Effect of black seeds, African nutmeg and Negro pepper on carcass characteristics and organoleptic properties of broiler chickens

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Abstract

The use of herbs and spices as natural feed additives in poultry diets has increased due to their ability to improve growth without the use of synthetic antibiotics. A total of 224 one-day old Arbor acre strain of broiler chickens were used to evaluate the effect of three herbal feed additives (black seed, African nutmeg and Negro pepper) as growth promoters in place of conventional antibiotics in the diet. The birds were randomly assigned to 7 treatments consisting of 4 replicates with 8 birds each in a completely randomized design experiment. Data were collected on carcass-organs characteristics and organoleptic properties of broiler chickens. The data were subjected to analysis of variance using SPSS version 25 and significant means were separated using Duncan's Multiple Range Test of the same software. Vaccines were given to the birds in all treatments. The treatments were T1 - control (0% additives but given conventional antibiotics), T2 - 0.5% black seed, T3 - 1.0% black seed, T4 - 0.5% African nutmeg, T5 - 1.0% African nutmeg, T6 - 0.5% Negro pepper, T7 - 1.0% Negro pepper. The inclusion of the phyto-additives had significant ($P < 0.05$) effect on carcass, organ and the sensory evaluation. However, at 0.5% inclusion level, the three phyto-additive did not have effect on the spleen and heart which indicates that this inclusion levels may not cause any detrimental effects on the organs of the birds but at 1% inclusion level, there was a slight increase in the organs which reflects the effects of high level of phytochemicals. It was concluded that inclusion of the three phyto-additives at 0.5% improved carcass quality parameters, reduces the abdominal fat and had no deleterious effect on the internal organs of the birds.

Keywords: Black seed, African nutmeg, Negro pepper, Carcass characteristics and organoleptic properties.

Efeito de sementes pretas, noz-moscada africana e pimenta negra nas características de carcaça e propriedades organolépticas de frangos de corte

Resumo

O uso de ervas e especiarias como aditivos naturais para ração em dietas de aves aumentou devido à sua capacidade de melhorar o crescimento sem o uso de antibióticos sintéticos. Um total de 224 frangos de corte da linhagem Arbor acre de um dia de idade foram usados para avaliar o efeito de três aditivos de ração à base de ervas (semente preta, noz-moscada africana e pimenta negra) como promotores de crescimento no lugar de antibióticos convencionais na dieta. As aves foram distribuídas aleatoriamente em 7 tratamentos consistindo de 4 repetições com 8 aves cada em um experimento de delineamento completamente casualizado. Os dados foram coletados sobre as características dos órgãos da carcaça e propriedades organolépticas de frangos de corte. Os dados foram submetidos à análise de variância usando o SPSS versão 25 e as médias significativas foram separadas usando o teste de múltiplos intervalos de Duncan do mesmo software. As vacinas foram administradas às aves em todos os tratamentos. Os tratamentos foram T1 - controle (0% de aditivos, mas recebendo antibióticos convencionais), T2 - 0,5% de semente preta, T3 - 1,0% de semente preta, T4 - 0,5% de noz-moscada africana, T5 - 1,0% de noz-moscada africana, T6 - 0,5% de pimenta negra, T7 - 1,0% de pimenta negra. A inclusão dos fitoaditivos teve efeito significativo ($P < 0,05$) na carcaça, órgão e avaliação sensorial. No entanto, no nível de inclusão de 0,5%, os três fitoaditivos não tiveram efeito no baço e no coração, o que indica que esses níveis de

inclusão podem não causar efeitos prejudiciais nos órgãos das aves, mas no nível de inclusão de 1%, houve um ligeiro aumento nos órgãos, o que reflete os efeitos do alto nível de fitoquímicos. Concluiu-se que a inclusão dos três fitoaditivos na concentração de 0,5% melhorou os parâmetros de qualidade da carcaça, reduziu a gordura abdominal e não teve efeito deletério nos órgãos internos das aves.

Palavras-chave: Semente preta, noz-moscada africana, pimenta negra, características da carcaça e propriedades organolépticas.

