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MEAT QUALITY AND MICROBIAL STUDIES OF BROILER CHICKEN FED DIETS SUPPLEMENTED WITH VITAMIN E AND SOYA BEAN OIL

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ABSTRACT
The experiment was designed to determine the effect of dietary vitamin E and soya oil supplementation on quality and microbial count of broiler chicken meat. Two hundred and twenty-five (225) day-old unsexed chicks were purchased from a reputable hatchery and randomly assigned into 5 treatments of 3 replicates per treatments with 15 birds in each replicate. They were assigned five dietary treatments; T1 - Basal diet + 0 % Soya oil and Vitamin E; T2 – Basal diet + 100 g/kg+100 mg/kg of soya oil and vitamin E respectively; T3 - Basal Diet + 100 g/kg + 200 mg/kg of Soya oil and vitamin E respectively, T4 (Basal diet + 100 g/kg + 300 mg/kg of soya oil and vitamin E respectively and T5 - Basal diet + 100 g/kg + 400 mg/kg of soya oil and vitamin E respectively in a completely randomized design. At the end of the 8th week of experimental feeding, one bird from each replicate was slaughtered and meat samples (breast muscle) were collected aseptically to determine meat microbial, technological, proximate and sensory parameters. Dietary supplements had significant effect ($p>0.05$) on meat Lipoprotein profile. The group had the highest ($p<0.05$) value for HDL, while the lowest value was observed in T4, T3, T2 and T1 (26.98 mg/dl, 28.80 mg/dl, 29.17 mg/dl and 29.30 mg/dl) respectively. Moreso, chickens fed with the basal diet had the best ($p<0.05$) meat lipid profile. T4 group had the highest vale for triglyceride (347.67 mg/dl), with T1 having the least value (193.67 mg/dl). T4 and T5 (147.60 and 125.17 mg/dl) groups had the highest value for cholesterol, while the lowest values for cholesterol was observed in T1 – T3 groups with values ranging from 92.90 – 115.10 mg/dl. All treatment diets influenced ($p>0.05$) proximate parameters of meat obtained from chickens fed dietary supplements. Results show that the dietary treatment had significant effect ($p<0.05$) on taste, tenderness, overall acceptability and meaty but there was no significant effect on the colour. Overall meat acceptability of sensory parameters was adjudged best for birds fed T5 (Basal diet + 100 g/kg + 400 mg/kg of soya oil and vitamin E respectively) diet, while meat lipid profile was best ($p<0.05$) in groups fed basal diet. For microbial analysis, data generated from this study were subjected to a 5 by 3 factorial arrangement in a completely randomized design. Escherichia coli amongst all other parameters measured were not found on the meat samples. Salmonella spp. Was significantly ($P<0.05$) different except in meat obtained from T3 (2.95 log10cfu/ml) and T4 (2.98 log10cfu/ml) groups on day 14 of refrigeration, which was not significantly ($P>0.05$) different. Though, all the treatments were capable of reducing microbial load on broiler meat if refrigerated, T2 effectively reduced microbial population on refrigerated broiler meat more than T1, T3, T4 and T5 groups with the consideration of shelf life.

Keywords: Vitamin E, Soya Oil, Broiler chicken Meat, Microbial, Lipid profile, and Sensory

Introduction

Studies demonstrated with chickens define saturation degree and source/type of diet that has influence on carcass quality improvement of animals (Rymer and Givens, 2005). Vitamin E, as D-alpha-tocopherol, is an excellent natural anti-oxidant that helps protect carotene and other oxidizable materials in feed and body. Oxidation of fat causes rancidity, spoiling the taste and flavour of the fats through the process known as lipid peroxidation or auto-oxidation. Chicken meat have been well documented to have high protein and low-fat content and deliberated as the principal source of polyunsaturated fatty acids (PUFA) with paramount concentration of n-3 PUFA (Engberg *et al*., 1994 and Howe *et al*., 2006). Broiler chickens have been considered an appropriate model

in lipid nutrition studies since it is highly sensitive to dietary fat modifications. Meat, an excellent source of protein in human diet is highly susceptible to microbial contamination, a major cause of spoilage and food borne infections in human, that result in economic losses (Komba *et al.,* 2012).

