EFFECTS OF NATURAL ANTIOXIDANT EXTRACTS SUPPLEMENTATION ON GROWTH PERFORMANCE, CARCASS TRAITS, MEAT QUALITY AND SHELF-LIFE OF BROILER CHICKENS

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ABSTRACT

Five experiments were conducted to evaluate the effect of three natural antioxidants: sweet orange peel extracts (SOPE); shaddock peel extracts (SHPE) and lemon peel extracts (LMPE) on performance, carcass traits, meat quality and lipid oxidation of broiler chickens. The peels of ripe sweet orange, shaddock and lemon fruits were oven-dried and extracted using standard methods. The qualitative and quantitative phytochemical contents and diphenyl 2- picrylhydrazyl (DPPH) abilities of SOPE, SHPE and LMPE, were also evaluated. Steroids, flavonoids, coumarins triterpenes and alkaloids were the phenolic compounds found in SOPE, SHPE and LMPE. While phlobatanin, anthocyanin and amino acid were not detected. SOPE contains significantly higher steroids (34.43 mg/100g), flavonoids (161.82 mg/100g), terpenoids (17.09 mg/100g), triterpenes (128.27µg/100g) and alkaloids (32.44 mg/100g) than SHPE and LMPE. SHPE contains significantly higher phenolics (26.76 mg/100g) than SOPE and LMPE. While LMPE contains significantly higher tannins (1.74 mg/100g), coumarins (18.15 µg/100g) than SOPE and SHPE. Three hundred Abhor acre- day old broiler chicks were assigned into 5 treatments (T) and 3 replicates each in a completely randomised design. The feeding trial was for 8 weeks period each. The birds were fed starter diets (23 % CP and 2879 Kcal/kg ME) for the first 4 weeks and finisher diets (20 % CP and 3000 Kcal/kg ME) for the remaining 4 weeks. The first feeding trial was based as follows; T1 (butylated hydroxy anisole BHA, 0.02 % per litre of water, + control), T2 (water OW as – control) and T3 (SOPE), T4 (SHPE), T5 (LMPE), 0.02 % per litre of water respectively. While in the second feeding trial, the supplementation was T1 (BHA as control), T2 T3, T4 and T5 (SOPE 0.02, 0.04, 0.06, 0.08 and 0.10 %) per litre of water respectively. In the first feeding trial, LMPE had better weight gain (WG) and FCR compared to other treatments. Treatment OW was significantly higher ($p<0.05$) in white blood cell (WBC) than other treatments. Whereas, red blood cell (RBC) of both SHPE and LMPE treatments were higher ($p<0.05$) than other treatments. Total protein (TP) and cholesterol (TC) were significantly higher ($p<0.05$) in OW and SOPE treatments. Treatment LMPE had a higher ($p<0.05$) carcass weight compared to other treatments. Sensory parameters of SOPE treatment were significantly higher ($p<0.05$) than other treatments. Thiobarbituric acid reactive substance (TBARS) value at day 0; for cooked meat in SHPE treatment and raw meat in OW and SOPE treatments were significantly lower ($p>0.05$) than other treatments. In storage day 2; the TBARS value in SHPE cooked meat was significantly lower ($p>0.05$) than other treatments. Whereas, for raw meat storage day 2, the TBARS values were similar in BHA, SOPE, SHPE and LMPE treatments but significantly lower ($p>0.05$) to OW treatment. In the second feeding trial, the WG and feed intake were significantly higher ($p<0.05$) in SOPE (0.04 %) compared to other treatments. Whereas, there were similarities in the FCR of BHA, SOPE (0.04 and 0.10 %) treatments and were significantly lower ($p>0.05$) to SOPE (0.06 and 0.08 %) treatments. Treatment SOPE (0.10 %) was significantly higher ($p<0.05$) in WBC compared to other treatments. Whereas, the RBC of SOPE (0.08 %) treatment was higher ($p<0.05$) than other treatments. Total cholesterol (TC) was significantly higher ($p<0.05$) in SOPE (0.06 %) treatment. Treatment SOPE (0.04 %) had a higher ($p<0.05$) carcass weight compared to other treatments. Sensory parameters of SOPE (0.10 %) treatment were significantly higher ($p<0.05$) compared to other treatments. TBARS value at day 0; for both cooked and raw meat in BHA, SOPE (0.04, and 0.08 %) with the exception of cooked meat of treatment SOPE (0.06 %), were significantly lower ($p>0.05$) than other treatments. It was concluded that SOPE had significant effect in most of the parameters of interest with better performance recorded in birds fed higher doses (0.04, 0.06 and 0.10 %) of SOPE in the second experimental feeding trial.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Meat from a broiler provides various nutritional benefits, including a high level of protein, low concentration of lipids, and a high polyunsaturated fatty acid concentration. When compared to red meat, this makes it a healthier option. During storage, however, broiler meat is especially sensitive to lipid oxidation and bacterial contamination (Dave and Ghaly, 2011). Meat quality criteria such as colour, juiciness, tenderness, and flavour, has been demonstrated to be harmed by lipid oxidation, resulting in a shorter shelf life for the meat (Min *et al*., 2008). Lima *et al*. (2013) suggested using dietary antioxidants to prevent lipid peroxidation in feed and animal products. Protein breakdown is unavoidable during the spoiling process. Temperature, pH, storage time, microbial protease, and endogenous variables all influence the degree of protein breakdown (Zhang *et al*., 2011). After a long period of storage, especially under unfavourable conditions, nitrogenous substances' autolysis becomes more apparent (Smith, 2001). Often, spoiling is accompanied by the creation of biogenic amines and the development of oxidative rancidity (Estevez, 2015). The use of synthetic chemicals has been discouraged as feed additives in recent years (Jonathan *et al.,* 2015). As a result, natural antioxidants generated from plants are becoming increasingly popular, which have gained appeal because they are thought to be safer than synthetic antioxidants (Moyo *et al*., 2011). Because of their ability to prevent lipid oxidation, synthetic antioxidants are utilised for enhancing the oxidative stability in meat. Natural components are increasingly preferred

by customers over synthetic compounds due to their inexpensive cost (Ahn *et al*., 2002). As a result, more data on the potency of various natural additives which enhances oxidative stability in meat is required. Several fruits have been found to have high levels of antioxidant chemicals like phenolic acids and flavanone glycosides (Abeysinghe *et al.*, 2007), which extended the shelf life of broiler meats under various storage settings by reducing lipid peroxidation through dietary supplementation in feed and water (Botsoglou *et al*., 2002; Rababah *et al*., 2004; Jang *et al*., 2007; Sahin *et al*., 2010b).

Bioactive compounds, such as phenolic acids and flavanone glycosides, can be found in fruit peels. Peels are the most common by-products of citrus juice production, and if they aren't utilised, they become waste and pollute the environment. Peels are the primary source of natural antioxidants in certain fruits (citrus, apples, berries and grapes), according to research (Rababah *et al*., 2004; Sallam *et al*., 2004; Lucia *et al*., 2008). Phenolic chemicals in peels and fruits are alternative to synthetic antioxidants in food preservation (Ignat *et al*., 2011). Many fruits include phenolic compounds, which are significant components (Miguel *et al*., 2004). According to Gelareh *et al*. (2009), there are about 5000 known plant phenolics, and investigations have shown that many of them have antioxidant properties. The redox characteristics of phenolics, which primarily are responsible for their antioxidant action, can act as reducing agents, hydrogen donors, singlet oxygen quenchers, and metal chelators (Kumar *et al*., 2015).

Physicochemical properties of meat, such as shape, structure, proteolysis, and enzyme activity, can be affected by protein oxidation (Wangang *et al*., 2013). These changes may influence the qualities of meat products and regulate the quality of fresh meat (Wangang, 2009). Meat quality is influenced by time and temperature. When the freezer temperature

is between -23 and -28 degrees Celsius, most meat will keep its good quality for long. Meat deteriorates more quickly at temperatures below -17 degrees Celsius (Emad *et al*., 2014).

Temperature fluctuations, such as those found in self-defrosting freezers, can degrade meat quality. The term "reactive oxygen species" refers to a group of chemicals that are responsible for the initiation of peroxidation processes (Kalam *et al*., 2012). Cellular damage caused by oxidation processes were linked to a number of degenerative health issues that have a negative impact on animal performance (Avanzo *et al*., 2001; Sharma *et al*., 2011; Yang *et al*., 2013;). The biological damage caused by lipid peroxidation is an important driver of food spoilage, significantly compromising food and meat qualities (Lima *et* al., 2013). Antioxidants are chemicals that considerably reduces or prevents oxidation in food (Srinvasan *et al.*,2008).

Food deterioration is caused by lipid oxidation (Lima *et al*., 2013). Due to their exposure to external elements such as light, temperature and air. Lipids oxidize and produce rancid odours and disagreeable flavours (Shah *et al*., 2014). Natural antioxidants are frequently provided with the chicken diet, while the body tissues synthesise some of the antioxidants in the body. Maintaining chicken health, productivity, and reproductive function requires a careful balance of antioxidants and pro-oxidants in cells, the digestive tract, and throughout the body (Fotina *et al*., 2013).

1.2 Statement of the Research Problems

The use of synthetic antioxidants such as butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT) in meat refrigeration has long been proved to improve oxidative stability and preserve meat quality. However, the concerns about the negative effects of synthetic preservatives on human health, including their toxicity, coronary heart disease, and carcinogenic effect, have led to a shift in study focus to natural preservatives.

The problem associated with chicken's productivity and reproduction as a result of oxidative stress which is caused by free radical generation or a lack of antioxidant defence, is also of a major concern in poultry industry and researchers.

1.3 Justification for the Study

Several research on the influence of dietary natural antioxidants on growth performance, oxidative stability, and meat quality in broiler chickens have been published (Li and Liu, 2012; Ismail *et al*., 2013; Loetscher *et al*., 2013; Saheed *et al*., 2015). The results, on the other hand, are mixed and inconsistent. As a result, more research in various production systems is needed to allow for customised decisions and informed choices in the utilisation of dietary antioxidants in broiler chicken.

Citrus peels have been found to be high in phenolic chemicals, according to the studies of the following authors; Velasco and Williams (2011); Ding *et al*. (2015); Kumar *et al*. (2015). Differences in climatic conditions, cultivars utilised, and agronomic practices have all been shown to alter the phytochemical composition of citrus peels, and hence their antioxidant capacity. As a result, evaluating the phenolic chemicals in fruit peels is justified.

In the meat and food industries, natural antioxidants have a lot of promises. Antioxidant capacity can be found in peel extracts, herbs, and spices. However, their use and usage in the meat business is limited due to a lack of evidence on their effectiveness and safety in large enough quantities to be used, necessitating more research in this field (Kumar *et al*., 2015). Sweet orange peel (SOP), shaddock peel (SHP), and lemon peel (LMP) are currently considered wastes and potential environmental hazards. Specifically, the peels of the aforementioned fruits, which, if left to amass over time, may pollute portable water, streams, and rivers, or provide as a haven for toxic reptiles and insects through run-off (Manthey and Grohmann 2001). As a result, a lot of money and time is spent getting rid of peels as a waste product. Similarly, any technology that converts these wastes into useful products will not only provide a valued product from a low-cost and widely available source, but will also aid in waste recycling. This would extend the raw material foundation for a low-cost supply of additional natural antioxidants.

1.4 Aim and Objectives of the Study

The aim of this research work is to evaluate the effect of dietary supplementation with natural antioxidant extracts on growth performance, carcass traits, meat quality and lipid oxidation of broiler chicken. The objectives are to determine the;

i. phenolic compounds (quality and quantity) in sweet orange, shaddock and lemon peel powder.
ii. use of SOP, SHP and LMP extracts on the growth performance and gut morphology of the broiler chickens.

iii. effect of using SOP, SHP and LMP extracts on the haematology and serum biochemistry of the broiler chickens.

iv. influence of SOP, SHP and LMP extracts on the carcass characteristics of the broiler chickens.

v. effect of using SOP, SHP and LMP extracts on the meat quality of the broiler chickens.

vi. suitability of SOP, SHP and LMP extracts on the oxidative stability of the broiler chicken meat.

vii. effect of graded levels of the most outstanding of the natural antioxidant extract used on growth performance, carcass trait and meat quality of broiler chickens.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Oxidation in Meat

Many studies have found that subjecting broiler meats to high temperatures increases free radical generation and accelerates oxidative damage, which is regarded as the primary cause of meat deterioration (Sahin and Kucuk, 2003; Zhao *et al*., 2019; Mujahid *et al*., 2007). Free radicals are highly reactive and unstable chemicals that can harm biologically important components in the body. When the generation of oxidants outpaces the antioxidant systems' ability to eliminate reactive oxygen species (ROS) in the body, peroxidation sets in. Increased tissue protein oxidation levels have been associated with health issues and aging in humans (Frighet, 2002).

2.1.1 Types of oxidations

Auto-oxidation and photo-oxidation are the two major oxidation reactions that can occur in meat products containing lipids, with auto-oxidation being the more prevalent. Free radical generation is due to auto-oxidation processes as a result of presence of oxygen in the medium. Antioxidants can delay the reactivity of lipid peroxide radicals, resulting in a mixture of compounds that are both non- propagating as well as non- radical (Frankel, 2005).

2.1.2 Mechanism of oxidative action in meat

The engagement of free radicals in lipid peroxidation events has the most significant impact on cellular metabolism. Oxidative stability can damage the tissue cell significantly. Poly unsaturated fatty acids represent the peroxidizable material in

membranes (PUFAs). The vulnerability of PUFAs to peroxidation has been found to be related to the number of double bonds in the compound. Docosahexaenoic acid (DHA) and arachidonic acid are two of the most important substrates for membrane oxidation. Lipid oxidation is divided into three stages: start, propagation, and termination (Madhavi *et al*., 1996). Free radicals start the process when exposed to these environmental conditions (Hamilton *et al*., 1997).

Hydroperoxides, which have no taste or odour, are produced predominantly through oxidation reactions. The hydroperoxides are broken down into carbonyl and other compounds as oxidation advances, resulting in a rancid flavour in oxidized lipids (Gordon, 1990). Lipid oxidation products are potentially hazardous and can have carcinogenic, mutagenic, and ageing effects (Lima *et al*., 2013). Chemical preservatives are currently utilised in meat and other fatty foods as synthetic antioxidants (Moure *et al.*, 2001). They stop the progression of lipid oxidation by interacting with hydroxyl group and inhibiting the propagative stage of lipid oxidation (Birch *et al*., 2001). Synthetic antioxidants' safety and toxicity in connection to their metabolism and build-up in bodily organs and tissues, however, is a source of worry (Cao *et al*., 1997). Due to lipid peroxidation in the tissues, the structure and functionality of these membranes are jeopardized. Proteins is also a major target for reactive oxygen species (ROS). Importantly, the production of reactive oxygen species (ROS) at targeted positions through oxidative metal ions and quasi-ROS-induced oxidation of organic molecules are the two mechanisms that cause oxidative alteration of proteins. A protein can be altered by oxidizing a specific protein or cleaving the protein framework. In both circumstances, the changed proteins' biological activity would be jeopardized. The severity of protein

damage is determined by a variety of factors; like reactive nitrogen species RNS and reactive oxygen species ROS activities (Fotina *et al*., 2013). At the moment, poultry accounts for roughly 30% of global meat consumption (FAO, 2006). Because of its ability to transfer beneficial nutrients from feed to poultry products and other desired nutritional and physiological features, like low lipid concentration and a substantial PUFA content, it has the potential to be regarded a functional meal (Igene *et al*., 1979). However, PUFAs' ability to serve as nutritionally helpful lipids in functional foods is limited by lipid oxidation. This usually results into quality loss and low shelf life in poultry meat. The amount of time it takes for food to become unfit for consumption is known as usable shelf life (Ashok *et al*., 2016). It is the suggested maximum storage duration for items, during which a set proportion of the goods maintains acceptable quality under expected (or stated) distribution, storage, and display conditions (Ashok *et al*., 2016). Due to the higher number of phospholipids in poultry meat, it is thought to be more susceptible to oxidative rancidity than red meat.

Phospholipids are polyunsaturated fatty acids that are found in the membrane structure. Colour and flavour deterioration, loss of nutritional value, shelf-life decline, and the formation of chemicals that may be harmful to consumers' health and safety are all caused by lipid peroxidation (Min *et al*., 2008). Polyunsaturated fatty acids are degraded to volatile short-chain oxidative products during lipid peroxidation, resulting in off-odour and off-flavour condition, loss of polyunsaturated fatty acids, essential amino acids, and fat-soluble vitamins, and the formation of off-odour and off-flavour (Fasseas *et al*., 2007). This significantly reduces the product's acceptance among consumers. Drip loss

can be caused by oxidative processes that decrease the membranes' ability to store water (Jensen *et al*., 1998).

2.1.3 Factors affecting oxidation

Proteins are vulnerable to oxidative damage due to their complicated structure and a range of oxidisable functional groups in amino acids. In fact, oxidized protein build-up has been linked to the aging process and other age-related diseases. In biological systems, a variety of oxidized proteins and amino acids have been studied (Wangang *et al*., 2013). In general, the balance between antioxidants, prooxidants, and removal/repair processes determines the build-up of oxidized proteins. Reversible disulphide bridges are formed when proteins are oxidized. Chemically changed derivatives are formed as a result of more severe protein oxidation, such as the shiff's base (Surai, 2006).

2.1.4 Preventive measures

To prevent lipid peroxidation process, antioxidants' potency has been examined and demonstrated, both *in vivo* and *in vitro*. Antioxidants can protect a tissue from oxidative damage by enhancing its natural defences, inhibiting ROS formation and other oxidative reaction activities (Verma *et al.,* 2009). The significance of the aforementioned endogenous enzymes in meat quality, particularly their impact on meat shelf life, has earlier been emphasized (Decker and Xu, 1998).

However, under crucial situations (oxidative stress, Ultra Violet exposure,) where the creation of free radicals greatly increases, the in vivo defence systems of animal tissues are unable to provide total antioxidant protection (Mondon *et al*., 1999). As a result, more

antioxidant sources are required (vitamins, carotenoids, polyphenols, sterols). By inhibiting antioxidative defence proteins and diminishing cellular antioxidant reserves such as retinol and carotene, oxidative stress accelerates the oxidative reaction (Jones *et al*., 2006). This is why it is critical to consume antioxidant-rich foods on a daily basis, especially during the restoration phase. Olive polyphenols, such as hydroxytyrosol, are potent phytochemicals that destroys harmful ROS in the body (Cicerale *et al*., 2010). In a review, Khan *et al*. (2012), reveal that feed additives were proven to be an efficient method for reducing lipid peroxidation in poultry meat and egg yolks, relieving stress conditions and boosting chicken productivity.

2.2 Meat Quality and Carcass Characteristics

The majority of variables that impact meat quality may be controlled during different stages of chicken production or during slaughtering and handling. Food and nutrients are directly linked to the production of broiler carcasses. Animals who have a sufficient quantity of nutrients will be able to build muscle properly. Colour, pH, water-holding capacity, and cooking loss are the key meat quality measurement factors (Mendes *et al*., 2003), and are closely related to proteins and meat colours. The final pH measurement at 24 hours post-mortem is crucial for quality meat. As a result, pH stability has an impact on physicochemical and sensory properties of the meat (Qiao *et al*., 2001). After slaughter, glycolysis is the primary cause of rapid meat pH drop, resulting in increased drip loss and deterioration of broiler meat (Ferket and Foegeding, 1994). Meat colour features are an intrinsic aspect of product appearance that influences consumer preference and acceptability. In addition, the colour of broiler meat changes over time as it is

processed and stored (Fletcher, 2002). Recent research has found that dietary antioxidants can improve the raw broiler carcass colour (Mihaela *et al.,* 2021). Dietary antioxidant supplementation improved the colour stability of broiler meat by increasing membrane-bound lipid stability and delaying the peroxidation activities (Bayraktar *et al.,* 2011). Tenderness in meat is a complex composition of tissues that is regulated by metabolism and tissue structures such as connective tissue content, tissue pH, rigor mortis, and proteolytic enzyme activity (Kun-ze *et al*., 2018).

In the first instance, consumers' biting effort appears to be used to determine meat tenderness. Mechanical instruments, on the other hand, have been created to assess meat softness. Although there are differences between mechanical and sensory tenderness evaluations, this is largely due to the fact that customer perceptions of meat tenderness are often impacted by factors other than the amount of biting force required. Shear force (peak and yield) values differed significantly between broiler bird flesh samples fed dietary antioxidants supplementation. By evaluating the variations in overall cutting force, force peak and yield are utilised to assess the variation in meat texture. The fluctuation in shear force values of broiler meat is linked to the water retaining capacity of meat (Alvarado and Sams, 2000). The meat's shear force levels are influenced by both endogenous and external causes (Destefanis *et al*., 2008). Dietary antioxidants supplementation administered to the birds had an effect on carcass and liver weights. Dietary antioxidant supplementation showed no discernible effect on carcass or liver weight, confirming previous findings that dietary supplementation had little effect on carcass characteristics; carcass and heart weight were unaffected by vitamin C supplementation (Celik and Ozturkcan, 2003; Konca *et al*., 2009). Vitamin C

supplementation does not affect the internal organ of the birds (Konca *et al*., 2009; Samar *et al*., 2014). Vitamin C supplementation, on the other hand, raised carcass weight and internal organs greatly, according to Sahin *et al*. (2002) and Lohakare *et al*. (2005). Breast weight were significant in broilers fed the dietary treatment ordinary water OW (negative control) and lower in broilers fed the shaddock peel extract treatment.

2.3 Antioxidants

Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tertiary butyl hydroquinone (TBHQ) are synthetic antioxidants that have been shown to successfully reduce lipid oxidation in preserved meats and meat products. However, there has been increased concern about their health impacts, particularly their carcinogenicity and toxicity (Van Esch, 1986; Chung, 1999; Dwyer-Nield *et al*., 2010). Hence, the emphasis on shifting research work to the use of antioxidants that are of natural origin.

Recently tocopherol, -carotene, vitamin C, and phenolic compound have all been found as possible antioxidants in bird's meat (King *et al*., 1995; Tisimidou *et al*., 1995; Jensen *et al*., 1998; Ozkan *et al*., 2004; DeBoer *et al*., 2005; Giovannini *et al*., 2006). Plant, herb, fruit, and spice extracts including rosemary, sage, and thyme have been examined for their antioxidant and antibacterial characteristics, which could help enhance the oxidative stability of tissues (Tisimidou *et al*., 1995; Gurdip *et al*., 2007). They have been linked to the synergistic activity of a number of antioxidants including vitamins (C and E), (Tsimidou *et al*., 1995; Gurdip *et al*., 2007; Sivropoulou *et al*., 1996; Pennington and Fisher *et al*., 2009). The presence of several active phytochemicals that are crucial to human nutrition, such as vitamins, flavonoids, terpenoids, carotenoids, cumarins,

curcumins, lingnin, saponin, and plant sterol, contributes to the antioxidant activity of orange (Citrus sinesis) (Lucia *et al*., 2008). Natural antioxidants introduced in the pre-slaughter and post-slaughter stages have been shown in several tests to improve the shelf-life and quality of meat. That is, adding natural antioxidants to animal meals or drinking water, applying them to the surface of meat, or using active packaging (Gavaris *et al*., 2004). Supplementation in feed or drinking water has been shown to be a better method than other strategies (Keshavamurthy, *et al*., 2013), as it offers the advantages of first, allowing the animals spread the substance effectively in the body, secondly, safe level of the compound tolerable will be absorbed by the animals (Ricard *et al.,* 2009).

Plant meals are high in phenolics, which are antioxidant compounds that help prevent heart disease and minimize meat lipid oxidation (Hoye *et al*., 2008; Wijngaard *et al*., 2009; Jin and Mumper, 2010), reduce inflammation (Jin *et al*., 2006; Zhang *et al*., 2011; Mohanlal *et al*., 2012), lessen cancer incidence (Slivova *et al*., 2005; Ramos, 2008; Pieme *et al*., 2010; Sawadogo *et al*., 2012), and lower cell genetical distortion (Gomez-Cordoves *et al*., 2001; Pedreschi and Cisnero- Zevallos *et al*., 2006; Sawadogo *et al*., 2012). Plant phenolic chemicals are classed as simple phenols or polyphenols based on the phenol numbers in the molecule (Soto-Vaca *et al*., 2012).

Flavonoids are example of phenolics and are dispersed widely in plant tissues which comes in different colourations (Rong, 2010; Ferreira and Pinho, 2012). Flavonoids contain three-ringed structures and are generated from phenylalanine (Routray and Orsat, 2012).

Phytochemicals have a variety of pharmacological and biological effects (Evans, 2002). The phenolic compounds determine the plant's distinctive odour and colouration. While

some of the plant provides flavour, herbs and spices for human benefits (Evans, 2002). Free radical scavengers phenolics, flavonoids, and alkaloids reduce oxidative cell damage and have potent anticancer activities (Pourmorad *et al*., 2006). They activate systems that influence cancer cells and stop tumours from spreading (Rafat *et al*., 2008). Tannins have been shown to be effective in the treatment of inflamed or ulcerated tissues as well as the prevention of cancer (Okwu and Emenike, 2006). Fruits include natural antioxidants that may be used as an anti-aging, therapeutic, anti-inflammatory, anti-analgesic, or anti-hyperlipidemic agent.

Saponins are found in the peels of fruits, and depending on the content of the peels, they can have a bitter or sweet flavour. Saponins are also recognized to be a stomach irritant with anti-carcinogenic and hypocholesterolaemia properties. However, in greater doses, they are hazardous, producing red blood cell haemolysis (Pirjo *et al*., 2018).

Tannins are anti-nutritional, but because they are also phenolic substances, they are thought to have health benefits. Lampart-Szcapa *et al*. (2003) found a tiny amount of total tannin in Lupin seed, concentrated largely in the cotyledon, however Ramilla *et al*. (2009) found no total tannin in Lupin. This could be due to differences in the geographical region where the plant (Lupin) grows, as well as the variety grown. Antibacterial activity is higher in fruits with more alkaloids than in fruits with fewer alkaloids (Davis and Riordan, 2004).

Various natural and synthetic antioxidants are commonly employed in the meat processing industry, and natural antioxidants are increasingly being used to replace synthetic antioxidants. Vitamin E, enhances oxidative stability in meat, thereby increasing the shelf life of the meat. For example, Nuez De Gonzalez *et al*. (2008) found

that 3% dried plum puree was beneficial in oxidative stability in pork meat. Similarly, grape seed extract has been demonstrated to increase the shelf life of bird's meat, inhibiting the development of TBARS (Brannan, 2008). At 0.02 per cent, grape seed extract has the ability to prevent lipid oxidation and enhanced the oxidative stability of the pork meat (Rojas and Brewer, 2007).

2.3.1 Antioxidant effect on fat composition of meat

Vitamin E, a lipid-soluble antioxidant found in animal tissues that protects them from oxidative damage. Lipid oxidation results from the decomposition of polyunsaturated fatty acids in tissue membranes, resulting in the conversion of the red muscle pigment myoglobin to brown metmyoglobin and the generation of rancid odours and flavours. Dietary supplementation with natural antioxidants boosts their deposition in tissue, delaying peroxidation and enhancing the oxidative stability of meat. This occurs not only in single-stomached species like pigs and chickens, which eat low-vitamin-E cereal-based diets, but also in ruminants that eat grass, which has a high natural vitamin E content. The natural antioxidant content of the feed has been shown to have a substantial impact on the lipid oxidation stability of chicken and turkey breast muscle in studies (Marusich *et al.* 1975). According to studies, the fatty acid content of broiler chicken meat is influenced by the fatty acid profile of the feed (Cherian *et al*., 1996; Bou *et al*., 2004). When compared to other meats, chicken meat has an abundance of poly unsaturated fatty acids (PUFA) as a result of broiler diets that are generally high in PUFA (Rhee *et al*., 1996). In most circumstances, the fatty acid composition of meat mirrors the fatty acid composition of the meal. Lipid peroxidation, sensory indicators, and post-mortem oxidative stability are all affected by increased PUFA concentration (Basmacioglu *et al*., 2004). According

to a study, the amount of saturated fatty acids (SFA) in poultry tissues is dependent on the amount of SFA in the diet and their synthesis in the liver (Sim and Qi, 1995). Vitamin E supplementation, identical to that given to turkeys, improves pig lipid oxidative stability while also retaining muscle redness and minimizing drip loss (Asghar *et al*., 1991). They can help minimize excessive drip loss from the pale, soft, exudative (PSE) muscle of stress-prone pigs (Cheah *et al*., 1995).

Lower membrane phospholipase activity appears to be the cause of this effect, which is thought to be mediated by higher vitamin E concentration in tissue membranes. Daily supplementation of concentrate diets with 2500mg vitamin E for 40 days resulted in a 7-10d extension of colour shelf-life when beef steaks were offered in modified-atmosphere packing (Taylor *et al*., 1994). The presence of 0.2 metmyoglobin in the muscle surface, as anticipated by spectrophotometer readings, signalled the end of shelf life.

2.3.2 Antioxidant and its protective mechanisms

To deal with reactive oxygen and nitrogen species (ROS and RNS), animals have evolved unique antioxidant defence mechanisms over time. As a result of the presence of natural antioxidants, animals can only thrive in an oxygen-rich environment. The phrase "antioxidant system" is used to characterize these systems. It has a wide range of functions and is responsible for protecting cells from free radical damage. This protective mechanism includes fat-soluble antioxidants (vitamins A, E, carotenoids, ubiquinones), water-soluble antioxidants (ascorbic acid, uric acid, taurine), and antioxidant enzymes such as glutathione peroxidase (GSH-Px), catalase (CAT), and superoxide dismutase (SOD).

Excess free radical generation in broiler chickens is influenced by a variety of dietary, environmental, and physiological factors, resulting in oxidative stress. Oxidative stress is caused by the interaction of free radicals with biologically inherent antioxidant activity in animals (Halliwell *et al*., 1990). In order to counteract this effect, the body system is equipped with innate antioxidants which consists of enzymes like SOD, CAT and GP_X and non- enzymes such as vitamins E and C. Surai (2007) examined the biological antioxidant properties in further depth. Antioxidants guard against free radicals by blocking pro-oxidant enzyme activity or directly scavenging free radicals in the body. (Irshad and Chaudhary, 2002; Abd Ellah, 2010). As a result, exogenous antioxidant supplementation is urgently needed to reduce the excess of free radicals in the animal's body. Oxidative stress is defined as an imbalance between the production and breakdown of reactive oxygen substances (ROS) such as superoxide anion, hydrogen peroxide, and lipid peroxides. SOD, CAT, and GP_X are enzymes that aid in the deactivation of reactive oxygen species in muscle tissue (ROS). SOD and CAT are direct radical-reacting antioxidant enzymes, whereas GP_X is an antioxidant enzyme.

Several studies have suggested that lipid peroxide absorption requires antioxidant defence enzymes that degrade lipid derivatives in the digestive tract mucosa and the liver system (Takahashi *et al*., 2002; Zalejska- Fiolka *et al*., 2010). The antioxidant enzyme glutathione peroxidase (GP_X) transforms hydrogen peroxide to water and lipid peroxides to alcohol molecules within animal cells (Sies, 1999). Organelles, subcellular compartments, and extracellular space contain antioxidant protecting chemicals, allowing for optimum cellular protection. In stressful situations, the body's antioxidant system is responsible for preventing free radical damage. As a result, dietary antioxidant

supplementation can help broiler production efficiency. Polyphenolic compounds, a class of plant-derived compounds that includes more than 8,000 different compounds with antioxidant and pro-oxidant properties in various situations, have been shown to be beneficial in antioxidant protection. Polyphenols, especially flavonoids, have a low bioavailability, which is one of their main drawbacks. They may have a high concentration in the diet, but their levels in the blood are modest, and their concentration in target tissues (liver and muscles) is usually minimal. As a result, the gut is the primary site of flavonoid action, where they can have health-promoting characteristics by helping to maintain the antioxidant-prooxidant balance (Surai *et al*., 2004; Surai, 2006). Flavonoids are antioxidants that have been found to improve the oxidative stability of meat (Ishola *et al*., 2017). Flavonoid consumption has also been associated to a reduced risk of coronary heart disease (Hertog *et al*., 1993). In addition to its antioxidant capabilities, flavonoid has biological effects such as protection against platelet aggregation, ulcers, free hydroxyl radical removal, inflammation, and allergies (Barakat *et al*., 1993). In comparison to 200 mg/kg vitamin C, a 100 mg/kg extract from the dried fruits of *Forsythia suspensa* (another herb used in traditional Chinese medicine that includes the lignan pinoresinol) reduced oxidative damage in poultry birds. (Sujatha *et al.*, 2010).

Furthermore, when compared to a control diet, Ramnath *et al*. (2008) and Sujatha *et al*. (2010) discovered that Ayurvedic polyherbal compositions Brahma rasayana extract and stress stroke could lower oxidative stress. Vitamin C and flavonoids supplemented diets are known to mitigate chronic heat challenged broilers with increasing dosage (Seven *et al*., 2009). In several tissues, the higher dose (3 g/kg) was able to improve oxidative

status in a similar way to 250 mg/kg vitamin C. When polyphenols from *Tamarindus indica* seed coatings were applied, oxidative damage did not improve when the temperature was elevated (Zhao *et al*., 2019). They also looked at the effects of genistein [an isoflavone flavonoid; (Onderci *et al*., 2004), lycopene [a carotene with no vitamin A activity; (Tuzcu *et al*., 2006), epigallocatechin-3-gallate [a flavanol flavonoid; (Sahin *et al*., 2011), tomato powder [containing 0.80 mg lycopene, 0.13 (Sahin *et al*., 2013a), in cyclic oxidative stress in Japanese quails, and Berberis vulgaris root extract [chemical composition stated in (Sahin *et al*., 2013a)]. They observed favourable effects, such as evidence that particular phytochemicals stimulated the host defence system at the cellular level, as indicated by up-regulation of Nrf2 and down-regulation of NF-B. (Sahin *et al*., 2010a; Sahin *et al*., 2013a). Sahin *et al*. (2013b) discovered significant correlations between feed intake and egg production on one hand, and Nrf2 (positive) and NF-B (negative) on the other, regardless of epigallocatechin-3-gallate supplementation, addressing their role in influencing oxidative stress responses. Akbarian *et al*. (2013) used ginger root powder (7.5 and 15 mg/kg) and an essential oil hydro distillate (75 and 150 mg/kg); the latter had 12 constituents identified, with major components zingiberene, -sesquiphellandrene, sabinene, ar-curcumene, and -bisabolene; all sesquiterpenes except sabinine, which is a monoterpene.

When compared to controls, all treatments improved serum oxidative status, including the positive control vitamin E (100 mg/kg), however only the ginger oil increased liver superoxide dismutase (SOD) activity, and both ginger root and oil reduced liver MDA. Recent research has found that phenolic compounds have beneficial effects on hens due to their antioxidant, anti-inflammatory, and antibacterial capabilities (Akbarian *et al*.,

2014; Akbarian *et al*., 2015). Surai (2013) and Akbarian *et al*. (2015) discovered that *Curcuma xanthorrhiza* essential oil, *Oreganum compactum* essential oil, and orange peel extracts can enhance the oxidative status of broiler chicks during cyclic chronic oxidative stress. Because of the modest quantity of active components in the extracts, Surai (2013) found that the significant effects of orange peel extracts were restricted to boosting erythrocyte GSH-Px activity for the 400 mg/kg treatment. Finding a regular pattern of potent antioxidant phytochemicals appears to be difficult. Terpenes/terpenoids, particularly sesquiterpenes/sesquiterpenoids, have been proven to be beneficial in several investigations while Lignans, as well as lycopene, are found in traditional Chinese medicinal herbs (Ryoiti, 2017). Nonetheless, other forms of flavonoids and related substances (proanthocyanidins and resveratrol) were found to be beneficial in the majority of trials, whether in combination or not with vitamin C or E. Surai (2013) questioned flavonoids' in vivo direct antioxidant effects, primarily because these compounds are poorly absorbed in the gut, quickly metabolized, and excreted, resulting in physiologically low concentrations in target tissues (e.g., typically less than 1 mol/L in healthy subjects' plasma). Most studies found that antioxidant phytochemicals were beneficial in improving oxidative stability in heat-stressed poultry, but were less effective or ineffective in non-heat-stressed poultry (Onderci *et al*., 2004; Sahin *et al*., 2013a). This supports the theory that antioxidant phytochemicals may have potential in challenging conditions. As a result, more research is needed in this area, particularly well-designed dose–response studies (dose impact is inadequately addressed in the aforementioned trials) that include non-heat stressed control treatments and study the mode of action.