1. Introduction

The use of herbs, spices, and their extracts as natural feed additives in poultry diets has increased in recent years due to their inherent multi-bioactive properties and ability to enhance performance, reduce pathogenic bacteria, and decrease antibiotic residues in meat and egg products (Dhama *et al*., 2015). When compared with antibiotic growth promoters which are also used to fight bacterial infection, medicinal plants are economical and readily available, edible and low in toxicity, rich in nutrients, and useful in improving the health status of animals and consumers (Youcef *et al*., 2018; Dhama *et al*., 2015). Applications of herbs in poultry diets have been reported to stimulate endogenous antioxidants, facilitate nutrient metabolism and improve meat quality by lowering cholesterol content and inhibiting peroxidation (Oloruntola *et al*., 2018). Interestingly, *Nigella sativa* (Black seeds), *Monodora myristica* (African Nutmeg) and *Xylopia aethiopica* (Negro pepper) are among the medicinal plants with a lot of potentials that can be utilized as feed additives or phytobiotics in poultry production.

Toghyani *et al.,* (2010) reported an increased carcass yield, liver, abdominal fat, breast, thigh, wing and neck weights in broiler chickens by feeding diet having 1% black cumin. However, Al-beitawi *et al.*, (2009) reported no improvement in carcass characteristics by feeding different levels of crushed as well as uncrushed *Nigella sativa* seed in broiler chickens. Broilers fed diet containing 1% whole grounded black cumin resulted in a significant decrease of dressing percentage as compared to the control, however, there were non-significant effects regarding liver, gizzard, heart and abdominal fat percentage by supplementation of whole *Nigella sativa* seeds (Abbas and Ahmed 2010). Shobiye *et al*., (2022) reported that phytobiotics had no significant effect on the carcass characteristics except the thigh percentage of broiler chicken. Also, there were significant variations in the meat flavour and tenderness of broiler chickens fed diets supplemented with different phytobiotics.

Research findings have confirmed the beneficial effect of dietary inclusion of black seeds, African nutmeg and Negro pepper in poultry diet either as therapeutic and nutritional purposes. Being good source of different nutrients, they improve the growth rate, increase the carcass yield of the animal and profitability to the farmer (Idowu *et al*., 2024a). In view of these, black seeds, African Nutmeg and Negro pepper have phytobiotic potential in poultry diets; they are relatively cheaper and with minimal residual effect on the product (Idowu *et al*., 2024b). Consequently, this research was conducted to evaluate the effect of black seed, African nutmeg and Negro pepper powder as phytobiotics on the carcass-organ characteristics and organoleptic property of meat type chickens.

2. Materials and Methods

2.1 Description of the experimental site

The experiment was carried out at the Poultry unit of Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. Ogbomoso is located in the derived savanna zone that lies on longitude $4^{0}10$ East of Greenwich meridian and latitude $8^{0}10$ North of the Equator. The latitude ranges from 300m and 600m above sea level while the mean temperature and annual rainfall are 27 ºC and 1247 mm (Google Earth Map, 2023).

2.2 Preparation of test ingredients

The black seed, African nutmeg, and Negro pepper were purchased from reputable and reliable markets within the Ogbomoso metropolis. The shells of African nutmeg were removed, and the seeds, Black seed, and Negro Pepper were ground with Eurolex Mixer/Grinder model MG1153 (a domestic blender) into the powdery form of 0.05mm and stored in air-tight containers.

2.3 Experimental diets

Seven experimental diets were formulated for the study such that Diet 1 (T1) serves as the control that neither contained black seed, African nutmeg, and negro pepper. T2 - 0.5% Black seed, T3 – 1.0% Black seed, T4 – 0.5% African nutmeg, T5 – 1.0% African nutmeg, T6 – 0.5% Negro pepper and T7 – 1.0% Negro pepper. The gross composition of the experimental diets is presented in Tables 1 and 2 for starter and finisher respectively.