Meat colour and lipid stability are major factors limiting the quality and acceptability of meat and meat products (Gatellier *et al*., 2001). The oxidative stability of muscle depends upon the balance between anti-oxidants, such as α-tocopherol and pro-oxidants including the free iron in the muscle and concentrations of PUFA (Yang *et al*., 2002). Quality characteristics affected in meat by lipid oxidation include; flavour, colour, texture and its nutritional value. Weber and Antipatis (2001) noted that the development of rancidity in meat by lipid oxidation begins at the time of slaughter and continues during storage.

Additionally, organisms such as *Escherichia coli*, *Salmonella spp and Shigella spp* can influence muscle quality and subsequent spoilage. Organisms in meat may infect the animal either while still alive (endogenous disease) or may contaminate the meat after slaughtering (exogenous disease) (Lawrie and Ledward, 2006). Microbes cause spoilage on meat if the meat is untreated, with rapid deterioration in a matter of hours or day. The use of anti-oxidants (vitamin E) limits this oxidative spoilage. Supplementations with antioxidants have been observed to mop up the free radicals that can retard fat oxidation and rancidity. Primary anti-oxidants are capable of interpreting and terminating free radical propagation steps. Antioxidants can be natural or synthetic. Examples of primary anti-oxidants are vitamin E (alpha-tocopherol), rosemary extract, carotenois and flavonoids which are naturally occurring and can be synthesized. Vitamin E have also been incorporated into animal feed to improve performance, strengthen immunological status, improve the quality of egg and to increase the vitamin E content of food of animal origin and thus increase the vitamin E intake of humans (Flachowsky, 2000).

Soybean oils are commonly used in poultry diets to increase energy density of feed. These oils are high in PUFA, which are more susceptible to deterioration by lipid oxidation. Oils have commonly been used as energy source in the diets of broilers. Advantages of utilizing oils in poultry diet include decrease of nourishment dust, increase in absorption and digestion of lipoproteins, significant amount of necessary fatty acids and their lower heat toward carbohydrates and proteins. Also, they assist vitamin A, vitamin E and Ca absorption (Leeson and Atteh, 1995). The need to do more research on these substances for methodology to produce high-quality broiler meat with improved shelf life, reduced rancidity and nutritious meat due to the demand for good animal protein is apt. Application of natural ingredients without compromise of the meat yield and quality is the balance craved for. An approach to overcoming the lipid oxidation and shelf life problem of the meat industry is the use of feed additives such as Vitamin E and soybean oil seed as supplement in the broilers diet which can increase lipid stability. Previous studies have shown that the inclusion of soybean oil seed and Vitamin E as antioxidant protected lipids from oxidizing (Estev *et al.,* 2015). Hence, this study is designed to show supplementation of broiler chicken diet with Soya Oil and vitamin E to improve meat quality as well as render it less susceptible to microbial contamination and spoilage.

Materials and Methods

Experimental Site

The chickens were raised at a poultry farm located in Aaya community, Camp, Abeokuta, Ogun State. Abeokuta is located in South-Western part of Nigeria which has prevailing climate with a mean average rainfall of about 1037mm. It has a dry season of about 5months (November- March). The mean ambient temperature ranges from 28^{o}C in December to 36^{o}C in February with a yearly average of 34^{o}C. Relative humidity ranges from 60% in January to 94% in August with a yearly average of about 82%. The vegetation represents an interphase between the tropical rain forest and derived savannah, Camp (7^{o}10'N and 3^{o}2'E) in Odeda Local Government Area of Ogun State Nigeria. Meat quality assessment was carried out at the Animal Product Laboratory of the Department of Animal Production and Health, Federal University of Agriculture Abeokuta, Ogun State. The second phase of the experiment (microbial load counting and identification) was carried out at the Department of Microbiology laboratory Federal University of Agriculture Abeokuta, Ogun State.

Management of Experimental Birds

Two hundred and twenty-five (225) day old broiler chicks were sourced from a reputable hatchery in Ibadan. Before the arrival of the birds, pens and other equipment were thoroughly cleaned, and disinfected. Dry wood shavings were spread on the floor of the pen as litter material. On arrival, birds were stabilized by orally giving them ant stress and multivitamins and brooded together for two weeks and were fed control diet. After brooding, they were randomly divided into five treatment groups of forty-five (45) birds per treatment. Each treatment was further sub-divided into three replicates of fifteen (15) birds per replicate and then fed the experimental diets as shown in Tables 1 and 2 which shows the (%) ingredients composition of the experimental diets for starter and finisher phases respectively. All other management practices such as routine vaccination and medication were administered appropriately.