2.4 Effects of Exogenous Antioxidants

Owing to the fundamental role of antioxidants in human life and health, and their general popularity due to increased media attention, the demand for these compounds by the general public has been increasing recently. Antioxidants are substances that, when present in low amounts compared to an oxidizable component (such as DNA, proteins, lipids, or carbohydrates), delay or prevent oxidative damage caused by reactive oxygen species ROS (Halliwell *and* Gutteridge,1990). At excessive doses, ROS can be harmful, causing pathophysiological effects, whereas at low doses, they can be useful for normal physiological functions (Grune, 2002; Martin and Barrett 2002; Valko *et al*., 2007; Elahi *et al*., 2009). Exogenous antioxidants are important in maintaining the delicate balance of oxidation and anti-oxidation in living systems (Valko *et al*., 2007).

2.4.1 Endogenous and exogenous anti-oxidation

Endogenous (enzymatic and non-enzymatic) antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP_X), and glutathione (GSH), among others, and exogenous antioxidants such as vitamin C, E, carotenoids, and polyphenols, with the diet being the main source, make up our antioxidant defence system (Bouayed, 2010; Ratman *et al*., 2006; Biehler and Bohn 2010; Andre *et al*., 2010). Endogenous and exogenous antioxidants work together to maintain or restore redox equilibrium, for as when vitamin E is regenerated by glutathione (GSH) or vitamin C is used to prevent lipid peroxidation (Valko *et al*., 2007), which can disrupt membrane integrity and damage membrane proteins by inactivating receptors, enzymes, and ion channels, as well as influence membrane fluidity and damage membrane proteins, resulting in cell death

(Bouayed *et al*., 2009). Vitamin E acts as a hydrogen donor for lipid peroxyl radicals (LOO) to prevent free radical chain reactions (chain-breaking antioxidant) (Lobo *et al*., 2010). Many health organizations, such as the "five a day" campaign, have advocated for the consumption of whole foods rich in naturally occurring antioxidants, including nutrients such as vitamins and phytochemicals such as polyphenols. (USFDA, 2009). Plant foods such as apples, plums, bananas, tomatoes, onions, and other fruits and vegetables provide a natural source of these antioxidant compounds because humans are unable to generate them on their own (Manach *et al*., 2004; Ratman *et al*., 2006; Andre *et al*., 2010; Bouayed, 2010; Biehler and Bohn, 2010). Fortification, supplementation with isolated components, and consumption of synthetic antioxidant additives such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were originally used to protect and preserve the nutritional quality of processed foods, as well as to extend their shelf life, in addition to their natural occurrence in foods (Shahidi, 2000; Moure *et al*., 2001). Endogenous antioxidants including phenolic compounds, vitamin E, and carotenoids have been found to work in tandem with innate antioxidant enzymes (superoxide dismutase SOD, glutathione peroxidase GP_X, and serum catalase CAT) in tissue maintenance and post-mortem ageing in both humans and animals (Praveen and Ashish, 2012). Antioxidants affect oxidative stress by interfering with free radical generation.

Higher amounts of malonaladehyde (MDA) were found in the blood and tissues of broiler chicks as lipid oxidation increased (Okutan *et al*., 2005; Ates *et al*., 2006; Samar *et al*., 2014). GP_X and SOD, which are found in the mitochondrial matrix, contribute to the catalysis and breakdown of different peroxides by oxidizing glutathione. Antioxidants,

such as phenolic compounds, are essential components of the glutathione peroxidase enzyme, which lowers peroxide and protects tissue cells from oxidative damage (Reddy *et al*., 2009). In their investigations, Jianhua *et al*. (2000); Payne and Southern (2005) found that selenium (Se), an antioxidant supplement, provided to broiler chickens enhanced plasma GP_X in the bird's tissues. An increase in ambient temperature causes cholesterol levels to rise and total protein levels to decline in the circulation of birds, according to Rashidi *et al*. (2010). An experimental trial in which the daytime ambient temperature reached 29 degrees Celsius may have resulted in increased blood fat content in birds. This is in agreement with Rashidi *et al*., (2010), who reported that an increase in blood lipids during heat stress could be due to high temperatures, which resulted in a reduction in feed consumption by the birds because broilers compensate for their energy needs through the process of lipolysis, which raises blood cholesterol and triglycerides.

2.5 In Vitro Evidence of Antioxidant Dosages

In vitro studies have demonstrated the cytoprotective efficacy of plant dietary ingredients such as polyphenols and combinations, as well as their ability to prevent cell death caused by oxidative stress (Heo *et al*., 2004). Although phytochemicals have been shown to have antioxidant properties (Ratman *et al*., 2006; Valko *et al*., 2007; Pandey and Rizvi, 2009; Bouayed 2010), they can also have pro-oxidant properties in specific circumstances, such as large doses or in the presence of metal ions (Azam *et al.*, 2004). The activity of pro-oxidants and antioxidants is directly proportional to their concentration. Some polyphenols that are already known to be antioxidants, such as quercetin, catechins including epicatechin and epigallocatechin-3-gallate (EGCG), and

gallic acid, have been shown to have pro-oxidative activity in recent cell model studies (Watjen *et al*., 2005). Quercetin (50 M), for example, has been shown to enhance superoxide radical (O_2^-) formation within isolated mitochondria and cultured cells at high concentrations (De Marchi *et al*., 2009). The antioxidant effect of quercetin was only identified at low doses (0.1–20 M) in another investigation, but higher doses (>50 M) lowered cell survival and viability, thiol content, total antioxidant capacity, and SOD, CAT, and glutathione S-transferase activities (Robaszkiewicz *et al*., 2007). At moderate concentrations (10–25 M), flavonoids (quercetin and fisetin) have similarly been found to protect rat H4IIE cells from H_2O_2-induced cytotoxicity, DNA strand breaks, and death, but high concentrations (50–250 M) caused cytotoxicity, DNA damage, and apoptosis (Watjen *et al*., 2005). Autoxidation and redox-cycling flavonoids like myricetin and quercetagetin, as well as redox-cycling flavonoids like quercetin, have been shown to produce large amounts of ROS (Ochiai *et al*., 1984; Gaspar *et al*., 1994). A single oral dose of 14C-BHT was given to male and female mice, resulting in rapid absorption and distribution of 14C to the tissues. The majority of 14C-BHT was eliminated in the faeces (41-65 %) and urine (26-50 %), with minor amounts in the expired air (6-9 percent). (Matsuo *et al.*, 1984). According to studies, day old chicks fed 14C-BHT at a dose of 200 mg/kg for 10 weeks had BHT and metabolite residues of 1-3 mg/kg. Similar laying hen diets resulted in egg residues of 2 mg/kg after 7 days, with the amount remaining constant after that (Frawley *et al*., 1965).

In the fat fraction of eggs from laying hens fed feed containing 500 or 100 mg/kg BHT, residues of 20 and 5 mg/kg were found, respectively. Body fat residues in broiler chicks

were 55 mg/kg on the 500 mg/kg diet and 5 mg/kg on the 100 mg/kg diet after a 21-week period (Van Stratum and Vos, 1965).

2.6 Protein Oxidation

Some blood components are predisposed to oxidative stress. RBCs, for example, are constantly exposed to ROS as a result of their function, whereas platelets are only exposed to ROS when coagulation occurs in areas of inflammation. Protein oxidation pathways are also important in transfusion therapy, as they have been linked to the "blood storage lesion" phenomena (Dumaswala *et al*., 2000; Kriebardis *et al*., 2006; Tinmouth *et al*., 2006; Kriebardis *et al*., 2007). It's unclear if blood product oxidation happens as a result of blood being exposed to oxidizing chemicals during puncture, handling, and blood product preparation (e.g., pathogen inactivation procedures), or only after storage due to aging or stress.

Hydroxyl radicals (OH) and hydrogen peroxide can cause protein side chain modifications, protein backbone breakage, the generation of carbonyl derivatives, and the development of cross-linked protein complexes (H_2O_2). Some responses are exclusive to and unique to individual residues, while others have broad and generic effects. Furthermore, reactive oxygen and nitrogen species damage DNA bases and sugar moieties, as well as lipid breakdown via peroxidation, which might modify proteins as a result of the by-products (Beckman and Koppenol, 1996). Proteins are important molecules that are harmed by ROS, and an accumulation of oxidized substances in muscle tissue causes meat quality to deteriorate. As a result, oxidative substance changes including tryptophan fluorescence decrease, sulfhydryl group losses, inter and intra

molecular cross connections, and the formation of carbonyl derivatives may affect meat quality (Xiong *et al.*, 2000).

2.6.1 Protein thiols and thio-ethers

Cysteine thiols can undergo a variety of oxidative modifications depending on their accessibility in the protein structure and the species with which they can interact. Furthermore, because cysteines generate disulphide connections that are essential for protein structure, their oxidation status is crucial for protein function. In not too long ago, cysteine oxidation has become more well acknowledged as a fundamental regulatory mechanism (Rhee *et al*., 2000). In a direct, reversible reaction, free sulphydryl groups can be oxidized to sulfenic acid, and then to sulphinic and sulphonic acid in an irreversible reaction. It's also feasible to nitrosylate free cysteines (Hogg, 2002).

Furthermore, in the protein environment, free sulphydryl groups can form disulphide bridges with low molecular weight sulphydryl molecules such free cysteine and glutathione (Bergenhem *et al*., 1986; Dormann *et al*., 1993; Fratelli *et al*., 2004; Dalle-Donne *et al*., 2007). In most cases, unless a second cysteine is present in close vicinity and may create a disulphide bridge, S-glutathionylation is a permanent alteration. Methionine is the most easily oxidized amino acid, along with cysteine (Lim *et al*., 2019). A key antioxidant process is methionine cyclic oxidation–reduction via NADPH-dependent thioredoxin reductase. (Levine *et al*., 1996; Levine *et al*., 1999; Stadtman *et al*., 2003). The methionine sulphoxide level of proteins has been demonstrated to rise with age in several tissues, particularly erythrocytes (Stadtman *et al*., 2005). Because the amount of protein in muscle determines the quality of chicken meat, protein oxidation is

significant in evaluating meat quality (Falowo *et al*., 2014). Due to the presence of oxidisable lipids and oxidative enzymes, muscle tissue is highly vulnerable to oxidation, leading in the generation of protein carbonyls (Lund *et al*., 2011).

Meat protein oxidation is determined by detecting the formation of protein disulphide cross-links and measuring the protein thiol concentration (sulfhydryl group SH) (Nieto *et al*., 2013). After death, changes in muscle tissue limit the meat's antioxidant activity (Baron and Anderson 2002). According to Xiong *et al*. (2000), protein oxidation can result in the loss of tryptophan and sulfhydryl groups, as well as the formation of molecular cross linkages, all of which can have a negative impact on meat quality.

2.7 Broiler Nutrition and its Effect on Meat Stability during Processing and Storage

One strategy to increase meat's oxidative stability is to add antioxidants either endogenously into the animal's diet or exogenously during processing (Decker and Xu, 1998). In experiments, increased vitamin E supplementation has been proven to improve meat quality in chickens, turkeys, cattle, pigs, and lambs (Buckley *et al*., 1995; Liu *et al*., 1995; Wulf *et al*., 1995; Sheehy *et al*., 1997).

Several studies have shown that polyphenols affect meat quality, although it appears that adding them directly to the meat during processing is more advantageous. Although polyphenols have the greatest direct effect in the chicken's intestines, and their concentrations in muscles are too low to exert direct antioxidant benefits, according to a recent study on the role of polyphenols in poultry nutrition, polyphenols have the greatest direct effect in the chicken's intestines (Surai, 2013). As a result, the favorable effects of plant extracts given to chickens, such as increased meat durability, are most likely due to

mechanisms other than antioxidant activity. Based on the aforementioned information about the beneficial effects of dietary antioxidants on protection against various stresses in broiler production, a number of anti-stress compositions/premixes have been developed and are available in the market. The original idea was based on the idea that giving antioxidants to birds in the form of feed or water could help them cope with stress more effectively. It has been proven that incorporating vitamin-gene-regulating substances in feed or water (carnitine, betaine, vitamin E, as well as various minerals, vitamins, electrolytes, and organic acids) can assist animals to cope with a variety of circumstances (Fisinin and Surai, 2012). This is advantageous during the chick's implantation, when the antioxidant system is crucial for the development of the digestive and immune systems (Fisinin and Surai, 2012). Specifically, putting an anti-stress composition (Anti stress Magic Mix, PerforMax) into the feed or drinking water enhanced chicken growth and feed conversion ratios, according to various experimental researches (Fotina *et al*., 2011). Using the same anti-stress composition under commercial conditions increased FCR every week throughout a 39-day broiler growth study. At the conclusion of the study, the improvement in FCR owing to the application of anti-stress composition during the first three days after hatch, as well as before and after vaccination, was extremely significant (Velichko and Surai, 2013). Furthermore, in broilers, the anti-stress component was shown to have immune-modulating effects (Fotina *et al*., 2011).

2.8 Haematological Effect of Dietary Antioxidants

Antioxidants in food help people and animals maintain optimal health by regulating immunological pathways that control and limit the harmful effects of high reactive oxygen substance activity in the body (Puertollano *et al*., 2011). White blood cells (WBCs) are important component of the blood. Animals with a high WBC have a higher propensity for antibody production, which promotes disease resistance, while animals with a low WBC are more susceptible to disease infection, according to research (Soetan *et al*., 2013). The high availability of both WBC and lymphocytes (LYM) may have been due to dietary antioxidant supplementation, which may contain specific phytochemicals, allowing the birds to maintain their good health. Asghar *et al*. (2018), discovered that boosting WBC, HGB, and LYM concentrations and strengthening the bird's antibody system against Newcastle disease improves the immune system and haematology of growing broiler chickens. The resistance to Newcastle disease in broilers fed a diet supplemented with genistein was revealed by antibody titres (Rasouli and Jahanian 2015). Furthermore, Hager- Theodorides *et al*. (2014) discovered that pigeons fed antioxidants quercetin produced more IgY antibodies. Antioxidant supplements used in this study are likely to have boosted B-cell activity, which is prone to oxidative damage and so impairs immunity (Catoni *et al*., 2008). Fruit peel-based extracts have been shown to have antioxidant, anti-inflammatory, and anti-microbial properties, resulting in improved broiler chick health (Havsteen 2002; Kamboh *et al*., 2015). The normal limits for haematological indicators in broiler chicks include RBC: 2.5-3.5Xx106ul, PCV: 22-35 percent, HGB: 7-13g/dl, and WBC: 12-30x 103ul (Bounous and Stedman, 2000).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experiment One: Determination of Antioxidant Potentials of Sweet Orange, Shaddock (Pomade) and Lemon Peel Extracts

3.2 Materials

Sieve with diameter 142.56 µm, Soxhlet apparatus, knives, Rotary evaporator, dark bottles and fridge. Chemicals used in this study were Folin-ciocalteu reagents, ethanol, aluminium chloride.

3.3 Methods

Sample collection

Ripe sweet oranges, shaddock and lemon fruits used in this study were collected from Lower Niger River Basin Authority Farm, Ilorin, Kwara State.

3.3.1 Preparation of extracts of sweat orange peel (SOP), shaddock peel (SHP) and lemon peel (LMP)

The experiment was carried out in the School of Agriculture and Agricultural Technology, Animal Production Laboratory, Federal University of Technology, Minna and Central Research Laboratory, Tanke, Ilorin. Ripe sweet orange, shaddock and lemon fruits were washed with water, peeled manually using a knife and their edible parts were separated. The peels were dried in a Gallenhamp oven (300 plus series model) at 40 ^{0}C for 48 hours until they reached a constant moisture level. According to the procedure described by Van-Acker *et al*. (2011), the dried samples were crushed to a fine powder with a blender (Smart Leaf VTCL, 18,000 rpm capacity, India) and passed through a 24 mesh - size sieve (142.56 µm). The phenolic components were extracted using an organic

solvent extraction technique. A total of 50 g of powdered samples were extracted with 400 ml ethanol at room temperature for 3 hours using the Soxhlet extraction method. To remove the particles, the mixture was decanted. The extraction operations were conducted twice under the same conditions to assure full extraction. Finally, using a rotary evaporator (RE-52A Model, England), the extracts were filtered and evaporated to dryness under decreased pressure at 60 ^{0}C. After that, the extracts were put in dark bottles and maintained in the refrigerator at 4 ^{0}C until they were used.

3.3.2 Qualitative analysis of the phytochemicals of the extracts

Tannins, phlobatannins, saponins, steroids, terpenoids, flavonoids, alkaloids, glycosides, phenolics, coumarins and titerpenes were analysed qualitatively as seen in Appendix A.

3.3.3 Quantitative analysis of the phytochemicals of the extracts

The following phytochemicals; tannins, phlobatannins, saponins, steroids, terpenoids, flavonoids, alkaloids, glycosides, phenolics, coumarins and titerpenes were analysed quantitatively as seen in Appendix B.

3.3.4 1, G1 – diphenyl 2- picrylhydrazyl (DPPH) determination

The ability of each of the peel extracts to produce DPPH was measured using the method described by Najafabad and Jamei (2014). A ml of each extract sample was mixed with 4 ml of a 0.1 mmol L-1 methanolic DPPH solution. A blank solution was made by combining 4 ml of 0.1 mmol L-1 methanolic DPPH solutions with 200 µl deionised distilled water. The absorbance was measured using a spectrophotometer at 517 nm against a prepared blank solution after 30 minutes of incubation in a dark room at room temperature. Inhibition of free radicals by DPPH in per cent was calculated as follows:

$$\%DPPH\ inhibitio = 100 - \frac{\{absorbance\ of\ sample - absorbance\ of\ blank\}}{control\ absorbance} x100$$

3.4 Experiment Two: Effects of Antioxidants Supplementation on Growth Performance, Haematology and Serum Biochemistry in Broiler Chickens

3.4.1 Broiler chicken managements and diets

The field experiment was conducted at Kwara State University Teaching and Research Farm, Malete. It is on a geographical location between latitude 08^{o} 71′ N to 08^{o} 96′ N and longitude 04^{o} 44′ E to 04^{o} 76′ E at 365 m above sea level. The climate of Malete is characterised by distinct wet and dry seasons with the annual mean rainfall of about 1,150 mm and an annual temperature that ranges from 25 – 28.9 ^{0}C (Olaniyan, 2003). A total of 300 day- old Abhor acre broiler chicks (mixed sex) of a commercial strain were used for this trial and reared under a deep litter system of production. Routine management and vaccination programmes were strictly adhered to. The broiler chicks were allowed access to feed and water *ad libitum* throughout the trial. The broiler birds were randomly assigned to five (5) treatment units using a completely randomised design. Each treatment unit was replicated three times with twenty (20) birds per replicate.

The birds were placed on formulated broiler starter diet containing 23 % CP and 2879 Kcal/kg ME (NRC, 2004) for the first four weeks and broiler finisher diet containing 20 % CP and 3000 kcal/kg ME (NRC, 2004) for the second four weeks. The composition and calculated analysis of the experimental diets for both starter and finisher stages are presented in Tables 3.1 and 3.2, respectively. The proximate analyses of the diets were carried out in accordance to the procedure outlined by Association of Official Analytical Chemists (AOAC, 2005), as shown in Appendix C.

3.4.2 Experimental design

A completely randomized design model with 5 treatments each replicated 3 times was used in this experiment. The treatments were based on the natural antioxidants supplementation in drinking water as stated below (USFDA, 2009)

1. Treatment one - Positive control (drinking water with 200 ppm butylated hydroxyanisole (BHA)/litre)
2. Treatment two - Negative control (drinking water with no antioxidant supplement)
3. Treatment three (drinking water with 200 ppm of sweet orange peel extract (SOPE)/litre)
4. Treatment four (drinking water with 200 ppm of shaddock peel extract (SHPE)/litre)
5. Treatment five (drinking water with 200 ppm of lemon peel extract (LMPE)/litre)

Key word: ppm (part per million)

1 mg is equivalent to 1 ppm.

USFDA, (2009) (United States Food and Drug Administration),

Table 3.1: Composition of broiler starter diet (% DM)

Ingredient	Composition %
Maize	57.50
Soybean meal	20.00
Groundnut cake	16.00
Fish meal	2.00
Bone meal	2.50
Limestone	1.00
Vitamin premix	0.25
Methionine	0.25
Lysine	0.25
Salt	0.25
Total (Kg)	100.00
Calculated analysis:	
CP %	23.00
ME Kcal/kg	2879.00
Crude Fibre %	3.91
Ether Extract %	4.63
Calcium %	1.18
Lysine %	1.31
Methionine + Cysteine%	0 .92
Available phosphorus %	0.50
Determined analysis	
Carbohydrate %	59.15
Crude protein %	22.55
Ether extract %	3.88
Crude fibre %	3.16
Ash %	6.12
Dry matter %	3.14
Moisture content %	2.00
Metabolisable energy (Kcal/kg)	2850.00

DM- Dry matter

Table 3.2: Composition of experimental broiler finisher diet (% DM)

Ingredient	Composition %
Maize	56.50
Palm oil	3.00
Wheat offal	2.00
Soybean meal	19.00
Groundnut cake	10.00
Fish meal	1.00
Bone meal	4.00
Oyster shell	3.50
Vitamin premix	0.25
Methionine	0.25
Lysine	0.25
Salt	0.25
Total (Kg)	100.00
Calculated analysis:	
CP %	20.00
ME Kcal/kg	3000.00
Crude fibre %	2.66
Ether extract %	4.64
Calcium %	2.75
Lysine %	0.93
Methionine + Cysteine%	0.47
Available phosphorus %	0.78
Determined analysis	
Carbohydrate %	61.05
Crude protein %	19.55
Ether extract %	3.98
Crude fibre %	2.16
Ash %	4.07
Dry matter %	2.14
Moisture content %	9.05
Metabolisable energy (Kcal/kg)	2998.50

DM- Dry matter

3.4.3 Data collection

3.4.3.1 Performance characteristics of the broiler chickens

Feed and water intake were recorded daily, weight gain was recorded weekly and feed conversion ratio (FCR) calculated. Nutrient digestibility was determined at 4^{th} and 8^{th} weeks in the second feeding trial. A quantity of 3 kg of feed was fed to the birds and faecal samples collected over a 72 hours' period. The excreta samples were weighed dried in the oven (Model: DHG-9053A, England) at 60 ^{o}C for 72 hrs, re-weighed, ground and proximate content analysed using (AOAC, 2005) procedure as was done for the feed.

$$Nutrient\ digestibility = \begin{pmatrix} nutrient\ consumed - nutrient \\ in\ faeces \end{pmatrix} x100$$

3.4.3.2 Physiological characteristics of the broiler chickens

3.4.3.2.1 Haematological parameters

At the end of the eight weeks feeding trial, three birds were randomly selected from each replicate for haematological and serum biochemistry test. The birds were fasted overnight, 3 ml of sample quantity of blood was collected via the left-wing veins into a bottle containing ethylene diamine tetra acetic acid (EDTA) and kept slant in crushed ice for 1 h, followed by centrifugation at 3000 g, 4 °C for 10 minutes. The recovered plasma fraction was separated into 1.5 ml aliquots and kept at 4 °C until analysis was performed to assess haematological parameters such as red blood cells (RBC), white blood cells (WBC), packed cell volume (PCV), and haemoglobin (Hb) concentration (Jain, 1986).

3.4.3.2.2 Serum biochemistry

Another 3 ml of blood sample from the same birds was collected into Vacutainer (BD Franklin Lakes, NJ, USA) serum tubes without anticoagulants and centrifuged for 10 minutes to separate the cell from the plasma before being refrigerated at - 4 °C for serum biochemical indices. The concentration of the purified serum protein fractions was used to determine;

(i) The total cholesterol, both the high-density lipoprotein (HDL) and low-density lipoprotein (LDL).

(ii) Triglycerides and

(iii) Total protein; both the Alanine amino transfarase (ALT) and Aspatate amino transfarase (AST), using the procedure described by Whitaker and Granum, (1980). A total of 0.02 ml serum was pipette into a cuvette, 0.01 ml biuret reagent was added, mixed, and incubated for 30 minutes between 20 and 25 degrees Celsius. Following that, 1.0 ml of blank reagent was produced and measured against water. Total protein = the absorbance of the sample - absorbance obtained.

3.4.3.2.3 Serum antioxidant enzyme activities

The serum from the above technique was also used to determine the serum biochemical parameters listed below: According to Cowell *et al*. (1994) serum catalase (CAT) activity was determined by detecting catalase degradation of H_2O_2 using a redox dye (ELISA Kit: QuantiChromTM, BioAssay Systems, USA, Catalog No. ECAT-100). The quantitative sandwich enzyme immunoassay technique is used in this assay. In each test plate (well), add 100 ml standard and plasma sample and incubate for 2 hours at 30 °C. Each well's liquid was withdrawn and left unclean. Each well received 100 l Biotin-antibody, which was incubated for 1 hour at 37 °C before being aspirated and cleaned three times. After

that, each well received 100 l HRP-avidin, which was incubated for 1 hour at 37 °C before being aspirated and washed 5 times. Each well was filled with 90 l TMB substrate and incubated for 15-30 minutes at 37 °C (protect from light). Each well received 50 l Stop solutions, and the colour development was completed in 5 minutes at 450 nm. The xanthine oxidase method (ELISA Kit: Cayman Chemical Company, USA, Catalog No. 706002) was used to test superoxide dismutase (SOD) activity, which checks the sample's inhibition of nitro blue tetrazolium reduction (Sun *et al*., 1988). The assay plasma samples and buffer were treated in a pre-coated assay plate (well) with SOD-HRP conjugate for 1 hour. The wells were decanted and washed five times after an hour. The wells were then treated with an HRP enzyme substrate. A blue-coloured complex was generated as a result of the enzyme-substrate reaction. To stop the reaction, a stop solution was added, which coloured the solution yellow.

In a microplate reader, the colour intensity was measured spectrophotometrically (Model T70 UV/VIS, PG Instrument Limited, UK.) at 450 nm. The colour intensity is inversely proportional to the concentration of SOD. The activity of glutathione peroxidase (GP_X) in the blood was measured using H_2O_2 and an electron donor dye that turns pink during the peroxide reaction (ELISA Kit: QuantiChrom^{TM}, BioAssay Systems, USA, Catalog No. DPOD-100), as described by Kokkinakis and Brooks, (1979). The GP_X ELISA kit uses a polyclonal anti- GP_X antibody and a GP_X -HRP conjugate to perform a competitive enzyme immunoassay. In a pre-coated assay plate (well), the assay plasma samples and buffer were treated for 1 hour with the GP_X -HRP conjugate. The wells were decanted and washed five times after the incubation period. The wells were then treated with an HRP enzyme substrate. A blue-coloured complex generated as a result of the enzyme-

substrate reaction. To stop the reaction, a stop solution was added, which coloured the solution pink. In a microplate reader, the colour intensity was measured spectrophotometrically (Model T70 UV/VIS, PG Instrument Limited, UK.) at 450 nm.

3.4.3.2.4 Statistical data analysis

Employing the statistical analysis of science program SAS, (2014) using analysis of variance (ANOVA), Data obtained from performance and physiological characteristics were submitted for analysis. At a 5 % significant level, the Tukey test was performed to separate the means.

3.5 Experiment Three: Influence of Antioxidants Supplementation on Carcass Traits and Meat Quality in Broiler Chickens

3.5.1 Experimental design

A completely randomized design model with 5 treatments each replicated 3 times was used in this experiment.

3.5.2 Carcass characteristics of the broiler chickens

Three birds from each replication (with no evident defects) were randomly selected at 8 weeks of age and slaughtered after fasting overnight, according to MSI 500:2009 Halal process (Department of standards Malaysia, 2009), deplumed and eviscerated. The carcass weight of each chicken was taken after the removal of the intestine and visceral organs. Weighed, documented, and recorded as a percentage of carcass weight were the primary cut components such as the thigh, drumstick, breast, back, and rib back. Internal organs were also calculated as a percentage of live weight, as well as the meat-to-bone ratio of the thigh and drumstick. The dressing percentage was calculated as the ratio of the carcass weight to the live weight of each chicken as shown in the equation below;

$$Dressing\ \% = \frac{CW}{LW} X100$$

CW = Carcass weight, LW = Live weight

3.5.3 Meat physical properties of the broiler chickens

The carcass dressed on day zero, after which 15 g of pectoralis major muscle was dissected from the outer surface of the breast meat of each of the chicken per replicate using the procedure described by Ishola *et al.* (2017), and divided into 3 equal parts. The first part and second part (50 g) were vacuum packaged (Petri dish) and stored in refrigerator at 4 ± 1 °C for meat quality (water holding capacity, shear force and colour coordinates) determination.

3.5.3.1 Water holding capacity of the broiler chickens

Drip loss was measured as described by Sabow *et al*. (2015). The fresh meat samples from the pectoralis muscle on day zero was weighed and recorded as initial weight (W1). The weighed samples were sealed in polyethylene plastic bags, labelled, and stored at 4 °C. The samples were retrieved from the bags after 2-, 4- and 6-days post mortem, gently blotted dried, weighed, and recorded as W 2. Drip loss was estimated and expressed as a percentage of the difference between the sample's original and final weight after storage, divided by the sample's starting weight, as given in the equation below:

Drip loss % = [W1- W2/W1] × 100

3.5.3.2 Colour coordinates (lightness, redness and yellowness) of the broiler chickens

The colour coordinates were determined in triplicate using Colour Flex Spectrophotometer (Hunter Lab Reston, VA, USA). The meat colour coordinates of pectoralis muscle samples from the treatments (BHA, OW, SOPE, SHPE, and LMPE)

were determined using the American Meat Science Association's (AMSA, 2012) method, as described by Sabow *et al*. (2015). A 1 cm height x 1 cm width x 2 cm length breast meat part from each of the five treatments was sliced and placed at the base of a Colour Flex spectrophotometer's colour flex cup with D65 illuminant and 10 degrees standard, tri stimulus values were taken for lightness (L*), redness (a*), and yellowness (b*) according to the International Commission on Illumination (CIE) (X, Y, Z) and reflectance at specific wavelength (400–700 nm). For each sample, triplicate readings for L*, a* and b* values were recorded and then averaged.

3.5.3.3 Shear force (tenderness) of the broiler chickens

The TA. HD plus® texture analyser (Stable Micro System, Surrey, UK) equipped with a Volodkevitch bite jaw was used to assess the texture of the treated breast meat. The equipment was calibrated to a weight of 5 kg, a height of 10 mm return distance, and a blade speed of 10 mm/sec. The protocol outlined by Sazili *et al*. (2005) was used to prepare the samples. Three replication blocks (1 cm height 1 cm width 2 cm length) were cut parallel to the direction of the muscle fibres from each sample, and each block was sheared perpendicular to the longitudinal direction of the fibres in the center. The average peak positive force values for all blocks of each treatment sample were reported as shear force.

3.5.4 Fatty acid composition of the broiler chickens

Total lipids were extracted from meat samples using a chloroform: methanol (2:1, v/v) mixture, as described by Adeyemi *et al*. (2015). According to AOAC (2007) guidelines, the fatty acids were transmethylated into fatty acid methyl esters (FAME) using 0.66 M potassium hydroxide (KOH) in methanol and 14 percent methanolic boron trifluoride

(BF3) and were separated in an Agilent 7890A gas chromatograph with a flame ionization detector (FID), which employs compressed air and high-quality hydrogen in the chromatograph. As a control, a heneicosanoic acid solution was employed. The fused silica capillary column was employed (Supelco SP-2560, 100 m, 0.25 mm ID, 0.20 mm film thickness).

At a rate of 40 ml/min, high purity nitrogen was employed as the carrier gas.

3.5.5 Sensory evaluation of the broiler chickens

According to Meilgaard *et al*, (2006), a consumer-type sensory evaluation was conducted. For each treatment, 20 grams of breast meat was trimmed of fat, labeled, and cooked in a water bath at 80 °C for 10 minutes. The meat samples were individually wrapped in aluminum foil and numbered. A consumer type sensory evaluation was conducted using thirty (30) assessors consisting of staff and students of Kwara State University, Malete, Nigeria. Assessors were taught on the sensory protocol and given characteristics to score using a 9-point hedonic scale (tenderness, juiciness, flavour, and overall acceptability) (Meilgaard *et al*., 2006). A score of nine denoted intense liking, while a score of one indicated extreme dislike.

3.5.6 Statistical data analysis

Employing the statistical analysis of science program SAS, (2014) using analysis of variance (ANOVA), Data obtained from physiological characteristics was submitted for analysis. At a 5% significant level, the Tukey test was performed to separate the means.

3.6 Experiment Four: Influence of Antioxidants Supplementation on Oxidative Stability of Broiler Chicken Meat

3.6.1 Materials and methods

Five birds per replicate were fasted overnight and slaughtered in accordance to the Halal procedure as outlined in MSI 500:2009 (Department of Standards Malaysia, 2009). A total of 5 g of pectoralis muscles from breast meat from three birds per treatment were divided into two parts, one of which was cooked in a microwave oven for five minutes while the other was left raw and stored in the refrigerator at 4 °C for oxidative stability (lipid and protein oxidation) testing.

3.6.2 Experimental design

The experiment was a 5*2*4 factorial arrangement in a completely randomised design with treatments (0 mg, 200 mg SOP, 200 mg SHP, 200 mg LMP and 200 mg BHA), meat state (cooked or raw) and storage period (day 0, 2, 4 and 6). Each treatment was replicated three times.

3.6.3 Lipid oxidation

Post rigor (cooked and raw) meat samples for the pectoralis muscles were refrigerated at 4 °C for 6 days, and lipid oxidation was measured using the QuantiChromTM TBARS (Thiobarbituric acid reactive substance) Assay Kit, DTBA-100, Bio-Assay Systems USA, according to the manufacturer's instructions (Adeyemi *et al*., 2016b). The raw and cooked meat sample treatments each contained two grams of meat. To prevent additional oxidation of the meat samples, they were re-suspended in PBS at 50 to 100 mg/ml. To reach the desired concentration, 100 ml of BHA solution was added. The meat samples were then homogenized in an ice-filled beaker using Teflon homogeniser (Model: Raider, Hamburg, Germany), then centrifuged for five minutes at 10,000 revolutions per minute to extract the supernatant. The TBARS content of the supernatant was then determined

directly (the supernatant and standard solution were reacted with thiobarbituric acid at 95 °C. After a 10-minute incubation period, the samples and standards were spectrophotometrically read at 532 nm wavelength (T70 UV/VIS, PG Instrument Limited, UK). Following that, the MDA value was compared to a pre-set MDA standard curve.

3.6.4 Protein oxidation

Carbonyl groups were calculated using the Protein Carbonyl Colorimetric Assay Kit cat # 10005020 (Cayman, USA) according to the manufacturer's instructions and expressed as nmol DNPH/mg protein as Adeyemi *et al*. (2016b) suggested. To collect the supernatant, the meat samples were homogenized in a beaker containing ice using Teflon homogeniser (Model: Raider, Hamburg, Germany) and centrifuged at 10,000 rpm for five minutes. Two aliquots of 1.5 ml of the resultant supernatant from each treatment were placed into micro-centrifuge tubes following centrifugation at 13,000 rpm for 20 minutes at 4 °C. For total protein content determination, the resulting supernatant was diluted 100-fold in extraction buffer (Bradford Assay). The residual supernatant was kept at -4 °C until examined.

3.6.4.1 Determination of protein concentration

Total protein concentration in samples supernatant was determined by Bradford Protein Assay Kit (Bio-Rad, USA). The Bradford protein test is a spectroscopic method for determining the amount of protein in a solution. 1.5 g/l test supernatants were diluted into six concentrations of 0.0, 0.1, 0.2, 0.3, 0.4, and 0.5 g/l in the same extraction buffer used for protein extraction to create the standard curve. In extraction buffer, protein samples

were diluted 100 times. In a 96-well plate with duplicate wells, 10 litre of each of the standards and samples were suspended, then 200 litre of Bradford dye reagent was added and properly mixed. The plates were stirred before being incubated at room temperature for 5 minutes. The absorbance was then measured at 595 nm using a spectrophotometer (GENESYSTM, Spectronic® 20; USA). Standard curve of each plate was constructed and used for the determination of total protein concentration of each sample supernatant.

3.6.5 Statistical data analysis

Employing the statistical analysis of science program SAS, (2014) using analysis of variance (ANOVA) General Model Procedure, in which treatments, state of meat samples, and the storage days' effects and their interactions were determined. At a 5 % significant level, the Tukey test was performed to separate the means.