Table 1. Gross composition of the experimental diets (broiler starter kg^{-1})

Ingredients (kg)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
Maize	54.00	54.00	54.00	54.00	54.00	54.00	54.00
Fishmeal	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Soyabean meal	32.00	32.00	32.00	32.00	32.00	32.00	32.00
Soya oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Wheat offals	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Lime stone	1.00	1.00	1.00	1.00	1.00	1.00	1.00
DCP	2.20	2.20	2.20	2.20	2.20	2.20	2.20
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
*Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	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**	**100.00**
Black seeds	-	+	++	-	-	-	-
African nutmeg	-	-	-	+	++	-	-
Negro pepper	-	-	-	-	-	+	++
Calculated Analysis							
Crude Protein (%)	22.13	22.29	22.34	22.21	22.29	22.22	22.31
Ether Extract (%)	3.67	3.86	4.09	3.91	4.13	3.83	3.98
Crude Fibre (%)	3.61	3.64	3.68	3.62	3.64	3.63	3.65
ME (Kcal/kg)	3065.18	3088.99	3112.79	3081.92	3098.64	3082.27	3099.36
Phosphorus (%)	1.20	1.20	1.20	1.20	1.20	1.20	1.20
Calcium (%)	1.09	1.09	1.09	1.09	1.09	1.09	1.09
Lysine (%)	1.34	1.34	1.34	1.34	1.34	1.34	1.34
Methionine (%)	0.54	0.54	0.54	0.54	0.54	0.54	0.54
Feed cost(₦)/100kg	35,668	37,168	38,668	37,418	39,918	36,918	38,168

***Premix composition: Vitamin A 12,500,000.00, Vitamin D3 2,500,000.00, Vitamin E 40,000.00 Vitamin K3 (mg) 2,000.00, Vitamin B1 (mg) 3,000.00, Vitamin B2 (mg) 5,500.00, Niacin (mg) 55,000.00, Calcium Pantothenate (mg) 11,500.00, Vitamin B6 (mg) 5,000.00, Vitamin B12 (mg) 25.00, Choline Chloride (mg) 500,000.00, Folic Acid (mg) 1,000.00, Biotin (mg) 80.00, Manganese (mg) 120,000.00, Iron (mg) 100,000.00, Zinc (mg) 80,000.00, Copper (mg) 8,500.00, Iodine (mg) 1,500.00, Cobalt (mg) 300.00, Selenium (mg) 120.00,Antioxidant (mg) 120,000.*

DCP = Di-calcium phosphate, ME = Metabolisable Energy, + = 0.5% inclusion level, ++ = 1.0% inclusion level

Table 2. Gross composition of Experimental diets (Broiler finisher)

Ingredients (kg)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
Maize	57.00	57.00	57.00	57.00	57.00	57.00	57.00
Fishmeal	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Soyabean meal	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Soya oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Wheat offals	4.60	4.60	4.60	4.60	4.60	4.60	4.60
Lime stone	3.00	3.00	3.00	3.00	3.00	3.00	3.00
DCP	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Bone meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
*Premix	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
Methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Black seeds	-	+	++	-	-	-	-
African nutmeg	-	-	-	+	++	-	-
Negro pepper	-	-	-	-	-	+	++
Calculated Analysis							
Crude Protein (%)	19.65	19.76	19.86	19.73	19.81	19.74	19.83
Ether Extract (%)	3.73	3.92	4.11	3.96	4.19	3.89	4.04
Crude Fibre (%)	3.71	3.74	3.78	3.72	3.74	3.73	3.76
ME (Kcal/kg)	3122.09	3145.90	3169.70	3138.83	3155.55	3139.18	3156.27
Phosphorus (%)	1.01	1.01	1.01	1.01	1.01	1.01	1.01
Calcium (%)	2.22	2.22	2.22	2.22	2.22	2.22	2.22
Lysine (%)	0.99	0.99	0.99	0.99	0.99	0.99	0.99
Methionine (%)	0.31	0.31	0.31	0.31	0.31	0.31	0.31
Feed cost(₦)/100kg	34,597	36,097	37,597	36,347	38,097	35,847	37,097

