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Article

Erythrocyte Osmotic Stability and Whole Blood Viscosity Profiles of Two Breeds of Broiler Chickens Fed Dietary Composite Sweet Orange (*Citrus sinensis*) Peels

[Estabilidade Osmótica dos Eritrócitos e Viscosidade Sanguínea em Frangos de Corte Alimentados com Cascas de Laranja-doce (*Citrus sinensis*)]

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ABSTRACT

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A fifty-six (56) day feeding trial was conducted to investigate the effect of sweet orange peels (CSOP) on the erythrocyte osmotic stability and whole blood osmotic profiles two breeds of meat-type chicken. 192 day-old chicks were randomly allocated to four (4) dietary treatments with each treatment replicated three (3) times in a 4 x 2 factorial experiment using a Completely Randomized Design. Four on-farm feed were formulated to contain 0% (control), 2.5%, 5.0%, and 7.5% inclusion level of CSOP. Each of the treatment had 48 birds comprising of 24 Arbor Acre (AA) and 24 Cobb500 (Cb) breeds such that each of the replicates had 8birds for AA and Cb, respectively. All data collected were subjected to analysis of variance (ANOVA) with the aid of SPSS. Significant differences were compared using Duncan's Multiple Range Test. Observed results showed that breed and interaction effects were not significantly ($P > 0.05$) different. Dietary effect was not significantly ($P > 0.05$) different in whole blood viscosity except for red blood osmotic stability which was significantly ($P < 0.05$) different between 0.70% to 0.90% saline concentrations. It was concluded that the inclusion of 7.50% CSOP in the diets of broiler would significantly improve red blood osmotic stability, thereby improve livability of the two breeds of meat-type chicken.

Keywords: broiler birds, citrus peels, erythrocyte osmotic stability, whole blood viscosity

RESUMO

Foi conduzido um experimento com duração de 56 dias com o objetivo de avaliar o efeito da inclusão de casca de laranja doce (CSOP) sobre a estabilidade osmótica dos eritrócitos e os perfis osmóticos do sangue total em duas linhagens de frangos de corte. Um total de 192 pintos de um dia, foram utilizados, distribuídos aleatoriamente em quatro tratamentos dietéticos, com três repetições cada, em um esquema fatorial 4 x 2, adotando-se um delineamento inteiramente casualizado. As dietas foram formuladas para conter 0% (controle), 2,5%, 5,0% e 7,5% de inclusão de CSOP. Cada tratamento incluiu 48 aves, sendo 24 da linhagem Arbor Acre (AA) e 24 da linhagem Cobb500 (Cb), de modo que cada repetição continha oito aves de cada linhagem. Os dados obtidos foram submetidos à análise de variância (ANOVA), utilizando-se o

software SPSS, e as médias foram comparadas pelo teste de Duncan. Não foram observadas diferenças significativas ($P>0,05$) para os efeitos de linhagem ou da interação. O efeito das dietas não foi significativo ($P>0,05$) para a viscosidade do sangue total, exceto para a estabilidade osmótica dos eritrócitos, que apresentou diferença significativa ($P<0,05$) entre as concentrações salinas de 0,70% a 0,90%. Conclui-se que a inclusão de 7,5% de CSOP na dieta de frangos de corte melhora significativamente a estabilidade osmótica dos eritrócitos, contribuindo para o aumento da viabilidade das duas linhagens avaliadas.

Palavras-chave: frangos de corte, polpa cítrica, estabilidade osmótica dos eritrócitos, viscosidade sanguínea

INTRODUCTION

The poultry industry plays a vital role in providing an efficient source of animal protein to meet the ever-growing world demand for meat (Kumar et al. 2022). For poultry researchers and producers, achieving efficient growth and optimum health in meat-type chickens while maintaining the quality of their meat is a constant pursuit. Also, the need to maximize chicken health, welfare, nutrition and production efficiency will grow as the demand for poultry products around the globe keep rising and as such, both small and large-scale poultry producers are interested in examining options for conventional feed sources as well as creating alternative, easily accessible and affordable feed sources.

