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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Heamatology and serum biochemistry of pullet grower chickens fed sweet orange (*Citrus sinensis*) fruit peel meal based diets

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Abstract

The experiment investigated the effect of feeding sun-dried sweet orange *(Citrus sinensis)* fruit peel meal (SOPM) on pullet grower chickens using haematological and serum biochemistry parameters. Three hundred and sixty (360) birds were randomly allotted to five treatment groups of 72 birds and four replicates of 18 birds each. SOPM was used to replace maize at 0, 10, 20, 30 and 40% for diets designated T_1 (control diet), T_2, T_3, T_4 and T_5 respectively. The birds were fed *ad libitum* from 11 to 20 weeks of age (10 weeks). Blood samples were collected from the birds through the brachial (wing) vein at the 10th week of the trial for haematological and serum biochemical studies. The result of blood analysis revealed that dietary treatment had no significant (P>0.05) effects on all the haematological and serum biochemical indices assessed. It was, therefore, concluded that utilization of SOPM in the diets of pullet grower chickens did not cause any harm to the physiological process controlling these blood traits.

Keywords: Sweet orange (C*itrus sinensis)*; peel; pullet grower chickens; haematology; serum biochemistry

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Introduction

The main constraint to livestock development in developing countries is the scarcity and fluctuation of the quality and quantity of year round animal feed supply. There is serious shortage in concentrated led animal feeds such as soya bean meal, groundnut meal and maize. The competing demand for grain as human food and poultry feed arises from inadequate local production. Indeed, the use of grain for feeding poultry when human needs have not been met raises moral questions. Search for alternatives to grain in poultry feed is imperative in order to reduce the cost of production of feed as well as reduce the competition between man and the poultry industry for grains (Oluyemi and Roberts, 2000). The novel approaches via the utilization of crop residues, agro-industrial by-products and non-conventional feed resources are required to bridge the gap between supply and demand of animal feeds. A number of agro-industrial by-products or wastes like citrus, pulp, citrus meals, citrus peels, citrus seed meal and citrus molasses are generated from fresh citrus after the main products of interest have been removed or extracted during processing or peeled for human consumption as in the case of developing countries. Clusters of the peel of the sweet orange are usually noticed on streets and along major roads in Nigeria because government and orange retailers have no strategic disposal program thus becoming an environmental problem (Oluremi et al., 2007). Sweet orange peel has been found to be a good source of energy comparable to maize and sorghum (Hill, 1988; Oluremi et al., 2006). Therefore, there exists a real potential in sweet orange peel meal to be explored as an animal feedstuff which can expand the feed resource base of the poultry industry and make

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poultry products cheaper. In considering the use of unconventional feedstuff for livestock feeding, it is very crucial and demanding to assay for effects of these feeding materials on the health condition of the birds/livestock. Haematological components of blood are valuable in monitoring feed toxicity especially with feed constituents that affect the formation of blood (Oyawoye and Ogunkunle, 1998). The haematological parameters most commonly used in nutritional studies include packed cell volume (PCV), red blood corpuscle (RBC), haemoglobin concentration (HBC), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV) and clotting time (Agbede and Aletor, 2003). Evaluation of both biochemistry tests and haematology together is essential for optimal recognition of many of the most characteristic disease patterns (Merck Veterinary Manual, 2010). Akinmutimi (2004) reported that both the haematological and biochemical components are influenced by the quantity and quality of feed and also the level of antinutritional elements or factors present in the feed. Ojabo et al. (2012) reported that the haematological parameters of rabbits fed sweet orange peel meal based diets showed no significant differences among the treatment diets in all the blood parameters measured.

The present study was carried out as a preliminary approach to evaluate the effects of feeding sun-dried sweet orange peel meal on the haematological and serum biochemical parameters of pullet grower chickens.