Research Policy
This study was conducted abiding to the Animal Ethics Committee guidelines of the Federal University of Agriculture, Abeokuta (FUNAAB, 2015)

Meat Quality Determination
Meat Proximate Analysis
Proximate analysis was carried out on the breast muscle of broiler chickens fed the experimental diets. Moisture content, Ether Extract, Ash, Crude protein and C1rude fibre were determined for each sample of each replicate following the procedure described by AOAC (2005)

Determination of Meat Lipoprotein Profile
Composite paste of thigh muscle from each replicate was prepared with known amount of chloroform and methanol mixture 1`: 1 (v/v). The resulting paste solvent mixture was filtered and rinsed with an additional volume of the combined homogenate and allowed to stand for 5 minutes. The filtered homogenate were equilibrated to remove non-lipid material, 2 % (0.32) w/v KCL solution was added to the aqueous layer (Folch *et al*., 1957). The filtrate was centrifuged and lipid extract decanted. The extract was made up to a final volume by addition of chloroform. The decanted mixtures obtained were used for the determination of cholesterol level, triglycerol, high- and low-density lipoprotein respectively.

Determination of Refrigerated Weight Loss of Chicken Meat
A 50g of meat obtained from each replicate of broiler chickens fed the experimental diets was weighed before and after refrigeration for 24 hours and the weight difference was determined as follows:
Refrigerated loss (g) = weight before refrigeration – weight after refrigeration
Refrigeration loss (%) = (weight before refrigeration – weight after refrigeration × 100)/Weight before refrigeration

Determination of Cook Weight Loss of Broiler Meat
A 50g of meat obtained from each replicate of broiler birds fed the experimental diets was cooked in the water bath at a temperature of 70ºC for 20 minutes. Final weights of the samples were taken after the boiled sample has been allowed to cool at room temperature of 15 minutes. Cook weight loss determination.
Cook loss (g) = weight before cooking (g) – weight after cooking (g)
Cook loss (%) = (Weight before cooking – Weight after cooking × 100)/Weight before cooking.

Water Absorptive Power
A 3 g each of the raw meat was weighed and placed in clean test-tubes; 10 mls of distilled water was also poured into each test-tube and left for an hour. The samples were removed and reweighed, and increase in weight was calculated to determine volume of water absorbed.

pH value of Meat Obtained from Birds fed Dietary Treatments
The pH was determined by using an ATC Pocket-sized pH meter model PH-108A on the raw meat samples.

Sensory Evaluation of Meat Obtained from Broiler Chickens fed Dietary Vitamin E and Soya Oil as Supplements
Each meat sample (10 g) of thigh muscle was cooked at 70ºC for 20 minutes and allowed to cool down to room temperature. A ten member panel evaluated some of the sensory characteristics such as colour, tenderness, flavour, meatiness and overall acceptability using a nine point hedonic scale, with varying degrees of acceptance such as Like extremely = 9, like very much=8, like moderately=7, slightly like = 6, neither like nor dislike=5, dislike slightly=4, dislike moderately=3, dislike very much=2, dislike extremely=1, as described by Sanwo *et al*.. (2012). A plenary briefing was held with the panelists, and each descriptive term was clearly explained. Water was served to the panelist to rinse their mouth after scoring each replicate sample of meat to minimize carry over effect. Samples were independent of one another.

Meat Microbial Analysis of Broiler Chickens Fed Dietary Supplements
Sample Collection
Fresh meat samples (breast muscle) were collected aseptically from broiler chickens fed experimental diets and taken to the laboratory for microbial evaluation after 0, 9 and 14 days of refrigeration using the method described by (Block, 1984; Bailey *et al*., 1988 and CDC/NIH 2007).

Statistical Analysis
Data generated for meat quality analysis in this study were subjected to one-way analysis of variance in a completely randomized design using the General Linear Model of SSPS (2011) version 20, while significant differences were separated using Duncan multiple Range Test within the same statistical package. For Microbial Analysis, the data generated were subjected to one-way ANOVA in a 5 by 3 factorial arrangement in a completely randomized design using the Statistical Package version 20 (SPSS, 2011) while significant ($P<0.05$) differences were separated using Duncan multiple Range Test within the same package.