***Premix composition: Vitamin A 12,500,000.00, Vitamin D3 2,500,000.00, Vitamin E 40,000.00 Vitamin K3 (mg) 2,000.00, Vitamin B1 (mg) 3,000.00, Vitamin B2 (mg) 5,500.00, Niacin (mg) 55,000.00, Calcium Pantothenate (mg) 11,500.00, Vitamin B6 (mg) 5,000.00, Vitamin B12 (mg) 25.00, Choline Chloride (mg) 500,000.00, Folic Acid (mg) 1,000.00, Biotin (mg) 80.00, Manganese (mg) 120,000.00, Iron (mg) 100,000.00, Zinc (mg) 80,000.00, Copper (mg) 8,500.00, Iodine (mg) 1,500.00, Cobalt (mg) 300.00, Selenium (mg) 120.00,Antioxidant (mg) 120,000.*

DCP = Di-calcium phosphate, ME = Metabolisable Energy, + = 0.5% inclusion level, ++ = 1.0% inclusion level

2.4 Experimental animals and management

Two hundred and twenty four (224) 1-day old Arbor Acre strain of broiler chicks purchased from a reputable hatchery less than 20 kilometer from the Teaching and Research farm (experimental location) were used for the experiment. The birds were weighed at the commencement of the experiment and randomly distributed into 7 Treatments of 32 birds each. Each treatment was replicated 4 times with 8 birds per replicate. Each replicate was housed in experimental pens measuring 125 x 125 x 185cm. The birds were managed intensively on deep litter with wood shavings as litter material, adequate feed, clean and fresh water were offered *ad-libitum* on daily basis throughout the experiment. Broiler starter diet was offered during the brooding phase for 3weeks while broiler finisher diet was offered at the rearing phase for another 3weeks. Routine vaccination programme and

medications as applicable to the environment were carried out.

2.5 Data collection

2.5.1 Carcass characteristics and Organ Analysis: At the end of the experiment, two birds having a representative weight of the treatment were selected from each replicate for carcass and organ evaluation. Prior to slaughtering feed was withdrawn while water was provided. The birds were slaughtered by severing the jugular vein after which they were bled. The bled weight was taken; a period of five minutes was allowed to elapse after bleeding before dry plucking of feather which was carried out manually. The plucked carcasses were eviscerated and the internal organs (lungs, heart, kidney, liver, pancreas, spleen and abdominal fat) were removed and weighed separately with a sensitive scale. The eviscerated carcasses were carefully cut into parts. The parts and interior organs were weighed using electronic scale and the weights converted to percentages of live weight.

2.5.2 Sensory evaluation of the meat: The sensory evaluation of the samples was determined using a ten (10) member trained panelist as reported by Akinwumi *et al.,* (2013). The samples from the breast muscle were washed in clean water and wrapped in impervious polythene pouches which cannot be destroyed by cooking process. The meat samples were cooked in boiling water for 20minutes using water bath at 80^0C with no spices added to the meat. The meat samples were served to ten (10) member panels that were drawn from students and staff in the Department of Animal Nutrition and Biotechnology, Ladoke Akintola University of Technology, Ogbomoso. Bite size portions of boiled meat sample from the breast muscle weighing about 10g was served at room temperature to the trained panelists. Each panelist was required to masticate one sample per replicate with ranked preferences in the following categories: colour, juiciness, meaty flavour, tenderness and over all acceptability. Scores were awarded using a 9 point Hedonic scale. (1= Dislike extremely, 2= Dislike very much, 3= Dislike moderately, 4= Dislike slightly, 5= Intermediate, 6= Like slightly, 7= Like moderately, 8= Like very much, 9= Like extremely.

2.6 Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) using IBM SPSS version 25, and significant means were separated using *Duncan's* multiple range test of the same software. The significance difference was determined at $p < 0.05$.

3. Results and Discussion

The relative carcass weight of broilers fed diet supplemented with black seed, African nutmeg and Negro pepper is shown in Tables 3. The parameters affected were the neck and back ($p<0.05$) while others were not significantly affected by the dietary treatments.

