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Haematology and Serum Biochemical Constituents of Finisher Broiler Chickens Fed Diets Containing Water Soaked Sweet Orange (*Citrus sinensis*) Peel Meal at Varying Durations

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Authors' contributions

This work was carried out in collaboration among all authors. Author OIAO designed the study and performed the statistical analysis. Author AOA performed the experiment, wrote the protocol and wrote the first draft of the manuscript. Authors CDT and FGK managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

A four week feeding trial was conducted using 180 5-week old Arbor acre broiler chickens to determine the effect of feeding various duration of water soaked sweet orange peels on haematology and serum biochemical indices with a view to determining the potential of soaked sweet orange peels as an alternative to maize. Six broiler finisher diets were formulated and fed to the chickens in groups of 30 birds per diet, subdivided into 3 replicates of 10 chickens each in a completely randomized design. Diet 1 (control) was maize-based, diets 2, 3, 4, 5 and 6, sweet orange peels replaced maize at various duration of water soaked 0, 24, 48, 72 and 96 hours respectively, and incorporated at 30% inclusion. White blood cell, red blood cell and mean corpuscular volume was depressed ($p<0.05$) by various duration of water soaked sweet orange peels, packed cell volume did not indicate anaemic tendency among treatment and white blood cell

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count did not indicate infection due to treatments. Serum protein was adequate and liver and kidney function were not compromised. Health status and nutrient absorption of birds were not compromised by replacing maize with various duration of water soaked sweet orange peels in broiler chickens.

Keywords: Broiler chicken; blood parameters; sweet orange peel.

1. INTRODUCTION

As the fastest means of bridging the protein deficiency gap prevailing in most developing countries, animal scientists, nutritionists and agriculturists have generally agreed that developing the poultry industries will provide sufficient animal protein for the growing human population [1]. This is based on the fact that poultry produces the much needed animal protein source; it is an efficient feed converter, grows quickly with broiler chickens attaining table size within 8 to 9 weeks and layers producing eggs within 18 to 24 weeks thereby yielding quick returns on investment [2]. Furthermore, poultry meat and products are one of the cheapest animal protein sources and are not associated with any religious or traditional taboo and are accepted to most people the world over [3]. Also, availability of vaccines and drugs against many poultry diseases has made it easy to curb some of the most prevalent poultry disease [3]. However, the feed industry is faced with a number of challenges not only regarding the availability of feed ingredients but also the ability to produce high quality products in a cost effective manner. According to [4], high cost and scarcity of compounded feed is one of the problems faced by livestock farmers in Nigeria. [5] stipulated that maize accounts for about 45 to 55% of poultry feeds. Therefore any effort to substitute maize for example in poultry feed with nutritionally adequate unconventional feedstuffs will significantly reduce the cost of production.

Sweet orange fruit (*Citrus sinensis*) production is significant in Nigeria with heavy direct consumption due to inadequate capacity of industries to convert the fruit to juice, concentrate and canned fruits [6]. The sweet orange peel is obtained from the pericap of orange fruit following the preparation (processing) of the fruit for direct consumption of the juice. The peel is removed with the aid of sharp knife or razor mostly by orange vendors. The peels are found mostly in large quantities indiscriminately after each day's sales on streets, drainage system and refuse dumps causing environmental pollution. These sweet orange fruit peels according to [7] contain some similarities with maize in the quantitative values of protein and metabolisable energy. Its crude protein and metabolisable energy contents are 10.73% CP and 3988.7 kcal ME/kg respectively, as against 9.00% CP and 3432 kcal ME/kg for maize. This proximate composition highlights the potential of sweet orange fruits peels as a feed resource capable of replacing maize.

The aim of this study was to determine the effect of feeding various duration of water soaked sweet orange peels on haematology and serum biochemistry of broiler chickens.

2. MATERIALS AND METHODS

2.1 Description of Experimental Site

The study was conducted in Osgood farm Welfare quarters Makurdi. Makurdi is the capital of Benue State and is located on longitude 8° 37^{1} East and latitude 7° 41^{1} North. Annual rainfall ranges from 609.9 mm to 1219.8 mm, the temperature ranges from 25.6°C to 39.6°C and the relative humidity is about 21% to 85% [8].

2.2 Preparation of Test Ingredients

Sweet orange fruit (*Citrus sinensis*) peels were collected from orange vendors within Makurdi metropolis who usually peel the orange fruit before selling to consumers, and analysed for its proximate composition (Table 1).