Results and Discussion
Table 3 shows the result of proximate analysis on breast muscle of broiler chickens fed diets containing soya oil and vitamin E. The dietary treatment significantly influenced ($p>0.05$) all parameters

measured. Treatment 3 had the highest ($p<0.05$) value for dry matter (29.66%), while treatment 4 had the lowest ($p<0.05$) value of (26.16%). The highest values for ether extract appeared in treatment 3 (5.28 %), while treatments 2, 4 and 5 were significantly least, with values ranging from 2.72 to 3.21 %. Ash value (0.99%) for Treatment 3 was highest, while chickens fed dietary supplement of 300 mg/kg Vitamin E and 4.5 g of Oil had the lowest ($p<0.05$) value (0.82%). Treatment groups 1, 2 and 5 had similar means ranging from (0.90 to 0.92%). The 0.21 % recorded for Birds fed 200 mg/kg of vitamin E and 4.5 g of Soya oil was highest ($p<0.05$), but meat obtained from groups 2, 4 and 5 had the least values (0.00%). Crude protein in birds fed 400 mg/kg and 4.5 g of Oil had 25.33 %, which was the highest ($p<0.05$), while other treatment groups had least values (22.44 - 23.73%). The highest value for nitrogen free extract was observed in treatment 5 (0.66) while treatment 4 (0.21) had the lowest value, with birds in groups 1 and 4 having similar ($p<0.05$) values (0.40 and 0.43).

Table 1: Composition of Experimental Diet of Broiler Chickens at Starter Phase

Treatments					
Ingredients (%)	Basal	1	2	3	4
Maize	48.00	48.00	48.00	48.00	48.00
Soya bean meal	35.00	35.00	35.00	35.00	35.00
Wheat offal	8.70	8.70	8.70	8.70	8.70
Fish meal	4.00	4.00	4.00	4.00	4.00
Bone meal	1.80	1.80	1.80	1.80	1.80
Oyster shell	1.50	1.50	1.50	1.50	1.50
Broiler start. Premix	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Lysine.	0.20	0.20	0.20	0.20	0.20
Methionine	0.30	0.30	0.30	0.30	0.30
Added supplements					
Vitamin E (mg/kg)	0	100	200	300	400
Soya oil (g/kg)	0	100	100	100	100
Determined Analysis					
Crude protein (%)	21.48	21.52	21.58	21.48	21.14
Crude Fibre (%)	3.60	5.89	5.82	5.92	5.92
Ether Extract (%)	6.03	5.89	5.82	5.92	5.92
(MJ/Kg)	11.72	11.89	11.82	11.82	11.86

A 2.5kg of premix contains vitamin A: 12,000,000IU, vitamin D3: 2,500,000IU, vitamin E: 30,000IU, vitamin K: 2,000mg, vitamin B1: 2,250mg, vitamin B2: 6,000mg, vitamin B6: 4,500mg, vitamin B12: 15mg, Niacin: 40,000mg, Pantothenic Acid: 15,000mg, Folic Acid: 1,500mg, Biotin: 50mg, Choline Chloride: 300,000mg, Manganese: 80,000mg, Zinc: 50,000mg, Iron: 20,000mg, Copper: 5,000mg, Iodine: 1,000mg, Selenium: 200mg, Cobalt: 500mg, Antioxidant: 125,000mg.

Table 2: Composition of Experimental Diet of Broiler Chickens at Finisher Phase

Treatments					
Ingredients (%)	**Basal**	**1**	**2**	**3**	**4**
Maize	55.00	48.00	48.00	48.00	48.00
Soya bean meal	28.00	35.00	35.00	35.00	35.00
Wheat offal	10.50	8.70	8.70	8.70	8.70
Fish meal	2.00	4.00	4.00	4.00	4.00
Bone meal	2.00	1.80	1.80	1.80	1.80
Oyster shell	1.60	1.60	1.60	1.60	1.60
Broiler Finisher. Premix	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Lysine.	0.20	0.20	0.20	0.20	0.20
Methionine	0.20	0.20	0.20	0.20	0.0
Added supplements					
Vitamin E (mg/kg)	0	100	200	300	400
Soya oil (g/kg)	0	100	100	100	100
Determined Analysis					
Crude protein (%)	19.10	19.50	19.58	19.60	19.60
Crude Fibre (%)	5.35	5.30	5.14	5.07	5.30
Ether Extract (%)	4.28	4.20	4.20	4.23	4.27
(MJ/Kg)	12.53	12.72	12.72	12.68	12.72

A 2.5kg of premix contains vitamin A: 12,000,000IU, vitamin D3: 2,500,000IU, vitamin E: 30,000IU, vitamin K: 2,000mg, vitamin B1: 2,250mg, vitamin B2: 6,000mg, vitamin B6: 4,500mg, vitamin B12: 15mg, Niacin: 40,000mg, Pantothenic Acid: 15,000mg, Folic Acid: 1,500mg, Biotin: 50mg, Cnoline Chloride: 300,000mg, Manganese: 80,000mg, Zinc: 50,000mg, Iron: 20,000mg, Copper: 5,000mg, Iodine: 1,000mg, Selenium: 200mg, Cobalt: 500mg, Antioxidant: 125,000mg.