Among the many dietary options that can impact poultry performance and health, feed additives and supplement have gained significant attention (Mahmoud et al. 2021). These additives are needed to improve feed efficiency, growth and overall health while also possibly enhancing the quality of poultry products. One of such feed additives that has gained interest is the addition of sweet orange peels in the diets of chickens (Mahmoud et al. 2021). Orange peel constitutes between 40 and 50% of the entire fruit mass and is obtained after the pulp and the juice have been extracted (Singh et al. 2020). The study opined that flavonoids, polyphenols and essential oils among other bioactive compounds present in sweet orange peels have been associated with possible health benefit. Orange peel meal is a potential source of important nutrients and natural antioxidants which help to neutralize free radicals and convert them to harmless molecules (Abd El-Latif et al. 2023). Nwobodo et al. (2020) reported that sweet orange peel meal can be used in non-ruminant animals' diets to partly replace cereals due to their low cost and easy availability, as they offer significant low-cost nutritional dietary supplements. According to Oyewole et al. (2018), sun-dried sweet orange peels of *Citrus sinensis* can substitute up to 20% of dietary corn in broiler diets without having side effects on their performance and 40% in layers without affecting their optimum performance while still in lay.

Understanding the effects of sweet orange peel inclusion on hematological parameter like whole blood viscosity and red blood cell osmotic stability/fragility can help poultry producers make informed decisions regarding feed formulation, ultimately leading to enhanced growth rates, feed efficiency, and product quality (Wu et al. 2014). If these compounds are transferred to poultry meat through dietary supplementation, it could result in meat products with enhanced nutritional and health-promoting attributes for consumers (Jensen; Jorgensen 1994).

This study hypothesized that the inclusion of composite sweet orange peel (CSOP) up to 7.5% in the diets of broiler birds can improve whole blood viscosity and erythrocyte osmotic stability in both Cobb500 and Arbor Acre Chickens. Despite the abundance of this fruit all year round in Nigeria, their peels are often discarded and left to decay, contributing to environmental pollution and degradation. A gap exists in the understanding of how CSOP can affect whole blood viscosity and red blood cell osmotic stability/fragility in broiler birds. Therefore, this present study was conducted to investigate the erythrocyte osmotic stability and whole blood viscosity profiles of two breeds of broiler chickens fed dietary composite sweet orange peels (CSOP). The findings of this study may provide practical basis for the application of CSOP as replacement for wheat offal.

MATERIAL AND METHODS

Experimental Site

This experiment was carried out at the Teaching and Research Farm of The Federal University of Technology, Akure, Ondo State, Nigeria. The study location lies between latitude 7.49° North of the Equator and 5.82° East of the Greenwich Meridian in the humid tropical rainforest region. The climatic condition of Akure follows the pattern of southwest Nigeria and it has an average annual rainfall of about 1300mm and 1650mm and annual daily temperature ranging between 21°C and 38°C (Daniel 2015).

Experimental Materials

Fresh sweet orange (*Citrus sinensis*) peels were collected from orange vendors around the FUTA community, Ilara-Mokin, Iju, and Akure which are the three major towns in the

contiguous local governments of Ifedore, Akure South and Akure North of Ondo State, Nigeria. The vendors were supplied with clean polythene bags for the storage and collection of the day's peels which were then sun dried for 3 weeks to reduce the moisture content to the barest minimum. The sweet orange peels were then milled and tagged as sun-dried composite sweet orange peels (CSOP) to remove the bias of factors of ecological location, variety and soil from the sampling components.