The collected sweet orange peels (SOP) were divided into five (5) equal parts, one part each was soaked in water for 0, 24, 48, 72, and 96 hours to obtain five different treated test ingredients. The peels were separately sun-dried on concrete floor until they become bristle. They were milled, coded SOP_0, SOP_{24}, SOP_{48}, SOP_{72}, and SOP_{96}, respectively, and used to replace maize in the control diet (CD) at 30% level to obtain test diets coded as SOP_0D, $SOP_{24}D$, $SOP_{48}D$, $SOP_{72}D$, $SOP_{96}D$, respectively (Table 2).

Table 1. Proximate composition and metabolisable energy of sweet orange peel meal (% DM) sweet orange peel meal

Parameters	SOP_0	SOP_{24}	SOP_{48}	SOP_{72}	SOP_{96}
Dry matter	93.17	93.52	94.77	94.12	93.58
Crude protein	8.01	9.20	9.36	10.69	9.69
Crude Fibre	12.99	11.63	11.03	10.44	9.81
Ether Extract	3.16	3.30	3.77	3.84	4.56
Ash	4.08	4.28	4.17	3.31	5.18
Nitrogen free extract	71.76	71.59	71.67	71.72	70.76
ME (Kcal/kg)	3627.13	3677.21	3726.82	3785.44	3768.87

ME: 53+38(%CP + 2.25 x %EE +1.1 x %NFE) Carpenter and Clegg (1957); ME: Metabolisable Energy; SOP_0: Sweet orange peels not soaked in water; SOP_{24}: Sweet orange peels soaked in water for 24 hours; SOP_{48}: Sweet orange peels soaked in water for 48 hours; SOP_{72}: Sweet orange peels soaked in water for 72 hours; SOP_{96}: Sweet orange peels soaked in water for 96 hours

Table 2. Gross composition of broiler finisher diet (g/kg)

Experimental Diets

Ingredients	CD	SOP_0D	$SOP_{24}D$	$SOP_{48}D$	$SOP_{72}D$	$SOP_{96}D$
Maize	57.23	40.06	40.06	40.06	40.06	40.06
Sweet orange peel	_	17.17	17.17	17.17	17.17	17.17
Groundnut cake	31.32	31.32	31.32	31.32	31.32	31.32
Brewer dried grain	5.00	5.00	5.00	5.00	5.00	5.00
Blood meal	1.50	1.50	1.50	1.50	1.50	1.50
Bone meal	2.50	2.50	2.50	2.50	2.50	2.50
Oystershell	1.50	1.50	1.50	1.50	1.50	1.50
*premix	0.25	0.25	0.25	0.25	0.25	0.25
Common salt	0.30	0.30	0.30	0.30	0.30	0.30
Methionine	0.30	0.30	0.30	0.30	0.30	0.30
Lysine	0.10	0.10	0.10	0.10	0.10	0.10
Total	**100**	**100**	**100**	**100**	**100**	**100**

Calculated values

ME(Kcal/kg)	2954.33	2987.84	2996.44	3004.95	3015.02	3012.17
Crude protein (%)	21.87	18.81	20.12	20.08	20.21	19.69
Crude fibre(%)	6.00	8.25	15.75	6.75	3.50	6.00
Crude fat (%)	5.25	3.75	3.50	3.25	4.50	3.25
Calcium (%)	1.55	1.55	1.55	1.55	1.55	1.55
Phosphorus(%)	0.79	0.74	0.74	0.74	0.74	0.74
Methionine(%)	0.29	0.26	0.26	0.26	0.26	0.26
Lysine (%)	0.75	0.71	0.71	0.71	0.71	0.71

*SOP= Sweet orange peel meal CD= Control diet. SOP_0D= Diet containing test ingredient not soaked in water. $SOP_{24}D$= Diet containing test ingredient soaked in water for 24 hrs. $SOP_{48}D$= Diet containing test ingredient soaked in water for 48 hrs. $SOP_{72}D$= Diet containing test ingredient soaked in water for 72 hrs. $SOP_{96}D$= Diet containing test ingredient soaked in water for 96 hrs; *0.25 kg of Premix supplied the following: Vitamin A 1500 IU, Vitamin D 300 IU, Vitamin E 300 IU, Vitamin K 0.25 g, Thiamine (B_4) 0.2 mg, Riboflavin (B_{11}) 0.6 mg, Pantothenic acid 1.00 mg, Pyridoxine(B_6) 0.4999 mg, Niacin 4.00 mg, Vitamin B_{12} 0.002 mg Folic acid 0.10 mg, Biotin 0.008 mg, Choline chloride 0.05 g, Antioxidant 0.0125 g, Manganese 0.0096 g, Zinc 0.006 g, Copper 0.0006 g, Iodine 0.00014 g, Selenium 0.024 mg, Cobalt 0.004 mg*

2.3 Experimental Birds and Management

One hundred and eighty (180) 5-week old (Arbor Acre) broiler chickens were balanced for weight and randomly assigned to six treatments in a completely randomized design. Each treatment was divided into 3 replications of 10 birds each. The study lasted for 28 days, during which feeds and clean cool water were supplied to the birds ad libitum, proper medication were administered, the litter material was maintained dry throughout the period of the experiment.