Table 3: Proximate Analysis of Broiler Meat Fed Diets Containing Vitamin E and Soya Oil as Supplement

Parameters (%)	T 1	T 2	T 3	T 4	T 5	S.E.M
Dry Matter content	27.90[ab]	27.65[ab]	29.66 [a]	26.16 [b]	27.66[ab]	0.40
Ether Extract	3.78[ab]	3.21 [b]	5.28[a]	2.72 [b]	3.20 [b]	0.32
ASH	0.90[ab]	0.90[ab]	0.99 [a]	0.82 [b]	0.92[ab]	0.21
Crude Fibre	0.16[ab]	0.00	0.21[a]	0.00	0.00	0.00
Crude Protein	22.73[b]	23.27[b]	22.59 [b]	22.44 [b]	25.33 [a]	0.36
Nitrogen Free extract	0.40[bc]	0.43[bc]	0.58[ab]	0.21[c]	0.66 [a]	0.50

[abc] Means along the same row with different superscripts are significantly different (P<0.05)
KEYS: T 1 – Control, T 2 – Vitamin E 100mg/kg and Oil 100 g/kg, T 3 - Vitamin E 200mg/kg and Oil 100 g/kg, T4 - Vitamin E 300mg/kg and Oil 100 g/kg, T 5 - Vitamin E 400mg/kg and Oil 100 g/kg, S.E.M – Standard Error of Mean

The result presented in Table 4 shows the effect of dietary treatment on the water absorptive power, refrigerated weight loss and cooking weight loss of breast muscles of broiler meat fed diets containing vitamin E and soya oil. However, the treatment diets had no influence on the water absorptive power, refrigeration weight loss, and cooking weight loss.

Table 4: Effect of Vitamin E and Soya Oil on the Water Absorptive Power, Refrigerated weight loss and cooking weight loss on Broiler Meat

Parameters	T 1	T 2	T 3	T 4	T 5	S.E.M
Water Absorptive Power(g)	0.10	0.10	0.09	0.11	0.10	0.03
Water Absorptive Power(g)	0.10	0.10	0.09	0.11	0.10	0.03
Cooking Weight Loss (g)	6.57	6.53	6.96	6.57	6.63	0.17
Cooking Weight Loss (%)	13.13	13.07	13.80	13.20	13.13	0.33
Refrigeration Weight loss(g)	0.88	0.84	0.80	0.55	0.86	0.78
Refrigeration Weight loss (%)	1.74	1.67	1.58	1.08	1.76	0.15

KEYS: T 1 – Basal,T 2 – Vitamin E 100mg/kg and Oil 100 g/kg, T 3, - Vitamin E 200mg/kg and Oil 100 g/kg, T 4 - Vitamin E 300mg/kg and Oil 100 g/kg, T 5 - Vitamin E 400mg/kg and Oil 100 g/kg , S.E.M – Standard Error of Mean

The result documented in Table 5 shows the effect of vitamin E and soya oil supplement on cholesterol, triglycerol, high- and low-density lipoprotein and pH of thigh muscle of broiler meat. The treatment diets had significant (p<0.05) effect on all parameters of meat lipoprotein profile, excluding meat pH. Treatments 4 and 5 had the highest value for cholesterol (147.60 and 125.17 mg/dl respectively) and the lowest value for cholesterol was recorded for treatment groups 1, 2 and 3 that ranged from 92.90 - 115.10 mg/dl. The highest (p<0.05) value for triglycerol was observed in chickens fed dietary supplementation of 300 mg/kg Vitamin E and 100 g/kg Soya Oil (347.67 mg/dl), with Treatment 1 having the least value (193.67 mg/dl). Treatment 1 also had the highest value for HDL, while other groups have low (p<0.05) values documented for treatments 4, 3, 2 and 1 (26.98 mg/dl, 28.80 mg/dl, 29.17 mg/dl and 29.30 mg/dl) respectively. The highest concentration of LDL was observed in treatments 4 and 5 (51.65 mg/dl and 47.75 mg/dl respectively), with the least recorded for birds in group 1 (17.75 mg/dl) while Treatment 2 and 3 had the same value (26.90 mg/dl).