3.7 Experiment Five: Effect of Graded Levels of Sweet Orange Peel Extracts on Performance, Meat Quality and Oxidative Stability of Broiler Chicken

3.7.1 Materials

Dietary graded levels of 0.04, 0.06 and 0.08 and 0.10 % of the extracts of the sweet orange peels were used in this experiment as a result of overwhelm best results of sweet orange extracts over other treatments obtained in experiments two, three and four based on the performance parameter indicators.

3.7.2 Methods

The experiment was conducted in the same location as in Experiment Two. A total number of 300-day old broiler chicks (mixed sex) Abhor acer strain were used for this trial under a deep litter system of production. Broiler chicken management and diets were adhered to as in experiment two.

3.7.3 Experimental design

Growth performance and meat quality determination were subjected to a completely randomised design with four (5) treatments in 3 replicates, with ten (20) birds per replicate. While the oxidative stability determination was done using a 5*2*4 (graded levels), (state of meat), (storage period), factorial arrangement in a completely randomised design.

3.7.4 Data collection

Data on growth performance was collected in line with the protocols in experiment two. Meat quality and oxidative stability parameters were also determined following the protocols in experiments three and four, respectively.

3.7.5 Statistical data analysis

All data obtained in completely randomised design experiments were subjected to analysis of variance (ANOVA) using statistical analysis of science package procedure of (SAS, 2014). While, data obtained in factorial arrangement in this experiment were subjected to analysis of variance (ANOVA) using the General Linear Model procedure of (SAS, 2014) in which treatments, state of meat samples, and the storage days' effects and their interactions were determined. Means were separated with Tukey HSD test at $p < 0.05$ level of significance.

CHAPTER FOUR

4.0 RESULTS

4.1 Phytochemical Compounds in Peel Extracts of Sweet Orange, Shaddock and Lemon Fruits

The results of the qualitative phytochemical screening of sweet orange, shaddock and lemon peel extracts are presented in Table 4.1. The results showed the presence of various secondary metabolites (saponin, tannins, phenolics, steroids, flavonoids, coumarins, terpenoids, glycosides, triterpenes and alkaloids). Sweet orange peel extract (SOPE) contained saponin, phenolics, steroids, flavonoids, coumarins, terpenoids, glycosides, triterpenes and alkaloids. Shaddock peel extracts (SHPE) contained saponin, tannins, phenolics, steroids, flavonoids, coumarins, terpenoids, triterpenes and alkaloids. While Lemon peel extracts (LMPE) contain tannins, phenolics, steroids, flavonoids, coumarins, glycosides, triterpenes and alkaloids. Phlobatannin is absent in all the peel extracts.

The quantitative results of phytochemical compounds in SOPE, SHPE and LMPE are presented in Table 4.2. There were significant differences ($p<0.05$) in all the phytochemical compounds measured in sweet orange, shaddock and lemon peel extracts. Sweet orange peel extract contains significantly higher steroids (34.43 mg/100g DW), flavonoids (161.82 mg/100g DW), terpenoids (17.09 mg/100g DW), glycosides (2.00 mg/100g DW), triterpenes (0.13 mg/100g DW) and alkaloids (32.44 mg/100g DW) than SHPE and LMPE. Shaddock peel extract (SHPE) had more saponin content (0.42

mg/100g DW) and phenolics (26.76 mg/100g DW) than SOPE and LMPE. Whereas, lemon peel extract (LMPE) had more tannins contents (1.74 mg/100g DW)

Table 4.1: Qualitative phytochemical screening of sweet orange peel, shaddock peel and lemon peel extracts.

Compounds	Extracts		
	SOPE	SHPE	LMPE
Saponin	+	+	-
Tannins	-	+	+
Phenolics	+	+	+
Phlobatanin	-	-	-
Steroids	+	+	+
Flavonoids	+	+	+
Coumarins	+	+	+
Terpenoids	+	+	-
Glycosides	+	-	+
Triterpenes	+	+	+
Alkaloids	+	+	+

+ = There is presence of the phytochemicals
- = Absence of the phytochemicals.
- SOPE – sweet orange peel extract
- SHPE – shaddock peel extract
- LMPE – lemon peel extract

and coumarins (0.05 mg/100g DW) than SOPE and SHPE (Table 4.2).

The results of the main effect of G1 – diphenyl 2- picrylhydrazyl (DPPH) inhibition of sweet orange, shaddock and lemon peel extracts (SOPE, SHPE and LMPE) are presented in Table 4.3. There were significant differences ($p<0.05$) in DPPH inhibition of the extracts and the concentration at different per cent inhibition level. Treatment SHPE had a higher per cent inhibition as compared to SOPE and LMPE, with SOPE having the least per cent inhibition. There were more concentration of the extracts SOPE, SHPE and LMPE measured of per cent inhibition level at 100. Whereas, the concentration of the extracts measured at inhibition level of 40 % was the lowest.

The results of the interactive effect among sweet orange, shaddock and lemon peel extracts and the concentration on the per cent inhibition are presented in Table 4.4. there were interactions ($p<0.05$) among the sweet orange, shaddock and lemon peel extracts and their concentrations measured at different per cent inhibition levels. At inhibition level measured at 20, 40 and 60 %, the concentration in mg/ml of shaddock peel extracts was significantly higher ($p<0.05$) than those of sweet orange and lemon peel extracts. However, at inhibition level measured at 80 and 100 per cent, the concentration in mg/ml of sweet orange peel extracts was significantly higher ($p<0.05$) than those of shaddock and lemon peel extracts. But at 100 per cent, the concentration of shaddock and lemon peel extracts were similar. Regardless of the extracts, the concentrations in mg/ml DPPH measured at inhibition level of 100 % was significantly higher ($p<0.05$) compared to concentrations of extracts measured at inhibition levels of 20, 40, 60 and 80 %.

Table 4.2: Quantitative phytochemical screening of sweet orange peel extracts, shaddock peel extracts and lemon peel extracts.

Compounds	Extracts				
	SOPE	SHPE	LMPE	SEM	P value
Saponin (mg/100 g DW)	0.19[b]	0.42[a]	0.00[c]	0.01	<0.0001
Tannins (mg/100 g DW)	0.00[c]	1.63[b]	1.74[a]	0.01	<0.0001
Phenolics (mg/ 100g DW)	2.68[b]	26.76[a]	1.79[c]	0.03	<0.0001
Steroids (mg/ 100g DW)	34.43[a]	25.60[c]	32.85[b]	0.02	<0.0001
Flavonoids (mg/ 100g DW)	161.82[a]	148.13[c]	160.34[b]	0.02	<0.0001
Coumarins (mg/ 100g DW)	0.03 [b]	0.01[c]	0.05[a]	0.01	<0.0001
Terpenoids (mg/ 100g DW)	17.09[a]	14.13[b]	0.00[c]	0.01	<0.0001
Glycosides (mg/ 100g DW)	2.00[a]	0.00[c]	0.88[b]	0.01	<0.0001
Triterpenes (mg/ 100g DW)	0.13[a]	0.11[c]	0.12[b]	0.01	<0.0001
Alkaloids (mg/ 100g DW)	32.44[a]	4.03[c]	6.55[b]	0.03	<0.0001

[a, b, c] means having different superscripts along the same row are significantly different ($p < 0.05$)

SOPE – sweet orange peel extract

SHPE – shaddock peel extract

LMPE – lemon peel extract

DW- dry weight, mg- milligram and g- gram

Table 4.3: G1 – diphenyl 2- picrylhydrazyl (DPPH) Activities of sweet orange peel, shaddock peel and lemon peel extracts.

Extracts	% DPPH Inhibition
SOPE	66.70^{c}
SHPE	83.68^{a}
LMPE	80.48^{b}
SEM	0.02
P- Value	<0.0001
% DPPH Inhibition	Concentration (mg/ml)
20	73.71^{d}
40	57.50^{e}
60	81.07^{c}
80	83.75^{b}
100	88.77^{a}
SEM	0.03
p-Value	<0.0001

^{a,b,c} means having different superscripts along the same column are significantly different ($p<0.05$)

SOPE – sweet orange peel extracts, SHPE – shaddock peel extracts, LMPE – lemon peel extracts. DPPH - 1, G1 – diphenyl 2- picrylhydrazyl

The result in Table 4.4 indicated that sweet orange peel extracts had the lowest concentration of 12.76 mg/ml measured at DPPH inhibition level of 40 per cent.

4.2 First Feeding Trial Results

Growth Performance

The results of dietary treatments on growth performance of the broiler chickens are presented in Table 4.5. There were no significant differences ($p>0.05$) on the initial body weight (IBW) of the birds across the treatments. However, significant differences ($p<0.05$) occurred on all the other growth parameters measured. The birds on LMPE treatment had higher ($p<0.05$) final body weight (FBW) and weight gain (WG) than other treatments. The FBW and WG of birds on BHA were higher ($p<0.05$) than those of the birds on SHPE which was in turn higher ($p<0.05$) that those of birds on SOPE treatment. The birds on OW treatment had the lowest FBW (2878.50 g/bird) and WG (50.67 g/bird/day) and were significantly lower ($p<0.05$) than all the other treatments. The feed intake (FI) followed the order of OW, SOPE, SHPE, LMPE and BHA, they were all significantly ($p<0.05$) different from one another. The water intake results showed that birds on OW, SOPE and LMPE were similar ($p>0.05$) but had higher ($p<0.05$) water intake when compared to those of birds on treatments BHA and SHPE which had similar values. The FCR of birds on BHA and LMPE were similar ($p>0.05$) they were, however, lower and better ($p<0.05$) than the other treatments. Birds on SHPE has better ($p<0.05$) FCR compared to birds on OW and SOPE treatments which had similar ($p>0.05$) values.

Table 4.4: Interactive effect between extracts and concentration on inhibition of sweet orange, shaddock and lemon peel extracts

Treatments	% DPPH Inhibition	Concentration (mg/ml)
SOPE	20	12.76^{n}
SHPE	20	79.83^{i}
LMPE	20	73.94^{l}
SOPE	40	63.37^{m}
SHPE	40	81.89^{f}
LMPE	40	77.85^{k}
SOPE	60	78.93^{j}
SHPE	60	83.50^{e}
LMPE	60	80.78^{h}
SOPE	80	85.21^{c}
SHPE	80	84.55^{d}
LMPE	80	81.50^{g}
SOPE	100	89.27^{a}
SHPE	100	88.67^{b}
LMPE	100	88.37^{b}
SEM		0.06
p-Value		<0.0001

^{a,b,c} means having different superscripts along the same column are significantly different ($p<0.05$)

SOPE – sweet orange peel extracts, SHPE – shaddock peel extracts, LMPE – lemon peel extracts. DPPH - 1, G1 – diphenyl 2- picrylhydrazy

The results of dietary antioxidants supplementation on the broiler chicken's haematological parameters are shown in Table 4.6. Dietary antioxidants supplementation showed a direct effect ($p<0.05$) on all the blood parameters measured, except the lymphocyte concentration distribution width (LCDW) that showed no significant differences ($p>0.05$). Birds on dietary treatment OW showed a higher ($p<0.05$) white blood cell (WBC) value compared to all the other treatments. Birds on SHPE treatment had higher ($p<0.05$) lymphocyte (LYM) value compared to other treatments. Birds on BHA, OW and SHPE treatments had similar ($p>0.05$) LYM values but were lower ($p<0.05$) than those on LMPE treatment.

Birds on SHPE treatment had higher ($p<0.05$) mid-sized cell (MID) value compared to other treatments. Similarly, birds on treatment LMPE have higher ($p<0.05$) MID value compared to BHA, OW and SOPE treatments. However, the MID values of birds on BHA and OW were similar ($p>0.05$) but significantly higher ($p<0.05$) than MID value of birds on treatment SOPE. Birds on BHA treatment had a higher ($p<0.05$) granulocyte (GRAN) value than all the other treatments which had similar ($p>0.05$) values. Dietary SHPE and LMPE treatments had similar effects ($p>0.05$) on the red blood cell (RBC) and haemoglobin (HGB) of the experimental birds but significantly higher ($p<0.05$) compared to other treatments. Similarly, dietary treatments OW and SOPE had the same effect ($p>0.05$) on the RBC and HGB of the experimental birds, however, their values were higher ($p<0.05$) than those of birds on BHA treatment. Birds on dietary treatment SHPE had higher ($p<0.05$) hemaloctil (HCT) compared to other treatments. Dietary OW and SOPE treatments had similar ($p>0.05$) effect on the HCT value but their values were lower ($p<0.05$) that those bird on LMPE treatment.

Table 4.5: Effect of dietary antioxidants on growth performance of broiler chickens

Parameter	Treatments (0.02 %)						
	BHA	OW	SOPE	SHPE	LMPE	SEM	P value
Initial body weight (g/b)	41.08	41.12	41.10	41.12	41.10	0.04	>0.93
Final body weight (g/b)	3337.00^{b}	2878.50^{e}	2888.50^{d}	3093.50^{c}	3390.00^{a}	0.58	<0.0001
Weight gain (g/b/d)	58.86^{b}	50.67^{e}	50.85^{d}	54.51^{c}	59.80^{a}	0.03	<0.0001
Feed intake (g/b/d)	89.86^{e}	93.27^{a}	92.34^{b}	90.93^{c}	90.48^{d}	2.71	<0.0001
Water intake (l/b/d)	0.24^{b}	0.25^{a}	0.25^{a}	0.24^{b}	0.25^{a}	0.02	>0.001
FCR	1.53^{c}	1.84^{a}	1.82^{a}	1.67^{b}	1.51^{c}	2.31	<0.0001

$^{a, b, c}$ means having different superscripts along the same row are significantly different ($P< 0.05$). FCR (feed conversion ratio).
BHA – butylated hydroxyanisole, OW – ordinary water, SOPE – sweet orange peel extract, SHPE – shaddock peel extract, LMPE – lemon peel extract
l – litre, g – gram, b – bird and d - day

The MCV results showed that birds on BHA, SOPE and LMPE had similar ($p>0.05$) values. Similarly, birds on OW and SOPE had similar ($p>0.05$) values. However, birds on BHA and LMPE had higher ($p<0.05$) MCV values than those birds on OW and SHPE. Birds on SHPE treatment had the lowest value. Birds on dietary treatment SHPE presented a higher ($p<0.05$) mean cell haemoglobin (MCH) than all the other treatments. Similarly, birds on LMPE had a higher ($p<0.05$) MCH value than those birds on BHA, OW and SOPE which had similar ($p<0.05$) MCH values.

Mean cell haemoglobin concentration (MCHC) results showed that birds on LMPE had the highest value and was significantly higher ($p<0.05$) than all the other treatments. Birds on OW and SOPE had similar ($p>0.05$) MCMC values their values were however, higher ($p<0.05$) than those of birds on BHA which in turn was significantly higher ($p<0.05$) than birds on SHPE treatment. Experimental birds on dietary treatments BHA, OW, SOPE and LMPE showed a higher ($p<0.05$) red blood cell (erythrocyte) distribution width (RDW-SD) compared to treatment SHPE. Birds on SHPE had higher ($p<0.05$) red blood cell (erythrocyte) concentration volume (RDW-CV) value compared to the other treatments. Birds on LMPE treatment had higher ($p<0.05$) RDW-CV value than those that were on OW and SOPE which had similar ($p>0.05$) values. Birds on BHA treatment had the least RDW-CV value. Birds on treatment BHA had the highest platelet (PLT) value and significantly higher ($p<0.05$) compared to birds on treatments SHPE, LMPE OW and SOPE which were also significantly different ($p<0.05$) in that order. Birds on OW, SOPE and SHPE had similar ($p>0.05$) mean platelet volume (MPV) values but were, however, higher ($p<0.05$) to those birds on BHA whose value was also higher ($p<0.05$) that those of the birds on LMPE treatment. Birds on LMPE treatment had a

higher ($p<0.05$) platelet distribution width (PDW) value compared to those birds on BHA, OW, SOPE and SHPE treatments. Similarly, birds on BHA had significantly higher ($p<0.05$) PDW value compared to those birds on OW, and SOPE which have similar ($p>0.05$) values. While birds on SHPE treatment had the lowest ($p>0.05$) PDW value. Birds on SHPE treatment had a highest platelet crit- large cell ratio (PLCR) value and significantly different ($p<0.05$) compared to those birds on OW, SOPE, BHA and LMPE treatments which were also significantly ($p<0.05$) different for each other in that order. Birds on SHPE treatment had a higher ($p<0.05$) packed cell volume (PCV) value compared to other treatments. However, birds on BHA, OW and SOPE treatments have similar ($p>0.05$) PCV values, their values were, however, higher ($p<0.05$) those birds on LMPE treatment.

The results of the effect of dietary butylated hydroxyanisole (BHA), water (OW), sweet orange, shaddock and lemon peel extracts (SOPE, SHPE and LMPE) on blood serum of broiler chickens are presented in Table 4.7. There were significant differences ($p<0.05$) in all the blood serum parameters measured. Birds on OW treatment had higher ($p<0.05$) total protein (TP) compared to those birds on SHEP, BHA, SOPE and LMPE which were also significantly different for each other in that order. Birds on SOPE treatment had a higher ($p<0.05$) total cholesterol (TC) compared to those on OW, LMPE, SHEP and BHA which were significantly ($P<0.05$) for each other in that order. The triglycerides (TRY) results showed significant ($p<0.05$) in all the treatments in the order of SHPE, OW, BHA, SOPE and LMPE. Similarly, the low-density lipoprotein concentration (LDL-C) results showed significant ($p<0.05$) variation among the different treatment in the order of OW, SHPE, SOPE, LMPE, and BHA.

Table 4.6: Effect of dietary antioxidants on haematological parameters of broiler chickens

Parameters	Treatments (0.02 %)						
	BHA	OW	SOPE	SHPE	LMPE	SEM	P value
WBC (10^9/l)	14.60^{c}	53.50^{a}	10.40^{d}	7.30^{e}	39.90^{b}	0.219	<0.0001
LYM (10^9/l)	8.00^{c}	12.20^{c}	7.00^{c}	35.20^{a}	26.10^{b}	1.267	<0.0001
MID (%)	1.30^{c}	1.80^{c}	0.30^{d}	10.40^{a}	7.10^{b}	0.148	<0.0001
GRAN (10^9/l)	1.10^{a}	0.40^{b}	0.40^{b}	0.20^{b}	0.05^{b}	0.118	0.0097
RBC (x 10^6/mm^3)	3.32^{c}	6.98^{b}	6.93^{b}	8.87^{a}	8.40^{a}	0.091	<0.0001
HGB (g/dl)	10.60^{c}	11.60^{b}	11.80^{b}	16.80^{a}	16.10^{a}	0.126	<0.0001
HCT (%)	31.80^{d}	34.80^{c}	35.40^{c}	50.40^{a}	48.30^{b}	0.167	<0.0001
MCV (fl)	63.80^{a}	56.80^{b}	60.00^{ab}	18.90^{c}	64.60^{a}	0.902	<0.0001
MCH (pg)	16.70^{c}	17.10^{c}	17.10^{c}	31.90^{a}	19.10^{b}	0.127	<0.0001
MCHC (g/l)	262.00^{c}	283.00^{b}	285.00^{b}	234.00^{d}	296.00^{a}	1.844	<0.0001
RDW-SD (%)	26.80^{a}	22.00^{a}	22.20^{a}	15.80^{b}	26.80^{a}	0.902	0.0021
RDW-CV (%)	13.40^{d}	14.80^{c}	14.80^{c}	50.90^{a}	15.90^{b}	0.167	<0.0001
PLT (10^9/l)	713.00^{a}	224.00^{d}	189.00^{e}	318.00^{b}	304.00^{c}	1.949	<0.0001
MPV (fl)	10.30^{b}	11.50^{a}	11.40^{a}	12.20^{a}	7.50^{c}	0.184	<0.0001
PDW (%)	8.90^{b}	7.90^{c}	7.90^{c}	5.30^{d}	9.80^{a}	0.148	<0.0001
PLCR (%)	50.10^{d}	62.90^{b}	60.40^{c}	66.60^{a}	17.70^{e}	0.195	<0.0001
PCV	0.19^{b}	0.24^{b}	0.24^{b}	0.85^{a}	0.03^{c}	0.015	<0.0001
LCDW	0.05	0.05	0.05	0.00	0.20	0.048	0.1689

$^{a, b, c}$ means having different superscripts along the same row are significantly different ($P< 0.05$). WBC (white blood cell), LYM (lymphocyte), MID (mid-sized cell), GRAN (granulocyte), RBC (red blood cell), HGB (haemoglobin), HCT (hemaloctil), MCV (mean corpuscular volume), MCHC (mean cell haemoglobin concentration), RDWSD (red blood cell (erythrocyte)distribution width), PLT (platelets), PCV (packed cell volume), MCH (mean cell haemoglobin), MPV (mean platelet volume), PDW (platelet distribution width), PLCR (platelet crit- large cell ratio), PCT (platelet crit). LCDW (lymphocyte concentration distribution width), BHA – butylated hydroxyanisole, OW – ordinary water, SOPE – sweet orange peel extracts, SHPE – shaddock peel extracts, LMPE – lemon peel extracts

Birds on dietary treatment SOPE was significantly higher ($p<0.05$) in high density lipoprotein concentration (HDL-C) compared to birds on other treatments. While birds on LMPE treatment had a higher ($p<0.05$) HDL-C value than those birds on OW treatment which in turn was significantly higher ($p<0.05$) than those birds on BHA treatment. Birds on SHPE treatment had the lowest ($p>0.05$) HDL-C value. Birds on OW treatment was significantly higher ($p<0.05$) in uric acid (UA) value compared to other treatments. Birds on SHPE treatment had a higher ($p<0.05$) UA value than those birds on BHA treatment which in turn had higher ($p<0.05$) UA value compared to birds in treatments SOPE and LMPE which had similar ($p>0.05$) UA values.

Aspartate amino transferase (AST) results showed that birds on dietary treatment OW was significantly higher ($p<0.05$) value compared to birds on other treatments. While birds on LMPE treatment had a higher ($p<0.05$) AST value than those birds on SHPE treatment which in turn was significantly higher ($p<0.05$) than those birds on SOPE treatment. Birds on BHA treatment had the lowest ($p>0.05$) AST value. Birds on SHPE treatment had significantly higher ($p<0.05$) alanine amino transferase (ALT) value compared to birds on other treatments. While birds on BHA treatment had a higher ($p<0.05$) ALT value than those birds on SOPE treatment which in turn was significantly higher ($p<0.05$) than those birds on OW treatment. Birds on LMPE treatment had the lowest ($p>0.05$) ALT value. Birds on SHPE treatment had significantly higher ($p<0.05$) superoxide dismutase (SOD) compared to birds on other treatments. While birds on OW treatment had a higher ($p<0.05$) SOD value than those birds on LMPE treatment which in turn was significantly higher ($p<0.05$) than those birds on SOPE treatment. Birds on

BHA treatment had the lowest ($p>0.05$) SOD value. Results showed that birds on BHA treatment had higher ($p<0.05$) in glutathione peroxidase (GP_X) compared to birds on other treatments. While birds on OW treatment had a higher ($p<0.05$) GP_X value than those birds on LMPE treatment which in turn had higher ($p<0.05$) GP_X value than those birds on SOPE treatment. Birds on SHPE treatment had the lowest ($p>0.05$) GP_X value. Glutathione S-transferase (GST) results indicated that birds on dietary treatment BHA had significantly higher ($p<0.05$) GST value compared to birds on OW, SOPE, SHPE and LMPE treatments. Birds on OW treatment had a higher ($p<0.05$) GST value compared to SOPE, SHPE and LMPE treatments. However, birds on SOPE, SHPE and LMPE treatments had a similar ($p>0.05$) GST value. Birds on LMPE treatment had significantly higher ($p<0.05$) serum catalase (CAT) compared to birds on other treatments. While birds on SHPE treatment had a higher ($p<0.05$) CAT value than those birds on SOPE treatment which in turn was significantly higher ($p<0.05$) than those birds on OW treatment. Birds on BHA treatment had the lowest ($p>0.05$) CAT value.

The results of effect of dietary butylated hydroxy anisole, water, sweet orange, shaddock and lemon peel extracts on carcass traits of broiler chicken are presented in Table 4.8. All the carcass parameters measured were influenced by the dietary treatments. Birds on LMPE treatment had a higher ($p<0.05$) live weight (LW) value compared to birds on other treatments. While the LW of birds on BHA treatment had a higher ($p<0.05$) value than those birds on SHPE treatment which in turn was significantly higher ($p<0.05$) than those birds on SOPE treatment. Birds on OW treatment had the lowest ($p>0.05$) LW value. Birds on LMPE treatment had a higher ($p<0.05$) bled weight (BW) value compared to birds on other treatments. While the BW of birds on BHA treatment had a

higher ($p<0.05$) value than those birds on SHPE treatment which in turn was significantly higher ($p<0.05$) than

Table 4.7: Effect of dietary antioxidants on blood serum parameters of broiler chickens

Parameters	Treatments (0.02 %)						
	BHA	OW	SOPE	SHPE	LMPE	SEM	P value
Total Protein (mg/dl)	8.18^{c}	8.87^{a}	7.71^{d}	8.47^{b}	7.54^{e}	0.021	<0.0001
Total Cholesterol (mg/dl)	366.91^{e}	433.62^{b}	468.16^{a}	383.58^{d}	426.47^{c}	0.015	<0.0001
Triglycerides (mg/dl)	116.60^{c}	130.10^{b}	112.45^{d}	133.9^{a}	104.15^{e}	0.111	<0.0001
LDL-C (mg/dl)	136.64^{e}	178.08^{a}	150.92^{c}	156.52^{b}	141.40^{d}	0.091	<0.0001
HDL-C (mg/dl)	191.40^{d}	212.17^{c}	279.76^{a}	182.43^{e}	250.35^{b}	0.095	<0.0001
UA (mg/dl)	2.48^{c}	2.69^{a}	2.34^{d}	2.57^{b}	2.29^{d}	0.015	<0.0001
AST (µ/l)	247.37^{e}	384.21^{a}	252.63^{d}	257.89^{c}	306.32^{b}	0.017	<0.0001
ALT (µ /l)	111.15^{b}	77.69^{d}	85.38^{c}	112.69^{a}	76.92^{e}	0.017	<0.0001
SOD (µ /l)	29.85^{e}	89.55^{b}	59.70^{d}	119.40^{a}	74.62^{c}	0.129	<0.0001
GP_X (µ /mg protein)	56.38^{a}	38.13^{b}	18.54^{d}	16.84^{e}	19.05^{c}	0.031	<0.0001
GST (nmole/CDMB)	71.57^{a}	61.34^{b}	20.45^{c}	20.45^{c}	20.45^{c}	0.034	<0.0001
CAT (µ /mg protein)	545.62^{e}	596.31^{d}	621.17^{c}	688.87^{b}	718.39^{a}	0.017	<0.0001

$^{a, b, c}$ means having different superscripts along the same row are significantly different ($p< 0.05$). T. Chl. (Total cholesterol), Trig. (Triglycerides), LDLP (low density lipoprotein), HDLP (high density lipoprotein), AST (aspartate amino transferase), ALT (alanine amino transferase), SOD (superoxide dismutase), GP_X (glutathione peroxidase), CAT (serum catalase), GST (glutathione S- transferase), UA (uric acid), BHA – butylated hydroxyanisole, OW – ordinary water, SOPE – sweet orange peel extracts, SHPE – shaddock peel extracts, LMPE – lemon peel extracts, µ/l- micron litre

those birds on OW and SOPE treatments. However, birds on OW and SOPE treatment had similar ($p>0.05$) BW values. Birds on BHA treatment had a higher ($p<0.05$) de-feathered weight (DFW) value compared to birds on other treatments. While the DFW of birds on LMPE treatment had a higher ($p<0.05$) value than those birds on SHPE treatment which in turn was significantly higher ($p<0.05$) than those birds on SOPE treatment. Birds on OW treatment had the lowest ($p>0.05$) DFW value. Birds on LMPE treatment had a higher ($p<0.05$) carcass weight (CW) value compared to birds on other treatments. While the CW of birds on BHA treatment had a higher ($p<0.05$) value than those birds on OW, SHPE and SOPE treatments. However, birds on treatments OW and SHPE had similar ($p>0.05$) CW values. Birds on SOPE treatment had the lowest ($p>0.05$) CW value.

Birds on OW treatment had a higher ($p<0.05$) dressing percentage (D %) value compared to birds on other treatments. While the D % of birds on LMPE treatment had a higher ($p<0.05$) value than those birds on BHA and SOPE and SHPE treatments. However, birds on treatments BHA and SOPE had a similar ($p>0.05$) D % values. Birds on SHPE treatment had the lowest ($p>0.05$) D % value. Birds on OW treatment had a higher ($p<0.05$) breast weight (BRW) value compared to birds on other treatments. While the BRW of birds on SOPE treatment had a higher ($p<0.05$) value than those birds on BHA treatment which in turn was significantly higher ($p<0.05$) than those birds on LMPE treatment. Birds on SHPE treatment had the lowest ($p>0.05$) BRW value. Birds on SOPE treatment had a higher ($p<0.05$) wing weight (WW) value compared to birds on other treatments. While the WW values of birds on SHPE and LMPE treatments were similar ($p>0.05$) but have higher ($p<0.05$) WW value than those birds on BHA treatment which

in turn had a higher ($p<0.05$) WW value than those birds on OW treatment. Birds on OW treatment had the lowest ($p>0.05$) WW value. Birds on SHPE treatment had a higher ($p<0.05$) thigh weight (TW) value compared to birds on other treatments. While the TW values of birds on SOPE have higher ($p<0.05$) TW value than those birds on BHA, OW and LMPE treatment. However, the TW values of birds on BHA, OW and LMPE treatments were similar significantly ($p>0.05$). Birds on SOPE and LMPE treatments had a similar ($p>0.05$) drumstick weight (DW) values but have significantly higher ($p<0.05$) DW values compared to birds on other treatments. While the DW values of birds on SHPE treatment was higher ($p<0.05$) than those birds on BHA and OW treatments. However, birds on BHA and OW treatments had a similar ($p>0.05$) DW values.

Birds on BHA and LMPE treatments had a similar ($p>0.05$) head weight (HW) values but have significantly higher ($p<0.05$) HW values compared to birds on other treatments. While the HW value of birds on SHPE treatment was higher ($p<0.05$) than those birds on SOPE and OW treatments. However, birds on SOPE treatment had a higher ($p<0.05$) HW value than those birds on OW treatment. Birds on OW treatment had the lowest ($p>0.05$) HW value. Birds on BHA treatment had a higher ($p<0.05$) leg weight (LGW) value compared to birds on other treatments. While the LGW of birds on LMPE treatment had a higher ($p<0.05$) value than those birds on SOPE treatment which in turn was significantly higher ($p<0.05$) than those birds on SHPE treatment. Birds on OW treatment had the lowest ($p>0.05$) LGW value. Birds on BHA treatment had a higher ($p<0.05$) neck weight (NW) value compared to birds on other treatments. While the NW of birds on LMPE treatment had a higher ($p<0.05$) value than those birds on OW treatment which in turn was significantly higher ($p<0.05$) than those birds on SOPE treatment. Birds on

SHPE treatment had the lowest ($p>0.05$) NW value. Birds on BHA treatment had a higher ($p<0.05$) liver weight (LIW) value compared to birds on other treatments. While the LIW of birds on LMPE treatment had a higher ($p<0.05$) value than those birds on SOPE treatment which in turn was significantly higher ($p<0.05$) than those birds on SHPE treatment. Birds on OW treatment had the lowest ($p>0.05$) LIW value. Birds on OW treatment had a higher ($p<0.05$) heart weight (HTW) value compared to birds on other treatments. While the HTW value of birds on SHPE was significantly higher ($p<0.05$) than those birds on BHA and SOPE treatments. However, birds on BHA and SOPE treatments have a similar ($p>0.05$) HTW value but have higher ($p<0.05$) HTW value than those birds on LMPE treatment. Birds on LMPE treatment had the lowest ($p>0.05$) HTW value. Birds on LMPE treatment had a higher ($p<0.05$) gizzard weight (GW) value compared to birds on other treatments. While the GW of birds on SHPE treatment had a higher ($p<0.05$) value than those birds on BHA treatment which in turn was significantly higher ($p<0.05$) than those birds on SOPE treatment. Birds on OW treatment had the lowest ($p>0.05$) GW value. Birds on BHA treatment had a higher ($p<0.05$) abdominal fat weight (AFW) value compared to birds on other treatments. While the AFW of birds on OW treatment had a higher ($p<0.05$) value than those birds on SHPE treatment which in turn was significantly higher ($p<0.05$) than those birds on LMPE treatment. Birds on SOPE treatment had the lowest ($p>0.05$) AFW value. Birds on SHPE treatment had a higher ($p<0.05$) intestinal weight (IW) value compared to birds on other treatments. While the IW of birds on BHA treatment had a higher ($p<0.05$) value than those birds on OW treatment which in turn was significantly higher ($p<0.05$) than

those birds on SOPE treatment. Birds on LMPE treatment had the lowest ($p>0.05$) IW value.

Table 4.8: Effect of dietary antioxidants on carcass traits of broiler chickens

Parameter	Treatments (0.02 %)						
	BHA	OW	SOPE	SHPE	LMPE	SEM	P value
Live weight (g)	3337.00^{b}	2878.00^{e}	2888.00^{d}	3093.00^{c}	3390.00^{a}	0.58	<0.0001
Bled weight (g)	3200.00^{b}	2770.00^{d}	2788.00^{d}	2990.00^{c}	3270.00^{a}	2.59	<0.0001
De-feathered weight(g)	3137.00^{a}	2578.00^{e}	2688.00^{d}	2843.00^{c}	3090.00^{b}	3.03	<0.0001
Carcass weight (g)	2387.00^{b}	2178.00^{c}	2088.00^{d}	2193.00^{c}	2466.00^{a}	9.89	<0.0001
Dressing (%)	71.53^{c}	75.68^{a}	72.30^{c}	70.90^{d}	72.74^{b}	0.11	<0.0001
Breast (%)	32.09^{c}	35.72^{a}	33.38^{b}	30.64^{e}	31.29^{d}	0.08	<0.0001
Wing (%)	10.33^{c}	9.71^{d}	11.21^{a}	11.07^{b}	11.10^{b}	0.1	<0.0001
Thigh (%)	14.26^{c}	14.48^{c}	15.12^{b}	16.10^{a}	14.39^{c}	0.58	<0.0001
Drumstick (%)	15.55^{c}	15.25^{c}	16.79^{a}	16.47^{b}	16.87^{a}	0.06	<0.0001
Head (%)	3.04^{a}	2.05^{d}	2.50^{c}	2.60^{b}	3.04^{a}	0.52	<0.0001
Shank (%)	5.52^{a}	3.68^{e}	5.00^{c}	4.94^{d}	5.12^{b}	0.13	<0.0001
Neck (%)	7.11^{a}	6.31^{c}	6.26^{d}	6.09^{e}	6.77^{b}	0.05	<0.0001
Liver (%)	2.10^{a}	1.33^{e}	1.83^{c}	1.60^{d}	1.88^{b}	0.03	<0.0001
Heart (%)	0.43^{c}	0.56^{a}	0.44^{c}	0.46^{b}	0.39^{d}	1.82	<0.0001
Gizzard (%)	1.34^{c}	1.20^{e}	1.22^{d}	1.77^{b}	1.89^{a}	0.08	<0.0001
Abdominal fat (%)	1.77^{a}	1.76^{b}	0.86^{e}	1.46^{c}	0.89^{d}	0.08	<0.0001
Intestinal weight (%)	3.14^{b}	2.95^{c}	2.69^{d}	3.23^{a}	2.67^{e}	0.1	<0.0001

$^{a, b, c}$ means having different superscripts along the same row are significantly different ($P<0.05$). BHA – butylated hydroxyanisole, OW – ordinary water, SOPE – sweet orange peel extracts, SHPE – shaddock peel extracts, LMPE – lemon peel extracts

The results of the effect of dietary BHA, OW, SOPE, SHPE and LMPE on the bird's meat quality is presented in Table 4.9. The results showed that dietary treatment influenced ($p<0.05$) the meat pH, lightness and redness. However, cooking loss, drip loss, WHC and yellowness were not affected ($p>0.05$) by the dietary treatments. Meat from birds on BHA treatment had the highest pH and was significantly higher ($p<0.05$) than other treatments. Meat from birds on treatments OW and SOPE had similar ($p>0.05$) pH value. The meat from birds on SOPE and SHPE also had similar ($p>0.05$) values. The lower pH was observed in meat from birds on LMPE treatment and was significantly lower ($p>0.05$) than those from BHA, OW, and SOPE treatments. Birds on dietary treatment OW and SHPE were significantly ($p<0.05$) lighter L* than other treatments. However, birds on SHPE treatment also showed some similarity ($p>0.05$) in its L* with birds on LMPE treatment. There were similarities ($p>0.05$) in the meat L* of birds on LMPE, BHA and SOPE treatments. However, the meats of birds on treatment LMPE were also slightly higher ($p<0.05$) in its L* value over the meats of birds on BHA and SOPE treatments.