Experimental Layout and Management of Animals

A total of 200-day-old broiler chickens comprising one hundred (100) Arbor Acre and one hundred (100) Cobb500 breeds were purchased from a reliable hatchery out of which 192 birds were randomly allocated to four (4) dietary treatments with each replicated three (3) times in a 4 x 2 factorial experiment using a Completely Randomized Design. Four on-farm feed were formulated to contain 0% (control), 2.5%, 5.0%, and 7.5% inclusion level of Composite Sweet Orange Peel (CSOP). Each of the treatment had 48 birds comprising of 24 Arbor Acre and 24 Cobb500 breeds with each treatment replicated three (3) times to have 8birds/replicate for Arbor Acre and Cobb500 breeds respectively. The birds were raised on deep litter, standard management practices were observed, i.e. vaccination (Lasota and Gumboro vaccines) and medication (Amprolium, keproceryl). Feed and drinking water were administered *ad libitum* throughout the experimental period of eight (8) weeks. The Gross composition of the experimental diets is presented in Table 1 and 2 below.

Data Collection and Laboratory Analysis

Red blood osmotic fragility/stability (Erythrocyte osmotic fragility/stability) determination.

At the end of 8 weeks, two birds per replicate with an average weight of 2kg were randomly selected from each treatment and euthanized. The blood samples (2mls) from birds were collected into a sterile EDTA bottle. The whole blood viscosity and erythrocyte osmotic stability assay were carried out at the Department of Animal Production and Health Laboratory of The Federal University of Technology, Akure. Erythrocyte osmotic fragility was determined as described by (Oyewale et al. 1991) as adapted by Aro et al. (2010) using NaCl and distilled water, 100ml of distilled water was measured into each of the test tubes, 0.0g

to 0.9g of NaCl were measured and dissolved into each of the test tube to give a saline concentration that ranged from 0.0 to 0.9g per 100ml of distilled. 0.5ml of the blood samples were collected into the haematocytometer and filled with the saline solution to a mark of 1.01 on the haematocytometer from which red blood cells were counted and presented as percentage of the red blood cells haemolysed per saline concentration and was used as a measure of red cells osmotic fragility/stability.

Blood viscosity

A whole blood sample was taken from the slaughtered birds. Blood viscosity analysis was conducted using a Chinese-made Baoshishan rotating viscometer, model 220V-NDJI-1. The blood viscosity was measured directly with the aid of the viscometer at room temperature. The blood viscosity was measured in decapascal (daPa).

Table 1: Gross composition (g/100g) of Broiler Starter Diets (0 – 4 weeks of age)

Ingredients (%)	Composite Sweet Orange Peel (CSOP) levels (%)			
	0.00	2.50	5.00	7.50
Maize	50.00	50.00	50.00	50.00
CSOP	0.00	2.50	5.00	7.50
Wheat Offal	7.50	5.00	2.50	0.00
Groundnut Cake	8.45	8.45	8.45	8.45
Soybean Meal	27.90	27.90	27.90	27.90
Fishmeal	1.30	1.30	1.30	1.30
Premix	0.15	0.15	0.15	0.15
Limestone	1.30	1.30	1.30	1.30
Bone meal	0.70	0.70	0.70	0.70
Methionine	0.10	0.10	0.10	0.10
Lysine	0.10	0.10	0.10	0.10
Salt	0.10	0.10	0.10	0.10
Vegetable oil	2.70	2.70	2.70	2.70
Total	100.00	100.00	100.00	100.00
Calculated Nutrient:				
Crude Protein (%)	20.94	20.74	20.55	20.36
Metabolizable Energy (kcal/kg)	3000.43	3019.94	3039.37	3058.84
Crude Fibre (%)	4.10	4.20	4.31	4.41
Ether Extract (%)	6.21	6.18	6.15	6.12
Calcium (%)	0.80	0.80	0.80	0.79
Phosphorus (%)	0.46	0.46	0.45	0.44
Lysine (%)	1.21	1.19	1.17	1.15
Methionine (%)	0.41	0.40	0.40	0.39

CSOP = Composite Sweet Orange Peel

Table 2: Gross Composition (g/100g) of Broiler finisher Diets (5-8 weeks of age)