Treatments 4 and 5 had highest cholesterol values of 147.60 and 125.17 mg/dl respectively. The lowest values documented were seen in Treatments 1, 3 and 2 (92.90 mg/dl, 97.50 mg/dl and 115.10 mg/dl respectively). Meat triglycerol, 347.67 mg/dl was documented as the highest (p<0.05) for Treatment 4, with 193.67 mg/dl least (p<0.05) in groups fed the basal diet. The birds in the Control group had the highest (p<0.05) meat HDL value of 39.95 mg/dl, while Treatments groups 4, 3, 2 and 1 (26.98, 28.80, 29.17 and 29.30 mg/dl respectively) have low (p<0.05) values respectively. On the other hand, the highest concentration of LDL was recorded in groups 4 and 5 (51.65 mg/dl and 47.75 mg/dl) respectively. 17.75 mg/dl was the least (p<0.05) LDL value in groups fed the basal diet, while Treatments 2 and 3 had the same value (26.90 mg/dl).

Table 5: Effect of Dietary Vitamin E and Soya oil Supplementation on Meat Lipoprotein Profile of Broiler Chickens

Parameters(mg/dl)	T 1	T 2	T 3	T 4	T 5	S.E.M
CHL	92.90 [b]	115.10[b]	97.50 [b]	147.60 [a]	125.17 [a]	6.47
TR	193.67 [d]	292.67 [b]	237.00 [c]	347.67 [a]	232.33 [c]	14.91
HDL	39.95[a]	29.17 [b]	28.80 [b]	26.98 [b]	29.30 [b]	1.15
LDL	17.75 [c]	26.90 [b]	26.90 [b]	51.65 [a]	47.75 [a]	6.62
Ph	6.50	6.60	6.60	6.43	6.53	0.03

[abcd] Means along the same row with different superscripts are significantly different (P<0.05)
KEYS: T 1 – Control, T 2 – Vitamin E 100 mg/kg and Oil 100 g/kg, T 3, - Vitamin E 200 mg/kg and Oil 100 g/kg, T 4 - Vitamin E 300 mg/kg and Oil 100 g/kg, T 4 - Vitamin E 400 mg/kg and Oil 100 g/kg, CHL – Cholesterol, TR – Triglycerol, HDL – High Density Lipo-protein.

The result presented in Table 6 shows the effect of dietary treatment on the sensory properties of breast muscle of broiler meat. The dietary treatment had significant effect (p<0.05) on colour, taste, tenderness, overall acceptability and meatiness, with colour not significantly different (p>0.05). Sensory analysis of meat obtained from broiler chickens fed diets containing vitamin E and soya oil is presented in Table 6. It was observed that all measured sensory parameters were significant (p<0.05) except colour. Colour score ranged from 5.90 - 6.43, labeled "slightly liked". Treatment 5 had the highest value (6.63) for colour. Meat samples of group had highest (p<0.05) value of 7.43 for tenderness which was "liked moderately", with Treatments 1 and 2 on similar scores of 7.27 and 7.23 respectively, while Treatment 3 had the least score of 5.00. For overall acceptability, 7.13, 7.03 and 6.53 scores were best (p<0.05) in Treatment groups 1, 2, and 5 respectively, but Treatment 3 had the lowest score of 5.83.

Table 6: Sensory Analysis of Meat Obtained from Broiler Chickens Fed Vitamin E and Soya Oil Diet as Supplement

Parameters	T 1	T 2	T 3	T 4	T 5	S.E.M
Colour	6.07	6.16	5.90	6.18	6.43	0.12
Taste	5.60[bc]	6.13[ab]	5.00[c]	6.00 [ab]	6.63 [a]	0.18
Tenderness	7.27[ab]	7.23 [ab]	6.33 [c]	6.70 [bc]	7.43 [a]	0.13
Meatiness	7.07 [a]	6.93 [a]	5.83 [b]	6.60 [a]	7.13 [a]	0.16
Overall Acceptability	6.53 [a]	7.13 [a]	5.73 [b]	6.37 [ab]	7.03 [a]	0.16

[abc] Means along the same row with different superscripts are significantly different (P<0.05)
KEY: T 1 – Control, T 2 – Vitamin E 100 mg/kg and Oil 100 g/kg, T 3, - Vitamin E 200 mg/kg and Oil 100 g/kg, T 4 - Vitamin E 300 mg/kg and Oil 100 g/kg, T 5 - Vitamin E 400 mg/kg and Oil 100 g/kg, S.E.M – Standard Error of Mean.

