had the least (28.70pg). The reference range of MCV may vary because of breed of animal, nutrition and the physiological status of the animal (Aba-Adulugba and Joshua, 1990; Esonu *et al.,* 2001). In laboratory evaluation of patients with anaemia the MCV is a commonly used parameter and an important classification and thus guiding further management (Janus and Mowrschel, 2010). Results obtained from this study showed that the broiler chickens were not anemic, normal MCV values indicated normocytic. It was observed that none of the haematological parameters measured had a particular sequence of variation.

The result on serum biochemical indices (Table 5) showed that globulin, cholesterol and SGOT/AST were significantly affected ($P < 0.05$) by the experimental diets among the treatment groups. Globulin values of birds in D5 were significantly higher ($P < 0.05$) than those of D6, while globulin values of birds in D1 (control group) and D4 were comparable. The globulin values obtained varied from 0.83mg/dl to 2.33mg/dl showing that biodegraded SOP increased globulin level. Increases in the globulin fraction usually results from an increase in immunoglobulins. Malnutrition and congenital immune deficiency can cause a decrease in total globulins due to decreased synthesis and nephrotic syndrome can cause a decrease due to protein loss through the kidney. The birds in D2 had significantly higher ($p < 0.05$) cholesterol value than the birds in the other treatment groups. Birds in D1 (control group), D3 and D4 had comparable cholesterol values. The serum cholesterol values of 127.43mg/dl to 154.90mg/dl obtained in this study were within normal reference of 52.00mg/dl to 148.00mg/dl (Mitruka and Rawnsley, 1977). The values of cholesterol in this study appeared to decrease as the replacement of maize by SOP increased. This could be attributed to decreased absorption and /or synthesis of cholesterol in the gastro – intestinal tract. This observation is in agreement with the findings of Petterson and Aman (1989) who reported that degraded dietary fibre fed to birds reduced serum cholesterol concentration by maintaining drain of the bile acid pool of the enterohepatic system. The SGOT/AST values were significantly different ($p < 0.05$) among the treatment groups. Birds in D2 and D5 had significantly higher ($p < 0.05$) SGOT/AST values while D1 (control group) had the least (20.70 µ/l). The AST values of 20.70 µ/l to 59.90 µ/l obtained in this study were within normal reference range (Mitruka and Rawnsley, 1977). Variation in AST did not show any particular pattern. Elevated AST values are related with myocardial infraction and are also associated with various forms of parenchymal liver disease. Total protein values of 2.56g/dl to 3.53g/dl obtained in this study were in agreement with Albokhadaim (2012) who observed total protein value between 3.30g/dl to 3.80g/dl for indigeneous chicken in Al-Ahsa, Saudi Arabia. Total protein showed marked though non-significant ($p > 0.05$) increase as SOP inclusion level increased. Increase in total protein probably was indicative of the improvement in protein synthesis in the liver. This indicates normal functioning of the liver. ALT values of 37.80 µ/l to 65.36 µ/l obtained in this study were within the range of 32 µ/l to 62 µ/l reported by Fasina *et al*. (2004) for broiler chickens. The values however tended to increase though not significantly different as the substitution of maize with SOP increased but were within normal reference values. According to Richards (2006) the level of SGPT is tested to find out and also to evaluate the damage of the liver. The ALT values obtained in this study suggested that the SOP did not exert any detrimental effect on the liver/or the normal healthy function of the birds.

In conclusion, the results support the view that biodegradation is essential in improving the nutritional quality of sweet orange peel. The utilization of biodegraded SOP did not show any apparent deleterious effect on haematological and serum biochemical parameters of broiler chickens. Biodegraded SOP can replace up to 10% of maize in broiler chicken diet.

Table 3: Ingredient and Dietary Composition of Broiler Finisher Diets containing Biodegraded SOP