Materials and Methods

The feeding trial was carried out at the Teaching and Research Farm of the Federal University of Agriculture, Makurdi, Benue State, Nigeria. Three hundred and sixty 10-week old Bovan black pullet growers were weighed and randomly allotted to five dietary treatments in a completely randomised design (CRD). The birds were divided into 20 groups of 18 birds each and four groups were randomly allotted to each of five treatments. Five experimental diets were formulated such that the first diet which had no inclusion of SOPM served as the control diet (T_1). The remaining four diets had varying levels of SOPM replacing maize and were labeled as T_2 (10% SOPM), T_3 (20% SOPM), T_4(30% SOPM) and T_5 (20% SOPM). The ingredient composition of the experimental diets is shown in Table 1. The sweet orange fruit peels were collected from orange fruit retail sellers in Wadata market, within Makurdi metropolis. The peels were immediately sun-dried on concrete platforms, until they became crispy after 48 hours of sun-drying. They were then milled to obtain the sweet orange peel meal (SOPM). The feeding trial lasted for 10 weeks. The birds were provided feed (offered as mash) and fresh clean water *ad libitum* throughout the feeding trial. The birds were reared on a deep litter under good hygienic conditions throughout the 10-week feeding period and necessary vaccines and prophylactic treatments as scheduled were administered.

Blood collection and analysis

At the 10th week of the feeding trial, 2ml blood samples were drawn, using syringes with 23g needles from the brachial veins of eight chickens per treatment (2/replicate) and dispensed into ethylene di-amine tetra-acetate (EDTA) coated bottles and used for the determination of haematological parameters. 2ml blood samples were put in bottles without anticoagulant and allowed to clot at room temperature, centrifuged and sera used for the determination of serum biochemical constituents. Haemoglobin concentration, erythrocyte counts and differential counts were done according to the methods of Brown (1976). The total leucocyte count and haemotocrit determination were performed with QBC_{11} machine (Centrifugal Haematology System, Beckton Dickson Co; USA). Mean corpuscular Volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) was computed using established formulae (Swenson, 1996). Serum constituents were determined commercially using kits, total protein (Henry et al., 1957); albumin (Doumas et al., 1991); globulin concentration was calculated as the difference between total protein and albumin; Bilirubin (Michaelson, 1961); alkaline phosphatase (Willard et al., 1989); alanin and aspartate amino transferase (Reitman and Frankel, 1957); Glucose (Matteinheimer, 1970); Tryglycerides (Noble and Cambel, 1970); Cholesterol (Allain et al., 1974); Urea (Willard et al., 1989) and creatinine (Slot, 1965).

Statistical analysis

Data obtained were subjected to one-way Analysis of Variance (ANOVA) technique outlined in the Minitab statistical software (2005).

Results

Table 2 shows the effect of diet on haematology values of birds. No significant ($P>0.05$) differences were observed in packed cell volume (PCV), red blood cells (RBC) counts and haemoglobin (Hb) concentration among the treatment groups. Also for MCV, MCH and MCHC, which were erythrocytic indices, the experimental diets did not produce significant variation ($P>0.05$) among the treatment means. Similarly no significant variation ($P>0.05$) was observed in leucocytes (WBC) and the differential counts measured among dietary groups. The data on serum parameters are shown in Table 3. The result

Table 1: Composition of pullet grower diets

Ingredients (%)	Experimental Diets T_1	T_2	T_3	T_4	T_5	
Maize	56.00	50.40	44.80	39.20	33.60	
Sweet orange peel meal	0	5.60	11.20	16.80	22.40	
Full fat Soybean meal	20.50	20.50	20.50	20.50	20.50	
Brewers dried grain	20.20	20.20	20.20	20.20	20.20	
Bone meal	2.70	2.70	2.70	2.70	2.70	
Premix*	0.25	0.25	0.25	0.25	0.25	
Common Salt	0.25	0.25	0.25	0.25	0.25	
Methionine	0.10	0.10	0.10	0.10	0.10	
Lysine	0.10	0.10	0.10	0.10	0.10	
Total	100.00	100.00	100.00	100.00	100.00	
Calculated nutrients						[c] Recommended Nutrient [c]
Energy(kcalME/Kg)[b]	3108.50	3139.70	3170.90	3202.02	3233.30	2650(min)
Crude protein (%)[b]	18.35	18.46	18.56	18.66	18.88	16 (min)
Crude fibre (%)[b]	4.88	5.17	5.46	5.90	6.04	7 (max)
Calcium (%)[b]	1.19	1.19	1.19	1.19	1.19	1.0
Total phosphorus (%)[b]	0.99	0.98	0.97	0.95	0.94	0.7
Lysine (%)[b]	0.92	0.90	0.89	0.87	0.86	1.8
Methionine (%)[b]	0.35	0.34	0.32	0.31	0.30	-
Cystine (%)[b] Methionine	0.29	0.28	0.27	0.26	0.25	-
+ Cystine (%)[b]	0.64	0.62[b]	0.59	0.57	0.55	0.5