The mechanical analysis and observation of dietary antioxidants effect on the tenderness of the experimental broiler meat are shown in Table 4.10. There were no significant differences ($p>0.05$) across the treatments on the stress peak, stress yield, width and thickness on the broilers' meat. However, force peak, force yield, strain peak and yield and Young's modulus were influenced ($p<0.06$) by dietary treatment. The positive control BHA (15.90 N) had higher force peak on the meat compared with other treatments. Birds on negative control OW (12.00 N) and LMPE (10.40 N) treatments had

similar (p>0.05) values. Their values were higher (p<0.05) than those on SOPE treatment which was in turn

Table 4.9: Effect of dietary antioxidants on meat quality (physicochemical properties) of broiler chicken's meat

Parameters	Treatments						
	BHA	OW	SOPE	SHPE	LMPE	SEM	P value
pH	5.6^{a}	5.4^{b}	5.35^{bc}	5.20^{cd}	5.05^{d}	0.03	0.0005
Cooking loss (%)	19.50	19.00	19.50	20.50	21.00	2.40	0.9705
Drip loss (%)	19.08	20.03	21.30	21.51	18.55	0.74	0.1212
WHC	25.10	26.20	26.80	26.80	24.20	0.64	0.1104
Lightness (L*)	20.03^{c}	38.23^{a}	18.47^{c}	34.62^{ab}	22.52^{bc}	2.733	0.0011
Redness(a*)	2.82^{b}	1.22^{b}	8.19^{a}	1.97^{b}	1.95^{b}	0.904	0.0017
Yellowness(b*)	5.33	4.67	3.12	5.11	2.33	0.827	0.1051

$^{a, b, c}$ means having different superscripts along the same row are significantly different ($p< 0.05$)
BHA – butylated hydroxyanisole, OW – ordinary water, SOPE – sweet orange peel extracts, SHPE – shaddock peel extracts, LMPE – lemon peel extracts
WHC- water holding capacity

higher ($p<0.05$) than those of birds on SHPE treatment. The negative control treatment OW (11.60) had the highest force yield and was significantly higher ($p<0.05$) than the other treatments. Birds on BHA and LMPE diets had similar ($p>0.05$) force yield values, their values were however higher ($p<0.05$) than those of birds on SOPE and SHPE diets which had similar ($p>0.05$) values. Birds on dietary treatment SHPE had a higher ($p<0.05$) strain peak value compared those birds on other treatments. While birds on BHA treatment was higher ($p<0.05$) in strain peak value compared to those birds on LMPE treatment which in

turn was significantly higher ($p<0.05$) in strain peak value to those in SOPE treatment.

Birds on SOPE treatment had a higher ($p<0.05$) strain peak value to those birds on OW treatment which had the lowest ($p>0.05$) strain peak value. Birds on dietary treatment BHA had a higher ($p<0.05$) strain yield value compared to those birds on other treatments. While birds on SHPE treatment was higher ($p<0.05$) in strain peak value compared to those birds on SOPE treatment which in turn were significantly higher ($p<0.05$) in strain yield value to those in LMPE treatment. Birds on LMPE treatment had a higher ($p<0.05$) strain yield value to those birds on OW treatment which had the lowest ($p>0.05$) strain yield value. Birds on dietary treatment OW had a higher ($p<0.05$) Young modulus value than those birds on other treatments. While birds on LMPE treatment was higher ($p<0.05$) in Young modulus value to those birds on BHA, SOPE and SHPE treatments. However, birds on BHA and SOPE treatments also showed some similarity ($p>0.05$) in their Young modulus values than those birds on SHPE treatment. Similarly,

birds on SOPE treatment also showed some similarity ($p>0.05$) in their Young modulus value with those birds on SHPE

Table 4.10: Effect of dietary antioxidants on meat quality (Tenderness) of broiler chickens

Parameters	Treatments (0.02 %)						
	BHA	OW	SOPE	SHPE	LMPE	SEM	P value
Force peak (N)	15.90^{a}	12.00^{b}	7.20^{c}	3.50^{d}	10.40^{b}	0.469	<0.0001
Force yield (N)	3.20^{b}	11.60^{a}	1.50^{c}	1.20^{c}	3.90^{b}	0.184	<0.0001
Stress peak(N/mm^2)	0.06	0.05	0.03	0.01	0.04	0.017	0.4029
Stress yield (N/mm^2)	0.01	0.05	0.01	0.01	0.02	0.01	0.1297
Strain peak (%)	71.91^{b}	10.18^{e}	52.31^{d}	95.09^{a}	59.22^{c}	0.013	<0.0001
Strain yield (%)	11.97^{a}	0.04^{e}	3.83^{c}	5.49^{b}	0.92^{d}	0.019	<0.0001
Young's modulus (N/mm^2)	0.1^{c}	2.13^{a}	0.05^{cd}	0.01^{d}	0.76^{b}	0.015	<0.0001
Width (mm)	25.00	25.00	25.00	25.00	25.00	1.0	1.0000
Thickness (mm)	10.00	10.00	10.00	10.00	10.00	1.0	1.0000

$^{a, b, c}$ means having different superscripts along the same row are significantly different ($p< 0.05$)
BHA – butylated hydroxyanisole, OW – ordinary water, SOPE – sweet orange peel extracts, SHPE – shaddock peel extracts, LMPE – lemon peel extracts

treatment. While, those birds on SHPE treatment had the lowest ($p>0.05$) Young modulus value.

The results of dietary antioxidants on the descriptive sensory perception of the broiler meat are as shown in Table 4.11. There were significant differences ($p<0.05$) in the taste, aroma and overall acceptability of the meat of birds fed dietary BHA, OW, SOPE, SHPE and LMPE treatments. The taste of meat for birds on BHA (7.40), OW (7.95), SOPE (8.00) and LMPE (7.75) treatments were not significantly different ($p>0.05$). Similarly, there were no significant differences ($p>0.05$) in the taste of meat of birds from BHA, OW, and SHPE treatments. However, meat from birds on SOPE and LMPE treatments had better ($p<0.05$) taste than those from SHPE treatment. The aroma of meat from birds on BHA (7.35), SOPE (7.80) and LMPE (7.45) treatments were not significantly different ($p>0.05$). Similarly, there were no significant differences ($p>0.05$) in the aroma of meat of birds from BHA, OW, and LMPE treatments. The aroma of meat from birds on dietary treatments BHA, OW and SHPE were significantly not different ($p>0.05$). However, meat from birds on SOPE treatment had higher ($p<0.05$) aroma than those from SHPE treatment. The overall acceptability of meat for birds on BHA (7.95), OW (7.15), SOPE (7.95) and LMPE (7.90) treatments were not significantly different ($p>0.05$). Similarly, there were no significant differences ($p>0.05$) in the overall acceptability of meat of birds from OW and SHPE treatments. However, meat from birds on BHA, OW, SOPE and LMPE treatments had higher ($p<0.05$) overall acceptability than those from SHPE treatment. There were no significant differences ($p>0.05$) on appearance and texture

sensory parameters across the treatments of the experimental broiler meat fed with dietary antioxidants.

Table 4.11: Effect of dietary antioxidants on sensory evaluation of broiler chickens.

Parameters	Treatments (0.02 %)						
	BHA	OW	SOPE	SHPE	LMPE	SEM	P value
Appearance	7.50	7.95	7.05	7.20	7.55	0.30	0.27
Taste	7.40^{ab}	7.00^{ab}	8.00^{a}	6.50^{b}	7.75^{a}	0.30	0.01
Texture	7.80	7.25	6.50	6.55	7.60	0.35	0.30
Aroma	7.35^{abc}	6.50^{bc}	7.80^{a}	6.30^{c}	7.45^{ab}	0.29	0.001
Overall Acceptability	7.95^{a}	7.15^{ab}	7.95^{a}	6.70^{b}	7.90^{a}	0.25	0.001

$^{a, b, c}$ means having different superscripts along the same row are significantly different ($p<0.05$). Those without superscripts are not significant

BHA – butylated hydroxyanisole, OW – ordinary water, SOPE – sweet orange peel extracts, SHPE – shaddock peel extracts, LMPE – lemon peel extracts

The results of the fatty acid profile of the broiler chicken fed dietary antioxidants shown in Table 4.12. revealed that all the fatty (Saturated, mono and poly unsaturated) acids were

influenced ($p<0.05$) by the treatments. Birds on dietary treatment OW had a higher ($p<0.05$) caproic acid (C6: O) value than those birds on other treatments. While birds on SHPE treatment was higher ($p<0.05$) in C6: O value to those birds on SOPE treatments. C6: O value of birds on SOPE treatment was higher ($p<0.05$) than birds on BHA treatment. Whereas, birds on BHA treatment were significantly higher ($p<0.05$) in C6: O than those birds in LMPE treatment which incidentally, had the lowest ($p>0.05$) C6: O value. Birds on dietary treatment OW had a higher ($p<0.05$) capric acid (C10:O) value than those birds on other treatments.

While birds on SHPE treatment was higher ($p<0.05$) in C10:O value to those birds on BHA treatments. C10:O value of birds on BHA treatment was higher ($p<0.05$) than those birds on LMPE treatment. Whereas, birds on LMPE treatment were significantly higher ($p<0.05$) in C10:O than those birds in SOPE treatment which had the lowest ($p>0.05$) C10:O value. Birds on dietary treatment SHPE had a higher ($p<0.05$) palmitic acid (C16:O) value than those birds on other treatments. While birds on BHA treatment was higher ($p<0.05$) in C16:O value to those birds on OW, SOPE and LMPE treatments. However, there were similarities ($p>0.05$) in the C16:O values of birds on dietary treatments OW, SOPE and LMPE. Birds on dietary treatment BHA had a higher ($p<0.05$) valeric acid (C5:O) value than those birds on other treatments. However, there were similarities ($p>0.05$) in the C5:O values of OW, SOPE, SHPE and LMPE treatments. Birds on dietary treatment SOPE had a higher ($p<0.05$) caprylic acid (C8:O) value than

those birds on other treatments. While birds on LMPE treatment was higher ($p<0.05$) in C8:O value to those birds on BHA treatments. C8:O value of birds on BHA treatment was higher ($p<0.05$) than those birds on SHPE treatment. Whereas, birds on SHPE treatment were significantly higher ($p<0.05$) in C8:O than those birds in OW treatment which had the lowest ($p>0.05$) C8:O value. Birds on dietary treatment SHPE had a higher ($p<0.05$) lauric acid (C12:O) value than those birds on other treatments. However, there were similarities ($p>0.05$) in the C12:O values of BHA, OW, SOPE and LMPE treatments. Birds on dietary treatment BHA had a higher ($p<0.05$) butyric acid (C4:O) value than those birds on other treatments. However, there were similarities ($p>0.05$) in the C4:O values of OW, SOPE, SHPE and LMPE treatments. Birds on dietary treatment OW had a higher ($p<0.05$) enanthic acid (C7:O) value than those birds on other treatments.

While birds on LMPE treatment was higher ($p<0.05$) in C7:O value to those birds on SHPE treatments. C7:O value of birds on SHPE treatment was higher ($p<0.05$) than those birds on SOPE treatment. Whereas, birds on SOPE treatment were significantly higher ($p<0.05$) in C7:O than those birds on BHA treatment which incidentally, had the lowest ($p>0.05$) C7:O value. Birds on dietary treatment LMPE had a higher ($p<0.05$) myristic acid (C14:O) value than those birds on other treatments. While birds on OW treatment was higher ($p<0.05$) in C14:O value to those birds on BHA treatment. C14:O value of birds on BHA treatment was higher ($p<0.05$) than those birds on SOPE and SHPE treatments. However, there were similarities ($p>0.05$) in C14:O values of SOPE and SHPE treatments. Birds on dietary treatment OW had a higher ($p<0.05$) propionic acid (C3:O) value than those birds on other treatments. However, there were similarities

($p>0.05$) in the C3:O values of BHA, SOPE, SHPE and LMPE treatments. Birds on dietary treatment SOPE had a higher ($p<0.05$) caproleic acid (C10:1Λ9) value than those birds on other treatments. While birds on LMPE treatment was higher ($p<0.05$) in C10:1Λ9 value to those birds on SHPE treatments. C10:1Λ9 value of birds on SHPE treatment was higher ($p<0.05$) than those birds on OW treatment. Whereas, birds on OW treatment was significantly higher ($p<0.05$) in C10:1Λ9 than those birds in BHA treatment which incidentally, had the lowest ($p>0.05$) C10:1Λ9 value. Birds on dietary treatment LMPE had a higher ($p<0.05$) palmitoleic acid (C16:1Λ9c) value than those birds on other treatments. While birds on OW treatment was higher ($p<0.05$) in C16:1Λ9c value to those birds on SOPE treatments. C16:1Λ9c value of birds on SOPE treatment was higher ($p<0.05$) than those birds on BHA and SHPE treatments. However, there were similarities ($p>0.05$) in C16:1Λ9c values of birds on BHA and SHPE treatments.

Birds on dietary treatment BHA had a higher ($p<0.05$) myristoleic acid (C14:1Λ9c) value than those birds on OW, SOPE, SHPE and LMPE treatments. However, there were similarities ($p>0.05$) in the C14:1Λ9c values of OW, SOPE, SHPE and LMPE treatments. Birds on dietary treatment SHPE had a higher ($p<0.05$) oleic acid (C18:1Λ9c) value than those birds on other treatments. Whereas, birds on LMPE treatment had a higher ($p<0.05$) C18:1Λ9c value than those birds on BHA treatment. Birds on BHA treatment was significantly higher ($p<0.05$) in C18:1Λ9c value than those birds in OW and SOPE treatments. However, there were similarities ($p>0.05$) in the C18:1Λ9c values of OW and SOPE treatments. Birds on dietary treatment LMPE had a higher ($p<0.05$) linoleic acid (C18:4(n-6)) value than those birds on other treatments. While birds on

SOPE treatment was higher ($p<0.05$) in C18:4(n-6) value to those birds on BHA treatments. C18:4(n-6) value of birds on BHA treatment was higher ($p<0.05$) than those birds on OW treatment. Whereas, birds on OW treatment was significantly higher ($p<0.05$) in C18:4(n-6) than those birds on SHPE treatment which incidentally, had the lowest ($p>0.05$) C18:4(n-6) value. Birds on dietary treatment OW had a higher ($p<0.05$) docosahexaenoic acid (C22:(6n-3)) value than those birds on BHA, SOPE, SHPE and LMPE treatments. However, there were similarities ($p>0.05$) in the C22:(6n-3) values of BHA, SOPE, SHPE and LMPE treatments. Birds on dietary treatment OW had a higher ($p<0.05$) acetic acid value than those birds on BHA treatment. The acetic acid value of birds on BHA treatment was higher ($p<0.05$) than those birds on SOPE, SHPE and LMPE treatments. However, there were similarities ($p>0.05$) in the acetic values of SOPE, SHPE and LMPE treatments. Birds on dietary treatment SHPE had a higher ($p<0.05$) eicosapentaenoic acid (ECA) and arachidonic acid (C20:4(n-6)) value than those birds on BHA, OW, SOPE and LMPE treatments. However, there were similarities ($p>0.05$) in the ECA and C20:4(n-6) values of BHA, OW, SOPE and LMPE treatments.

Regardless of the antioxidants fed, the most abundant fatty acid in the broiler meat was C10: O followed by C8: O. For saturated fatty acids like C6:O (7.71), C10:O (39.66), C7:O (29.48) and C3:O (3.90) in treatment OW, were significantly different ($p>0.05$) as compared to other treatments. While BHA varied significantly ($p>0.05$) in C5:O (2.96) and C4:O (4.18) when compared to treatments OW, SOPE, SHPE and LMPE. C8:O in SOPE (15.96) differ significantly ($p>0.05$) from treatments BHA, OW, SHPE and LMPE. Meanwhile, SHPE was the only treatment that contains C12:O also significantly ($p>0.05$) having higher amount of C16:O (4.25) compared to other treatments.

(C10:1Λ9), an example of mono unsaturated fatty acids in treatment SOPE was significantly different at p>0.05 compared to treatments BHA, OW, SHPE and LMPE. While treatment LMPE has more content of (C16:1Λ9) compared to other treatments. BHA was the only treatment with (C14:1Λ9c) (1.84), a mono unsaturated fatty acid and significantly different (p>0.05) from other treatments. Also, (C18:1Λ9c) of SHPE (6.00) is significantly different (p>0.05) compared to treatments BHA, OW, SOPE and LMPE. The poly unsaturated fatty acid content especially the (C18:4(n-6)) for LMPE (14.62) is significantly varied at p>0.05 to treatments BHA, OW, SOPE and SHPE. Whereas, treatment SHPE was the only treatment with (C20:4(n-6)) (2.13) and differed significantly (p>0.05) to other treatments.

The results of dietary treatments fed to the broiler chicken on the serum antioxidant enzyme activities in the broiler meat are presented in Table 4.13. The superoxide dismutase (SOD), serum catalase (CAT) and serum glutathione peroxidase (GP_X) of the meat of broiler chicken fed dietary antioxidants supplementation differ significantly (p<0.05) among the treatment means. Birds on dietary treatment SHPE had a higher (p<0.05) superoxide dismutase (SOD) value than those birds on other treatments. While birds on OW treatment was higher (p<0.05) in SOD value to those birds on BHA treatments. SOD value of birds on BHA treatment was higher (p<0.05) than those birds on LMPE treatment. Whereas, birds on LMPE treatment were significantly higher (p<0.05) in SOD value than those birds on SOPE treatment which incidentally, had the lowest (p>0.05) SOD value. Birds on dietary treatment BHA had a higher (p<0.05) serum catalase (CAT) value than those birds on other treatments. While birds on SOPE

treatment was higher ($p<0.05$) in CAT value to those birds on OW treatments. CAT value of birds on OW treatment was higher ($p<0.05$) than those birds on SHPE treatment. Whereas, birds on SHPE treatment were significantly higher ($p<0.05$) in CAT value than those birds on LMPE treatment which had the lowest

Table 4.12: Effect of dietary antioxidants on fatty acid profile of broiler chickens.

Parameters	Treatments (0.02 %)						
	BHA	OW	SOPE	SHPE	LMPE	SEM	P value
Saturated Fatty Acids							
Caproic acid (C6:O)	1.72^{d}	7.71^{a}	2.41^{c}	6.94^{b}	0.00^{e}	0.0007	<0.0001
Capric acid(C10:O)	25.28^{c}	39.66^{a}	12.10^{e}	30.99^{b}	20.19^{d}	0.027	<0.0001
Palmitic acid(C16:O)	0.79^{b}	0.00^{c}	0.00^{c}	4.25^{a}	0.00^{c}	0.0006	<0.0001
Valeric acid(C5:O)	2.96^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.0005	<0.0001
Caprylic acid(C8:O)	12.71^{c}	5.72^{e}	15.96^{a}	12.33^{d}	15.50^{b}	0.001	<0.0001
Lauric acid(C12:O)	0.00^{b}	0.00^{b}	0.00^{b}	2.00^{a}	0.00^{b}	0.0005	<0.0001
Butyric acid(C4:O)	4.18^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.0003	<0.0001
Enanthic acid(C7:O)	0.00^{e}	29.48^{a}	1.14^{d}	1.61^{c}	1.90^{b}	0.026	<0.0001
Myristic acid(C14:O)	1.01^{c}	1.48^{b}	0.00^{d}	0.00^{d}	3.07^{a}	0.007	<0.0001
Propionic acid(C3:O)	0.00^{b}	3.90^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.026	<0.0001
Mono Unsaturated Fatty Acids							
Caproleic acid (C10:1Λ9)	1.07^{e}	1.23^{d}	9.17^{a}	2.01^{c}	4.86^{b}	0.001	<0.0001
Palmitoleic acid (C16:1Λ9c)	0.00^{d}	1.69^{b}	1.29^{c}	0.00^{d}	3.50^{a}	0.004	<0.0001
Myristoleic acid (C14:1Λ9c)	1.84^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.0005	<0.0001
Oleic acid (C18:1Λ9c)	1.07^{c}	0.00^{d}	0.00^{d}	6.00^{a}	2.07^{b}	0.009	<0.0001
Poly Unsaturated Fatty Acids							
Linoleic acid (C18:4(n-6))	7.98^{c}	3.58^{d}	9.08^{b}	0.00^{e}	14.62^{a}	0.0005	<0.0001
DHA(C22:(6n-3))	0.00^{b}	0.84^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.0005	<0.0001

Acetic acid	0.93[b]	1.47[a]	0.00[c]	0.00[c]	0.00[c]	0.0006	<0.0001
ECA	0.00[b]	0.00[b]	0.00[b]	5.55[a]	0.00[b]	0.0005	<0.0001
Arachidonic acid(C20:4(n-6))	0.00[b]	0.00[b]	0.00[b]	2.13[a]	0.00[b]	0.0005	<0.0001

[a, b, c] means having different superscripts along the same row are significantly different ($p < 0.05$). BHA – butylated hydroxyanisole, OW – ordinary water, SOPE – sweet orange peel extracts, SHPE – shaddock peel extracts, LMPE – lemon peel extracts, DHA- Docosahexaenoic acids, ECA- Eicosapentaenoic acid

($p>0.05$) CAT value. Birds on dietary treatment BHA had a higher ($p<0.05$) glutathione peroxidase (GP_X) value than those birds on other treatments. While birds on SHPE treatment was higher ($p<0.05$) in GP_X value to those birds on LMPE treatments. GP_X value of birds on LMPE treatment was higher ($p<0.05$) than those birds on OW treatment. Whereas, birds on OW treatment was significantly higher ($p<0.05$) in GP_X value than those birds on SOPE treatment which had the lowest ($p>0.05$) GP_X value.

Table 4.14 shows the results of antioxidant dietary effect on state of meat and storage days on both the lipid and protein oxidation of broiler chicken meat. There was significant effect ($p<0.05$) on the lipid oxidation of the meat of the birds on BHA, OW, SOPE, SHPE and LMPE treatments. The lipid oxidation of meat for birds on SOPE and LMPE treatments were not significantly different ($p>0.05$). Similarly, there were no significant differences ($p>0.05$) in the lipid oxidation of meat of birds on BHA, OW, SHPE and LMPE treatments. However, meat from birds on SOPE treatments had higher ($p<0.05$) lipid oxidative values than those from BHA, OW and SHPE treatments. Dietary antioxidants had no significant effect ($p>0.05$) on post mortem protein oxidation of the broiler meat. There were significant differences ($p<0.05$) on the state of meat (cooked and raw) as a result of dietary treatments (BHA, OW, SOPE, SHPE and LMPE) fed to

the birds. The lipid oxidation of the cooked bird's meat was significantly higher ($p<0.05$) than the raw meat. Whereas, the protein oxidation of the raw bird's meat was significantly higher ($p<0.05$) than the cooked meat. The lipid oxidation of the bird's meat on post mortem storage day 6 was significantly higher ($p<0.05$) than other storage days. Whereas, meat on post mortem storage day 4 had a higher ($p<0.05$) lipid oxidative value than the meat on storage day 2. However, bird's

Table 4.13: Effect of dietary antioxidants on serum antioxidant enzyme activities of broiler chicken meat

Parameters	Treatments (0.02 %)						
	BHA	OW	SOPE	SHPE	LMPE	SEM	P value
SOD (U/l)	380.62^{c}	398.65^{b}	332.68^{e}	608.52^{a}	345.55^{d}	0.04	<0.0001
CAT (U/mg protein)	273.33^{a}	223.34^{c}	260.00^{b}	196.67^{d}	166.67^{e}	0.52	<0.0001
GP_X (U/mg protein)	850.37^{a}	723.92^{d}	709.70^{e}	828.81^{b}	816.16^{c}	0.07	<0.0001

$^{a, b, c}$ means having different superscripts along the same row are significantly different ($p< 0.05$). SOD (superoxide dismutase), CAT (serum catalase), GP_X (glutathione peroxidase)
BHA – butylated hydroxyanisole, OW – ordinary water, SOPE – sweet orange peel extract, SHPE – shaddock peel extract, LMPE – lemon peel extract

meat on storage day 2 had a higher ($p<0.05$) lipid oxidative value compared to the on-storage day 0, which has the lowest ($p>0.05$) lipid oxidative value. The protein oxidation of the bird's meat on post mortem storage day 6 was significantly higher ($p<0.05$) than other storage days. However, there were similarities in the protein oxidative values of bird's meat on post mortem storage day 2 and 4. Similarly, bird's meat on storage days 2 and 4 were significantly higher ($p<0.05$) in protein oxidative value than the meat on storage day 0, which incidentally had the lowest ($p>0.05$) protein oxidative value.

Table 4.15 shows the results of the interactive effect of dietary antioxidants supplementation on broiler chickens and their impact on the state of meat (cooked or raw) on both the lipid and protein oxidation of broiler meat. There were significant differences ($p<0.05$) of the treatments BHA, OW, SOPE, SHPE and LMPE on lipid and protein oxidative stability of the broiler meat. The lipid oxidative values of cooked bird's meat on BHA, OW, SOPE, LMPE and raw bird's meat on SOPE treatments were similar ($p>0.05$). There were similarities in the lipid oxidative values of cooked bird's meat on SHPE, raw bird's meat on BHA, OW, SOPE, SHPE and LMPE treatments. However, the lipid oxidation in cooked bird's meat on OW and SOPE treatments were significantly higher ($p<0.05$) than the raw bird's meat on BHA and OW treatments. The protein oxidative values of cooked bird's meat on SOPE, LMPE and raw bird's meat on BHA, OW, SOPE, SHPE and LMPE treatments were similar ($p>0.05$). There were similarities in the protein oxidative values of cooked bird's meat on BHA, OW, SOPE, SHPE, LMPE and raw bird's meat on OW treatments. However, the protein oxidation in raw bird's meat on SOPE and

Table 4.14: Main effect of dietary antioxidants, state of meat and storage days on lipid and protein oxidation of broiler chicken meat

Factors		TBARS (mgMDA/kg)	Carbonyl (nmol/mg protein)
Treatments	BHA	0.74^{b}	0.45
	OW	0.74^{b}	0.46
	SOPE	0.82^{a}	0.50
	SHPE	0.69^{b}	0.46
	LMPE	0.75^{ab}	0.50
	SEM	0.018	0.018
	P value	0.004	0.166
State of meat	Cooked	0.80^{a}	0.42^{b}
	Raw	0.70^{b}	0.53^{a}
	SEM	0.011	0.011
	P value	<0.0001	<0.0001
Storage days	0	0.19^{d}	0.26^{d}
	2	0.35^{c}	0.34^{c}
	4	0.51^{b}	0.42^{c}
	6	1.95^{a}	0.87^{a}
	SEM	0.016	0.016
	P value	<0.0001	<0.0001

$^{a, b, c}$ means having different superscripts along the same column are significantly different ($p< 0.05$)

BHA – butylated hydroxyanisole, OW – ordinary water, SOPE – sweet orange peel extract, SHPE – shaddock peel extract, LMPE – lemon peel extract

TBARS- Thiobarbituric acid reactive substance

Table 4.15: Interactive effect of dietary antioxidants and state of meat on lipid and protein oxidative stability of broiler chicken meat

Treatments	State of Meat	TBARS (mgMDA/kg)	Carbonyl (nmol/mg protein)
BHA	Cooked	0.81^{ab}	0.39^{c}
OW		0.83^{a}	0.42^{bc}
SOPE		0.87^{a}	0.44^{abc}
SHPE		0.69^{bc}	0.39^{c}
LMPE		0.81^{ab}	0.46^{abc}
BHA	Raw	0.67^{c}	0.52^{ab}
OW		0.66^{c}	0.51^{abc}
SOPE		0.77^{abc}	0.55^{a}
SHPE		0.69^{bc}	0.52^{ab}
LMPE		0.70^{bc}	0.55^{a}
SEM		0.0265	0.0245
P value		<0.0001	<0.0001

$^{a, b, c}$ means having different superscripts along the same column are significantly different ($p < 0.05$)

BHA – butylated hydroxyanisole, OW – ordinary water, SOPE – sweet orange peel extract, SHPE – shaddock peel extract, LMPE – lemon peel extract

TBARS - Thiobarbituric acid reactive substance

LMPE treatments were significantly higher ($p<0.05$) than the cooked bird's meat on BHA and SHPE treatments.

Table 4.16 shows the results of the effect of dietary antioxidants supplementation fed to broiler chickens on the storage days (0, 2, 4 and 6) of the broiler meat. Regardless of dietary antioxidants supplementation fed to broiler chickens, the lipid and protein oxidative stability on storage days (0, 2, 4 and 6) were significantly difference ($p<0.05$). Although, lipid oxidative stability of antioxidants supplementation fed to the broiler chickens on storage day (6) was not different significantly ($p>0.05$). The lipid oxidation of meat on SOPE treatment and storage day 4 was significantly higher ($p<0.05$) than the meat on other treatments and storage days. However, there were similarities ($p>0.05$) in the lipid oxidative values of bird's meat on SHPE and day 4, LMPE and day 4, OW and day 2, BHA, SOPE and LMPE day 2, and BHA and OW day 4.

Similarly, there were similarities in the lipid oxidation of bird's meat on LMPE day 0, BHA day 2 and 4, SOPE day 2, SHPE day 2, LMPE day 2, and OW day 4. There was also similarity in the lipid oxidative values of bird's meat on BHA day 0 and SOPE day 0. The lipid oxidative values of bird's meat on SHPE day 0 had the lowest ($p>0.05$) value. There were no significant differences ($p>0.05$) in the lipid oxidation of bird's meat on BHA, OW, SOPE, SHPE and LMPE on storage day 6. There were similarities ($p>0.05$) in the protein oxidation of bird's meat on OW, BHA, SOPE and SHPE on storage day 6. There was also similarity ($p>0.05$) in the protein oxidative values of bird's meat on SOPE, SHPE and LMPE day 6. There were also similarities ($p>0.05$) in the protein oxidative values of bird's meat on LMPE day 4 and 6. There were also similarities ($p>0.05$) in the protein oxidative values of bird's meat on LMPE and OW

treatments on day 4. Similarly, there were similarities ($p>0.05$) in the protein oxidation of bird's meats on SOPE on day 0, SHPE and LMPE on day 2, BHA on day 4. Similarities also ($p>0.05$) occurred in the protein oxidative values of bird's meats on OW, SHPE and LMPE on day 0, BHA, OW and SOPE treatments on day 2, SOPE and SHPE on day 4. Finally, the protein oxidative stability of bird's meat on OW day 6 was significantly higher ($p<0.05$) than the bird's meat on BHA day 0. The protein oxidation of the bird's meat on BHA day 0 had the lowest ($p>0.05$) value.

Table 4.17 shows the results of the interactive effect of state of meat (cooked or raw) and the storage days (0,2,4 and 6) and their impact on the lipid and protein oxidative stability of meats of broiler chickens fed dietary antioxidants supplementation. There were significant differences ($p<0.05$) on the state of meat (cooked or raw) and the storage days (0,2,4 and 6) on the broiler chicken meat fed dietary antioxidants supplementation. The lipid oxidation of both the cooked and raw bird's meat on storage day 6 were similar ($p>0.05$), but significantly higher ($p<0.05$) than both the cooked and raw bird's meat on storage days 0, 2 and 4. The lipid oxidative value of cooked bird's meat on storage day 4 was higher ($p<0.05$) than either cooked or raw bird's meat on storage days 0,2 and 4 There were also similarities ($p<0.05$) in the lipid oxidative values of both cooked bird's meats on storage day2 and raw meat on storage day 4. Similarly, similarities also occurred in the lipid oxidation of cooked meat on storage day 0, 2 and raw meat on storage day 2. However, lipid oxidation in both cooked and raw meat on day 6 were significantly higher ($p<0.05$) than the cooked meat on day 4 and raw meat on day 0. Lipid oxidation of raw bird's meat on storage day 0 had the lowest ($p>0.05$) value. The protein oxidative value of raw bird's meat on storage day 6 was higher ($p<0.05$) than either

cooked or raw bird's meat on storage days 0, 2, 4 and raw meat on storage day 6. The protein oxidative value of cooked bird's

Table 4.16: Interactive effect of antioxidants and storage days on lipid and protein oxidative stability of broiler chicken meat

Treatments	Storage days	TBARS (mgMDA/kg)	Carbonyl (nmol/mg protein)
BHA	0	0.24^{efg}	0.19^{f}
OW		0.14^{fg}	0.23^{ef}
SOPE		0.23^{efg}	0.40^{e}
SHPE		0.05^{g}	0.23^{ef}
LMPE		0.28^{def}	0.29^{ef}
BHA	2	0.30^{cdef}	0.34^{ef}
OW		0.48^{c}	0.27^{ef}
SOPE		0.37^{cde}	0.31^{ef}
SHPE		0.28^{def}	0.38^{e}
LMPE		0.31^{cdef}	0.39^{e}
BHA	4	0.44^{cd}	0.40^{e}
OW		0.39^{cde}	0.41^{de}
SOPE		0.75^{b}	0.38^{ef}
SHPE		0.49^{c}	0.35^{ef}
LMPE		0.49^{c}	0.59^{cd}
SEM		0.03	0.03
P Value		<0.0001	<0.0001
BHA	6	1.97	0.90^{ab}
OW		1.96	0.94^{a}
SOPE		1.93	0.92^{ab}
SHPE		1.95	0.87^{ab}
LMPE		1.95	0.74^{bc}
SEM		0.02	0.03

P value	>0.06	<0.0001

[a, b, c] means having different superscripts along the same column are significantly different ($p < 0.05$). BHA – butylated hydroxyanisole, OW – ordinary water, SOPE – sweet orange peel extract, SHPE – shaddock peel extract, LMPE – lemon peel extract

meat on storage day 6 was higher ($p<0.05$) than the raw meat on storage day 4. The protein oxidation of raw bird's meat on storage day 4 was higher ($p<0.05$) than the raw meat on storage day 2. There were similarities ($p<0.05$) in the protein oxidative values of both cooked bird's meat on storage day 0, 2,4 and raw meat on storage day 2. Similarly, similarities also occurred in the protein oxidation of raw meat on storage day 0 and cooked meat on storage days 0, 2 and 4. Protein oxidation of raw bird's meat on storage day 0 had the lowest ($p>0.05$) value.

The results of the interactive effect of treatments (BHA, OW, SOPE, SHPE and LMPE), state of meat (cooked or raw) and storage days (0, 2, 4 and 6) on lipid and protein oxidative stability of the broiler meat are shown in Table 4.18. There were significant differences ($p<0.05$) on lipid and protein oxidative stability of broiler meat fed dietary treatments (BHA, OW, SOPE, SHPE and LMPE) on state of meat (cooked or raw) and storage days (0, 2, 4 and 6). However, there were no significant differences ($p>0.05$) on protein oxidative stability of broiler chicken's meat fed dietary treatments for state of meat (raw) and storage days 4 and 6. Similarly, meat of broiler chickens fed dietary treatments (BHA, OW, SOPE, SHPE and LMPE) showed no significant difference ($p>0.05$) on lipid oxidative stability of the meat on state of meat (cooked and raw) and storage day 6. The interactive effects of the dietary SOPE were significantly higher ($p<0.05$) in the lipid oxidation on raw bird's meats on storage day 4 compared to BHA cooked bird's meat on day 0, OW and SOPE raw bird's meat on day 0. The interactive effects of the dietary SOPE raw bird's meat on day 4 were similar ($p>0.05$) in lipid

oxidative value of LMPE cooked bird's meat on day 4. However, there were similarities ($p>0.05$) in the interactive effect of

Table 4.17: Interactive effect of state of meat and storage days on lipid and protein oxidative stability of broiler chicken meat

State of Meat	Storage Days	TBARS (mgMDA/kg)	Carbonyl (nmol/mg protein)
Cooked	0	0.28^{d}	0.28^{de}
	2	0.37^{cd}	0.31^{de}
	4	0.57^{b}	0.31^{de}
	6	1.98^{a}	0.78^{b}
Raw	0	0.09^{e}	0.25^{e}
	2	0.32^{d}	0.36^{d}
	4	0.46^{c}	0.54^{c}
	6	1.92^{a}	0.97^{a}
SEM		0.0245	0.0237
P value		<0.0001	<0.0001

$^{a, b, c}$ means having different superscripts along the same column are significantly different ($p< 0.05$)
BHA – butylated hydroxyanisole, OW – ordinary water, SOPE – sweet orange peel extract, SHPE – shaddock peel extract, LMPE – lemon peel extract
TBARS - Thiobarbituric acid reactive substance

the dietary BHA, OW and SOPE cooked bird's meat on storage day 4. Similarly, there was also similarities ($p>0.05$) in the interactive effects of dietary BHA, OW, SOPE, SHPE and

LMPE on the lipid oxidation of both cooked and raw bird's meat on storage days 0, 2 and 4. Furthermore, there were also similarities ($p>0.05$) in the interactive effects of dietary BHA, OW, SOPE, SHPE and LMPE on the lipid oxidation of cooked meats on days 0 and 2, and raw bird's meat on storage days 2 and 4. There were also similarities ($p>0.05$) in the interactive effects of dietary OW and LMPE on the lipid oxidation of raw bird's meat on storage days 0 and 4. There was similarity ($p>0.05$) in the interactive effects of dietary BHA and SHPE on the lipid oxidation of raw bird's meat on storage day 0. The interactive effect of dietary OW, SOPE and SHPE on the lipid oxidation of both cooked and raw bird's meat, storage day 0 had the lowest ($p>0.05$) value.