2.4 Blood Analysis

Two finisher broiler chickens per replicate were slaughtered by slitting their throats [9] to collect blood samples. Blood from the slaughtered birds was allowed to run under gravity as the birds were held with their heads downward into sample bottles containing about 2 mg of ethylene diamine tetraacetic acid (EDTA). Haematological indices analysed were red blood cell count (RBC), haemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), packed cell volume (PCV), using Mindray Automatic Blood Analyzer. To determine serum biochemical indices, 2.0 ml of blood was collected from the birds into separate set of EDTA free blood sample tubes. The parameters determined were total protein, albumin, total bilirubin and direct bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST). Samples were taken immediately to the Federal Medical Centre, Makurdi for laboratory analysis.

2.5 Data Analysis

All data collected were subjected to one-way analysis of variance (ANOVA) using SPSS statistical software (version 20).

3. RESULTS

3.1 Proximate and Energy Constituents of Sweet Orange Fruit (*Citrus sinensis*) Peel Meal (SOP), Soaked in Water

The proximate composition and metabolizable energy of sweet orange fruit (*Citrus sinensis*) peel meal (SOP), soaked in water for 0 hours (SOP_0), 24 hours (SOP_{24}), 48 hours (SOP_{48}), 72 hours (SOP_{72}) and 96 hours (SOP_{96}) is shown in Table 1. The crude protein (CP), crude fibre (CF), ether extract (EE), ash, nitrogen free extract (NFE), and metabolisable energy values were expressed on dry matter basis.

The dry matter of each of the processed sweet orange peel was very high above 93%. It was observed that dry matter was least in SOP_0 (93.17%) and was comparatively higher in SOP_{24} to SOP_{96} in which the peels were fermented before sun drying. The crude protein content of the peels was between 8.01% and 10.69%, again the least CP of 8.01% was in SOP_0, while relatively higher CP content of 9.20% to 10.69% was found in the fermented SOP. The crude fibre for the fermented peels groups (9.81% to 11.63%) were however, lower than CF of 12.99% in SOP_0 which was not fermented before sun drying. The ether extract (EE) varied from 3.16 to 4.56% as the duration of fermentation increased from 0 to 96 hours. For ash it varied from 3.31 to 5.18% and SOP_{72} had the least amount of 3.31%. The nitrogen free extract content was above 70% irrespective of the duration of fermentation of the peels. The metabolisable energy (ME) was also observed to be high varying between 3627.13 to 3785.44 kcal/kg. Nevertheless SOP_0 had least ME of 3627.13 kcal/kg while SOP_{72} had the highest ME of 3785.44 kcal/kg.

3.2 Haematological Parameters of Broiler Chickenss Fed Diets Containing Various Duration of Water Soaked Sweet Orange Peel Meal

The result of the haematological parameters of broiler chickenss fed diets containing various duration of water soaked sweet orange peel meal is as shown in Table 3. White blood cell (WBC), Red blood cell (RBC) and mean corpuscular volume (MCV) differed significantly ($p<0.05$) but without a sequence in variation. The diets had no significant variation ($p>0.05$) on Haemoglobin (Hb), packed cell volume (PCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and Platelet among the groups. Table 3, shows the results of serum biochemical constituent of broilers fed diets containing various duration of water soaked sweet orange peel meal. Significant different ($p<0.05$) were recorded in total bilirubin and serum triglyceride among the treatment groups, without any particular trend, while all other parameters were not significant ($p>0.05$).

Table 3. Effect of diets containing duration of water soaked sweet orange peel meal on haematological indices of finisher broiler chickens

Experimental Diets

Indices	CD	SOP_0D	$SOP_{24}D$	$SOP_{48}D$	$SOP_{72}D$	$SOP_{96}D$	SEM
WBC(10^9/l)	201.77^{ab}	204.93^{ab}	202.43^{ab}	207.63^{ab}	215.43^{a}	168.57^{b}	13.42*
Hb(g/dl)	12.10	12.40	12.57	11.93	13.33	11.57	0.66^{ns}
RBC(10^{12}/l)	2.30^{ab}	2.34^{ab}	2.34^{ab}	2.22^{ab}	2.59^{a}	1.50^{b}	0.30*
PCV (%)	29.20	28.53	29.73	28.77	32.03	26.033	1.87^{ns}
MCV(fl)	127.23^{ab}	126.60^{ab}	127.23^{ab}	129.77^{a}	124.10^{b}	127.8^{ab}	159*
MCH (pg)	52.57	53.00	53.70	53.73	51.43	53.83	1.52^{ns}
MCHC (g/dl)	41.30	41.70	42.23	41.43	41.53	42.42	0.17^{ns}
Platelet (g/l)	7.00	6.33	7.00	6.33	8.33	7.33	0.98^{ns}