Table 7 shows the main effect of days of refrigeration and treatment on meat samples obtained from broiler chicken fed diets containing vitamin E and soya oil as supplements, which was significantly (P<0.05) different. *Salmonella spp.* has an initial count 32.20 Log10cfu/ml at day 9 of refrigeration. *Salmonella spp.* count increased to 44.39 Log10cfu/ml and at day 14 of refrigeration, reduced to 37.77 Log10cfu/ml. TPC counts were 31.15 Log10cfu/ml, 18.36 Log10cfu/ml and 22.46 Log10cfu/ml on days 0, 9 and 14 of refrigeration respectively. *Escherichia coli* were not found on the meat sample. For the effect of dietary supplementation of Vitamin E and Soya oil on meat samples of broiler chickens, groups fed T_1 (25.08) and T_4 (25.08) diets had the same (p<0.05) value, but no obvious effect of *Escherichia coli on* meat samples obtained from birds fed experimental diets.

Table 7: The Main Effect of Days of Refrigeration and Dietary Treatments on Microbial Population of Meat Samples obtained from Broiler Chickens Fed Diets containing Vitamin E and Soya Oil as Supplements

	Days of Refrigeration/Dietary Treatments (Log10cfu/ml)									
Microbes	**0**	**9**	**14**	**SEM**	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**SEM**
Escherichia coli	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Salmonella spp.	32.20[c]	44.39[a]	37.77[b]	1.37	25.08[c]	56.93[b]	23.83[d]	25.08[c]	59.67[a]	1.76
TPC	31.15[a]	18.36[c]	22.46[b]	0.38	23.08[b]	18.35[e]	20.50[d]	22.32[c]	35.70[a]	0.49

[a,b,c] Means along the same row with different superscripts are significantly different (P<0.05)
SEM = Standard Error of Mean
TPC = Total Plate Count

The interactive effect of days of refrigeration and treatment on microbial population of meat samples obtained from broiler chicken fed diets containing vitamin E and soya oil as supplements is presented in Table 8. *Salmonella spp.* significantly (P<0.05) influenced meat samples fed dietary supplements. *Salmonella spp. in* meat obtained from T_3 (29.50 Log10cfu/ml) and T_4 (29.85 Log10cfu/ml) groups on day 14 of refrigeration was similar (P>0.05). TPC was also significantly (P<0.05) different except for T_1 (18.30 Log10cfu/ml) and T_2 (18.20 Log10cfu/ml) groups at days 0 and 9 of refrigeration respectively which were not significant (P>0.05).

Similarly, on the 14th day of storage, groups fed 400 mg/kg Vitamin E and 100 g/kg of Soya Oil had the highest (p<0.05) Salmonella count (98.00 Log10cfu/ml) with groups fed dietary supplement of 100 mg/kg Vitamin E and 100 g/kg of Soya Oil next (95.00), on the 9th day of storage. A 13.55 and 11.80 Log10cfu/ml *Salmonella spp* counts were recorded for groups T1andT2 on days 9 and 14 respectively. Birds fed supplementary diet of 100 g/kg and 100 mg/kg Vitamin E and Soya Oil had the least (p<0.05) *Salmonella spp* count.

Table 8: The Interactive Effect of Days of Refrigeration and Treatment on Microbial Population of Meat Samples Obtained from Broiler Chicken Fed Diets Containing Vitamin E and Soya Oil as Supplement (Log10cfu/ml)

	T_1			T_2			T_3			T_4			T_5			SE
Days	0	9	14	0	9	14	0	9	14	0	9	14	0	9	14	M
Microbes																
E. coli	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S.spp.	42.00^{e}	13.55^{m}	19.70^{j}	64.00^{d}	95.00^{b}	11.80^{n}	18.35^{k}	23.65^{h}	29.50^{f}	20.65^{i}	24.75^{g}	29.85^{f}	16.00^{l}	65.00^{c}	98.00^{a}	3.05
TPC	18.30^{k}	22.55^{e}	28.40^{b}	15.45^{l}	18.20^{k}	21.40^{f}	18.60^{j}	19.85^{h}	23.05^{d}	19.40^{i}	20.75^{g}	26.80^{c}	84.00^{a}	10.45^{n}	12.65^{m}	0.84