Regardless of the dietary treatments, there were no significant differences ($p<0.05$) in the lipid oxidation of both cooked and raw bird's meat on storage day 6. The interactive effects of the dietary OW and SOPE cooked bird's meat on day 6 were similar ($p>0.05$) in the protein oxidative value. The was a similarity ($p>0.05$) in the interactive effects of the dietary BHA and SHPE cooked bird's meat on day 6. There was a significant difference ($p<0.05$) in the interactive effect of the dietary LMPE cooked bird's meat on storage day 6 as compared to the protein oxidation of LMPE cooked bird's meat on storage day 4. Similarly, there were also similarities ($p>0.05$) in the interactive effects of protein oxidation of cooked bird's meat on dietary SOPE day 0, raw meat on SOPE

treatment storage day 0, cooked meat on SHPE and LMPE storage day 2, raw meat on BHA, SOPE, SHPE and LMPE storage day 2. BHA, OW, SOPE, SHPE and LMPE on the protein oxidation of both cooked and raw bird's meat on storage days 0, 2 and 4. Furthermore, there were also similarities ($p>0.05$) in the interactive effects of protein oxidation of cooked bird's meat on dietary SHPE and LMPE on day 0, raw meat on LMPE treatment of storage day 0, cooked meat of BHA, OW and SOPE treatments on storage day 2, raw meat of OW on storage day 2, cooked meats of BHA and OW on storage day 4. There were also similarities ($p>0.05$) in the interactive effects of protein oxidation of both cooked and raw bird's meat on dietary OW storage day 0, raw meat on SHPE treatment storage day 0 and cooked meat on SOPE storage day 4. Finally, there were also similarities ($p>0.05$) in the interactive effects of protein oxidation of both cooked and raw bird's meat on dietary BHA on storage day 0 and cooked meat of chickens on SHPE storage day 4. Interactive effect of both cooked and raw bird's meat on BHA treatment storage day 0 and cooked bird's meat on SHPE treatment storage day 4 had a lower ($p>0.05$) protein oxidative value. There were no significant differences ($p>0.05$) in the protein oxidation of raw bird's meat from BHA, OW, SOPE, SHPE and LMPE treatments on storage days 4 and 6.

Table 4.18: Interactive effect of dietary antioxidants, state of meat and storage days on lipid and protein oxidative stability of broiler chicken meat.

Factors		Treatments											
State of meat	Storage days	BHA		OW		SOPE		SHPE		LMPE		P VALUE	
		TBARS	CARB	TBARS	CARB	TBARS	CARB	TBARS	CARB	TBARS	CARB	TBARS	CARB
Cooked	0	0.37^{cde}	0.18^{h}	0.28^{def}	0.24^{gh}	0.46^{cde}	0.43^{efg}	0.01^{g}	0.25^{fgh}	0.30^{def}	0.30^{fgh}	<0.0001	<0.001
Raw	0	0.11^{fg}	0.19^{h}	0.05^{g}	0.21^{gh}	0.05^{g}	0.35^{efg}	0.08^{fg}	0.21^{gh}	0.25^{efg}	0.28^{fgh}	<0.0001	<0.0001
Cooked	2	0.31^{def}	0.30^{fgh}	0.50^{cde}	0.25^{fgh}	0.46^{cde}	0.25^{fgh}	0.28^{def}	0.38^{efg}	0.32^{def}	0.39^{efg}	<0.0001	<0.0001
Raw	2	0.28^{def}	0.37^{efg}	0.46^{cde}	0.29^{fgh}	0.28^{def}	0.36^{efg}	0.28^{def}	0.38^{efg}	0.30^{def}	0.38^{efg}	<0.0001	<0.0001
Cooked	4	0.56^{cd}	0.25^{fgh}	0.56^{cd}	0.28^{fgh}	0.58^{cd}	0.21^{gh}	0.50^{cde}	0.15^{h}	0.63^{bc}	0.64^{bcd}	<0.0001	<0.0001
Raw	4	0.32^{def}	0.54	0.22^{efg}	0.54	0.92^{b}	0.54	0.48^{cde}	0.54	0.34^{cde}	0.54	<0.0001	0.84
Cooked	6	1.98	0.82^{abc}	1.98	0.90^{ab}	1.98	0.88^{ab}	1.98	0.79^{abc}	1.98	0.50^{def}	0.92	<0.0001
Raw	6	1.95	0.98	1.94	0.98	1.88	0.95	1.91	0.94	1.91	0.98	0.92	0.84

$^{a, b, c}$ means having different superscripts along the row are significantly different ($p< 0.005$)

BHA – butylated hydroxy anisole, OW – Ordinary water, SOPE – sweet orange peel extract, SHPE – shaddock peel extract, LMPE: - lemon peel extract, TBARS – thiobarbituric acid reactive substance (mgMDA/kg), CARB – Carbonyl (nmol/mg protein)

4.3 Second Feeding Trial Results

Growth performance

The results of dietary graded levels of sweet orange peel extracts (SOPE) and butylated hydroxy anisole (BHA) on growth performance of the broiler chickens are presented in Table 4.19. Regardless of the birds on dietary treatments BHA, SOPE (0.04, 0.06, 0.08 and 0.10 %), there were significant differences ($p<0.05$) on the final body weight (FBW), weight gain (WG), feed intake (FI), water intake (WI) and feed conversion ratio (FCR). There were no significant differences ($p>0.05$) on the initial body weight (IBW) of the birds across the treatments. Birds on dietary SOPE (0.04 %) treatment had a higher ($p<0.05$) FBW and WG value than birds on other treatments. Birds on SOPE (0.10 %) was significantly higher ($p<0.05$) in FBW and WG than those birds on BHA treatment, which in turn was higher ($p<0.05$) than those birds on SOPE (0.08 %). Birds on SOPE (0.08 %) treatment had a higher ($p<0.05$) FBW and WG values than birds on SOPE (0.06 %). However, birds on SOPE (0.06 %) treatment had the lowest ($p<0.05$) FBW and WG values. Birds on dietary SOPE (0.04 %) treatment had a higher ($p<0.05$) FI value than birds on other treatments. Birds on SOPE (0.08 %) was significantly higher ($p<0.05$) in FI than those birds on SOPE (0.10 %) treatment, which in turn was higher ($p<0.05$) than those birds on BHA treatment. Birds on BHA treatment had a higher ($p<0.05$) FI value than birds on SOPE (0.06 %). However, birds on SOPE (0.06 %) treatment had the lowest ($p<0.05$) FI value. Birds on dietary SOPE (0.04, 0.08 and 0.10 %) treatment had a similar ($p<0.05$) WI value and were significantly higher ($p<0.05$) in WI than those birds on SOPE (0.06 %) treatment. Birds on SOPE (0.06 %) treatment had a higher ($p<0.05$) WI value compared to those birds on BHA treatment. However, birds on BHA treatment

had the lowest ($p>0.05$) WI value. There were similarities ($p<0.05$) in the FCR of the birds on SOPE (0.06 and 0.08 %) treatments but were significantly higher ($p<0.05$) in FCR than those birds on BHA, SOPE (0.04 and 0.10 %) treatments. Similarly, birds on BHA, SOPE (0.04 and 0.10 %) treatments had similar ($p>0.05$) FCR values.

The result of the effect of graded levels of sweet orange peel extract supplementation on residual nutrient retention of broiler chicken at 4^{th} week of age is presented on Table 4.20. Regardless of the dietary treatments fed to the experimental birds, there were significant differences ($p<0.05$) in the dry matter (DM), total protein (TP), crude fibre (CF), crude lipids (CL), ash (AS), caloric value (CV) and carbohydrate (CHO) of the retained nutrients. Birds on BHA, SOPE (0.04, 0.06 and 0.08 %) treatments had similar ($p>0.05$) DM values but were significantly higher ($p<0.05$) in DM values compared with those birds on SOPE (0.10 %) treatment. However, birds on SOPE (0.10 %) treatment had the lowest ($p>0.05$) DM value. Birds on BHA, SOPE (0.04 and 0.06 %) treatments had similar ($p>0.05$) TP values but were significantly higher ($p<0.05$) in TP values compared with those birds on SOPE (0.08 and 0.10 %) treatments. Similarly, birds on SOPE (0.08 and 0.10 %) treatments had similar ($p>0.05$) TP values. However, birds on SOPE (0.08 and 0.10 %) treatments had the lowest ($p>0.05$) TP values. Birds on SOPE (0.10 %) treatment had a higher ($p<0.05$) CF value compared to those birds on BHA, SOPE (0.04, 0.06 and 0.08 %) treatments, which were in turn not significantly ($p<0.05$) different from each other. There was similarity ($p>0.05$) in the CL value of the birds on SOPE (0.04 %) and BHA treatments. Similarly, there were similarities ($p>0.05$) in the CL value of the birds on SOPE (0.08 and 0.10 %)

Table 4.19: Effect of dietary graded levels of antioxidants on growth performance of broiler chickens

Parameter	Treatments (%)						
	BHA (0.02)	SOPE (0.04)	SOPE (0.06)	SOPE (0.08)	SOPE (0.10)	SEM	P value
Initial body weight (g/b)	41.10	41.12	41.14	41.13	41.13	0.01	>0.56
Final body weight (g/b)	3300.00^{c}	3500.00^{a}	3200.00^{e}	3250.00^{d}	3400.00^{b}	7.30	<0.0001
Weight gain (g/b/d)	58.19^{c}	61.77^{a}	56.41^{e}	57.30^{d}	59.98^{b}	0.03	<0.0001
Feed intake (g/b/d)	96.74^{d}	101.73^{a}	96.32^{e}	99.17^{b}	98.18^{c}	0.01	<0.0001
Water intake (l/b/d)	0.25^{c}	0.27^{a}	0.26^{b}	0.27^{a}	0.27^{a}	0.01	<0.0001
FCR	1.66^{b}	1.65^{b}	1.71^{a}	1.73^{a}	1.64^{b}	0.01	<0.0001

$^{a, b, c}$ means having different superscripts along the same row are significantly different ($p < 0.05$)

BHA – Butylated hydroxyanisole, SOPE – Sweet orange peel extract, FCR – Feed conversion ratio, g - gram, b - bird, d - day

Gut Morphology

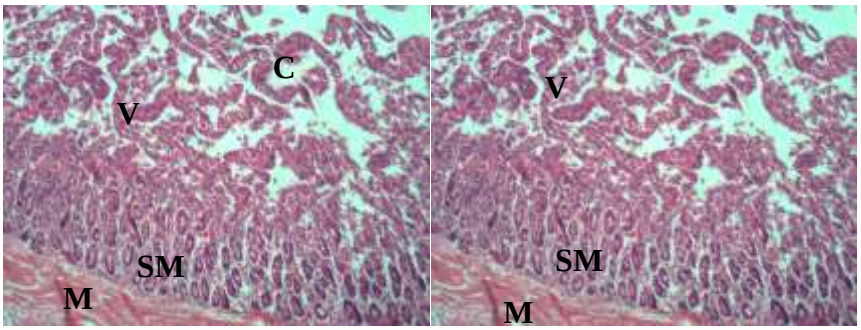

Plate I: (SM – Submucosa, M – Mucosa, : C - crypts of Lieberkuhn, V – villi) (Haematoxylin and Eosin x40).

Plate I: Representative photomicrograph of gut of experimental animal on BHA (0.02 %) treatment showing typical gut morphology with characteristic staininng properties. The cellularity and morphological delineation apear normal.

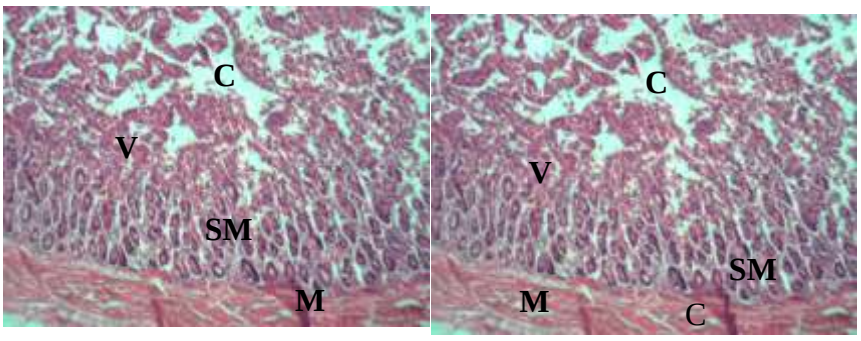

Plate II: (SM – Submucosa, M – Mucosa, : C - crypts of Lieberkuhn, V – villi) (Haematoxylin and Eosin x40).

Plate II: Representative photomicrograph of gut of experimental animal on SOPE (0.04 %) treatment showing typical gut morphology with characteristic staininng properties.

The cellularity and morphological delineation apear normal. The crypt of Lieberkuhn appear longer compared to treatments BHA.

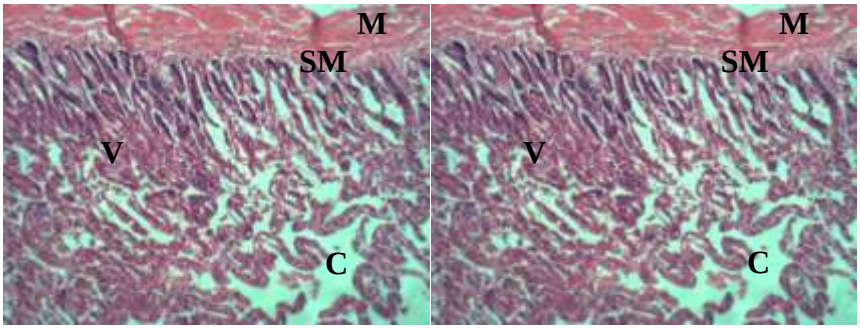

Plate III: (SM – Submucosa, M – Mucosa, : C - crypts of Lieberkuhn, V – villi) (Haematoxylin and Eosin x40).

Plate III: Representative photomicrograph of gut of experimental animal on SOPE (0.06 %) treatment showing typical gut morphology with characteristic staininng properties. The cellularity appear normal. the crypt of Lieberkuhn appear longer compared to treatment BHA.

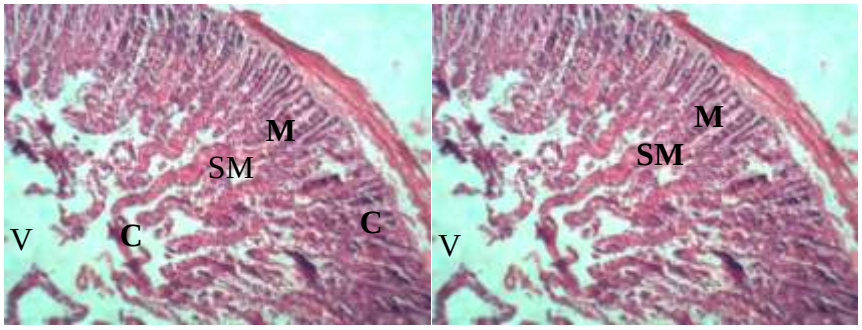

Plate IV: (SM – Submucosa, M – Mucosa, : C - crypts of Lieberkuhn, V – villi) (Haematoxylin and Eosin x40).

Plate IV: Representative photomicrograph of gut of experimental animal on SOPE (0.08 %) treatment showing typical gut morphology with characteristic staininng properties. The cellularity appear normal, the crypt of Leiberkuhn appear longer compared to treatment BHA.

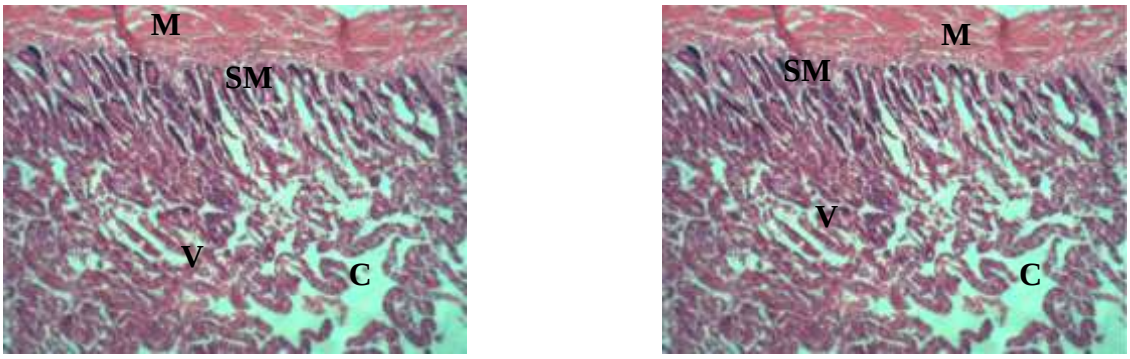

Plate V: (SM – Submucosa, M – Mucosa, : C - crypts of Lieberkuhn, V – villi) (Haematoxylin and Eosin x40).

Plate V: Representative photomicrograph of gut of experimental animal on SOPE (0.10 %) treatment showing typical gut morphology with characteristic staininng properties. The cellularity appear normal, the crypt of Leiberkuhn appear longer compared to treatment BHA.

treatments. Furthermore, birds on SOPE (0.04 %) treatment had a higher ($p<0.05$) CL value than those birds on SOPE (0.06 %) treatment. However, birds on SOPE (0.06 %) treatment had the lowest ($p>0.05$) CL value. Birds on dietary SOPE (0.06, 0.08 and 0.10 %) treatments had similar ($p>0.05$) AS values and are significantly higher than those birds on SOPE (0.04 %) treatment, which the birds on BHA had the least AS value of 1.16 %. Birds on dietary BHA treatment had a higher ($p<0.05$) CV value than those birds on any SOPE treatment. Whereas, birds on SOPE (0.04 %) were significantly higher ($p<0.05$) in CV value than those birds on SOPE (0.08 and 0.10 %). However, birds on SOPE (0.08 and 0.10 %) treatments were similar ($p>0.05$) in CV values, but were significantly higher ($p<0.05$) in CV value compared to those birds on SOPE (0.06 %) treatment. Birds on SOPE (0.06 %) treatment had the lowest ($p>0.05$) CV value. Birds on dietary BHA, SOPE (0.08 and 0.10 %) treatments were similar ($p>0.05$) CHO values and are significantly higher than those birds on SOPE (0.04 %) treatment. Whereas, birds on SOPE (0.04 %) treatment had a higher ($p<0.05$) CHO value than those birds on SOPE (0.06 %) treatment. However, birds on SOPE (0.06 %) treatment had the lowest ($p>0.05$) CHO value.

The result of the effect of graded levels of sweet orange peel extract supplementation on residual nutrient retention of broiler chicken at 8^{th} week of age is presented on Table 4.21. Regardless of the dietary treatments fed to the experimental birds, there were significant differences ($p<0.05$) in the total protein (TP), crude lipids (CL), ash (AS), caloric value (CV) and carbohydrate (CHO) of the retained nutrients. However, there were no significance differences ($p<0.05$) in the dry matter (DM) and crude fibre (CF) of the birds.

Table 4.20: Effect of dietary graded levels of antioxidants on residual nutrient retention of broiler chickens at 4th week of age

Parameters	Treatments (%)						
	BHA		SOPE				
	0.02	0.04	0.06	0.08	0.10	SEM	P Value
DM (%)	9.40[a]	8.49[a]	8.68[a]	8.56[a]	5.70[b]	0.41	>0.0008
Total protein (%)	12.57[a]	12.78[a]	13.53[a]	7.71[b]	7.49[b]	0.35	<0.0001
Crude fibre (%)	2.09[b]	1.31[b]	2.37[b]	1.93[b]	4.02[a]	0.32	>0.0016
Crude lipid (%)	2.51[ab]	2.73[a]	1.83[d]	2.01[cd]	2.30[bc]	0.07	< 0.0001
Ash (%)	1.16[c]	5.50[b]	8.74[a]	7.30[a]	8.06[a]	0.35	<0.0001
Caloric value (kg/100g)	1497.23[a]	1459.01[b]	1359.83[d]	1396.10[c]	1404.03[c]	6.90	<0.0001
Carbohydrate (%)	71.18[a]	67.89[b]	63.28[c]	71.00[a]	71.00[a]	0.46	<0.0001

[a,b,c] means having different superscripts along the same row are significantly different ($p<0.05$).

DM- Dry matter, BHA- Butylated hydroxyanisole, SOPE – sweet orange peel extract.

There were similarities ($p<0.05$) in TP values of birds on BHA, SOPE (0.04, 0.08 and 0.10 %) treatments. Similarly, there were similarities ($p>0.05$) in TP values of birds on BHA, SOPE (0.06, 0.08 and 0.10 %) treatments. However, birds on SOPE (0.04 %) treatment had a higher ($p<0.05$) TP value than those birds on SOPE (0.06 %) treatment. Whereas, birds on SOPE (0.06 %) treatment had the lowest ($p>0.05$) TP value. Birds on dietary SOPE (0.08 %) had a higher ($p<0.05$) CL value than those birds on other treatments. However, birds on BHA, SOPE (0.04, 0.06 and 0.10 %) treatments were similar ($p>0.05$) in CL values. The birds on BHA, SOPE (0.04 and 0.10 %) treatments were similar ($p>0.05$) in AS values and significantly higher than birds on SOPE (0.06 and 0.08 %) treatments. There were also similarities ($p>0.05$) in AS values of those birds on SOPE (0.04 and 0.06 %) treatments. However, birds on SOPE (0.06 %) treatment had a higher ($p<0.05$) AS value compared to birds on SOPE (0.08 %) treatment. While birds on SOPE (0.08 %) treatment had the lowest ($p>0.05$) AS value. There were similarities ($p>0.05$) in CV values of birds on SOPE (0.08, 0.06 and 0.04 %) treatments. There were also similarities ($p>0.05$) in CV values of birds on BHA, SOPE (0.08, 0.06 and 0.04 %) treatments. However, birds on SOPE (0.08 %) treatment were significantly higher ($p<0.05$) in CV value compared to birds on BHA and SOPE (0.10 %) treatments. There were similarities ($p>0.05$) in the CHO values of birds on SOPE (0.06, 0.08 %) and BHA treatments. There were also similarities ($p>0.05$) in CHO values of birds on SOPE (0.04, 0.10, 0.08 %) and BHA treatments. However, birds on SOPE (0.06 %) treatment were significantly higher ($p<0.05$) in CHO value compared to birds on SOPE (0.04 and 0.10 %) treatments.

Table 4.21: Effect of dietary graded levels of antioxidants on residual nutrient retention of broiler chickens at 8th week of age

Parameters	Treatments (%)						
	BHA		SOPE				
	0.02	0.04	0.06	0.08	0.10	SEM	P Value
DM (%)	10.58	11.05	11.57	11.75	10.25	0.48	>0.2109
Total protein (%)	14.91^{ab}	16.49^{a}	13.42^{b}	14.85^{ab}	14.80^{ab}	0.42	>0.0067
Crude fibre (%)	2.71	2.57	2.27	3.28	2.61	0.46	>0.6385
Crude lipid (%)	2.32^{b}	2.73^{b}	2.55^{b}	3.97^{a}	2.19^{b}	0.16	<0.0001
Ash (%)	4.41^{a}	3.51^{ab}	2.89^{b}	1.19^{c}	4.44^{a}	0.29	<0.0001
Caloric value (kg/100g)	1404.68^{b}	1423.69^{ab}	1427.10^{ab}	1468.73^{a}	1403.74^{b}	13.55	>0.0398
Carbohydrate (%)	63.64^{ab}	62.27^{b}	65.86^{a}	63.76^{ab}	63.56^{b}	0.48	>0.0056

^{a,b,c} means having different superscripts along the same row are significantly different ($p<0.05$). DM- Dry matter, BHA- Butylated hydroxyanisole, SOPE – sweet orange peel extract.

The results of effect of dietary butylated hydroxy anisole, and graded levels of sweet orange peel extract on carcass traits of broiler chicken are presented in Table 4.22. All the carcass parameters measured except the meat – bone ratio (drumstick), were influenced by the dietary treatments. Birds on SOPE (0.04 %) treatment had a higher ($p<0.05$) live weight (LW) and bled weight (BW) values compared to birds on other treatments. While the LW and BW of birds on SOPE (0.10 %) treatment had a higher ($p<0.05$) value than those birds on BHA treatment which in turn was significantly higher ($p<0.05$) than those birds on SOPE (0.08 %) treatment. Birds on SOPE (0.06 %) treatment had the lowest ($p>0.05$) LW and BW values. Birds on SOPE (0.04 %) treatment had a higher ($p<0.05$) de-feathered weight (DFW) value compared to birds on other treatments. While the DFW of birds on SOPE (0.10 %) treatment had a higher ($p<0.05$) value than those birds on BHA treatment which in turn was significantly higher ($p<0.05$) than those birds on SOPE (0.08 %) treatment. Birds on SOPE (0.06 %) treatment had the lowest ($p>0.05$) DFW value. Birds on SOPE (0.04 %) treatment had a higher ($p<0.05$) carcass weight (CW) value compared to birds on other treatments. While the CW of birds on SOPE (0.10 %) treatment had a higher ($p<0.05$) value than those birds on SOPE (0.06 %) treatment. While birds on SOPE (0.06 %) treatment had a higher ($p<0.05$) CW value compared to birds on BHA and SOPE (0.08 %) treatment. However, birds on treatments BHA and SOPE (0.08 %) had similar ($p>0.05$) CW values and the lowest ($p>0.05$) CW value. Birds on SOPE (0.06 %) treatment had a higher ($p<0.05$) breast weight (BRW) value compared to birds on other treatments. While the BRW of birds on SOPE (0.10 %) treatment had a higher ($p<0.05$) value than those birds on SOPE (0.04 and 0.08 %) and BHA treatments. However, there were similarities ($p>0.05$) in the BRW values of birds

on SOPE (0.04 and 0.08 %) treatments which in turn were significantly higher ($p<0.05$) than those birds on BHA treatment. Birds on BHA treatment had the lowest ($p>0.05$) BRW value. Birds on SOPE (0.08 %) treatment had a higher ($p<0.05$) thigh weight (TW) value compared to birds on other treatments. While the TW values of birds on SOPE (0.04 and 0.10 %) have a similar ($p>0.05$) TW value and are higher ($p<0.05$) than those birds on BHA and SOPE (0.06 %) treatments. However, the TW values of birds on BHA and SOPE (0.06 %) had a lower ($p>0.05$) TW value. Birds on SOPE (0.08 %) treatment had a higher ($p<0.05$) drumstick (DW) value compared to birds on other treatments. While the DW values of birds on SOPE (0.04 and 0.10 %) have a similar ($p>0.05$) DW value and are higher ($p<0.05$) than those birds on BHA and SOPE (0.06 %) treatments. However, the DW values of birds on BHA and SOPE (0.06 %) had a lower ($p>0.05$) DW values. Birds on SOPE (0.06 %) treatment had a higher ($p<0.05$) wing weight (WW) value compared to birds on other treatments. While the WW values of birds on SOPE (0.08 and 0.10 %) treatments were similar ($p>0.05$) but have higher ($p<0.05$) WW values than those birds on BHA and SOPE (0.04 %) treatments. Birds on SOPE (0.06 %) was significantly higher ($p<0.05$) in WW value than those birds on BHA and SOPE (0.04 %) treatments. However, birds on BHA and SOPE (0.04 %) treatment had the lowest ($p>0.05$) WW value. Birds on SOPE (0.08 %) treatment were similar ($p>0.05$) in backbone weight (BBW) with those birds on SOPE (0.06 %) treatment but were significantly higher ($p<0.05$) in BBW values compared to other treatments. Birds on SOPE (0.04 and 0.10 %) treatments have a similar ($p>0.05$) BBW values and were significantly higher ($p<0.05$) than birds on BHA treatment. However, birds on BHA treatment had the lowest ($p>0.05$) BBW value. Birds on SOPE (0.08 %) treatment had a

higher ($p<0.05$) liver weight (LIW) value compared to birds on other treatments. While the LIW of birds on SOPE (0.04 and 0.10 %) treatments had a similar ($p>0.05$) value and were significantly higher ($p<0.05$) than those birds on BHA and SOPE (0.06 %) treatments. Whereas, birds on BHA and SOPE (0.06 %) treatments were similar ($p>0.05$) in LIW value and had the lowest ($p>0.05$) LIW value. Birds on SOPE (0.04 %) treatment had a higher ($p<0.05$) heart weight (HTW) value compared to birds on other treatments and similar ($p>0.05$) in HTW value with those birds on SOPE (0.08 and 0.10 %) treatments. There were similarities ($p>0.05$) in HTW values of birds on BHA and SOPE (0.06 %) treatments. However, birds on BHA and SOPE (0.06 %) treatments had the lowest ($p>0.05$) HTW values. Birds on SOPE (0.06 %) treatment had a higher ($p<0.05$) abdominal fat weight (AFW) value compared to birds on other treatments and have similarities ($p>0.05$) in AFW value to those birds on BHA and SOPE (0.08 %) treatments. While birds on BHA, SOPE (0.08 and 0.10 %) treatments have some similarities ($p>0.05$) in their AFW values. The AFW of birds on SOPE (0.10 %) treatment had a higher ($p<0.05$) AFW value than those birds on SOPE (0.04 %) treatment. However, birds on SOPE (0.04 %) treatment had the lowest ($p>0.05$) AFW value. Birds on SOPE (0.04 %) treatment had a higher ($p<0.05$) dressing percentage (D %) value compared to birds on other treatments. While the D % of birds on SOPE (0.06 and 0.08 %) treatments were similar ($p>0.05$) in D % values but had a higher ($p<0.05$) D % value than those birds on BHA and SOPE (0.10 %) treatments. However, birds on treatments BHA and SOPE (0.10 %) had a similar ($p>0.05$) D % values. Birds on SHPE treatment had the lowest ($p>0.05$) D % value. Birds on SOPE (0.04 %) treatment had a higher ($p<0.05$) intestinal weight (IW) value compared to birds on other treatments.

While the IW of birds on SOPE (0.10 %) treatment had a higher ($p<0.05$) IW value than those birds on BHA and SOPE (0.08 %) treatments. Birds on BHA and SOPE (0.08 %) treatments were similar ($p>0.05$) in IW values and were also significantly higher ($p<0.05$) than the IW value obtained in birds on SOPE (0.06 %) treatment. However, birds on treatment SOPE (0.06 %) had the lowest ($p>0.05$) IW value. The meat to bone ratio of thigh muscles (M: B, T) of birds on SOPE (0.10 %) treatment were significantly higher ($p<0.05$) compared to birds on other dietary treatments. Birds on SOPE (0.04 %) treatment had a higher ($p<0.05$) M: B, T value than birds on BHA treatment which in turn was higher ($p<0.05$) than the birds on SOPE (0.06 %) treatment. Whereas, birds on SOPE (0.06 %) treatment were significantly higher ($p<0.05$) in M: B, T than birds on SOPE (0.08 %) treatment, which had the lowest ($p>0.05$) M: B, T value. Regardless, of the dietary treatments on the birds, there were no significant differences ($p>0.05$) in their meat to bone (M: B) of the drumstick part.

The results of BHA and graded levels of dietary SOPE supplementation on the broiler chicken's haematological parameters are shown in Table 4.23. Dietary antioxidants supplementation showed a direct effect ($p<0.05$) on all the blood parameters measured. Birds on dietary treatment SOPE (0.10 %) showed a higher ($p<0.05$) white blood cell (WBC) value compared to birds on other dietary treatments. Whereas, birds on SOPE (0.08 %) treatment had a higher ($p<0.05$) WBC value than birds on BHA, SOPE (0.04 and 0.06 %) treatments. However, there were similarities ($p>0.05$) in WBC values of birds on BHA, SOPE (0.04 and 0.06 %) treatments and were also significantly lower ($p>0.05$) in the WBC value. Birds on SOPE (0.08 %) treatment were significantly higher

(p<0.05) in lymphocyte (LYM) value compared to other treatments and were also similar (p>0.05) in LYM value of birds on BHA treatment.