**(p<0.05) significant difference; ^{a,b,c} Means in the same row with different superscripts are significantly different (p<0.05); ns= Not significant (p>0.05)SEM= Standard Error of Means; CD= Control diet, SOP_0D = Diet containing test ingredient not soaked in water. $SOP_{24}D$ = Diet containing test ingredient soaked in water for 24 hrs. $SOP_{48}D$ = Diet containing test ingredient soaked in water for 48 hrs. $SOP_{72}D$ = Diet containing test ingredient soaked in water for 72 hrs. $SOP_{96}D$ = Diet containing test ingredient soaked in water for 96 hrs. WBC= white blood cell, Hb= haemoglobin, RBC = Red blood cell, PCV= packed cell volume, MCV= mean corpuscular volume, MCH= mean corpuscular haemoglobin, MCHC= mean corpuscular haemoglobin concentration*

Table 4. Effect of diets containing duration of water soaked sweet orange peel meal on serum biochemical constituents of finisher broiler chickens

Experimental Diets

Indices	CD	SOP_oD	$SOP_{24}D$	$SOP_{48}D$	$SOP_{72}D$	$SOP_{96}D$	SEM
Glucose (mmol/l)	12.11	12.09	12.08	12.71	12.87	12.72	0.36^{ns}
Total protein (g/dl)	3.42	3.33	3.28	3.49	3.72	3.36	66.54^{ns}
AST(u/l)	264.57	351.13	225.43	260.93	237.70	237.00	66.54^{ns}
ALT (u/l)	1.07	1.40	0.37	1.43	0.07	1.20	0.64^{ns}
Total bilirubin (umol/l)	1.40^{abc}	1.82^{a}	1.25^{b}	1.42^{abc}	1.62^{ab}	1.00^{c}	0.17*
Albumin (g/l)	12.00	11.00	10.67	11.00	11.33	11.67	0.75^{ns}
Urea (umol/l)	0.63	0.67	0.60	0.67	0.60	0.60	0.14^{ns}
Cholesterol (mg/dl)	99.06	90.87	93.99	109.98	97.89	96.72	0.20^{ns}
Triglyceride (mg/dl)	36.08^{a}	23.76^{b}	32.56^{ab}	27.28^{ab}	29.92^{ab}	29.04^{ab}	0.04*

**(p<0.05) = Significant difference; ^{a,b,c} Means in the same row with different superscripts are Significantly different (p<0.05); ns= Not significant (p>0.05) SEM = Standard Error of Means; CD= Control diet, SOP_0D = Diet containing test ingredient not soaked in water. $SOP_{24}D$ = Diet containing test ingredient soaked in water for 24 hrs. $SOP_{48}D$ = Diet containing test ingredient soaked in water for 48 hrs. $SOP_{72}D$ = Diet containing test ingredient soaked in water for 72 hrs. $SOP_{96}D$ = Diet containing test ingredient soaked in water for 96 hrs. AST = Aspartate aminotransferase, ALT = Alanine transaminase*

4. DISCUSSION

4.1 Proximate Composition of Sweet Orange (*Citrus sinensis*) Fruit Peel Meal

The laboratory determination of proximate composition of sweet orange (*Citrus sinensis*) fruit peel (SOP) obtained in this study showed both variations and similarities with previous studies. The values in this study deviated from the proximate constituent ranges of 86.64% to 86.90% DM, 6.71% to 7.22%CP, 1.66% to 1.95%EE, 3.24% to 3.67% Ash, 61.49% to 62.43% NFE and 3175 to 3161 kcal/kg ME for soaked sweet orange fruit peel reported by [10]. The result of this study did not agree with the proximate constituents of 9.3% and 10.96% CP reported by [11] and 9.24% to 12.82%CF reported by [12]. The differences in reports on the proximate composition by different researchers could be due to the differences in varieties of orange fruits, the processing and handling methods of the peel and the stage of maturity (ripening) at which the fruits were harvested.