*Premix: 1kg of premix supplied the following: Vitamin A (stabilized) 6,670,000 I.U, Vitamin D_3(stabilized) 150,0000 I.U, Vitamin E (stabilized) 3,3401 I.U, Menadione sodium bisulphate (vitamin (stabilized)), 1,349mg, Vitamin $B_{12,}$ B_{12} 3,000mg, Vitamin B_6 20,000mg, Nicotinic acid 1,467mg, Calcium d-pantothenate 400mg, VitaminB_{12} 8mg,Choline chloride 1,3340mg, D.O.T. (3,5 dinotro-ortholuamide) 66,700mg, Manganese 53,340mg, Iron 33,340mg, Zinc 2,6670gm, Copper 1,600mg, Iodine 93mg, Cobalt 134mg, Selenium 34mg. [b] Calculated values from: The Tropical Feed Stuff analysis Table (Aduku, 1993); [c]Recommended nutrients from Nigeria Industrial Standard NIS 259 (1989).

Table 2: Haematology indices of pullet grower (11-20 weeks) fed diets containing sweet orange (*Citrus sinensis*) fruit peel meal

Haemogram	Experimental Diets T_1	T_2	T_3	T_4	T_5	SEM	Literature range
Packed cell volume (%)	31.38	30.18	29.50	29.95	29.13	0.32^{ns}	24.9 – 45.2
Erythrocytes (x 10^6/µl)	2.68	2.53	2.63	2.58	2.50	0.03^{ns}	1.5 – 3.8
Haemoglobin (g/dl)	9.75	9.36	9.45	9.35	9.13	0.19^{ns}	7.4 - 13.1
MCV (fl)	117.93	119.43	113.46	116.22	116.50	0.61^{ns}	102 - 139
MCH (pg)	33.51	36.95	35.54	36.28	36.46	0.50^{ns}	31.9 – 40.5
MCHC (g/dl)	30.64	30.99	31.33	31.18	31.43	0.31^{ns}	25.9 – 33.9
Leucocytes (x10^3/µl)	20.63	21.93	22.10	21.25	21.60	0.30^{ns}	19.2 – 23.6
Hetrophils (%)	29.95	32.18	32.05	29.18	29.68	0.05^{ns}	15.6 – 32.8
Lymphocytes (%)	0.092	0.088	0.093	0.093	0.098	0.01^{ns}	0.04 – 0.12
Monocytes (%)	0.46	0.44	0.46	0.43	0.44	0.12^{ns}	0.06 – 0.78
Eosinophils(%)	3.40	3.40	3.55	3.35	3.43	0.22^{ns}	6.25 – 8.25
Basophils (%)	1.58	1.33	1.78	1.70	1.78	0.15^{ns}	2.5 – 5.30

ns = Not significant (P> 0.05); SEM = Standard error of mean; MCV = Mean corpuscular volume; MCH = Mean corpuscular haemoglobi; MCHC = Mean corpuscular haemoglobin concentration; [1]Source: Mitruka and Rawnsley (1977).

obtained showed that no single blood metabolite was significantly affected (P>0.05) by the experimental diets among the groups.

Discussion

Evaluation of haemotology and blood chemistry status of chickens fed SOPM, an unconventional feed was with a view to ascertain its safety to the health status of the birds. Haematology and serum biochemistry of livestock are indices of physiological disposition of the animal to their nutritional and health status (Madubuike and Ekenyem, 2006). Mitruka and Rawnslay (1977) stated that disease states in experimental animals are often accompanied by biochemical changes. Esonu et al. (2001) stated that haematological parameters indicate responsiveness of the animal to its internal and external environments, which includes feeds and feeding. The haematology and serum biochemistry results showed that SOPM at all levels of maize replacement of 0 to 40% in pullet grower diets had no deliterious effect because all the