Table 4.22: Effect of dietary graded levels of antioxidants on carcass trait of broiler chicken

Parameters	Treatments (%)						
	BHA		SOPE				
	0.02	0.04	0.06	0.08	0.10	SEM	P-Value
Live weight(g)	3300.00^{c}	3500.00^{a}	3200.00^{e}	3250.00^{d}	3400.00^{b}	7.30	<0.0001
Bled weight(g)	3200.00^{c}	3400.00^{a}	3100.00^{e}	3150.00^{d}	3300.00^{b}	6.35	<0.0001
De-feathered weight (g)	3000.00^{c}	3200.00^{a}	2900.00^{e}	2980.00^{d}	3100.00^{b}	6.33	<0.0001
Carcass weight (g)	2800.00^{d}	3160.00^{a}	2830.00^{c}	2800.00^{d}	2870.00^{b}	3.41	<0.0001
Breast (%)	20.22^{d}	24.86^{bc}	30.74^{a}	21.89^{cd}	27.34^{b}	0.70	0.0001
Thigh (%)	10.99^{c}	12.17^{ab}	10.84^{c}	12.88^{a}	11.10^{bc}	0.24	0.0004
Drumstick (%)	11.64^{c}	12.17^{bc}	11.41^{c}	13.11^{a}	12.62^{ab}	0.19	0.0005
Wing (%)	7.91^{b}	7.75^{b}	8.83^{a}	8.42^{ab}	8.57^{ab}	0.19	0.0141
Backbone (%)	12.37^{c}	15.58^{b}	16.56^{ab}	16.98^{a}	15.45^{b}	0.28	0.0001
Liver (%)	1.88^{c}	2.21^{b}	1.84^{c}	2.72^{a}	2.17^{b}	0.06	0.0001
Heart (%)	0.44^{b}	0.58^{a}	0.45^{b}	0.56^{ab}	0.52^{ab}	0.03	0.0120
Abdominal fat (%)	0.69^{ab}	0.54^{c}	0.72^{a}	0.60^{ab}	0.69^{bc}	0.03	0.0037
Dressing (%)	84.85^{c}	90.29^{a}	88.44^{b}	86.15^{b}	84.41^{c}	1.43	0.0153
Intestinal weight (%)	70.72^{c}	98.40^{a}	65.72^{d}	63.72^{c}	76.72^{b}	0.01	0.0001
Meat: Bone (thigh)	6.14^{c}	6.52^{b}	5.76^{d}	4.55^{e}	6.77^{a}	0.05	0.0001
Meat: Bone(drumstick)	4.02	3.91	3.71	3.83	3.58	0.13	0.2313

$^{a, b, c}$ means having different superscripts along the same row are significantly different (p< 0.05)
BHA – butylated hydroxyanisole, SOPE – sweet orange peel extract

: ratio

Birds on BHA treatment have similar ($p>0.05$) LYM value with birds on SOPE (0.04 %) treatment but were significantly higher ($p<0.05$) than SOPE (0.06 %) treatment LYM value. Birds on SOPE (0.06 %) treatment had a higher ($p<0.05$) LYM value compared to birds on SOPE (0.10 %) treatment. However, birds on SOPE (0.10 %) treatment had a lower ($p>0.05$) LYM value. Birds on SOPE (0.10 %) treatment had higher ($p<0.05$) mid-sized cell (MID) value compared to other treatments. However, birds on BHA, SOPE (0.04, 0.06 and 0.08 %) treatments were significantly similar ($p>0.05$) in their MID values. Birds on SOPE (0.10 %) treatment had a higher ($p<0.05$) granulocyte (GRAN) value than all the other treatments. There were similarities in the GRAN of birds on SOPE (0.08 and 0.06 %) treatments. Similarities ($p>0.05$) were also observed in the GRAN values of birds on BHA, SOPE (0.04 and 0.06 %) treatments. Birds on BHA and SOPE (0.04 %) treatments had the lowest ($p>0.05$) GRAN values. Birds on SOPE (0.08 %) treatment had a higher ($p<0.05$) red blood cell (RBC) value than other treatments. Birds on SOPE (0.06 %) treatment was significantly higher ($p<0.05$) than birds on SOPE (0.04 and 0.10 %) treatments which were in turn similar ($p<0.05$) in their RBC values. Dietary treatments SOPE (0.04 and 0.10 %) were significantly higher ($p<0.05$) in RBC values compared to BHA treatment which had the lowest ($p>0.05$) RBC value. Birds on SOPE (0.10 %) treatment had a higher ($p<0.05$) haemoglobin value than all the other treatments. Birds on BHA treatment was significantly higher ($p<0.05$) in HGB value than birds on SOPE (0.04 %) treatment. There were some similarities in the HGB of birds on SOPE (0.04 and 0.08 %) treatments. Birds on SOPE (0.04 %) treatment had a higher

($p<0.05$) HGB value than birds on SOPE (0.06 %) treatment which in turn had the lowest ($p>0.05$) HGB value. Birds on dietary treatment SOPE (0.08 %) had higher ($p<0.05$) hemaloctil (HCT) compared to other treatments. Similarly, birds on SOPE (0.04 and 0.06 %) treatments were similar ($p>0.05$) in their HCT values and were significantly higher ($p<0.05$) in HCT value compared to SOPE (0.10 %) treatment. However, birds on SOPE (0.10 %) treatment had a higher ($p<0.05$) HCT value than those birds on BHA treatment which in turn had the lowest ($p>0.05$) HCT value. The mean corpuscular volume (MCV) results showed that birds on SOPE (0.04 %) treatment had higher ($p<0.05$) values compared to other treatments and was also similar ($p>0.05$) to birds on SOPE (0.08 %) treatment. Similarly, birds on SOPE (0.06 and 0.08 %) had similar ($p>0.05$) HCT values. However, birds on SOPE (0.06 %) had higher ($p<0.05$) HCT values than those birds on SOPE (0.08 %). Birds on SOPE (0.10 %) treatment were significantly higher ($p<0.05$) in HCT value compared to BHA treatment which had the lowest ($p>0.05$) HCT value. Mean cell haemoglobin concentration (MCHC) results showed that birds on BHA treatment had the highest value and was significantly higher ($p<0.05$) than all the other treatments. Birds on SOPE (0.10 %) were significantly higher ($p<0.05$) in MCHC than those birds on SOPE (0.04 %) which in turn was higher ($p<0.05$) in MCHC value than birds on SOPE (0.06 %) treatment, which in turn was higher ($p<0.05$) in MCHC value than birds on SOPE (0.08 %) which had the lowest ($p>0.05$) MCHC value. Birds on BHA, SOPE (0.04, 0.06 and 0.10 %) treatments had similar ($p>0.05$) red blood cell (erythrocyte)distribution width (RDWSD) values, their values were however, higher ($p<0.05$) than those of birds on SOPE (0.08 %) treatment. Birds on BHA treatment were significantly higher than birds on SOPE (0.08 %) treatment which had the lowest

(p>0.05) RDWSD value. Birds on SOPE (0.08 %) treatment had a higher (p<0.05) platelet (PLT) value to other treatments. However, birds on SOPE (0.04 and 0.06 %) treatments have similar (p>0.05) PLT values. Whereas, birds on LMPE treatment have the lowest PCV value, their values were however, higher (p<0.05) than those birds on BHA treatment. Birds on BHA treatment were significantly higher (p<0.05) in PLT values compared to birds on SOPE (0.10 %) treatment which had the lowest (p>0.05) PLT value. Birds on SOPE (0.08 %) treatment had a higher (p<0.05) packed cell volume (PCV) value than birds on other treatments. There were similarities (p>0.05) in the PCV values of birds on BHA, SOPE (0.04 and 0.06 %) treatments, there values were however, higher (p<0.05) than those birds on SOPE (0.10 %) treatment, which had the lowest (p>0.05) PCV value.

The results of the effect of dietary butylated hydroxyanisole (BHA) and graded levels of dietary SOPE supplementation on the broiler chicken's blood serum of broiler chickens are presented in Table 4.24. There were significant differences (p<0.05) in all the blood serum parameters listed in Table 4.24 on the birds fed dietary treatments BHA and graded levels of SOPE. Birds on BHA treatment were significantly higher (p<0.05) in total protein (TP) compared to those birds on other treatments. There were similarities (p>0.05) in TP values of birds on SOPE (0.04%, 0.06 and 0.08 %) treatments which in turn were higher (p<0.05) than those in treatment SOPE (0.10 %). However, birds on treatment SOPE (0.10 %) had the lowest (p>0.05) TP value. Birds on SOPE (0.06 %) treatment had a higher (p<0.05) total cholesterol (TC) to those birds on other treatments. While the TC value of birds on SOPE (0.08 %) treatment were significantly higher (p<0.05) than those on BHA and SOPE (0.10 %) treatment that have similar (p>0.05) TC

values, which in turn were higher than those on SOPE (0.04 %) treatment. However, birds on SOPE (0.10 %) treatment had the lowest ($p>0.05$) TC value.

Table 4.23: Effect of dietary graded levels of antioxidants on haematological indices of broiler chicken

Parameters	Treatments (%)						
	BHA		SOPE				
	0.02	0.04	0.06	0.08	0.10	SEM	P-Value
WBC (10^9/l)	8.20^{c}	7.40^{c}	6.60^{c}	10.50^{b}	19.67^{a}	0.88	0.0001
LYM (10^9/l)	8.10^{ab}	7.40^{bc}	6.07^{c}	9.23^{a}	2.90^{d}	0.35	0.0001
MID (%)	0.00^{b}	0.00^{b}	0.37^{b}	0.40^{b}	2.77^{a}	0.11	0.0001
GRAN (%)	0.10^{c}	0.00^{c}	0.40^{bc}	0.63^{b}	2.77^{a}	0.11	0.0001
RBC (10^6/mm^3)	0.96^{d}	1.68^{c}	1.99^{b}	2.40^{a}	1.50^{c}	2.57	0.0001
HGB (g/dl)	8.13^{b}	5.10^{c}	2.80^{d}	3.00^{cd}	11.12^{a}	0.49	0.0001
HCT (%)	5.47^{d}	19.80^{b}	20.61^{b}	26.36^{a}	12.00^{c}	0.63	0.0001
MCV (fl)	58.90^{d}	117.70^{a}	101.73^{b}	111.00^{ab}	73.47^{c}	2.42	0.0001
MCHC (g/l)	1500.33^{a}	259.00^{c}	135.00^{d}	112.33^{e}	922.00^{b}	27.22	0.0321
RDWsd (%)	38.20^{a}	34.40^{ab}	36.00^{ab}	31.80^{b}	35.30^{ab}	1.15	0.0005
PLT (10^9/l)	692.00^{c}	820.00^{bc}	894.00^{b}	1598.00^{a}	144.00^{d}	29.94	0.0004
PCV	0.70^{b}	0.69^{b}	0.75^{b}	1.24^{a}	0.19^{c}	2.98	0.0060

$^{a, b, c}$ means having different superscripts along the same row are significantly different ($p< 0.05$). WBC (white blood cell), LYM (lymphocyte), MID (mid-sized cell), GRAN (granulocyte), RBC (red blood cell), HGB (haemoglobin), HCT (hemaloctil), MCV (mean corpuscular volume), MCHC (mean cell haemoglobin concentration), RDWSD (red blood cell (erythrocyte)distribution width), PLT (platelets), PCV (packed cell volume)

BHA – butylated hydroxyanisole, SOPE – sweet orange peel extract

Birds on BHA and SOPE (0.10 %) treatments had a similar ($p>0.05$) triglycerides (TRY) value and are significantly higher ($p<0.05$) in TRY values compared to birds on other treatments. While birds on SOPE (0.06 %) treatment were significantly higher ($p<0.05$) in TRY value than birds on SOPE (0.08 %) treatment which in turn were higher ($p<0.05$) than those birds on SOPE (0.04 %) treatment. However, birds on SOPE (0.04 %) treatment had the lowest ($p>0.05$) TRY value. Birds on dietary treatment SOPE (0.06 %) were significantly higher ($p<0.05$) in low density lipoprotein concentration (LDLP) compared to birds on other treatments. While birds on SOPE (0.08 %) treatment had a higher ($p<0.05$) LDLP value than those birds on BHA treatment which in turn were significantly higher ($p<0.05$) than those birds on SOPE (0.10 %) treatment. Whereas, birds on SOPE (0.10 %) had a higher ($p<0.05$) LDLP value than those birds on SOPE (0.04 %) treatment. Birds on SOPE (0.04 %) treatment had the lowest ($p>0.05$) LDLP value. There were similarities in the high-density lipoprotein concentration (HDLP) of birds on BHA and SOPE (0.10 %) treatments which in turn were higher than birds on SOPE (0.06 %) treatment. Birds on dietary treatment SOPE (0.06 %) were significantly higher ($p<0.05$) in HDLP compared to birds on SOPE (0.04 and 0.08 %) treatments, which were similar ($p>0.05$) in HDLP values and with the least ($p>0.05$) HDLP value. Birds on dietary treatment SOPE (0.08 %) were significantly higher ($p<0.05$) in aspartate amino transferase (AST) compared to birds on other treatments. While birds on BHA treatment had a higher ($p<0.05$) AST value than those birds on SOPE (0.06 %) treatment

which in turn were significantly higher ($p<0.05$) than those birds on SOPE (0.10 %) treatment. Birds on SOPE (0.10 %) treatment had a higher ($p<0.05$) AST value than birds on SOPE (0.04 %) treatment which had the lowest ($p>0.05$) AST value. Birds on BHA treatment were significantly higher ($p<0.05$) in alanine amino transferase (ALT) compared to birds on other treatments. While birds on SOPE (0.08 %) treatment had a higher ($p<0.05$) ALT value than those birds on SOPE (0.04 and 0.06 %) treatments which were similar ($p>0.05$) in ALT values but were however, significantly higher ($p<0.05$) in ALT than those birds on SOPE (0.10 %) treatment. Birds on SOPE (0.10 %) treatment had the lowest ($p>0.05$) ALT value. Birds on SOPE (0.06 %) treatment was significantly higher ($p<0.05$) in superoxide dismutase (SOD) compared to birds on other treatments. While birds on BHA treatment had a higher ($p<0.05$) SOD value than those birds on SOPE (0.04 and 0.10 %) treatments which are similar ($p>0.05$) in SOD values but were however, significantly higher ($p<0.05$) in SOD than those birds on SOPE (0.08 %) treatment. Birds on SOPE (0.08 %) treatment had the lowest ($p>0.05$) SOD value. Birds on BHA and SOPE (0.08 %) treatments were similar ($p>0.05$) in glutathione peroxidase (GP_X) and were significantly higher ($p<0.05$) in GP_X compared to birds on SOPE (0.04, 0.06 and 0.10 %) treatments. While birds on SOPE (0.04, 0.06 and 0.10 %) treatments were similar ($p<0.05$) and had the lowest ($p>0.05$) GP_X value. Birds on dietary treatment BHA were significantly higher ($p<0.05$) in serum catalase (CAT) value compared to birds on other treatments. While birds on SOPE (0.04 %) treatment had a higher ($p<0.05$) CAT value than those birds on SOPE (0.08 %) treatment which in turn were significantly higher ($p<0.05$) than those birds on SOPE (0.10 %) treatment. Birds on SOPE (0.10 %)

treatment had a higher ($p<0.05$) CAT value than birds on SOPE (0.06 %) treatment which had the lowest ($p>0.05$) CAT value.

Table 4.24: Effect of dietary graded levels of antioxidants on blood serum indices of broiler chicken

Parameters	Treatments (%)						
	BHA		SOPE				
	0.02	0.04	0.06	0.08	0.10	SEM	P-Value
T.Chl.(mg/dl)	169.50^{c}	118.21^{d}	279.01^{a}	221.04^{b}	165.32^{c}	1.18	0.0001
Trig(mg/dl)	101.28^{a}	38.98^{d}	65.50^{b}	50.48^{c}	102.87^{a}	0.56	0.0001
LDLP (mg/dl)	59.78^{c}	49.67^{e}	192.71^{a}	144.78^{b}	54.00^{d}	0.78	0.0001
HDLP (mg/dl)	90.23^{a}	60.75^{c}	73.20^{b}	66.13^{c}	90.75^{a}	1.24	0.0001
AST (µ/l)	181.24^{b}	93.68^{e}	158.95^{c}	244.23^{a}	140.95^{d}	0.93	0.0001
ALT(µ/l)	58.46^{a}	31.92^{c}	31.16^{c}	46.15^{b}	25.09^{d}	0.45	0.0001
SOD (µ/l)	391.79^{b}	369.32^{c}	433.25^{a}	348.26^{d}	373.13^{c}	0.94	0.0001
GP_X (µ/l)	3108.10^{a}	1175.70^{b}	1250.60^{b}	3143.80^{a}	1020.00^{b}	149.12	0.0001
CAT (µ/l)	4433.33^{a}	4353.30^{b}	1506.60^{e}	3066.67^{c}	1806.67^{d}	2.38	0.0001

$^{a, b, c}$ means having different superscripts along the same row are significantly different ($p< 0.05$). T. Chl. (Total cholesterol), Trig. (Triglycerides), LDLP (low density lipoprotein), HDLP (high density lipoprotein), AST (aspartate amino transferase), ALT (alanine amino transferase), SOD (superoxide dismutase), GP_X (glutathione peroxidase), CAT (serum catalase)
BHA – butylated hydroxyanisole, SOPE – sweet orange peel extracts

The results of the effect of dietary graded levels of sweet orange peel extracts on the bird's meat quality are presented in Table 4.25. The results showed that the dietary treatments influenced ($p<0.05$) the meat pH, cooking loss, drip loss, WHC, lightness and redness of the meat. However, the yellowness of the meat was not affected ($p>0.05$) by the dietary treatments. Meat from birds on SOPE (0.04,0.08 and 0.10 %) treatments showed some similarities ($p>0.05$) in their pH values and were significantly higher ($p<0.05$) than the pH value of birds on BHA and SOPE (0.06 %) treatments. There were also some similarities in the pH values of birds on SOPE (0.10 and 0.06 %) treatments, which in turn were significantly higher ($p<0.05$) in pH value of birds on BHA treatments. However, birds on BHA treatment had the lowest ($p>0.05$) pH value. Birds on dietary treatment BHA were significantly higher ($p<0.05$) in cooking loss and water holding capacity (CL and WHC) values compared to birds on other treatments. While birds on SOPE (0.04 %) treatment had a higher ($p<0.05$) CL and WHC values than those birds on SOPE (0.06 %) treatment which in turn were significantly higher ($p<0.05$) than those birds on SOPE (0.10 %) treatment. Birds on SOPE (0.10 %) treatment had a higher ($p<0.05$) CL and WHC values than birds on SOPE (0.08 %) treatment which had the lowest ($p>0.05$) CL and WHC values. Birds on dietary treatments BHA and SOPE (0.04 %) were similar ($p>0.05$) in drip loss (DL) values and also significantly higher ($p<0.05$)

in DL values compared to birds on other treatments. While birds on SOPE (0.06 %) treatment had a higher ($p<0.05$) DL value than those birds on SOPE (0.10 %) treatment which in turn were significantly higher ($p<0.05$) than those birds on SOPE (0.08 %) treatment. Birds on SOPE (0.08 %) treatment had the lowest ($p>0.05$) DL value.

Birds on dietary treatment SOPE (0.04 and 0.08 %) were similar ($p>0.05$) in their lightness (L*) and were significantly ($P<0.05$) lighter L* than other treatments. There were also some similarities ($p>0.05$) in the L* of birds on SOPE (0.08 and 0.10 %) treatments and were also significantly higher ($p<0.05$) in L* value than birds on BHA and SOPE (0.06 %) treatments. However, birds on BHA and SOPE (0.06 %) treatments had the lowest ($p>0.05$) L* values. Birds on SOPE (0.08 %) treatment were significantly higher ($p<0.05$) in redness (a*) value than those birds on BHA and SOPE (0.04, 0.06 and 0.10 %) treatments. However, birds on BHA and SOPE (0.04, 0.06 and 0.10 %) treatments have a similar ($p>0.05$) a* values and the lowest ($p>0.05$) a* values.

The results of the effect of graded levels of sweet orange peel extracts and BHA on meat tenderness of the experimental birds are presented in Table 4.26. There were significant differences ($p<0.05$) on the force (peak and yield), stress (peak and yield), strain (peak and yield), Young modulus, width and thickness of the broiler meat on dietary treatments (BHA, 0.04, 0.06, 0.08 and 0.10 % of SOPE). Birds on dietary SOPE (0.08 %) treatment were significantly higher ($p<0.05$) in force peak (FP) value compared to birds on other treatments. Whereas, there were similarities ($p>0.05$) in the FP value of birds on BHA and SOPE (0.06 %) treatments which in turn were significantly higher ($p<0.05$) than those birds on SOPE (0.10 %) treatment. While birds on SOPE (0.10 %) treatment had a

higher ($p<0.05$) FP value compared to SOPE (0.04 %) treatment. However, birds on SOPE (0.04 %) treatment had the lowest ($p>0.05$) FP value. Birds on dietary SOPE (0.08 %) treatment were significantly higher ($p<0.05$) in force yield (FY) value compared to birds on other treatments. Whereas, birds on SOPE (0.10 %) treatment had a higher ($p<0.05$) FY

Table 4.25: Effect of dietary graded levels of antioxidants on physicochemical properties of broiler chickens

Parameters	Treatments (%)						
	BHA	SOPE				SEM	P-Value
	0.02	0.04	0.06	0.08	0.10		
pH	5.85^{c}	6.15^{a}	5.95^{cb}	6.18^{a}	6.05^{ab}	0.01	<0.0001
Cooking Loss (%)	35.62^{a}	29.88^{b}	20.59^{c}	9.53^{e}	13.18^{d}	0.13	<0.0001
Drip Loss (%)	28.42^{a}	28.86^{a}	24.40^{b}	18.03^{d}	21.15^{c}	0.05	<0.0001
WHC	64.04^{a}	58.74^{b}	44.99^{c}	27.56^{e}	34.33^{d}	0.11	<0.0001
Lightness (L*)	20.00^{c}	38.23^{a}	18.47^{c}	34.62^{ab}	22.52^{bc}	1.22	<0.0011
Redness(a*)	2.82^{b}	1.22^{b}	1.97^{b}	8.19^{a}	1.95^{b}	0.41	<0.0017
Yellowness(b*)	5.33	4.67	3.12	5.11	2.32	0.37	<0.1051

$^{a, b, c}$ means having different superscripts along the same row are significantly different ($p< 0.05$)
BHA – butylated hydroxyanisole, SOPE – sweet orange peel extract, WHC – water holding capacity

value compared to birds on BHA and SOPE (0.06 %) treatments. There were similarities ($p>0.05$) in FY values of birds on BHA and SOPE (0.06 %) treatments and were significantly higher ($p<0.05$) in FY values compared to birds on SOPE (0.04 %) treatment. However, birds on SOPE (0.04 %) treatment had the lowest ($p>0.05$) FY value. Birds on dietary SOPE (0.08 %) treatment were significantly higher ($p<0.05$) in stress peak (SP) value compared to birds on other treatments. There are some similarities ($p>0.05$) in the SP values of birds on SOPE (0.06 %) and BHA treatments. Similarity also occurred in the SP values of birds on BHA and SOPE (0.04 %) treatments, which in turn were significantly higher ($p<0.05$) in SP values compared to birds on SOPE (0.10 %) treatment. However, birds on SOPE (0.10 %) treatment had the lowest ($p>0.05$) SP value. Birds on dietary SOPE (0.08 %) treatment were significantly higher ($p<0.05$) in stress yield (SY) value compared to birds on other treatments. There were similarities ($p>0.05$) in the SY values of birds on BHA and SOPE (0.04, 0.06 and 0.10 %) treatments and they had the lowest ($p>0.05$) SY values. There were some similarities ($p>0.05$) in the strain peak (SRP) values of birds on BHA and SOPE (0.08 %) treatments. Some similarity ($p>0.05$) also occurred in the SRP values of birds on SOPE (0.06 and 0.08 %) treatment which in turn were significantly higher ($p<0.05$) in SRP values than those birds on SOPE (0.04 and 0.10 %) treatments. Similarly, birds on SOPE (0.04 and 0.10 %) treatments had a similar ($p>0.05$) SRP values and had the lowest ($p>0.05$) SRP values. Birds on BHA

and SOPE (0.06 %) were similar in their strain yield (SRY) and in turn significantly higher ($p<0.05$) in SRY than those birds on SOPE (0.04, 0.08 and 0.10 %) treatments. Similarly, birds on SOPE (0.04, 0.08 and 0.10 %) treatments had a similar ($p>0.05$) SRY values and also had the lowest ($p>0.05$) SRY values. Birds on SOPE (0.10 %) treatment had a higher ($p<0.05$) Young modulus (YM) value compared to those birds on BHA and SOPE (0.04, 0.06 and 0.08 %) treatments. However, birds on BHA and SOPE (0.04, 0.06 and 0.08 %) treatments had a similar ($p>0.05$) YM values and the lowest ($p>0.05$) YM values. Birds on BHA and SOPE (0.10 %) treatments were similar ($p>0.05$) in their width (WT) and significantly higher ($p<0.05$) in WT compared to birds on SOPE (0.06 %) treatment. Birds on SOPE (0.06 %) treatment had a higher ($p<0.05$) WT size than birds on SOPE (0.04 and 0.08 %) treatments. Birds on SOPE (0.04 and 0.08 %) treatments were similar ($p>0.05$) in their WT and equally had the lowest ($p>0.05$) WT values. There were similarities in the thickness (TN) of birds on SOPE (0.06, 0.10 %) and BHA treatments which in turn were significantly higher ($p<0.05$) than those birds on SOPE (0.04 and 0.08 %) treatments. Similarly, similarity occurred in the TN values of birds on SOPE (0.04 and 0.08 %) treatments which in turn had the lowest ($p>0.05$) TN values.

The results of the effect of graded levels of sweet orange peel extracts and BHA on sensory evaluation of broiler meat are presented in Table 4.27. Regardless of the dietary treatments fed to the experimental birds, there were significant differences ($p<0.05$) on the appearance, taste, texture, aroma and overall acceptability of the bird's meat. Meats of the birds on dietary treatment SOPE (0.10 %) were rated significantly higher ($p<0.05$) in appearance (AP) and aroma (AR) a sensory perception of the assessors compared to

birds on other treatments. While bird's meat on BHA treatment had a higher ($p<0.05$) AP and AR assessor's perception than those bird's meat on SOPE (0.08 %) treatment which in turn was significantly higher ($p<0.05$) than those bird's meat on SOPE (0.04 %) treatment.

Table 4.26: Effect of dietary graded levels of antioxidants on tenderness of broiler chickens

Parameters	Treatments (%)						
	BHA		SOPE			SEM	P-Value
	0.02	0.04	0.06	0.08	0.10		
Force peak (N)	17.80^{b}	5.30^{d}	17.90^{b}	24.50^{a}	8.30^{c}	0.22	<0.0001
Force yield (N)	4.00^{c}	1.30^{d}	4.10^{c}	10.66^{a}	5.20^{b}	0.10	<0.0001
Stress peak(N/mm^2)	0.02^{bc}	0.01^{cd}	0.02^{b}	0.06^{a}	0.00^{d}	0.00	<0.0001
Stress yield (N/mm^2)	0.00^{b}	0.00^{b}	0.00^{b}	0.02^{a}	0.00^{b}	0.00	<0.0001
Strain peak (%)	66.51^{a}	27.67^{c}	56.54^{b}	61.31^{ab}	29.33^{c}	0.64	<0.0001
Strain yield (%)	13.00^{a}	3.88^{b}	16.50^{a}	0.54^{b}	0.22^{b}	0.37	<0.0001
Young's modulus (N/mm^2)	0.03^{b}	0.04^{b}	0.05^{b}	0.03^{b}	1.39^{a}	0.02	<0.0001
Width (mm)	50.33^{a}	25.00^{c}	35.00^{b}	25.00^{c}	50.00^{a}	0.58	<0.0001
Thickness (mm)	18.00^{ab}	15.00^{b}	19.00^{a}	15.00^{b}	20.33^{a}	0.35	<0.0022

$^{a, b, c}$ means having different superscripts along the same row are significantly different ($p< 0.05$)
BHA – butylated hydroxyanisole, SOPE – sweet orange peel extract.

Birds on SOPE (0.04 %) treatment had a higher ($p<0.05$) AP and AR assessor's perception than bird's meat on SOPE (0.06 %) treatment which had the lowest ($p>0.05$) AP and AR assessor's sensory perception. Meats of the birds on dietary treatments SOPE (0.08 and 0.10 %) were similar ($p>0.05$) in their taste (TT) sensory perception of the assessors and were significantly higher ($p<0.05$) in TT perception of the assessors compared to bird's meat on other treatments. While bird's meat on BHA treatment had a higher ($p<0.05$) TT assessor's perception than those bird's meat on SOPE (0.04 %) treatment which in turn was significantly higher ($p<0.05$) than those bird's meat on SOPE (0.06 %) treatment. Bird's meat on SOPE (0.06 %) treatment had the lowest ($p>0.05$) TT assessor's sensory perception. Meat of the birds on dietary treatment BHA were significantly higher ($p<0.05$) in texture (TE) sensory perception of the assessors compared to birds on other treatments.

While bird's meat on SOPE (0.10 %) treatment had a higher ($p<0.05$) TE assessor's perception than those bird's meat on SOPE (0.08 %) treatment which in turn was significantly higher ($p<0.05$) than those bird's meat on SOPE (0.04 %) treatment. Birds on SOPE (0.04 %) treatment had a higher ($p<0.05$) TE assessor's perception than bird's meat on SOPE (0.06 %) treatment which had the lowest ($p>0.05$) TE assessor's sensory perception. Meat of the birds on dietary treatment SOPE (0.10 %) was significantly higher ($p<0.05$) in overall acceptability (OA) sensory perception of the assessors compared to birds on other treatments. While bird's meat on SOPE (0.08 %) treatment had a higher ($p<0.05$) OA assessor's perception than those bird's meat on BHA treatment

which in turn was significantly higher ($p<0.05$) than those bird's meat on SOPE (0.04 and 0.06 %)

Table 4.27: Effect of dietary graded levels of antioxidants on sensory evaluation of broiler chickens

Parameters	Treatments (%)						
	BHA			SOPE		SEM	P- Value
	(0.02)	(0.04)	(0.06)	(0.08)	(0.10)		
Appearance	7.50^{b}	7.05^{d}	7.00^{e}	7.30^{c}	7.60^{a}	0.25	<0.001
Taste	7.40^{b}	7.35^{c}	7.00^{d}	7.45^{a}	7.45^{a}	0.26	<0.001
Texture	7.80^{a}	7.20^{d}	6.75^{e}	7.45^{c}	7.55^{b}	0.27	<0.001
Aroma	7.35^{b}	7.15^{d}	6.65^{e}	7.20^{c}	7.45^{a}	0.26	<0.001
Overall acceptability	7.40^{c}	7.25^{d}	7.25^{d}	7.50^{b}	7.95^{a}	0.21	<0.001

$^{a, b, c}$ means having different superscripts along the same row are significantly different ($p< 0.05$)
BHA – butylated hydroxyanisole, SOPE – sweet orange peel extract.

treatments. However, bird's meat on SOPE (0.04 and 0.06 %) treatments had a similar ($p>0.05$) OA assessor's perception which in turn had the lowest ($p>0.05$) OA assessor's perception.

The results of the effect of graded levels of sweet orange peel extracts and BHA on fatty acid compositions of the experimental broiler meat are presented in Table 4.28. Regardless of the dietary treatments (BHA, 0.04, 0.06, 0.08 and 0.10 % SOPE) fed to the birds there were significant differences ($p<0.05$) on the saturated fatty acids (SFA) (palmitic acid [C16:O], caproic acid [C6:O], myristic acid [C14:O] and lauric acid [C12:O], mono unsaturated fatty acids (MUFA) (palmitoleic acid [C16:1Λ9c], oleic acid [C18:1Λ9c] and caproleic acid [C10:1Λ9c] and poly unsaturated fatty acids (PUFA) (linoleic acid [C18:2(n-6)] and docosahexaenoic acids [C22:(6n-3)] of the broiler meat. The palmitic acid (C16:O) of broiler meat on dietary treatment SOPE (0.10 %) was significantly higher ($p<0.05$) compared to other treatments.

While bird's meat on SOPE (0.06 %) treatment had a higher ($p<0.05$) C16:O value than those bird's meat on SOPE (0.08 %) treatment which in turn was significantly higher ($p<0.05$) than those bird's meat on BHA treatment. Birds on BHA treatment had a higher ($p<0.05$) C16:O value than bird's meat on SOPE (0.04 %) treatment which had the lowest ($p>0.05$) C16:O value. There were similarities ($p>0.05$) in the caproic (C6:O) and palmitoleic (C16: 1Λ9c) acids of the bird's meat on BHA and SOPE (0.06 %) treatments and are significantly higher ($p<0.05$) than bird's meat on SOPE (0.04, 0.08 and 0.10 %) treatments. Similarly, similarity ($p>0.05$) occurred in the C6:O and C16: 1Λ9c of the

bird's meat on SOPE (0.04, 0.08 and 0.10 %) treatments which in turn had the lowest ($p>0.05$) C6:O and C16: 1Λ9c values. Bird's meat on BHA and SOPE (0.06%) treatments had similar ($p>0.05$) myristic acid (C14:O) values and are significantly higher ($p<0.05$) than those bird's meat on other treatments. Bird's meat on SOPE (0.08 %) treatment had a higher ($p<0.05$) C14:O value compared to bird's meat on SOPE (0.04 and 0.10 %) treatments. However, there were similarities in C14:O value of bird's meat on SOPE (0.04 and 0.10 %) treatments which also had the lowest ($p>0.05$) C14:O values. There were similarities ($p>0.05$) in the lauric (C12:O) and linoleic (C18:2(n-6)) acids of the bird's meat on SOPE (0.04 %) treatments and are significantly higher ($p<0.05$) than bird's meat on BHA and SOPE (0.06, 0.08 and 0.10 %) treatments. Similarly, similarity ($p>0.05$) occurred in the C12:O and C18:2(n-6) values of the bird's meat on BHA and SOPE (0.06, 0.08 and 0.10 %) treatments which in turn had the lowest ($p>0.05$) C12:O and C18:2(n-6) values. The oleic acid (C18:1Λ9c) of broiler meat on dietary treatment BHA was significantly higher ($p<0.05$) compared to other treatments.

While bird's meat on SOPE (0.04 %) treatment had a higher ($p<0.05$) C18:1Λ9c value than those bird's meat on SOPE (0.06 %) treatment which in turn was significantly higher ($p<0.05$) than those bird's meat on SOPE (0.10 %) treatment. Birds on SOPE (0.10 %) treatment had a higher ($p<0.05$) C18:1Λ9c value than bird's meat on SOPE (0.08 %) treatment which had the lowest ($p>0.05$) C18:1Λ9c value. The caproleic acid (C10:1Λ9c) value of broiler meat on dietary treatment SOPE (0.10 %) treatment was significantly higher ($p<0.05$) compared to other treatments. While bird's meat on SOPE (0.04 %) treatment had a higher ($p<0.05$) C10:1Λ9c value than those bird's meat on BHA

and SOPE (0.06 and 0.08 %) treatments. Bird's meat on BHA and SOPE (0.06 and 0.08 %) treatments

Table 4.28: Effect of dietary graded levels of antioxidants on fatty acid profile of broiler chicken

Parameters	Treatments (%)						
	BHA		SOPE			SEM	P-value
	0.02	0.04	0.06	0.08	0.10		
Saturated fatty acids							
Palmitic acid (C16:O)	20.09^{d}	9.93^{e}	35.73^{b}	28.26^{c}	98.09^{a}	0.030	<0.001
Caproic acid (C6:O)	3.02^{a}	0.00^{b}	3.02^{a}	0.00^{b}	0.00^{b}	0.002	<0.001
Myristic acid (C14:O)	15.64^{a}	0.00^{c}	15.66^{a}	9.15^{b}	0.00^{c}	0.022	<0.001
Lauric acid (C12:O)	0.00^{b}	2.28^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.001	<0.001
Mono unsaturated fatty acids							
Palmitoleic acid (C16:1Λ9c)	1.43^{a}	0.00^{b}	1.43^{a}	0.00^{b}	0.00^{b}	0.011	<0.001
Oleic acid (C18:1Λ9c)	100.65^{a}	84.35^{b}	62.94^{c}	17.10^{e}	53.78^{d}	0.03	<0.001
Caproleic acid (C10:1Λ9)	0.00^{c}	2.28^{b}	0.00^{c}	0.00^{c}	56.24^{a}	0.022	<0.001
Poly unsaturated fatty acids							
Linoleic acid (C18:2(n-6))	0.00^{b}	40.82^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.001	<0.001
DHA (C22:(6n-3))	0.00^{b}	0.00^{b}	0.00^{b}	5.30^{a}	0.00^{b}	0.077	<0.001

$^{a, b, c}$ means having different superscripts along the same row are significantly different ($p< 0.05$)

BHA – butylated hydroxyanisole, SOPE – sweet orange peel extract, DHA-docosahexaenoic acids

had a similar ($p>0.05$) C10:1Λ9c values and at the same time the lowest ($p>0.05$) C10:1Λ9c values. The docosahexaenoic acids (C22:(6n-3)) value of bird's meat on SOPE (0.08 %) treatment were significantly higher ($p<0.05$) than those bird's meat on BHA, SOPE (0.04, 0.06 and 0.10 %) treatments. However, there were similarities in the C22:(6n-3) values of bird's meat on BHA, SOPE (0.04, 0.06 and 0.10 %) treatments which in turn had the lowest ($P>0.05$) C22:(6n-3) values.

The results of the effect of graded levels of sweet orange peel extracts and BHA on serum antioxidant enzyme activities of the experimental broiler meat are presented in Table 4.29. The superoxide dismutase (SOD), serum catalase (CAT) and serum glutathione peroxidase (GP_X) of the meat of broiler chicken fed dietary antioxidants supplementation were different significantly ($p<0.05$). The superoxide dismutase (SOD) of broiler meat on dietary treatment SOPE (0.08 %) was significantly higher ($p<0.05$) compared to other treatments. While bird's meat on BHA treatment had a higher ($p<0.05$) SOD value than those bird's meat on SOPE (0.04 %) treatment which in turn was significantly higher ($p<0.05$) than those bird's meat on SOPE (0.06 %) treatment. Birds on SOPE (0.06 %) treatment had a higher ($p<0.05$) SOD value than bird's meat on SOPE (0.10 %) treatment which had the lowest ($p>0.05$) SOD value.

The serum catalase (CAT) of broiler meat on dietary treatment BHA was significantly higher ($p<0.05$) compared to other treatments. While bird's meat on SOPE (0.06 %) treatment had a higher ($p<0.05$) CAT value than those bird's meat on SOPE (0.04 %) treatment which in turn was significantly higher ($p<0.05$) than those bird's meat on

SOPE (0.08 %) treatment. Birds on SOPE (0.08 %) treatment had a higher ($p<0.05$) CAT value

Table 4.29: Effect of dietary graded levels of antioxidants on serum antioxidant enzyme activities of broiler chicken

Parameters	Treatments (%)						
	BHA		SOPE			SEM	P-Value
	(0.02)	(0.04)	(0.06)	(0.08)	(0.10)		
SOD ((µ/l)	442.05[b]	394.24[c]	276.89[d]	480.78[a]	250.71[e]	0.08	<0.0001
CAT (µ /mg protein)	443.33[a]	366.67[c]	420.00[b]	313.33[d]	306.67[e]	0.52	<0.0001
GP_X (µ /mg protein)	885.81[a]	757.22[d]	842.98[b]	774.46[c]	728.76[e]	0.02	<0.0001

[a, b, c] means having different superscripts along the same row are significantly different ($p< 0.05$)

BHA – butylated hydroxyanisole, SOPE – sweet orange peel extract, SOD -superoxide dismutase, GPX- glutathione peroxidase, CAT – serum catalase

than bird's meat on SOPE (0.10 %) treatment which had the lowest ($p>0.05$) CAT value. The glutathione peroxidase (GP_X) value of broiler meat on dietary treatment BHA was significantly higher ($p<0.05$) compared to other treatments. While bird's meat on SOPE (0.06 %) treatment had a higher ($p<0.05$) GP_X value than those bird's meat on SOPE (0.08 %) treatment which in turn was significantly higher ($p<0.05$) than those bird's meat on SOPE (0.04 %) treatment. Birds on SOPE (0.04 %) treatment had a higher ($p<0.05$) GP_X value than bird's meat on SOPE (0.10 %) treatment which had the lowest ($p>0.05$) GP_X value.