[a,b] Means along the same row with different superscripts are significantly different (P<0.05)

T_1 = Formulated broiler feed
T_2 = Formulated broiler feed + 100 g/kg soya oil + 100 mg/kg vitamin E
T_3 = Formulated broiler feed + 100 g/kg soya oil + 200 mg/kg vitamin E
T_4 = Formulated broiler feed + 100 g/kg soya oil + 300mg/kg vitamin E
T_5 = Formulated broiler feed + 100 g/kg soya oil + 400mg/kg vitamin E
TPC = Total Plate Count, SEM = Standard Error of Mean
S. spp. = *Salmonela spp.*
E. coli = *Escherichia coli*

Proximate findings from this trial does not agree with Adebiyi *et al*. (2011) who included vitamin C into the diets of broiler birds at 50 mg/kg, 100 mg/kg and 150mg/kg. This could be as a result of the different levels of inclusion and the high stocking density. The effects of vitamin E and oxidized oil on cholesterol, HDL, LDL and triglyceride concentrations are largely speculative. While some researchers obtained no significant effects in rats (Eder, 1999a) or birds (Juskiewicz, 2000), others reported that oxidized dietary oil reduces cholesterol and triglyceride concentrations (Eder & Kirchgessner, 1999; Eder, 1999b; Eder *et al.*, 2003). HDL and LDL was best in chickens fed the control diet with Treatments 4 and 5 having poor (p<0.05) values. It could thus be stated that inclusion of vitamin E at 300 and 400 mg/kg did not reduce the LDL in the meat. LDL, termed bad cholesterol, when in high concentration in a living system can cause fatty acid deposits in blood vessel that sometimes can break suddenly and form clots, with subsequent hardening of arteries. The values obtained for the pH were non-significant values. The values were in agreement with results obtained by Adebiyi *et al*. (2011) who fed broiler chickens diets containing vitamin C as supplements at inclusion levels of 50, 100 and 150 mg/g. The non-significant values obtained in this experiment were in agreement with the same result obtained by Adebiyi (*ibid*) who fed broiler birds diets containing vitamin C at inclusion levels of 50mg/g, 100mg/g and 150mg/g. Treatments 1, 2, and 5 (7.13, 7.07, 6.93 and 6.60 respectively) had best overall acceptability scores, with Treatment 3 (5.83) least (p<0.05). This conform with the findings of Chaves *et al*. (2008) who also observed that antioxidants had no significant effect on sensory characteristics of sirloins which were fed diets containing cinnamaldehyde with inclusion level of 0 and 0.2 g/kg. The result of this present study also agrees with the microbiological specification of standard set for frozen chickens (105 - 106 Log10cfu/ml), and within the maximum acceptable microbiological limits (Gracey *et al.*, 1999). When the microbial count of frozen chicken is < 106 (Log10cfu/ml), the product is denoted satisfactory and could thus be used for human consumption, but when level ranges between 106- < 108 (Log10cfu/ml), the

product is accepted as moderately satisfactory for human consumption. However, for microbial count that exceeds 108 (Log10cfu/ml), the product is considered as unfit or unsatisfactory for human consumption (HPA, 2009). This present study also shows that freezing can be detrimental to *Salmonella spp.* survival but does not guarantee the destruction of the organism. There is an initial rapid decrease in the number of viable organisms at temperature close to freezing point as a result of freezing damage. However, at lower temperatures, *Salmonella spp.* has the mechanism to survive long term frozen storage (Jay *et al.*, 2003). Furthermore, Strawn and Danyluk (2010) showed that *Salmonella spp.* was able to survive on frozen mangoes and papayas stored at -20ºC for at least 180 days.

Conclusion

Vitamin E and soya oil dietary supplementation had no significant influence on meat proximate parameters. However, Sensory evaluation was significantly affected by the experimental diet. There was high reduction in total bacteria count of meat samples obtained from the chickens at eight weeks of age when experimental diets were fed, *Escherichia coli* was absent on all the treatment samples of meat obtained. Refrigeration had little or no effect in reducing microbial population. Broiler chickens should be fed the basal diet to obtain meat with best lipid profile, but if flavour is to be induced, supplementing with 400 mg/kg of Vitamin E and 100 g/kg of Soya oil is best. If refrigeration is not to be considered, treatments 3, 4 and 5 which had vitamin E supplemented for 200 mg/kg – 400 mg/kg gave the best results of reduced *Salmonella spp.* population on unrefrigerated meat.

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