Ingredients (%)	Composite Sweet Orange Peel (CSOP) levels (%)			
	0.00	2.50	5.00	7.50
Maize	51.40	51.40	51.40	51.40
CSOP	0.00	2.50	5.00	7.50
Wheat Offal	7.50	5.00	2.50	0.00
Groundnut Cake	18.15	18.15	18.15	18.15
Soybean Meal	15.00	15.00	15.00	15.00
Premix	0.15	0.15	0.15	0.15
Limestone	1.55	1.55	1.55	1.55
Bone meal	0.90	0.90	0.90	0.90
Methionine	0.10	0.10	0.10	0.10
Lysine	0.10	0.10	0.10	0.10
Salt	0.15	0.15	0.15	0.15
Vegetable oil	5.00	5.00	5.00	5.00
Total	100.00	100.00	100.00	100.00
Calculated Nutrient:				
Crude Protein (%)	18.80	18.60	18.41	18.21
Metabolizable Energy (kcal/kg)	3132.01	3151.48	3170.95	3190.42
Crude Fibre (%)	3.44	3.55	3.66	3.76
Ether Extract (%)	8.98	8.95	8.92	8.89
Calcium (%)	0.82	0.82	0.82	0.82
Phosphorus (%)	0.45	0.44	0.43	0.43
Lysine (%)	0.98	0.96	0.94	0.91
Methionine (%)	0.37	0.37	0.36	0.35

CSOP = Composite Sweet Orange Peel

Statistical Analysis

All data collected were subjected to analysis of variance (ANOVA) with the aid of Statistical Package for Social Sciences (SPSS) where significant difference was observed, the means were compared using Duncan's Multiple Range Test.

The following model was used for the statistical analysis;

$$Y_{ijk} = \mu + A_i + B_j + AB_{ij} + \epsilon_{ijk}$$

Where;

Y_{ijk} = Any individual observation of the experimental unit

μ = Grand population mean

A_i = Treatment or diet effect

B_j = Breed effect

AB_{ij} = Interaction of the diet and breed of the birds

ϵ_{ijk} = Random error

RESULTS AND DISCUSSION

The red blood cell (RBC) osmotic Stability profile of the two breeds of Meat-type chickens fed dietary inclusion of composite sweet orange peel is presented in Table 3 below. The values for percentage red blood cell (RBC) osmotic stability per saline concentration among diets, breeds and their interaction effects showed a progressive increase in the number of red blood cells haemolysed as saline concentration increased from 0.00%NaCl to 0.90%NaCl. This result agrees with the report of (Oyewale 1994) and (Aldrich et al. 2006) that complete haemolysis of blood cannot be ensured even at the least saline concentration as far as osmotic fragility of the avian and reptilian red blood cells are concerned, which is as a result of the nucleated red blood cells of these species that confers better resistance to fragility in hypotonic medium than their mammalian counterpart. Observed results showed no significant ($P > 0.05$) dietary effect on red blood cell haemolysed from 0.00% to 0.60% saline concentration levels.

Birds on dietary CSOP (diets B, C and D) had a significantly ($P < 0.05$) higher red blood osmotic stability relative to diet A (control) across 0.70%, 0.80%, and 0.90% saline concentrations. The trend was a significant decrease in red blood cell osmotic stability in the control relative to other dietary treatments which made diets B, C, and D to be statistically ($P > 0.05$) similar across saline concentrations of 0.70% to 0.90% NaCl. The increase in the rate of red blood osmotic stability across the concentration levels can be attributed to the increasing levels of CSOP in the diets of the birds, as birds on the CSOP diets (B, C and D) had a lower red blood cell osmotic fragility when compared to birds on the control diet. Thus, CSOP in the diets conferred a lower osmotic fragility and hence a higher osmotic stability of the RBC relative to the control diet.