Table 3: Blood chemistry of pullet growers (11-20 weeks) fed diets containing sweet orange (*Citrus sinensis)* peel meal

Blood Metabolites	Experimental Diets					SEM	Literature range[1]
	T_1	T_2	T_3	T_4	T_5		
Total protein (g/dl)	6.20	5.48	5.65	6.00	6.23	0.20^{ns}	5.2 – 6.9
Albumin (g/dl)	2.71	2.70	2.70	2.68	2.68	0.22^{ns}	2.1 – 3.45
Globulin (g/dl)	3.49	2.78	2.95	3.32	3.32	-	-
Albumin/globulin	0.78	0.97	0.92	0.81	0.78	-	0.58 – 1.30
Glucose (mg/dl)	164.25	163.50	164.75	157.25	164.50	0.18^{ns}	152 - 182
Urea N (mg/dl)	4.63	4.73	4.68	4.65	4.60	0.22^{ns}	2.47 – 8.08
Creatinine (mg/dl)	1.36	1.38	1.40	1.38	1.30	0.22^{ns}	0.90 – 1.85
Total bilirubin (mg/dl)	0.14	0.14	1.14	0.14	0.14	-	0.00 – 0.20
Conjugated bilirubin (mg/dl)	0.11	0.11	0.11	0.11	0.11	-	-
Unconjugated bilirubin(mg/dl)	0.03	0.03	0.03	0.03	0.03	-	-
Cholesterol (mg/dl)	82.05	82.60	82.50	81.65	81.98	0.22^{ns}	52 - 148
Triglycerides(mg/dl)	30.68	29.50	31.10	31.20	31.35	0.22^{ns}	-
Aspartate aminotransferase (Iμ/l)	133.75	137.25	136.25	135.00	135.00	0.22^{ns}	88 - 208
Alanine aminotransferase (Iμ/l)	2.50	12.50	13.00	12.50	12.75	0.22^{ns}	9 – 37.2
Alkaline Phosphatase (Iμ/l)	31.00	31.25	32.50	32.00	31.50	0.22^{ns}	24.5 – 44.4

ns = Not significant (P> 0.05); SEM = Standard error of mean; [1]Source: Mitruka and Rawnsley (1977).

values showed no significant differences between treatments. PCV values obtained in this study are within the reference ranges reported by Mitruka and Rawnsley (1977) and Hewitt et al. (1989) for normal healthy chickens. This indicates that the nutritional adequacy of the experimental diets was maintained inspite of the substitution of maize by SOPM in the test diets, consequently the birds were not anaemic. Observed Hb values are within normal references range for healthy birds as reported by Mitruka and Rawnsley (1977). This may suggest that utilization of SOPM in growing pullet diets did not affect nutrient availability in the diets such as to compromise protein and iron intake thereby causing anaemia. Observed RBC count are within the range reported by MacDonald (1996) and Mitruka and Rawnsley (1977) for normal chickens. The normal values of RBC obtained in this study are an indication that there was no malnourishment among chickens fed the experimental diets.

Erythrocyte indices values in this study appear to be normal, an indication that SOPM based diets were significantly high in quality protein, with adequate vitamins and minerals especially iron. Irons in conjuction with high quality protein are essential for the formation and maintenance of RBC and the prevention of anaemia. Normal values of Hb, PCV, RBC, MCH, MCV and MCHC could be related to nutritional adequacy and safety of the test ingredient (Olabanji et al., 2007).

Neither leucopenia nor leucocytosis was observed in this study. This indicates absence of disease or toxic substances which symbolizes good health of the birds (Heath and Olusanya, 1985). The implication is that SOPM fed at various levels does not have any negative effect on the health of the birds. The birds remained healthy throughout the experimental periods of 11-20 weeks of age with no mortality recorded. Dietary replacement of maize with SOPM had no significant effect on the serum metabolites. Their values were all within normal reference range reported by Mitruka and Rawnsley (1977) for normal chickens.

Conclusion

The results of this experiment showed that dried sweet orange (*Citrus sinensis)* fruit peel meal (SOPM) did not have any deleterious effect on the haematological and serum biochemical indices of pullet grower chickens.

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