Table 3. Relative carcass weight of broiler chickens fed diet containing Black seed, African nutmeg and Negro pepper

Parameters	T1 Control	T2 0.5% BS	T3 1.0% BS	T4 0.5% AN	T5 1.0% AN	T6 0.5% NP	T7 1.0% NP	SEM	P-value
Final weight (g)	1899.63^{b}	2032.46^{a}	1991.82^{a}	1959.21^{a}	1910.43^{b}	1970.86^{a}	1820.25^{c}	0.18	0.04
% of live weight									
Dressed weight	70.22	69.67	71.23	70.07	69.29	70.11	68.54	0.57	0.95
Head	2.69	2.74	2.62	2.54	2.68	2.59	2.60	0.04	0.92
Neck	3.61^{ab}	3.40^{b}	3.46^{b}	4.70^{a}	4.0^{ab}	3.82^{ab}	4.35^{ab}	0.15	0.02
Wing	8.03	7.85	8.33	8.47	8.38	8.22	8.11	0.13	0.88
Back	15.75^{a}	14.73^{ab}	15.60^{a}	13.46^{bc}	14.58^{ab}	13.76^{bc}	13.13^{c}	0.29	0.04
Breast	19.40^{b}	20.25^{ab}	19.71^{b}	21.87^{a}	20.41^{ab}	23.72^{a}	21.55^{a}	0.19	0.02
Thigh	12.14	11.96	10.53	11.04	9.93	11.10	10.55	0.29	0.62
Drumstick	10.40	10.78	11.92	10.83	9.77	9.94	10.82	0.26	0.35
Shank	4.26	14.18	4.45	4.31	4.11	4.09	4.53	0.09	0.76

^{abc}Means within each row with different subscripts are significantly different ($p<0.05$)

Table 4 shows the relative organ weight of broilers fed diets supplemented with black seeds, African nutmeg and Negro pepper at 6 weeks. The dietary treatments did not have significant ($P>0.05$) effect on the relative pancreas, spleen, kidney, heart and lung weight but the relative liver weight and abdominal fat were significantly ($P<0.05$) affected across the treatments.

Table 4. Relative organ weight of broilers fed diet containing Black seeds, African nutmeg and Negro pepper at 6 weeks of age

Parameters	T1 Control	T2 0.5% BS	T3 1.0% BS	T4 0.5% AN	T5 1.0% AN	T6 0.5% NP	T7 1.0% NP	SEM	P-value
% of live weight									
Pancreas	0.27	0.27	0.22	0.30	0.35	0.28	0.21	0.02	0.31
Spleen	0.12	0.10	0.13	0.10	0.18	0.09	0.11	0.01	0.23
Liver	2.34^{a}	2.20^{a}	1.59^{b}	2.27^{a}	2.42^{a}	2.17^{a}	2.39^{a}	0.07	0.01
Kidney	0.42	0.29	0.30	0.38	0.41	0.33	0.31	0.03	0.83
Heart	0.55	0.49	0.51	0.54	0.55	0.46	0.54	0.02	0.51
Lung	0.53^{ab}	0.55^{ab}	0.35^{ab}	0.67^{a}	0.51^{ab}	0.51^{ab}	0.43^{ab}	0.04	0.03
Abdominal fat	1.12^{a}	0.83^{ab}	0.55^{b}	0.72^{ab}	0.94^{ab}	0.50^{b}	0.92^{ab}	0.06	0.05

^{ab}Means within each row with different subscripts are significantly different ($p<0.05$)

The sensory evaluation of broilers fed diets containing black seeds, African nutmeg and Negro pepper at the finisher phase is shown in Table 5. The colour and tenderness of the meat were significantly influenced ($p<0.05$) across the treatments while other parameters were not significantly affected ($p>0.05$).