The minimum and maximum level of red blood cell osmotic fragility in this study for birds on diet A (Control) were at 0.30% and 0.00%, while those on the CSOP based diets (B, C and D) were at 0.40% and 0.00% saline concentration levels. Breed and interaction effects were not significantly ($P > 0.05$) different. Cobb 500 showed a numerically lower progressive red blood cell osmotic stability across all the saline concentration levels. The minimum and maximum level of red blood cell osmotic fragility of Cobb500 breed were 0.40% and 0.00% compared to Arbor Acre breed with 0.30% and 0.00%, respectively. The disparity observed in osmotic fragility/stability could have resulted from intrinsic factors such as age, genes, species, breed, phenotype, gender, size, and differences in erythrocyte membrane composition (Habibu et al. 2016; Igbokwe et al. 2016).

The thickness or stickiness of blood is measured by blood viscosity. It provides an accurate indication of how well blood flows through blood vessels. Higher plasma levels of coagulation factors and fibrinogen, as well as dehydration, can all contribute to increased blood viscosity (Aro; Akinlemimu 2015; Aro 2018; Aro et al. 2018). The whole blood viscosity profile as presented in Table 4 in this study was not significantly ($P > 0.05$) influenced by dietary, breed and their interaction effects. Birds on the diet B (2.50% CSOP) showed a slightly higher value of 0.39 decaPascal while birds on diets A (Control), C (5.00% CSOP) and D (7.50% CSOP) showed similar values of 0.38 decaPascal.

This observation agrees with the report of (Oluwapelumi et al. 2022) who reported an insignificant effect in layers whole blood viscosities reared in large furnished, small furnished, and conventional battery cages, and further concluded that stress levels of the birds in the different cages were similar, but differ from the report of (Aro et al. 2018) who reported a significant decrease in whole blood viscosity across the treatments relative to the concentration of NaCl in the diets of Isa White and Barred Plymouth Rock cocks. However, the non-significant ($P > 0.05$) dietary, breed and interaction effects observed in this study suggest that feeding broiler birds with CSOP as reported in this trial had no deleterious effect on the birds' whole blood viscosity either at dietary treatment levels, between the two breeds or their interactions.

Table 3: Red Blood Cell Osmotic Stability of Two Breeds of Meat-Type Chickens Fed Dietary Inclusion of Composite Sweet Orange Peels (CSOP)

		Red blood Cell Osmotic Stability ($\times 10^6/\text{mm}^3$)									
		Saline Concentration (%)									
Diet	Breed	0.00	0.10	0.20	0.30	0.40	0.50	0.60	0.70	0.80	0.90
A		1.13	1.28	1.39	1.51	1.63	1.73	1.84	1.93 ^b	2.01 ^b	2.07 ^b
B		1.44	1.60	1.75	1.88	2.04	2.22	2.40	2.51 ^a	2.63 ^a	2.70 ^a
C		1.38	1.55	1.71	1.86	1.96	2.16	2.31	2.45 ^a	2.57 ^a	2.65 ^a
D		1.46	1.64	1.82	1.96	2.01	2.27	2.40	2.51 ^a	2.62 ^a	2.64 ^a
	±SEM	0.91	0.15	0.13	0.13	0.33	0.14	0.53	0.55	0.59	0.61
	P-Value	0.076	0.104	0.084	0.090	0.093	0.068	0.058	0.047	0.039	0.037
	Arbor acre	1.27	1.41	1.56	1.68	1.82	1.97	2.11	2.21	2.31	2.37
	Cobb500	1.44	1.62	1.78	1.92	2.05	2.22	2.36	2.49	2.61	2.69
	±SEM	0.65	0.74	0.82	0.92	0.94	1.01	1.08	1.10	1.13	1.14
	P-value	0.077	0.062	0.080	0.077	0.101	0.098	0.117	0.087	0.078	0.071
Breed X Diet (Interaction)											
A	Arbor Acre	1.12	1.26	1.39	1.50	1.60	1.67	1.78	1.86	1.94	2.00
	Cobb500	1.15	1.31	1.40	1.51	1.67	1.80	1.90	2.00	2.08	2.15
B	Arbor Acre	1.43	1.57	1.71	1.83	1.99	2.18	2.40	2.51	2.62	2.69
	Cobb 500	1.46	1.63	1.78	1.93	2.07	2.25	2.40	2.51	2.64	2.72
C	Arbor Acre	1.26	1.41	1.54	1.69	1.79	1.99	2.14	2.25	2.36	2.43
	Cobb 500	1.50	1.69	1.87	2.03	2.13	2.33	2.49	2.65	2.78	2.85
D	Arbor Acre	1.27	1.42	1.59	1.71	1.89	2.04	2.14	2.21	2.30	2.37
	Cobb 500	1.67	1.87	2.06	2.21	2.33	2.51	2.66	2.80	2.94	3.02
	±SEM	0.13	0.15	0.17	0.18	0.19	0.21	0.22	0.22	0.25	0.23
	P-value	0.441	0.485	0.471	0.522	0.692	0.732	0.625	0.549	0.535	0.537