The results of dietary antioxidants effect on state of meat and storage days on both the lipid and protein oxidation of broiler meat are presented in Table 4.30. There was significant effects ($p<0.05$) on both the lipid and protein oxidation of the meat of the birds on BHA, SOPE (0.04, 0.06, 0.08 and 0.10 %) treatments. The lipid oxidation of meat for birds on BHA, SOPE (0.04, 0.06 and 0.10 %) treatments were similar ($p>0.05$) and significantly higher ($p<0.05$) than the bird's meat on treatment SOPE (0.08 %). The lipid oxidation of bird's meat on SOPE (0.08 %) had the lowest ($p>0.05$) lipid oxidation value. There were similarities ($p>0.05$) in the post mortem protein oxidation of the broiler meat on dietary treatments BHA and SOPE (0.04 %), which in turn had a similarity ($p>0.05$) with the protein oxidative stability of bird's meat on SOPE (0.06 %) treatment. However, bird's meat on BHA treatment was significantly higher ($p<0.05$) in protein oxidative value than bird's meat on treatment SOPE (0.08 %) which in turn had a higher ($p<0.05$) protein oxidation than bird's meat on SOPE (0.10 %) treatment. Bird's meat on SOPE (0.10 %) treatment had the lowest ($p>0.05$) protein oxidative value. There were

significant differences ($p<0.05$) in both the lipid and protein oxidation of bird's meat on the state of meat (Cooked and Raw) regardless of the dietary treatments fed to the birds. The lipid oxidation of the cooked bird's meat was significantly higher ($p<0.05$) than the raw meat. Whereas, the protein oxidation of the raw bird's meat was significantly higher ($p<0.05$) than the cooked meat. The lipid oxidation of the bird's meat on post mortem storage day 6 was significantly higher ($p<0.05$) than other storage days. Whereas, meat on post mortem storage day 4 had a higher ($p<0.05$) lipid oxidative value than the meat on storage day 2. However, bird's meat on storage day 2 had a higher ($p<0.05$) lipid oxidative value compared to the on-storage day 0, which has the lowest ($p>0.05$) lipid oxidative value. The protein oxidation of the bird's meat on post mortem storage day 4 was significantly higher ($p<0.05$) than other storage days. Whereas, the protein oxidative value of bird's meat on storage day 6 was significantly higher ($p<0.05$) than the bird's meat on storage days 0 and 2. However, there were similarities ($p>0.05$) in the protein oxidative values of bird's meat on post mortem storage day 0 and 2, which in turn had the lowest ($p>0.05$) protein oxidative values.

The results of the interactive effect of graded levels of sweet orange peel extracts and BHA and their impact on the state of meat (cooked or raw) on both the lipid and protein oxidative stability of broiler meat are presented in Table 4.31. There were significant differences ($p<0.05$) of the treatments BHA, SOPE (0.04, 0.06, 0.08 and 0.10 %) on lipid and protein oxidative stability of the broiler meat. The lipid oxidative values of cooked bird's meat on BHA, SOPE (0.04, 0.06 and 0.10 %) treatments were similar ($p>0.05$) and significantly higher ($p<0.05$) than the lipid oxidation of cooked bird's meat on SOPE (0.08 %) treatment which in turn had the lowest ($p>0.05$) lipid oxidative value. The lipid

oxidation stability of raw bird's meat on dietary SOPE (0.06 %) treatment was significantly higher ($p<0.05$) than

Table 4.30: Main Effect of dietary graded levels of antioxidants, state of meat and storage days on lipid and protein oxidation of broiler meat

Factors		TBARS (mgMDA/kg)	Carbonyl (nmol/mg protein)
Treatments	BHA (0.02 %)	0.37^{a}	0.47^{a}
	SOPE (0.04 %)	0.35^{a}	0.45^{ba}
	SOPE (0.06 %)	0.38^{a}	0.41^{bc}
	SHPE (0.08 %)	0.27^{b}	0.42^{b}
	SOPE (0.10 %)	0.33^{a}	0.36^{c}
	SEM	0.01	0.01
	P value	<0.0001	<0.0001
State of Meat	Cooked	0.43^{a}	0.28^{b}
	Raw	0.25^{b}	0.57^{a}
	SEM	0.01	0.01
	P value	<0.0001	<0.0001
Storage Days	0	0.06^{d}	0.15^{c}
	2	0.12^{c}	0.12^{c}
	4	0.51^{b}	0.96^{a}
	6	0.67^{a}	0.46^{b}
	SEM	0.01	0.01
	P value	<0.0001	<0.0001

$^{a, b, c}$ means having different superscripts along the same column are significantly different ($p< 0.05$)
BHA – butylated hydroxyanisole, SOPE – sweet orange peel extracts, TBARS - Thiobarbituric acid reactive substance

the raw meat of birds on other treatments. However, there were similarities ($p>0.05$) in the lipid oxidative stabilities of raw bird's meat on BHA, SOPE (0.04, 0.08 and 0.10 %) treatments, which in turn had the lowest ($p>0.05$) lipid oxidative values. There were similarities in the protein oxidative values of cooked bird's meat on BHA, SOPE (0.04, 0.08 and 0.10 %) treatments. Meanwhile, the protein oxidation of cooked bird's meat on BHA treatments was significantly higher ($p<0.05$) than the protein oxidation of cooked bird's meat on SOPE (0.06 %) treatment, which in turn had the lowest ($p>0.05$) protein oxidative value. There were similarities in the protein oxidative values of raw bird's meat on BHA, SOPE (0.04, 0.06 and 0.08 %) treatments, which in turn were significantly higher ($p<0.05$) than the protein oxidative value of the raw bird's meat on SOPE (0.10 %) treatment. However, the protein oxidation in raw bird's meat on SOPE (0.10 %) treatment had the lowest ($p>0.05$) protein oxidative value.

The results of the interactive effect of graded levels of sweet orange peel extracts and BHA and storage days (0, 2, 4 and 6) on both the lipid and protein oxidative stability of broiler meat are presented in Table 4.32. Regardless of dietary antioxidants supplementation fed to broiler chicken, the lipid oxidative stability on storage days (2, 4 and 6) and protein oxidative stability on storage day (4), were significantly different ($p<0.05$). Whereas, the lipid oxidative stability of bird's meat on storage days (0) and protein oxidative stability of bird's meat on storage days (0, 2 and 6) were not significantly different ($p>0.05$). The lipid oxidation of meat on SOPE (0.06 %) treatment on storage day 2 was significantly higher ($p<0.05$) than the meat on other treatments and

storage days. However, there were similarities (p>0.05) in the lipid oxidative values of bird's meat on BHA, SOPE (0.04, 0.10 %) on storage day 2 and were significantly higher than bird's meat on SOPE (0.08 %) on

Table 4.31: Interactive effect of dietary graded levels of antioxidants and state of meat on lipid and protein oxidative stability of broiler chicken meat

Treatments (%)	State of Meat	TBARS (mgMDA/kg)	Carbonyl (nmol/mg protein)
BHA (0.02)	Cooked	0.47^{a}	0.33^{c}
SOPE (0.04)		0.47^{a}	0.30^{dc}
SOPE (0.06)		0.46^{a}	0.22^{d}
SOPE (0.08)		0.30^{b}	0.28^{dc}
SOPE (0.10)		0.44^{a}	0.28^{dc}
BHA (0.02)	Raw	0.28^{b}	0.63^{a}
SOPE (0.04)		0.22^{b}	0.60^{a}
SOPE (0.06)		0.30^{a}	0.59^{a}
SOPE (0.08)		0.23^{b}	0.55^{a}
SOPE (0.10)		0.23^{b}	0.45^{b}
SEM		0.02	0.02
P value		<0.0001	<0.0001

$^{a, b, c}$ means having different superscripts along the same column are significantly different ($p< 0.05$)

BHA – butylated hydroxyanisole, SOPE – sweet orange peel extract, TBARS - Thiobarbituric acid reactive substance

storage day 2. However, lipid oxidative stability of the bird's meat on SOPE (0.08 %) storage day 2 had the lowest ($p>0.05$) value. There were similarities ($p>0.05$) in the lipid oxidative stability of the bird's meat on BHA, SOPE (0.04, 0.08 and 0.10 %) on storage day 4 which in turn were significantly higher ($p<0.05$) than the bird's meat on SOPE (0.06 %) and day 4. However, bird's meat on SOPE (0.06 %) storage day 4 had the lowest ($p>0.05$) lipid oxidative value. There were similarities ($p>0.05$) in the protein oxidative stability of the bird's meat on BHA, SOPE (0.04 %) storage day 4 which in turn were significantly higher ($p<0.05$) than the bird's meat on SOPE (0.06 and 0.08 %) and day 4. However, bird's meat on SOPE (0.06 and 0.08 %) storage day 4 were significantly higher ($p<0.05$) in protein oxidative values than the bird's meat on SOPE (0.10 %) on day 4. The bird's meat on SOPE (0.10 %) on storage day 4 had the lowest ($p>0.05$) protein oxidative value. There were some similarities ($p>0.05$) in the protein oxidative values of bird's meat on SOPE (0.06 %) and BHA, storage day 6 and were significantly higher than other treatments. The protein oxidative stability of bird's meat on SOPE (0.04 and 0.10 %) have some similarities ($p>0.05$) and were significantly higher ($p>0.05$) than those bird's meat on SOPE (0.08 %) on storage day 6 treatment. However, the bird's meat on SOPE (0.08 %) on storage day 6 had the lowest ($p>0.05$) protein oxidative value.

The results of the interactive effect of graded levels of sweet orange peel extracts and BHA and their impact on the state of meat (cooked or raw) and the storage days (0,2,4 and 6) on both the lipid and protein oxidative stability of broiler meat are presented in

Table 4.33. There were significant differences ($p<0.05$) on the state of meat (cooked or raw) and the storage days (0,2,4 and 6) on the broiler chicken meat fed graded levels of dietary

Table 4.32: Interactive effect of graded levels of antioxidants and storage days on lipid and protein oxidative stability of broiler chicken meat

Treatments (%)	Storage Days	TBARS (mgMDA/kg)	Carbonyl (nmol/mg protein)
BHA (0.02)	0	0.05	0.15
SOPE (0.04)		0.06	0.15
SOPE (0.06)		0.07	0.15
SOPE (0.08)		0.05	0.15
SOPE (0.10)		0.08	0.15
P-Value		>0.10	0.07
BHA (0.02)	2	0.16^{fg}	0.14
SOPE (0.04)		0.12^{fg}	0.13
SOPE (0.06)		0.21^{f}	0.15
SOPE (0.08)		0.03^{g}	0.10
SOPE (0.10)		0.11^{fg}	0.10
P Value		<0.0001	0.07
BHA (0.02)	4	0.55^{cde}	1.13^{a}
SOPE (0.04)		0.55^{cde}	1.07^{a}
SOPE (0.06)		0.42^{e}	0.93^{b}
SOPE (0.08)		0.51^{cde}	0.92^{b}
SOPE (0.10)		0.51^{cde}	0.75^{c}
P Value		<0.0001	0.0001
BHA (0.02)	6	0.74^{ab}	0.50
SOPE (0.04)		0.66^{abc}	0.46
SOPE (0.06)		0.82^{a}	0.41
SOPE (0.08)		0.49^{de}	0.49
SOPE (0.10)		0.64^{bcd}	0.47
P value		<0.003	0.07

$^{a, b, c}$ means having different superscripts along the same column are significantly different ($p< 0.05$)

BHA – butylated hydroxyanisole, SOPE – sweet orange peel extract, TBARS - Thiobarbituric acid reactive substance

antioxidants supplementation. The lipid oxidation of the cooked bird's meat on storage day 6 was significantly higher ($p<0.05$) than both the cooked bird's meat on storage days (0, 2

4) and raw bird's meat at storage days (0, 2, 4 and 6). The lipid oxidative value of cooked bird's meat on storage day 4 was higher ($p<0.05$) than raw bird's meat on storage day 6 which in turn was higher ($p<0.05$) than the lipid oxidative value of raw bird's meat on storage day 4. Whereas, the lipid oxidative value of raw bird's meat on storage day 4 was significantly higher ($p<0.05$) than cooked bird's meat on storage day 2.

However, there were some similarities ($p>0.05$) in the lipid oxidative values of cooked bird's meat on storage days (0 and 2) and raw bird's meat on storage day 2. Similarly, there were also similarities ($p>0.05$) in the lipid oxidative values of both cooked bird's meats on storage day 0 and raw meat on storage days 0 and 2. However, lipid oxidation in raw meat on day 0 had the lowest ($p>0.05$) value. The protein oxidative value of raw bird's meat on storage day 4 was higher ($p<0.05$) than either cooked on storage days (0, 2, 4 and 6) or raw bird's meat on storage days (0, 2 and 6). Whereas, the protein oxidative value of raw bird's meat on storage day 6 was higher ($p<0.05$) than the cooked meat on storage days 4 and 6. There were similarities ($p>0.05$) in the protein oxidative values of cooked bird's meat on storage days 4 and 6 and are also higher ($p<0.05$) than both the cooked and raw bird's meat on storage days 0 and 2. However, the protein oxidation of both cooked and raw bird's meat on storage days 0 and 2 had the lowest ($p>0.05$) value.

The results of the interactive effect of the dietary treatments and their impact on the state of meat (cooked or raw) and the storage days (0,2,4 and 6) on both the lipid and protein oxidative stability of broiler meat are presented in Table 4.34. Regardless of the dietary

Table 4.33: Interactive effect of state of meat and storage days on lipid and protein oxidative stability of broiler chicken meat

State of Meat	Storage Days	TBARS (mgMDA/kg)	Carbonyl (nmol/mg protein)
Cooked	0	0.08^{ef}	0.15^{d}
	2	0.16^{e}	0.13^{d}
	4	0.65^{b}	0.45^{c}
	6	1.83^{a}	0.39^{c}
Raw	0	0.04^{f}	0.15^{d}
	2	0.09^{ef}	0.11^{d}
	4	0.36^{d}	0.46^{a}
	6	0.51^{c}	0.54^{b}
SEM		0.019	0.019
P value		<0.0001	<0.0001

[a, b, c] means having different superscripts along the same column are significantly different ($p< 0.05$)
BHA – butylated hydroxyanisole, SOPE – sweet orange peel extract, TBARS - Thiobarbituric acid reactive substance

treatments fed to broiler chickens, the lipid oxidative stability on state of meat (cooked or raw) and storage days (0,2,4 and 6) were significantly different ($p<0.05$). Whereas, irrespective of the dietary treatments fed to broiler chickens, the protein oxidative stability on state of meat (cooked or raw) and storage days (2,4) and cooked bird's meat on storage day 6 were significantly different ($p<0.05$). However, the protein oxidative value of both cooked and storage day 0 and raw bird's meat on storage days 0 and 6 based on dietary treatments, were not significantly different ($p>0.05$).

There were similarities ($p>0.05$) in the lipid oxidative stability of raw broiler meat fed dietary treatments BHA, SOPE (0.04 and 0.08 %) on storage days 0, which in turn were significantly higher ($p<0.05$) than the cooked bird's meat fed dietary BHA, SOPE (0.04, 0.06, 0.08 %) and raw bird's meat fed dietary treatments SOPE (0.06 and 0.10 %) on storage day 0. Meanwhile, there were some similarities ($p>0.05$) in the lipid oxidative stability of cooked bird's meat fed dietary BHA, SOPE (0.04, 0.06, 0.08 %) and raw bird's meat fed dietary treatments SOPE (0.06 and 0.10 %) on storage day 0, which in turn were significantly higher ($p<0.05$) than cooked bird's meat fed dietary SOPE (0.10 %) treatment on storage day 0. However, cooked bird's meat fed dietary SOPE (0.10 %) treatment on storage day 0 had the lowest ($p>0.05$) lipid oxidative value. The lipid oxidative value of cooked bird's meat fed dietary SOPE (0.06 %) treatment on storage day 2 was significantly higher ($p<0.05$) than other bird's meat (cooked or raw) fed dietary treatments. There were similarities ($p>0.05$) in the lipid oxidative stability of cooked broiler meat fed dietary treatments BHA, SOPE (0.04 and 0.10 %) on storage day 2 and raw bird's meat fed treatment SOPE (0.06 %) on storage day 2, which in turn were significantly higher ($p<0.05$) than the raw bird's meat fed dietary BHA on storage day 2.

The lipid oxidative stability of raw bird's meat fed dietary BHA on storage day 2 was significantly higher($p<0.05$) than raw bird's meat fed SOPE (0.04 and 0.08 %) treatments on storage day 2. Meanwhile, there were similarities ($p>0.05$) in the lipid oxidative stability of raw bird's meat fed dietary SOPE (0.04 and 0.08 %) treatments on storage day 2. Which in turn were significantly higher ($p<0.05$) than the cooked bird's meat fed SOPE (0.08 %) and raw bird's meat fed SOPE (0.10 %) treatments both on storage day 2. Meanwhile, there were similarities ($p>0.05$) in the lipid oxidative stability of cooked bird's meat fed dietary SOPE (0.08 %) treatment on storage day 2 and raw bird's meat fed dietary treatments SOPE (0.10 %) on storage day 2. However, cooked bird's meat fed dietary SOPE (0.08 %) treatment and raw bird's meat fed treatment SOPE (0.10 %) both on storage day 2 had the lowest ($p>0.05$) lipid oxidative values. There were similarities ($p>0.05$) in the protein oxidative stability of cooked broiler meat fed dietary treatments BHA, SOPE (0.04, 0.06 and 0.10 %) on storage day 2 and raw bird's meat fed treatment BHA, SOPE (0.06 %) on storage day 2, which in turn were significantly higher ($p<0.05$) than the cooked bird's meat fed SOPE (0.08 %) treatment and raw bird's meat fed dietary SOPE (0.04 and 0.08 %) treatments both on storage day 2. Similarly, there were also similarities ($p>0.05$) on the protein oxidative stability of the cooked bird's meat fed SOPE (0.08 %) treatment and raw bird's meat fed dietary SOPE (0.04 and 0.08 %) treatments both on storage day 2 which in turn were higher ($p<0.05$) than the raw bird's meat fed dietary treatment SOPE (0.10 %) on storage day2. However, raw bird's meat fed dietary treatment SOPE (0.10 %) on storage day2 had the lowest ($p>0.05$) protein oxidative values. There were some similarities ($p>0.05$) in the lipid oxidative stability of cooked broiler meat fed dietary treatments BHA, SOPE (0.04 %) on storage day 4, which

in turn were significantly higher ($p<0.05$) than the cooked bird's meat fed SOPE (0.08, 0.06 and 0.10 %) treatment and raw bird's meat fed dietary BHA, SOPE (0.04, 0.06, 0.08 and 0.10 %) treatments both on storage day 4.The lipid oxidative stability of cooked bird's meat fed SOPE (0.08 %) on storage day 4 was higher ($p<0.05$) than cooked bird's meat fed SOPE (0.06 and 0.10 %) storage day 4 and raw bird's meat fed SOPE (0.10 %) on storage day 4. However, there were similarities in the lipid oxidative values of cooked bird's meat fed SOPE (0.06 and 0.10 %) and raw bird's meat fed SOPE (0.10 %) treatments both on storage days 4 which in turn were higher ($p<0.05$) than raw bird's meat fed SOPE (0.06 and 0.08 %) storage day 4. Similarly, there were some similarities ($p>0.05$) in the lipid oxidative stability of raw bird's meat fed SOPE (0.06 and 0.08 %) on storage day 4, and also had a higher ($p<0.05$) lipid oxidative value than raw bird's meat fed SOPE (0.04 %) on storage day 4. Raw bird's meat fed SOPE (0.04 %) storage day 4 had a higher ($p<0.05$) lipid oxidative value than the raw bird's meat fed BHA treatment and storage day 4. However, raw bird's meat fed BHA treatment on storage day 4 had the lowest ($p>0.05$) lipid oxidative value. There were some similarities ($p>0.05$) in the protein oxidative stability of raw broiler meat fed dietary treatments BHA, SOPE (0.04 and 0.06 %) on storage day 4, which in turn were significantly higher ($p<0.05$) than the raw bird's meat fed SOPE (0.08 and 0.10 %) treatments and on storage day 4. Meanwhile, there were similarities in the protein oxidative values of the raw bird's meat fed SOPE (0.08 and 0.10 %) treatments and on storage day 4 and are in turn had a higher ($p<0.05$) protein oxidative value than cooked bird's meat fed BHA and SOPE (0.04 %) on storage day 4. However, there were some similarities in the protein oxidative stability of cooked bird's meat fed SOPE (0.08 and 0.10 %) storage day 4, which are in turn

significantly higher ($p<0.05$) in the protein oxidative value than cooked bird's meat fed SOPE (0.06 %) on storage day 4. However, the cooked bird's meat fed SOPE (0.06 %) had the lowest ($p>0.05$) protein oxidative. There were similarities in the lipid oxidative stability of cooked bird's meat fed SOPE (0.06, 0.04 and 0.10 %) treatments on storage day 6 which in turn had a higher ($p<0.05$) lipid oxidative value than the cooked bird's meat fed BHA treatment on storage day 6. The cooked bird's meat fed BHA treatment and storage day 6 had a higher ($p<0.05$) lipid oxidative value than raw bird's meat fed BHA treatment on storage day 6. Meanwhile, the raw bird's meat fed BHA treatment on storage day 6 had a higher ($p<0.05$) lipid oxidative value than cooked bird's meat fed SOPE (0.08 %) treatment and raw bird's meat fed (SOPE (0.04 and 0.06 %) treatments both on storage day 6. There were similarities ($p>0.05$) in the lipid oxidative stability of cooked bird's meat fed SOPE (0.08 %) treatment and raw bird's meat fed (SOPE (0.04 and 0.06 %) treatments both on storage day 6, which in turn had a higher ($p<0.05$) lipid oxidative value than raw bird's meat fed SOPE (0.08 and 0.10 %) treatments on storage day 6. However, the raw bird's meat fed SOPE (0.08 and 0.10 %) treatments on storage day 6 had the lowest ($p>0.05$) lipid oxidative value. There were similarities ($p>0.05$) in the protein oxidative stability of cooked bird's meat fed BHA, SOPE (0.04, 0.08 and 0.10 %) on storage day 6 which in turn were significantly higher ($p<0.05$) in protein oxidative value than cooked bird's meat fed SOPE (0.06 %) treatment on storage day 6. However, the cooked bird's meat fed SOPE (0.06 %) treatment on storage day 6 had the lowest ($p>0.05$) protein oxidative value.

Table 4.34: Interactive effect of dietary graded levels of antioxidants, state of meat and storage days on lipid and protein stability of broiler chicken meat.

Factors		Treatments (%)											
State of Meat	Storage days	BHA		SOPE (0.04)		SOPE (0.06)		SOPE (0.08)		SOPE (0.10)		P VALUE	P VALUE
		TBARS	CARB	TBARS	CARB	TBARS	CARB	TBARS	CARB	TBARS	CARB	TBARS	CARB
Cooked	0	0.08^{mn}	0.15	0.07^{mn}	0.15	0.08^{mn}	0.14	0.08^{mn}	0.15	0.09^{lmn}	0.15	<0.0002	0.082
Raw	0	0.02^{n}	0.15	0.04^{n}	0.15	0.06^{mn}	0.15	0.02^{n}	0.15	0.06^{mn}	0.15	<0.0001	0.082
Cooked	2	0.18^{klm}	0.14^{ghi}	0.18^{klm}	0.14^{ghi}	0.24^{gkl}	0.15^{ghi}	0.01^{n}	0.09^{hi}	0.19^{klm}	0.14^{ghi}	<0.0001	<0.0001
Raw	2	0.13^{lmn}	0.13^{ghi}	0.06^{mn}	0.11^{hi}	0.17^{glm}	0.14^{ghi}	0.06^{mn}	0.11^{hi}	0.03^{n}	0.05^{i}	<0.003	<0.0001
Cooked	4	0.78^{bc}	0.56^{d}	0.74^{bcd}	0.52^{de}	0.49^{efg}	0.32^{efg}	0.63^{bef}	0.44^{def}	0.16^{efg}	0.43^{def}	0.002	0.002
Raw	4	0.32^{hlm}	1.70^{a}	0.35^{gkl}	1.61^{a}	0.36^{gk}	1.54^{ab}	0.38^{fgk}	1.06^{c}	0.41^{efg}	1.06^{c}	0.002	0.001
Cooked	6	0.83^{abc}	0.45^{def}	1.88^{ab}	0.37^{def}	1.05^{a}	0.28^{fgh}	0.50^{fgi}	0.44^{def}	0.88^{ab}	0.39^{def}	0.002	0.002
Raw	6	0.65^{bde}	0.55	0.44^{fgi}	0.54	0.59^{dfg}	0.54	0.47^{efh}	0.54	0.40^{efj}	0.54	<0.0001	0.06

^{a,b,c} means having different superscripts along the same row are significantly different ($p< 0.005$)

BHA – butylated hydroxyanisole, SOPE – Sweet Orange Peel Extract, TBARS – Thiobarbituric Acid Reactive Substance, CARB – Carbonyl

CHAPTER FIVE

5.0 DISCUSSION

5.1 Phenolic Compounds in Peel Extracts of Sweet Orange, Shaddock and Lemon Fruits

From the study, the phenolics, steroids, flavonoids, coumarins, triterpenes and alkaloids that are present in sweet orange, shaddock and lemon peel extracts, are due to the fact that practically all fruit plant tissues can manufacture phenolic chemicals in their pulp or peels (Shahidi and Naczk, 2004). The findings of this study are consistent with those of Hafiz *et al*. (2020), who found polyphenol content and antioxidant potential in twenty different fruit peel samples using an ethanolic extraction method, as well as detailed characterisation and quantification using LC-MS and HPLC. In a study to investigate the qualitative presence of natural antioxidants in grape seed peels, faba bean peels, buck-wheat peels, and oil hemp seed peels, Medina (2011) discovered the presence of certain natural antioxidants in bulk wheat, grape seed peels, faba bean peels, and oil hemp seed peels.

5.1.1 Total phenolics compounds present in sweet orange, shaddock and lemon peel extracts

The total phenolic compounds in saponins and phenolics that was found to be higher in shaddock peel extracts (SHPE) (0.42 and 26.76 mg/100 g DW) as compared to sweet orange peel extracts (SOPE) (0.19 and 2.68 mg/100 g DW) and LMPE (0.00 and 1.79 mg/100 g DW), could be owing to the abundant betacyanin pigments found in shaddock plants, which have been linked to the formation of phenolic compounds in plant tissue by raising the phenolic content (Shahidi *et al*., 2019). This study was consistent with the findings of Nurliyana *et al*. (2010), who discovered that dragon fruit peel contains more

phenolic chemicals than grape and mango fruit peel. Tannins are a type of phenolic substance that can be divided into two categories: hydrolysable and condensed tannins. The increased tannin content of lemon peel extract LMPE (1.74 mg/100 g DW) compared to SHPE (1.63 mg/100 g DW) and SOPE could be attributed to the long ripening process in lemon fruit, which can slow the rate at which tannin is hydrolysed. Overall, the findings are consistent with those of (Hafiz *et al*.,2020), who discovered that avocado peel had a greater total tannin concentration (9.01 0.20 mg CE/g) than mango, sweet orange, and lemon peel.

The low value of SOPE (0.00 mg/100 g DW) may be related to the hydrolysable kind of tannin it contains, which frequently drops during the ripening process (Masibo and He, 2008). The highest amount of flavonoid was found in the SOPE (161.82 mg/100 mg DW), followed by LMPE (160 mg/100 mg DW) and SHPE (148.13 mg/100 mg DW), corroborate with those of Marina and Noriham (2014), who found that mango peel contains more flavonoids than other tropical fruit peels including guava and pineapple peels. Flavonoids are found in higher concentrations in tropical fruits that ripen quickly. However, the flavonoid content variation observed in this study varies from that reported by Ayala – Zavala *et al*. (2011), who discovered increased flavonoid content in lemon and other tropical fruits. This could be due to changes in growing regions, environmental circumstances, variety differences, and extraction methods. Fruits grown in different climates have variable flavonoid content in their peels, with the peels being the exterior part of the fruit body exposed to more sunlight than the pulp, resulting in the synthesis of flavonoids that are plentiful and diversified. The flavonoid profile of the same fruits cultivated in different regions under varied climatic circumstances, soil qualities, and

cultivation practices has a variable flavonoid profile (Loh *et al*., 2017). Furthermore, the extraction efficiency of flavonoids varies depending on extraction parameters such as solvent type, solvent concentration, extraction duration and temperature, and solvent-to-solid ratio (Ruiz- Montanez *et al*., 2014). Glycosides, triterpenes and alkaloids that were higher in SOPE (2.00, 0.13 and 32.44 mg/100 mg DW) compared with LMPE (0.88, 0.12 and 6.55 mg/100 mg DW) and SHPE (0.00, 0.11 and 4.03 mg/ 100 mg DW), suggest that secondary metabolites such as glycosides, triterpenes, and alkaloids were simpler to extract from sweet orange peel than lemon or shaddock peel using the methanol extraction method. The findings of this study correspond with those of Amin *et al*. (2017), who found that utilising the methanol extraction method, the contents of metabolites such as alkaloids were simpler to extract in orange peel than in lemon peel.

5.1.2 Diphenyl 2- picrylhydrazyl (DPPH) inhibition of sweet orange, shaddock and lemon peel extracts

When compared to other extracts, the SOPE had a higher DPPH per cent inhibition concentration of 89.27 mg/ml at 100 % inhibition level. This contradicts the findings of Ajila *et al*. (2007), who found that grapefruit peels had a better ability to scavenge DPPH radicals than sweet orange and mango peels (9.17 ± 0.19, 8.67 ± 0.49 and 8.67 ± 0.44 mg AAE/g, respectively). This could be due to the freeze-drying procedure used on the fruit peels. Free radicals are scavenged and neutralized by the freeze-drying process, which produces redox-active metabolites (Castro- Vazquez *et al*., 2016). In other words, SOPE will be more effective at chelating the hydroxyl free radicals in glutathione peroxidase, preventing aging in people and animals and reducing oxidative damage in meat products.

This study adds to Sara *et al*. (2008) findings, which indicated that there was a link between glutathione peroxidase, antioxidant enzymes, disease, and aging in humans.

5.2 First Feeding Trial

5.2.1 Growth performance of broiler chickens fed dietary antioxidants

The highest final body weight (3390.00 g) and weight gain (59.80 g) observed in LMPE treatment compared to both positive (BHA treatment) and negative (OW) controls, could be attributed to the presence of phenolic compounds, which aid in nutritional digestion and use by birds. The findings of this study agree with those of Papa-dopoulou *et al*. (2005), who found that phenolic grape extracts influence microflora by reducing the number of propionibacteria, bacteroides, and clostridia and increasing the population of lactobacilli and bifido bacteria in the gastro intestinal tracts (GIT) of birds, which aids in nutrient digestion and utilisation. This finding was also consistent with a study to assess the effect of aqueous *Moringa oleifera* (Lam) leaf extracts on growth performance and carcass characteristics of hubbard broiler chicken, Alabi *et al*. (2017) found that birds fed AMOLE0+ treatment had the greatest FBW and DBWG when compared to other treatments. Interestingly, the feed intake (93.27 g/b/d) and water intake (0.25 l/b/d) of birds on negative control treatment (OW) were high but did not translate into gain in weight. This contradicts the assumption that as feed intake of an animal is increased, the weight gain should also increase (Ishola and Atteh, 2018; Atteh 2002). This might mean the feed is not properly digested or not utilised. The high figure observed in both feed and water intake in the negative control (OW) may also be attributable to the presence of some phytochemicals like tannins and saponins that exhibit bitter taste perception in the extracts containing treatments, which eventually affect the bird's feeding. The similarity

of feed consumption in this study is consistent with the findings of Samar *et al*. (2014), who supplemented antioxidants such as butylated hydroxytoulene (BHT) and vitamin C. In this study, the feed conversion ratio (FCR) of birds on dietary treatment LMPE had the lowest FCR value (1.51) as compared to high FCR of birds on negative control OW (1.84). This indicated that birds on LMPE treatment utilised their feeds efficiently as compared to birds on negative control OW. This could be due to the presence of beneficial phenolic chemicals that aid in digestion, nutrient utilisation, and may have functioned as a growth stimulant in the feed. This discovery is in line with the findings of a study conducted on broiler chicks fed a diet low in Alphamune G. (Bolu *et al*., 2012).

5.2.2 Haematology parameters of broiler chickens fed dietary antioxidants

The birds on OW a negative control treatment had the highest leukocytes, white blood cell (WBC) (53.50 10^9/l) contents in the present study. This could be owing to stress-induced glucocorticoids, which are used to keep pigeons on the nutritional therapy OW immune-competent. This finding contradicts Cirule *et al*. (2012), who found that adding antioxidants, genistein, and hesperidin to the broiler feed boosted the bird's WBC. Birds on dietary treatment SHPE, had the highest lymphocyte value of (35.20 10^9/l) compared to other treatments. This could indicate that SHPE has the ability to cause a fast influx of leukocytes from the birds' bone marrow into the bloodstream. This result was consistent with Cirule *et al*. (2012) findings. The high haemoglobin (Hb) levels associated with dietary supplementation with SHPE (16.80 g/dl) and LMPE (16.10 g/dl) could be linked to fat digestion, because dietary anti-oxidants promote fat digestion by increasing the availability of substrates for sz-oxidation and succinyl- CoA production via the Krebs cycle (Cunningham and Klein, 2005), both of which are linked to increased haemoglobin

production. this study is consistent with that reported in antibody titres of broilers dietary supplemented with genistein, an antioxidant (Rasouli and Jahanian, 2015). Mean corpuscular haemoglobin (MCH), an average amount of Hb per red blood cell (RBC) that is high in birds on treatment LMPE (8.40 x 106/mm3), is a helpful measure for estimating the degree of anaemia (Aguihe *et al*., 2017), which corresponds with the findings of the study (Rasouli and Jahanian, 2015). Furthermore, dietary treatments of SHPE with the highest value of (31.90 pg) increased the MCH index of broiler chickens to some extent, indicating that these compounds have the ability to improve the health of growing birds. Natural antioxidants aid in the maintenance of optimal health in both animals and humans by up-regulating immunological pathways that control and mitigate the negative effects of excessive ROS generation (Puertollano *et al*., 2011). Antioxidant supplementation increased the generation of antibodies against Newcastle disease in the current investigation. The MCH finding in this study is similar to that seen in antibody titres of broilers fed a genistein-rich diet (Rasouli and Jahanian 2015). The immune-stimulatory, anti-inflammatory, and antibacterial capabilities of the dietary antioxidants used in this study (Havsteen 2002; Kamboh *et al*., 2015) could explain the health impacts reported in the birds.

5.2.3 Serum biochemistry (blood) parameters of broiler chickens fed dietary antioxidants

In the present study, the total protein value was observed to be higher in the negative control treatment OW (8.87 mg/dl) as compared to other treatments supplemented with antioxidants. Despite the fact that all of the treatments' total protein contents are within the usual range of 25.00 g/l for broiler chicks (Harr, 2002). However, the high value seen

in the OW therapy could be due to the absence of antioxidants, which are known to boost serum protein synthesis in the liver. This study's findings are consistent with those of Mehdi *et al*. (2018), who found variance in total protein in broiler chicks fed dietary ginger.

Total cholesterol and triglycerides levels in supplemented dietary SOPE (468.16 mg/dl) and SHPE (133.90 mg/dl) were found to be high. This rise in total cholesterol could be due to the presence of phenolic chemicals in the blood, which enhance lipoprotein activity.

Lipoproteins are involved in the transfer of cholesterol in the blood of animals. Furthermore, because triglycerides are produced in the intestinal mucosa and liver as a result of the digestion of dietary components and the absorption of fatty acids, antioxidants play an important role in nutrient digestion and absorption.