Table 5. Sensory evaluation of broiler chickens fed diet containing black seeds, African nutmeg and Negro pepper at 6 weeks

Parameters	T1 Control	T2 0.5% BS	T3 1.0% BS	T4 0.5% AN	T5 1.0% AN	T6 0.5% NP	T7 1.0% NP	SEM	P-value
Colour	5.75^{b}	6.85^{a}	6.70^{a}	7.00^{a}	5.55^{b}	6.80^{a}	6.85^{a}	0.19	0.01
Flavour	6.60	6.80	6.90	6.65	6.95	7.15	6.20	0.20	0.32
Juiceness	6.40	6.20	6.85	6.85	6.75	7.20	6.70	0.20	0.80
Tenderness	5.75^{c}	5.70^{c}	6.20^{b}	6.15^{b}	6.45^{b}	6.10^{bc}	7.45^{a}	0.22	0.02
Overall acceptability	6.70	7.05	7.30	6.80	6.90	7.85	6.60	0.18	1.10

^{ab}Means within each row with different subscripts are significantly different ($p<0.05$)

SEM: Standard Error of Mean

The values obtained for the relative carcass parameters were within range of values reported for broiler chickens (Kana *et al*., 2017 and Ingweye *et al*., 2021). However, the lower abdominal fat recorded across the treatments except the control could be due to inclusion of the phyto-additives in the diets. This could be so because supplementing broiler's diets with phyto-additives could minimize fat deposition in the body of the birds as they are capable of modifying fat metabolism pathway in the birds (Karaskova *et al*., 2015). Therefore, to achieve low fat in broilers, a quality desired by consumers in this era of healthy eating, may require the inclusion of spices in the diets of birds (Ingweye *et al*., 2021).

The relative liver weight was significantly affected across the treatments, these result agrees with the findings of Woyengo *et al*., (2011) who reported increase in liver weights when canola meal was fed to broilers. Durrani *et al*. (2007) also reported an increase in liver weight when he fed 4% of black seeds to broilers. Moreover, 0.5% inclusion level of black seed, African nutmeg and Negro pepper did not have effect on the spleen and heart which indicates that this inclusion level may not cause any detrimental effects on the organs of the birds but at 1% inclusion level, there was a slight increase in the organs which reflects the effect of high level of phytochemicals. Fat deposition in the abdominal area of broilers is regarded as waste in poultry production because it represents a loss in the market and reduced consumer's acceptability. The result of this study showed that supplementation of broiler's diet with black seeds, African nutmeg and Negro pepper has the potential to reduce this waste by reducing the abdominal fat content.

Chicken breast is the most preferred part of broiler carcass in addition to the drumsticks because it is the site for most muscle deposition. Faster growth and deposition of muscle is one of the results of adding feed additives to diets (Karaskova *et al*., 2015). Hence, the higher deposition of muscle in the breast could be attributed to the inclusion of these phyto-additives. However, their inclusion had no significant effect on the percentage dressed weight as well as thighs, wings and drumsticks of the carcasses.

Sensory evaluation is an important tool for quality assessment of meat. Colour, flavour, tenderness and juiciness are the most important meat quality that consumers are seeking for before accepting any meat product (Omojola, 2007). Inclusion of these phyto-additives in the diet of broiler birds enhanced the meat colour and improved the tenderness which is advantageous to the meat industry.

4. Conclusions

It can be concluded that supplementation of black seed, African nutmeg and Negro pepper especially at 0.5% inclusion level improved the dressing percentage and had no deleterious effect on the internal organs of the birds but also decreased the abdominal fat and improved the meat tenderness. Also, supplementing broiler's diet with these phyto-additives suppressed abdominal fat deposition which is a desirable meat quality for the current anti-fat meat culture.

5. Acknowledgments

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6. Authors' Contributions

Idowu Adijat Oyeyemi: conceptualized the study and wrote the article. *Ojebiyi Olusegun Ojeniyi*: designed the study and proofread the writing. *Ademola Sodiq Gbolagade*: Proofread the writing. *Olakojo Adewale Olusegun*: experimented.

7. Conflicts of Interest

No conflicts of interest.

8. Ethics Approval

Not applicable.

9. References

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