The unsoaked sweet orange peel meal (SOP_0) was lowest in crude protein (CP) and soaked orange peels for 72 hours was highest. It was

observed in this study that longer duration of water soaked sweet orange peel improved %CP. According to [13] soaking exposes feed material to attack by microorganisms via chance inoculation. [14] observed higher CP level in fermented copra meal over the unfermented batch. [15] observed similar findings with mango peels. [16] reported similar trend with sweet orange fruit peel meal. The increased in crude protein in this study from 8.01 to 10.69% could be attributed to bacteria colonization of the media thereby causing fermentation activities and also it could be due to bacteria cell protein which may have multiplied during inoculation [17].

The ether extract (EE) observed in this study (3.16 to 4.56%) increased as the duration of water soaking of the orange peel increased. This is in line with the report of [15] and [16] when mango peels and sweet orange peels were fermented.

The observed crude fibre (CF) in this study decreased as the duration of water soaked of orange peels increased. The observed trend could be due to the activities of some micro-organism which led to the breakdown of non-starch polysaccharides (NSPs) in the sweet orange peels. The effect of which became more pronounced as the duration of water soaking of the orange peels increased. The ability of fungi to degrade cellulose has been reported [18].

NFE in this study was observed to decrease as the duration of water soaking of the orange peels increased. This is in order because at this time the microorganisms must have multiplied appreciably and had started utilizing NFE for their metabolic activities. This is in line with the report of [10] when sweet orange peels were fermented.

4.2 Haematological Indices of Broiler Fed Diets Containing Duration of Water Soaked Sweet Orange Peel Meal

The finisher broiler chickens on the sweet orange peel (SOP) based diets had haematological variables satisfactorily comparable to that of chickens in the maize based diet group (control). The obtained white blood cell (WBC) values were higher than the range reported by [19] and [20]. However, the observed values were similar irrespective of the diets; it could be suggested that all the diets had same effect on the concentration of WBC in the blood. Haemoglobin (Hb) values were within the range of 7.40 g/dl to 13.10 g/dl reported for normal birds [21]. This implies that utilization of SOP in finisher broiler diets did not impair dietary iron availability in the diets such as could cause anaemia and that protein intake was adequate. Red blood cell (RBC) values indicated that haemopoiesis was not suppressed by dietary treatment. This is because birds in other dietary groups had similar RBC values with those fed control diet. The observed range is however, comparable to the range of 1.29 to $2.54x10^6$ reported by [20] and 2.08 to 2.18 $x10^6$ mm^3 obtained by [19] for normal birds.

There was no significant effect on the packed cell volume (PCV) among the treatment groups. This is an indication that the birds were not anaemic and were not under stress due to dietary treatment. However, the PCV values observed were within the range of 28 to 37% reported by [22] and 26.33 to 36.00% reported by [20] as normal range for healthy broilers. MCV values of 124.10 to 129.77fl observed in this study were within the range of 126 to 163fl [22] and 97.07 to 166.45fl [23] as normal range for healthy broilers.

4.3 Serum Biochemical Indices of Broiler Fed Diets Containing Duration of Water Soaked Sweet Orange Peel Meal

Serum biochemical indices of birds are presented in Table 3. Glucose and total serum protein were not significantly affected among the treatment groups. This result indicated that the quality of SOP based diets was adequate and therefore their consumption did not cause stress, starvation or malnutrition to the birds [24]. Also, it has been observed that serum protein depends on quality and quantity of protein supplied in the diets [25]. The total protein observed is similar to 3.20 to 10.55 g/100 ml reported by [26], and 3.80 to 4.36 g/dl reported by [27]. In addition, there was no significant difference on aspartate aminotransferase (AST) and Alanine transaminase (ALT) among the treatment groups. This implied that the birds did not suffer from kidney or liver damage [28]. Significant difference was observed in Total bilirubin, however, the observed values with birds on the test diets (SOP) were similar to those on the control. This implies that the liver function was not hindered. None significant difference observed in Albumin and urea indicates that the birds were healthy and utilized protein in the respective diets adequately and that the renal functions were not compromised such that uric

acid excretion was adequate. The triglyceride values observed with birds on the test diets were comparable to those of the control. This indicates that the utilization of the SOP did not interfere with the digestion of dietary fatty acids.

5. CONCLUSION AND APPLICATION

1. Haematological and serum constituents concentrations indicate that water soaked sweet orange peels (SOP) incorporated at 30% in broilers diets did not cause any harm to the physiology process thereby proving that sweet orange peels is suitable for incorporation in broilers chicken diets.
2. Further research should be conducted to investigate the effect of deliberate inoculation of water soaked SOP with fermentation microbes in other to reduce fibre fraction and phyto-nutrients in the peels.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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