^{ab}Means of the same column but different superscripts are statistically significant ($P < 0.05$); ±SEM = Standard Error of Mean; A = Diet with 0.00% CSOP; B = Diet with 2.50% CSOP; C = Diet with 5.00% CSOP; D = Diet with 7.50% CSOP.

Table 4: Whole Blood Viscosity Profile of Two Breeds of Meat-type Chicken Fed Dietary Inclusion of Composite Sweet Orange Peels (CSOP).

Diet	Breed	Blood Viscosity (Decapascal)
A		0.38
B		0.39
C		0.38
D		0.38
±SEM		0.04
P-value		0.287
	Arbor Acre	0.39
	Cobb500	0.38
	±SEM	0.003
	P-value	0.067
Breed X Diet (Interaction)		
A	Arbor Acre	0.39
	Cobb500	0.38
B	Arbor Acre	0.38
	Cobb 500	0.39
C	Arbor Acre	0.40
	Cobb500	0.37
D	Arbor Acre	0.38
	Cobb 500	0.38
	±SEM	0.005
	P-value	0.335

A = Diet with 0.00% CSOP; B = Diet with 2.50%CSOP; C = Diet with 5.00%CSOP; D = Diet with 7.50%CSOP; ±SEM = Standard Error of Mean.

CONCLUSION AND RECOMMENDATIONS

The study showed that no significant breed and interaction effects exist between Cobb500 and Arbor Acre for the parameters measured. However, Arbor Acre breed tends to show a higher numerical value for all whole blood viscosity except for red blood cell osmotic fragility, which suggest that Arbor Acre had better health profile than Cobb500. Red blood osmotic stability was significantly influenced by the dietary inclusion levels of composite sweet orange peel in the diets of the birds, except for whole blood viscosity. Observed trend showed that dietary inclusions of composite sweet orange peels were beneficial to the health profile of the birds decreasing its osmotic fragility. However, based on the observed physiological response of the birds, the inclusion of 7.50% CSOP in the diets of broiler would significantly improve red blood osmotic stability by reducing their fragilities. Further studies on higher dietary levels of CSOP especially on serum biochemistry, antioxidant and meat quality profile of broiler chickens is also recommended.

Conflict of interest

The authors have no conflict of interest to declare.

Ethical Approval

Principles of laboratory animal care" (NIH publication No. 85- 23, revised 1985) were followed, as well as specific national laws where applicable as the animals used in research were cared for and experimental protocols were followed as approved by the Institutional Animal Ethical and Use Committee (FUTA/ETH/2023)

Authors' contributions

Author Michael Adinoyi Joseph wrote the original draft of the manuscript and also performed the field work. Author Adewale Johnson Atansuyi wrote the protocol for the study. Author Toyin Victoria Abegunde performed the data curation and statistical analysis. Author Ronke Stella Atansuyi performed the literature review. Author Samuel Olarewaju Aro conceptualized and designed the study.

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