	Experimental Diets					
Ingredients	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**
Maize	56.30	55.17	54.05	52.92	50.80	50.67
SOPM	0.00	1.13	2.25	3.38	4.50	5.63
SBM	32.45	32.45	32.45	32.45	32.45	32.45
BDG	5.00	5.00	5.00	5.00	5.00	5.00
Bone ash	2.20	2.20	2.20	2.20	2.20	2.20
Limestone	1.50	1.50	1.50	1.50	1.50	1.50
Blood meal	1.50	1.50	1.50	1.50	1.50	1.50
Methionine	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.30	0.30	0.30	0.30	0.30	0.30
Common salt	0.25	0.25	0.25	0.25	0.25	0.25
Vit./Min. Premix*	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**
Calculated Nutrients						
Crude Protein %	21.77	21.76	21.82	21.73	21.62	21.70
Crude fibre %	4.23	4.35	4.49	4.60	4.68	4.82
Fat %	3.76	3.75	3.78	3.74	3.68	3.82
ME (Kcal/Kg)	2,065.86	2,385.98	1,865,09	3,019.622	2,019.61	2,050.94
Methionine (%)	0.61	0.60	0.61	0.60	0.60	0.59
Lysine (%)	1.45	144	1.44	1.44	2.43	1.43
/Calcium (%)	1.44	1.44	1.44	1.44	1.43	1.43
Phosphorus (%)	0.62	0.63	0.65	0.62	0.61	0.61

Vitamin – mineral premix* (animal care ®) supplied the following nutrients per kg of feed. Vitamin: A (1,200,000 I.U) D_3 (300,000 I.U), E (3,000mg), K_3 (250mg), folic acid (10mg), Niacin (4,000mg), Calpan

(1,000mg), B_2 (500mg), B_{12} (2mg), B1 (200mg), B_6 (350mg), Biotin (8mg), Antioxidant (12,500mg), Minerals; Cobalt (25mg), Selenium (25mg), Iodine (120mg), Iron (4,000mg), Manganese (700mg), Copper (800mg), Zinc (6,000mg), and Chlorine chloride (20,000mg).

Table 4: Effect of Graded Dietary Level of Biodegraded Sweet Orange Peel on Haematological Indices of Finisher Broiler Chickens

	Experimental Diets						
Haematological indices	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**SEM**
Packed cell volume (%)	32.00^a	30.66^a	31.00^a	30.33^a	30.33^a	31.00^a	3.20
Red blood cell (x 10^{12}/L)	2.50^a	2.66^a	3.40^a	3.00^a	3.56^a	3.36^a	1.01
White blood cell (x 10^{12}/L)	1.90^a	1.86^a	2.00^a	1.86^a	2.16^a	2.13^a	0.81
Haemoglobin (g/L)	10.66^a	10.23^a	10.33^a	10.10^a	10.10^a	10.36^a	1.85
MCV (fl)	136.93^a	115.96^a	97.30^a	111.26^a	84.13^a	92.80^a	5.95
MCH (pg)	45.63^a	38.73^a	32.36^a	37.00^a	28.70^a	31.03^a	3.44
MCHC (g/fl)	33.20^a	33.33^a	33.30^a	33.26^a	33.26^a	33.40^a	3.33

^{a,b} Means with different superscripts in the same row are significantly different ($P < 0.05$), SEM = Standard error of mean
MCV = Mean corpuscular volume
MCH = Mean corpuscular haemoglobin
MCHC = Mean corpuscular haemoglobin concentration

Table 5: Effect of Graded Dietary level of Biodegraded Sweet Orange Peel on Serum Biochemical Constituents of Broiler Finisher Chickens

	Experimental Diets						
Serum indices	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**SEM**
Total protein (g/dL)	2.28^a	2.56^a	3.06^a	3.23^a	3.53^a	3.26^a	1.01
Albumin (g/dL)	1.06^a	1.73^a	2.00^a	1.80^a	1.20^a	1.20^a	0.70
Globulin (mg/dL)	1.72^{abc}	0.83^c	1.06^{bc}	1.43^{abc}	2.33^a	2.06^{ab}	0.72
Cholesterol (mg/dL)	150.93^{ab}	154.90^a	135.75^{ab}	139.73^{ab}	128.83^b	127.43^b	6.81
SGPT/ALT (μ/L)	37.80^a	51.63^a	58.70^a	58.33^a	59.96^a	65.36^a	4.21
SGOT/AST (μ/L)	20.74^b	59.73^a	45.90^{ab}	45.36^{ab}	59.90^a	47.70^{ab}	3.88

^{a,b,c} Means with different superscripts in the same row are significantly different ($P < 0.05$), SEM = Standard error of mean
SGPT/ALT = Serum glutamic pyruvate transaminase/Alanine aminotransferase
SGOT/AST = Serum glutamic oxalacetic transaminase/Aspartate aminotransferase

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