As a result, the SHPE treatment, which contains phenolic compounds, has a higher triglyceride value than the negative control (OW) treatment. However, the results of this study contrast those of Musa *et al*. (2007), who found no significant differences in cholesterol and triglyceride levels in 12-week-old Anka breed hens fed supplementary antioxidant dietary feed. This could be due to the types and quantities of phytochemicals found in fruit peels, which will impact the phytochemicals' efficacy in lowering lipid levels and components in the blood (Onakpoya *et al*., 2013). Because the presence of phenolic compounds in dietary supplemented antioxidant feeds has a tendency to modify HDL and low-density lipoprotein (LDL) concentrations by decreasing HDL and increasing LDL in the blood, the high value of high-density lipoprotein (HDL) observed in SOPE treatment (279.76 mg/dl) is understandable (Emilia *et al*., 2020). The findings

of this study correspond with those of Alizadeh-Navaei *et al*. (2008), who found that supplementing broiler chicks with ginger powder raises low-density lipoprotein levels while decreasing high-density lipoprotein levels in the bloodstream. Aspartate amino transferase (AST) and alanine amino transferase (ALT) were significantly influenced by the dietary supplementation with antioxidants. Antioxidants in the diet considerably lower AST levels, with the negative control OW (384.21 u/l) having the greatest AST level in the blood. All dietary antioxidant treatments, with the exception of SHPE (112.69 u/l), diminish the amount of ALT in the birds' bloodstream. Because AST and ALT are sensitive, non-specific biomarkers of liver disease in birds, the low levels of AST and ALT found in diets supplemented with antioxidants could attest to the ability of the experimental diets to potentially treat liver damage when compared to the negative control OW treatment. The findings of this study are similar to those of Akram *et al*. (2010), who found that turmeric and curcumin can reverse biliary hyperplasia and necrosis in rats' afflicted livers.

In the present study, there was increased activities of superoxide dismutase (SOD), glutathione peroxidase (GP_X) and serum catalase (CAT) in SHPE (119.40 u/l), BHA (156.38 u/mg) and LMPE (718.39 u/mg protein) as compared to the negative control OW, could be attributed to the activities of phytochemical compounds present in SHPE which enables the enzymes to actively function as it was expected. The findings of this study agree with those of Gutowicz *et al*. (2008), who found that a rise in the activity of SOD, GP_X, and CAT in the blood of birds is induced by the environmental burden they are exposed to during their development. The activity of antioxidant enzymes such as

superoxide dismutase (SOD), GP_X, and CAT, as well as other antioxidant enzymes, is dependent on the number of antioxidants in the diet.

5.2.4 Carcass traits of broiler chickens fed dietary antioxidants

In the present study, the live weight before slaughter, bled and carcass weight after slaughter were observed to be high in LMPE treatment (3390.00 g), (3270.00 g) and (2466.00 g), respectively as compared to other treatments and especially, the negative control, OW. The high values reported could be due to the high quantity of tannins and coumarin in the LMPE treatment, which may have reduced parasite microorganisms in the birds' gastro intestinal tract (GIT), hence increasing the amount of nutrients absorbed by the bird's intestinal mucosa. This finding is similar to that of Jiya *et al*. (2014), who found that the cut-up parts of the experimental broiler's breast and back differed considerably between experimental groups fed beniseed powder and *Moringa oleifera* drumstick leaves as a source of lysine. However, it contradicts the findings of Ebrahimi *et al*. (2013), who found that the effect of several treatments supplemented with dried *C. sinesis* peel on broiler final weight and carcass % was not substantially different from the control group.

The discrepancy could be due to the management strategies used and the feeding experiments' geographic location. The results of this study show that birds fed SHPE had a larger (3.23 %) intestinal weight than birds fed other treatments, including the negative control OW. The presence of some metabolites in SHPE could impact hypothalamic peptides implicated in appetite regulation, resulting in an increase in the size of the small intestine and its absorptive capacity (Hai *et al*., 2000). In this study, the abdominal fat

level was lower in the birds fed SOPE (0.86) treatment compared with the positive control BHA (1.77) that had the highest level of abdominal fat. The low amount of abdominal fat in SOPE treatment could be due to a phytochemical component found in SOPE that inhibited pancreatic lipase, reducing lipid digestion and absorption and so decreasing body fat accumulation or precipitation. The findings of this study are congruent with those of Hossin, (2009), who found indications of pomegranate peel extracts interfering with obesity in hypercholesterolemic rats. This was also confirmed by Lei *et al*. (2007), who discovered evidence of an anti-obesity benefit of pomegranate leaf extract in obese mice fed a high-fat diet.

5.2.5 Meat quality (physicochemical properties) of broiler chickens fed dietary antioxidants

The ability of meat to hold its inherent and additional moisture during preparation and storage is known as water holding capacity (WHC). The water holding capacity, cooking, and drip loss in broiler meat were not significantly affected by dietary antioxidant supplementation on the birds in this study. However, this contradicts the findings of Berg and Allee (2001); Maddock *et al*. (2002), who found that the pectoralis muscle of chickens fed creatine-glucose supplements had a substantial impact on the animal's cooking and drip loss. The pH value in birds fed BHA treatment was higher (5.6) than in birds fed other treatments. The pH in the pectoralis muscles of the experimental broiler chicken fed nutritional antioxidants (SOPE, SHPE, and LMPE) was lowered. The pH drop could be due to glycogen production triggered by myotube enlargement (Low *et al*., 1996). The results of this study correspond with those of Jette *et al*. (2004), who assessed the pectoralis muscle in chickens by feeding them nutritional creatine supplementation.

Pigments in haemoglobin, pre-slaughter, slaughter, and processing methods are all essential determinants in broiler meat colour. Lower pH on pectoralis muscles in hens has been linked to lighter colour (L*) and reduced water retention capacity (Fletcher, 1999). (Dransfield and Sosnicki, 1999). Meat quality features (low pH, WHC, and lighter coloration (L*) of the meat) were more prominent in SOPE (18.47) treatments, indicating that phenolic chemicals responsible for increasing meat quality had been deeply incorporated. The findings of this study agree with those of Jette *et al*. (2004), who looked at the colour properties of chicken flesh provided dietary creatine supplementation. Furthermore, the high redness (a*) value reported in treatment SOPE (8.19), showed that the meat quality of the birds in treatment SOPE was preserved during post mortem ageing. The findings of this study are consistent with those of Jette *et al*. (2004), who found that dietary creatine supplementation impacted the colour characteristics of chicken flesh in broiler chicks.

5.2.6 Meat quality (shear force analysis) of broiler chickens fed dietary antioxidants

When a meat is chopped and torn, the force peak and yield are indications that determine the shear force. The current study found that supplementing broiler chickens' diets with SHPE (3.50 and 1.20 N) and SOPE (7.20 and 1.50 N) improved meat tenderness. The fundamental mechanism, however, is not well understood. However, it is thought that a meat's tenderness is determined by its shear force. According to Lyon and Lyon (2001), shear force values between 3.2 to 30.61 N for chicken meat were considered soft. This contradicts the findings of Alfaig *et al*. (2014), who found that the shear force value of pectoralis muscle in the probiotic-supplemented condition was 26.3 0.28 N. This study's

fluctuation in shear force values is similar to Harris *et al*, (2001)'s study, which found that high amounts of vitamin E in beef improved the rate of softness. The shear force values of pectoralis muscles of the experimental broiler birds' strain (peak and yield) were higher in dietary SHPE (95.09 %) and BHA (11.97 %) supplementation. This is in line with the findings of Rebecca (2013), who found that the tensile strength of the bird's meat fed with supplemented antioxidants was high, and that the stress with which the cutting material slices through the meat tissue and the strain that occurs as a result of this procedure on the meat tissue were both high.

5.2.7 Meat quality (sensory evaluation) of broiler chickens fed dietary antioxidants

The panellists' perceptions of taste (8.00), aroma (7.80), and overall acceptability (7.95) in broiler meat fed dietary SOPE were all high in this study's findings. This could be due to the presence of aroma-producing phytochemicals in the dietary SOPE therapy, which could stimulate the olfactory sensual response to the boiled beef (Pawer *et.al*., 2007). In essence, the findings reveal that the panellists gave the dietary SOPE supplementation on broiler meat greater scores on taste, aroma, and overall acceptability. The results showed that panellists preferred broiler chicken meat from SOPE-supplemented diets above meat from control treatments BHA and OW, as well as treatments SHPE and LMPE. This finding is consistent with Zdunczyk *et al*. (2010), who found that adding 30 mg/kg of Macleaya cordata plant extracts, which belongs to the same alkaloid family as black pepper, to breast flesh resulted in a powerful smell without any peculiar odour. Regardless of whether the birds in this study received dietary antioxidant treatment, the appearance and texture criteria were unaffected. This contrasts the findings of Kim *et al*.

(2009), who found that adding dietary garlic bulb and husk supplementation to chicken meat improved the texture and flavour of the meat.

5.2.8 Fatty acids composition of broiler chickens fed dietary antioxidants

Animal diets influence fatty acid profile, which is frequently used for meat fatty acid profile modification with excellent effectiveness in single-stomached birds (Wood *et* al., 2004). The findings of the treatments SOPE, SHPE, and LMPE showed that dietary antioxidant supplementation on broiler chicks reduced the amount and number of fatty acids in the broiler meat significantly. In the present study, the effect of dietary supplementation of antioxidants on the fatty acid profile of broiler pectoralis muscles were clarified as follows:

Saturated fatty acids profile (SFA) - The high levels of caproic (7.71), capric (39.66), enanthic (29.48), and propionic (3.90) acids found in the negative control OW compared to other treatments supplemented with dietary antioxidants could be due to the lack of phytochemicals in the dietary treatment, which could either inhibit or aid in the oxidation of the lipids to either be metabolised or precipitated in the tissue. The high levels of palmitic (4.25) and lauric (2.00) acids seen in the SHPE treatment could indicate that the broiler tissue has a limited capacity for some of the saturated fatty acid changes caused by dietary antioxidant supplementation. Supplementation with butylated hydroxyanisole (BHA) resulted in an increased precipitation level of valeric (2.96) and butyric (4.18) acids and decreased the contents of lauric, enanthic and propionic acids to (0.00) level. This could be due to BHA's antioxidant activity, which prevents the oxidation of valeric and butyric acids in muscle tissue, whereas the high levels of Caprylic (15.96) and myristic (3.07) in SOPE and LMPE treatments could be due to the presence of phenolic

compounds, which prevent caprylic and myristic acids from being metabolised in the birds' tissue. The results of this study's saturated fatty acid profile are comparable to those of Kim *et al*. (2009), who found that dietary supplementation with varied levels of garlic bulb and garlic husk influenced the fatty acid composition of the meat when compared to the control treatment. Mono unsaturated fatty acids (MUFA) – The high levels of caproleic (9.17), palmitoleic (3.50), myristoleic (1.84) and oleic (6.00) acids observed in dietary treatments SOPE, LMPE, BHA and SHPE may be caused by increased storage of polyunsaturated fatty acids (PUFA), which inhibits the synthesis of MUFA by inhibiting antioxidants and the 9-desaturase complex, a crucial enzyme in the conversion of SFA to MUFAs. The findings of this study are consistent with those of Ayerza *et al*. (2002), who found that antioxidants have an effect on the fatty acid composition of broiler chicken meat fed antioxidant-supplemented diets.

Polyunsaturated fatty acids (PUFA); the high levels of linoleic (14.62), eicosapentaenoic (5.55), and arachidonic (2.13) acids in LMPE and SHPE treatments could be due to the phenolic compounds present in both LMPE and SHPE treatments, which are known to limit PUFA oxidation in the tissue. Antioxidants added to the diet have been demonstrated to boost long-chain n-3 fatty acids like ECA and DHA in broiler thigh meat (Saleh *et al*., 2017). The high quantities of DHA (0.84) and Acetic (1.47) acids found in the negative control OW, however, contradict the findings of Jung *et al*. (2010), who found elevated DHA levels in the breast meat of broilers fed a dietary mixture of gallic acid and linoleic acid.

5.2.9 Serum biochemistry (meat) of broiler chickens fed dietary antioxidants

The dietary antioxidant supplementation on broiler birds in this study increases the level of superoxide dismutase, SOD (608.52) in SHPE treatment, serum catalase, CAT (273.33) and glutathione peroxidase, GP_X (850.37) in meat sample of BHA treatment, as compared to the negative control OW. This could be due to antioxidant (natural or synthetic) activities that protect against oxidative stress by catalytically eliminating free radicals and other reactive species from the system, consequently enhancing SOD, CAT, and GP_X activities in the tissue or blood system. This finding is consistent with Bayraktar *et al*. (2011), who found that adding 200 mg kg-1 vitamin E to the diet of broiler chickens increased SOD, CAT, and GP_X levels, minimizing the deleterious impact on dietary oxidised oil. This finding is also consistent with Bayraktar *et al*. (2011), who found that adding 200 mg/ kg vitamin E to the diet of broiler chickens improves SOD, CAT, and GP_X levels, minimizing the deleterious impact on dietary oxidised oil.

5.2.10 Lipid oxidation of broiler chickens fed dietary antioxidants

Oxidative rancidity in broiler meat is a sign of nutrient loss, flavour degradation, colour changes, and the development of hazardous substances (Sola- Ojo *et al*., 2013). The oxidative stability of lipids in broiler meat administered dietary antioxidants was considerably impacted by post-mortem ageing. In the present study, cooked broiler chicken meat at storage day 0 (0.01) fed dietary SHPE treatment had the lowest TBARS values, followed by raw broiler chicken meat at storage day 0, fed dietary treatments SOPE (0.05) and OW (0.05), a negative control. Demonstrating that the treatments SHPE, SOPE, and OW inhibited lipid oxidation in broiler meat post mortem (storage day 0) better than the BHA and LMPE treatments. This could mean that the phenolic chemicals in SHPE and SOPE slowed lipid oxidation at storage day 0 compared to

storage days 2, 4, and 6. The findings of this study corroborate those of Lau and King (2003), who found that when experimental birds were fed grape seed extracts as a dietary supplement, the TBARS levels of poultry flesh were low. This discovery is also in line with the findings of Olorunsanya *et al*. (2011), who found that exogenous application of bamboo and elephant grass leaf extracts on broiler meat affected the oxidative stability of cooked and raw meat. The BHA containing synthetic antioxidants, on the other hand, promotes rather than inhibits lipid oxidation in broiler meat.

Koreleski *et al*. (2003) hypothesized that synthetic antioxidants (BHA) would protect feed from oxidation during storage but would have no antioxidant effect in muscle tissue in vivo or post mortem. Interestingly, the low TBARS level of the negative control treatment OW (0.05) at storage day 0, (Table 4.18) raw state could not be explained since it is considered that the OW treatment does not have any antioxidant characteristics that would require the meat to be kept from oxidising. Despite the high mean TBARS value across the storage period, the TBARS value on days 0, 2, and 4 post mortems were lower than the value (0.6 mg MDA/kg) of broiler meat with unpleasant taste (O'Grady *et al*., 2006). TBARS levels for natural antioxidants in cooked and raw beef; SOPE, SHPE, and LMPE increase as storage days increase. In contrast, there was no such pattern in the control treatments BHA and OW, where the TBARS levels did not increase in days 0 and 2, but only increased in days 4 and 6. This showed that raw broiler meat has higher TBARS levels than cooked meat, indicating that raw broiler meat has more oxidative processes. Because the lipid-free radicals that are soluble in the lipid fraction are more soluble at lower temperatures and at the same time, this supports the findings of Smet and

De Smet, (2008), who evaluated the oxidative stability of broiler breast muscle administered dietary natural antioxidant supplementation.

5.2.11 Protein oxidation of broiler chickens fed dietary antioxidants

Carbonyl is a common end product of protein oxidation that has been utilised as a food and biological protein oxidation indicator (Lund *et al*., 2011; Popova and Marinova, 2013). Analysis of carbonyl in pectoralis muscle of the broiler chicken, showed a significant effect of post mortem conditioning. Regardless of the dietary regimens, the content of carbonyl in raw meat increased as the storage days increased in this investigation. The carbonyl level of broiler chicken cooked meat at storage day 4 (0.15) fed dietary SHPE treatment and both cooked (0.18) and raw (0.19) meat at storage day 0 on dietary BHA treatment was also the lowest. This contradicts the findings of Adeyemi *et al*. (2016a), who found that the carbonyl content of carprine longissimus lumborum muscle decreased from 41.72 nmol/mg protein on day 0 to 34.58 nmol/mg protein on day 7, representing a 17.11 per cent decrease in caprine longissimus lumborum during post-mortem ageing. The carbonyl content of pectoralis muscle tissue protein in broiler chicken meat increased during storage for all dietary antioxidants supplemented in the current investigation. The natural antioxidants SOPE, SHPE, and LMPE have higher thiol content in cooked and raw meat as storage days increases. This is in line with Petron *et al*. (2007), who found no significant variations in carbonyl oxidation during storage of turkey, mutton, and pork when thiol levels were tested over a 10-day period.

5.3 Second Feeding Trial

5.3.1 Growth performance of broiler chickens fed dietary graded levels of antioxidants

In the present study, the final body weight FBW (3500.00 g/b), daily weight gain DWG (61.77 g/b/d), feed intake FI (101.73 g/b/d) and water intake WI (0.27 l/b/d) of birds fed treatment SOPE 0.04 % were observed to be higher than other graded level treatments and butylated hydroxyanisole (BHA), the control treatment. This could be due to the reasons given by Basmacioglu *et al*. (2010); Brenes and Roura (2010); Lee *et al*. (2003), to the efficacy of plant or fruit extracts on animal performance is dependent on a variety of factors, including the dose of the extracts used, the concentration and profile of active ingredients present in the extracts, the physiological state of the animal, the background diet, and the housing conditions. By enhancing the digestibility and absorption of nutrients from the feed, the dosage level of 0.04 per cent is thought to have had a good impact on the FBW, DWG, FI, and WI of broiler chickens. The findings of this study accord with those of Steven *et al*. (2008), who found that employing large dosages of plant extracts rich in phenolics and vitamin C might partially reverse growth and carcass quality depression in broiler chickens. It is also consistent with the findings of Portugaliza and Fernandez (2012); John and Kenaleone (2014), who found that the FBW of birds on aqueous *Moringa oleifera* leaf extract treatments was significantly lower ($p>0.05$) than the control treatment. This conclusion, however, contradicts the findings of Ishola *et al*. (2020), who found that the FBW and WG of BHA, the positive control treatment, were higher than those of sweet orange peel extracts (SOPE) treatment of birds administered dietary natural antioxidant extracts supplementation. It also contradicts Alabi *et al*. (2017), who found that increasing the dose of aqueous *Moringa oleifera* leaf

extracts food supplementation increased the birds' FBW and daily body weight gain (WG). Furthermore, treatment SOPE 0.04 % may include an adequate amount of bioactive phytochemical substances that may impact physiological parameters such as nutritional digestibility (Wallace *et al*., 2010). The feed conversion ratio (FCR) of treatment SOPE 0.08 % (1.73) appeared to be higher compared with SOPE 0.10 % (1.64) and BHA (1.66), the control treatment. This indicates that the birds in SOPE (0.10 %) treatment performed better compared to the control treatment BHA and SOPE 0.04, 0.06 and 0.08 %. This indicates that SOPE 0.10 % contains a phenolic molecule that, in addition to its antioxidant properties, acts as a growth stimulator. This result is consistent with the findings of Safa and Tazi (2012); Ebenebe *et al*. (2012), who discovered that birds fed *Moringa oleifera* diets had a higher FCR than birds fed the control diet.

Gut Morphology

The hair-like structure from the surface (finger-shaped villi) and circular folds of the mucosa and submucosa (kerckring valves) and the crypts of lieberkuhn are shown in the gut morphology of the graded levels of dietary antioxidants supplementation on the experimental broiler birds as shown on plates 1-5, (which lie between the villi). SOPE 0.10 % (Plate 5) has a stronger effect than BHA and SOPE (0.04, 0.06 and, 0.08 %). Treatment with SOPE at 0.10 % inclusion level had good nutritional absorption in the duodenum and jejunum, as seen on the plate, resulting in a substantial feed efficiency as reflected in its low FCR when compared to other treatments. This conclusion is consistent with the findings of Nasir *et al*. (2012), who found that the heights of jejunum villi were considerably greater in birds fed a graded level of 100 percent dietary supplementation of curcumin on growth performance.

5.3.2 Nutrient retention of broiler chickens fed dietary graded levels of antioxidants

The high nutrient retention values of dry matter DM (9.40 %), caloric value CV (1497.23 kg/ 100g) and carbohydrate CHO (71.18 %) observed in BHA the control treatment over the other treatments, could be because of the synthetic substances in the BHA, which could have prevented the enzymes from breaking down the nutrients in the feed. This result is consistent with Jiya *et al*. (2016), who found that dietary regimens containing graded levels of NatuzymeTM treated groundnut shell had a substantial impact on the nutrient digestibility of broiler chicks. The high levels of crude lipid retention in birds fed treatment SOPE 0.04 % (2.73 %) (Table 4.20) could be attributable to the low digestion of ground nut cake and other fats and proteins in the diet. Some phenolic chemicals found in the SOPE 0.04 % may have blocked the enzymes needed to break down the protein and lipids in the food, making the nutrients difficult to absorb. This discovery is identical to what was previously reported by Oluyemi and Roberts (2000). The high levels of total protein TP (13.53 %) and ash (8.74 %) reported in the SOPE 0.06 % treatment could be attributable to the birds' failure to absorb many amino acids due to phytochemical inhibition in the extracts. This is consistent with the findings of Ishola and Atteh (2018), who hypothesized that birds utilise protein content faster than energy source feed.

Crude fibre retention either in 4^{th} (Table 4.20) or 8^{th} week (Table 4.21) by birds fed dietary antioxidants supplementation were lower than other nutrients retained by the birds. This could be owing to the complicated architectures of non-starch polysaccharides in the diets and the birds' inability to utilise them effectively (Oldale and Hoffman, 1996). Monogastric animals, such as broiler chickens, are thought to lack enzymes that can hydrolyse non-starch polysaccharides, which typically contain water insoluble fractions

of pentosanes and D-glucans that are difficult for monogastric animals to hydrolyse (Choct and Kocher, 2002). The high values of crude lipid, carbohydrates and total protein in the 4th week (neonate phase) of the birds fed dietary antioxidants supplementation as compared to the 8th (grower phase), could be due to the inability of neonates' intestinal walls and enzymes to digest and absorb the nutrients, as opposed to grower birds, who have well-developed villi, intestinal mucosa, and crypts to manage the digestion and absorption of nutrients. The outcomes of this study support the findings of Ishola and Atteh (2018), who conducted research to assess broiler responses to early dietary energy and protein levels.

5.3.3 Carcass characteristics of broiler chickens fed dietary graded levels of antioxidants

In the present study, the values of the carcass weight (3160.00 g), dressing percentage (90.29 %) and intestinal weight (98.40 %) of SOPE 0.04 % treatment was observed to be higher compared to BHA, the control treatment. The results reveal those birds on the dietary treatment SOPE (0.04 %) were able to successfully use the nutrient and deposit muscles, as seen by their final body weight (3500.00 g). This could be due to the moderate number of phenolic compounds contained in this diet, which aided in nutrient digestion and absorption. This finding is consistent with that of Heidarisafar *et al*. (2016), who supplemented broiler chicken with processed apple peel waste. The acceleration of metabolic activity by the phenolic compounds present in the dietary antioxidant supplement could potentially be responsible for the increase in intestinal weight. However, this result also indicated that inclusion level of 0.04 % SOPE supplementation to the diets of broiler chickens reduces the abdominal fat content (0.54

%) compared to treatments BHA (control) and SOPE 0.06, 0.08 and 0.10 %. This study's findings are similar to those of Adeyemi *et al*. (2008), who found that abdominal fat weight in broilers given 25 % cassava root meal fermented with rumen filtrate decreased as compared to controls. Zhang *et al*. (2016), on the other hand, found that dietary fermented feed enhanced the belly fat content of 8-week-old broiler chicks by 6 %. The quality and amount of fermented feed addition, as well as the research settings, may explain why these inconsistencies exist. The findings of this study contrast those of Mehala and Moorthy (2008), who found no significant effect of turmeric powder on the carcass percentage of broiler chickens grown to 42 days of age. The high breast weight (30.74 %) and abdominal fat (0.72 %) values observed in dietary treatment SOPE (0.06 %), high thigh (12.88 %) and drumstick weight (13.11 %) observed in dietary treatment 0.08 %, in this study contradicts the findings of Durrani *et al*. (2006), who found that broiler chicks fed a diet containing 5 g/kg turmeric powder had a high breast weight. This discrepancy could be due to varying quantities of phenolic chemicals in the diet. The reduced liver weight in all treatments compared to the liver weight of broiler chicks fed cassava pulp fermented with *Acremonium charticola* (Sugiharto *et al*., 2017) could be due to the presence of an antioxidant added in the diet, which could also have antibacterial and anti-inflammatory properties. As a result, the population of microorganisms in the intestine decreases, and toxin production decreases. consequently, the liver's detoxifying activities are reduced, and liver hypertrophy is reduced (Mehr *et al*., 2007). The liver weight in percentage of birds fed dietary treatment SOPE 0.06 % is smaller compared to other treatments which shows that the birds in treatment SOPE 0.06 % could be healthier than those in treatments BHA (control) and SOPE 0.04, 0.08 and

0.10 %. This is consistent with the findings of Mehr *et al*. (2007) and Bozkurt *et al.* (2009), who found a reduction in liver weight in broilers provided dietary probiotics supplementation.

5.3.4 Haematological indices of broiler chickens fed dietary graded levels of antioxidants

The total white blood cell (WBC), red blood cell (RBC), lymphocytes, and other blood parameters in this study on all treatments (Table 4.23) are normal and correspond to the values obtained by Imaseun and Ijeh (2017) on broiler birds fed a diet supplemented with two sources of antioxidants as feed additives. Again, this demonstrated that the anti-oxidative properties of the dietary antioxidants supplied in this feeding trial alter the immune response system in birds. When compared to the control and other treatments, birds on the dietary treatment SOPE (0.10 %) had the greatest white blood cell WBC (19.67 10^9/l), mid-sized cell MID (2.77 %), granulocyte GRAN (2.77 %), and haemoglobin HGB (11.12 g/dl) values. The high values of these blood parameters on SOPE (0.10 %) treatment may not be due to inflammation or disease, which would normally increase leucocytes, but rather to the high dosage of antioxidative properties of SOPE (0.10 %) treatment, which was responsible for the birds' immune system response. As a result, the WBC value in SOPE (0.10 %) treatment is quite high.

The findings of this study support the findings of Ademola *et al*. (2009), who found that a ginger-supplemented diet impacted white blood cell and other haematological parameters in broiler chickens. However, the WBC result in treatment SOPE 0.06 % (6.60 10^9/l) was the lowest among the treatment groups, suggesting that the inclusion level of 0.06 % of

SOPE reduces WBC amount. This suggests that this medication at this level (0.06 %) may be less harmful to the bird's immune system. This conclusion shows that care should be made against high dosage of the antioxidant usage. Also, in the present study, the values of lymphocytes, LYM (9.23 10^9/l), red blood cells RBC (2.40 10^6/mm3) and packed cell volume PCV (1.24) were observed to be greater in birds on dietary therapy SOPE (0.08 %) as compared to other treatments and the control (BHA). The high RBC levels on dietary level of SOPE (0.08 %) could be correlated with dietary antioxidants which accelerate fat digestion by increasing the availability of substrates for sz- oxidation and the synthesis of succinyl- CoA via Kreb's cycle (Cunningham and Klein, 2005), which has been linked to enhanced haemoglobin production (Cunningham and Klein, 2005). The results obtained in this study correspond with those obtained by Asghar *et al*. (2018), who found a greater haemoglobin concentration in birds fed 10 mg of Genisten + Hesperidin in their food.

5.3.5 Blood serum of broiler chickens fed dietary graded levels of antioxidants

In the present study, the results of total cholesterol, triglyceride, low density lipoprotein LDLP (bad cholesterol) and high-density lipoprotein HDLP (good cholesterol) were influenced by the antioxidative supplements across the treatment groups. Treatment SOPE (0.04%) was observed to have the lowest values of total cholesterol (118.21 mg/dl), serum triglyceride (38.98 mg/dl), LDLP (49.67 mg/dl) and HDLP (60.75 mg/dl). This could indicate that SOPE (0.04 %) is a potent antioxidant in the fight against lipid peroxidation, as evidenced by the lower total cholesterol. The findings on the effect of dietary antioxidant supplementation in broiler diets are consistent with report of Ademola *et al.* (2009). When fatty acids, protein, and glucose levels rise over the body's needs,

triglycerides are generated in the liver and eventually deposited in adipose tissue (Esubonteng, 2011). Broiler birds in the SOPE 0.04 % dietary treatment had lower triglycerides than those in the BHA (control), SOPE 0.06, 0.08 and 0.10 % dietary treatments. This suggests that in the feeding treatment SOPE 0.04 %, there were fewer fat deposits in the adipose tissue of the birds. This study's findings are similar to those published by Wayas *et al*. (2018). The liver enzymes alanine transaminase (ALT) and aspartate transaminase (AST) are crucial in assessing how well the liver functions (Ambrosy *et al*., 2015). An increase in the level of ALT and AST, which indicate the liver's functional status, could be the result of damaged liver cells. In birds, the optimal concentrations of liver enzymes are 70-220 u/l for AST and 568-8831 u/l for ALT (Meluzzi *et al*., 1992). With the exception of treatment SOPE 0.08 %, the AST readings in this study are normal. The low levels of AST in the dietary treatments BHA (control), SOPE 0.04, 0.06 and 0.10 % (Table 4.24) indicate that the birds in these dietary treatments remained healthy during the feeding trial. The findings of this study are consistent with those of Wayas *et al*. (2018), who studied the haematological and serum biochemistry responses of Ovambo chickens fed pro-vitamin A bio-fortified maize. The level of superoxide dismutase SOD (433.25) was found to be higher in the SOPE (0.06 %) treatment than in the other treatments and the control. This meant that the dietary treatment SOPE 0.06 % was able to reduce free radicals in the birds' plasma and liver more effectively than other treatments, resulting in increased SOD activity in the birds' livers. Jiang *et al.* (2007)'s findings that the addition of varied amounts of soybean isoflavone boosted SOD activity in the liver of broilers were verified by this study. Glutathione peroxidase GP_X (3143.80), (3108.10) for treatment SOPE (0.08 %), BHA,

and serum catalase CAT (4433.33) for BHA, the control therapy, were all shown to be greater than the other treatments. This revealed that the dietary treatments SOPE (0.08 per cent) and BHA were more effective than other treatments at inhibiting hydroxyl radicals in the plasma and liver of the birds, consequently increasing GP_X and CAT activity in the birds' livers by converting hydrogen peroxide to water. This finding is consistent with that of Saheed *et al*. (2015), who found that dietary antioxidants improved broiler oxidative stress, performance, and meat quality.

5.3.6 Meat quality (physicochemical properties) of broiler chickens fed dietary graded levels of antioxidants

It is common knowledge that peroxidation in meat reduces the quality of broiler meat by reducing the colour, water holding capacity, and increasing drip loss (Holownia *et al*., 2003; Zhang *et al*., 2011). In the present study, dietary treatment SOPE 0.04 and 0.08 % (Table 4.25) had increased pH value of pectoralis muscles. In this study, the lowest pH value observed in the control treatment BHA (5.85) could be as a result of increase in post mortem glycolysis. As expected, this resulted in increase of water holding capacity WHC (64.04), cooking loss (35.62 %) and drip loss (28.42 %) of the control treatment BHA. The result observed in this study agrees with the findings of Zhang *et al*. (2011), who found that dietary oxidized oil administered to broilers lowers the sarcoplasmic reticulum Ca2+ ATPase (SERCA) activity of the pectoralis muscle as a result of enhanced post-mortem glycolysis. The conclusions of this study are likewise consistent with the findings of the report of Reiche *et al*. (2019).

Supplementing broiler diets with SOPE 0.04 per cent (Table 4.25) increased the lightness L* (38.23) of broiler meat colour as compared to other treatments, including BHA, the control treatment. This could be due to a less content of phytochemicals responsible for regulating the level of metmyoglobin in meat, which influences the meat's discolouration after slaughter. The findings of this study support those of Jiang *et al*. (2007), who found that supplementing broiler diets with 40 or 80 soybean isoflavone ISF kg-1 diet increased the lightness of the flesh colour substantially. In this study, supplementation of broiler diets with SOPE 0.08 % (Table 4.25) increased the redness a* (8.19) of the broiler meat colour compared to other treatments including BHA, the control treatment. This could be due to a sufficient number of phytochemicals responsible for the stability of membrane-bound lipids, which often plays a key role in the conversion of red oxymyoglobin to brown metmyoglobin, increasing the colour stability of meat after slaughtering. The findings of this study are consistent with those of Bayraktar *et al*. (2011), who discovered high antioxidant activity of -tocopherol in the feed supplied to broiler birds and its post-mortem colour.

5.3.7 Meat quality (shear force analysis) of broiler chickens fed dietary graded Levels of antioxidants

Several studies have found a link between increasing protein carbonyl concentration and decreased mechanical texture (tenderness) in meat (Rowe *et al*., 2004; Zakrys *et al*., 2008). Protein oxidation has been discovered to alter (decrease) meat softness by inactivating endogenous enzymes, reducing proteolytic breakdown, and increasing protein cross-linkages via disulphide bond formation (Lund *et al*., 2007). The force peak and yield on the flesh are used to determine the shear force values of the pectoralis

muscles of the experimental broiler birds. When dietary treatment SOPE 0.08 % was compared to dietary control BHA and other treatments, the force peak and yield of birds on SOPE 0.08 % had higher values (24.50 N) and (10.66 N). The high value of force peak and yield could be due to a high phytochemical content in the tissue of the bird's meat, which promotes protein oxidation and proteolytic disintegration. The result of The SOPE 0.08 % force peak and yield were somewhat greater than Malovrh *et al*. (2009)'s results, which showed an average force of 21.22 N. Surprisingly, the findings of this investigation revealed that SOPE 0.08 % treatment improved the softness of breast meat. This conclusion is in line with the investigation of Rebecca (2013).

5.3.8 Meat quality (sensory evaluation) of broiler chickens fed dietary graded levels of antioxidants

The sensory parameters as perceived by the panellist showed that SOPE 0.10 % inclusion level improved the appearance, taste, aroma and overall acceptability of bird's meat compared to the control BHA and other treatments (Table 4.27). This could be due to the dietary SOPE 0.10 % high antioxidant action on the breakdown of the meat fibre matrix, the release of flavour-juices, and the volatile scent components into the mouth. The findings in this study agreed with Sampaio *et al*. (2012), who showed nice flavour and acceptability in cooked chicken meats enhanced with sage, oregano, and honey as natural antioxidants. The sensory qualities (taste, aroma, texture, and appearance) of broiler chicken meat are considerably affected by dietary antioxidant supplementation. The findings of this study contrast those of Zaneta *et al*. (2016), who found that better juiciness and taste perception by consumers were associated with higher WHC and less cooking loss. An increased concentration of SOPE in broiler tissues as a result of dietary

antioxidant supplementation may effectively inhibit peroxidation and, as a result, affect the consumer's sensory impression (Ruiz *et al.,* 2001). The sensory quality of broiler meat is thought to be affected by the time of storage (Sheldon *et al*., 1997). Antioxidants like SOPE limit the oxidative process in meat, lowering the production of oxidation products that could lead to meat deterioration (taste, aroma, and texture) (Kennedy *et al*., 2005).

5.3.9 Meat quality (fatty acid profile) of broiler chickens fed dietary graded levels of antioxidants.

The Palmitic (98.09) and Caproleic (56.24) acids in dietary treatment SOPE (0.10 %) were higher compared to the control BHA and other treatments. This could be due to the ability of birds in the SOPE (0.10 %) dietary treatment to absorb and deposit palmitic and caproleic acids into muscle more efficiently than birds in the control BHA and other treatments. It has been discovered that the fatty acid composition of broiler meat is influenced by the fatty acid composition of the ration (Azman *et al*., 2005). This finding agreed with the result observed by Surai (2003), who found that an increase in tocopherol absorption impacts the digestibility and fat absorption in broiler bird rations. It also supports the findings of Katleen *et al*. (2008), who found that dietary antioxidant supplementation altered broiler meat lipid and protein oxidation. In this study, the dietary therapy SOPE 0.06 % had higher caproic, myristic, and palmitoleic values than the other treatments. Surprisingly, the BHA control treatment had similar levels of caproic and palmitoleic acid as the SOPE 0.06 % treatment (Table 4.28). Also, when

compared to the control BHA and other treatments, the lauric and linoleic content of SOPE 0.04 % were higher.

The dietary therapy SOPE 0.08 % (5.30) had greater docosahexaenoic acids than the control BHA and other treatments. All of the differences in the fatty acid composition of broiler meat reported in this investigation could be due to the doses of phytochemicals used in each of the dietary regimens, which indicated their lipid-lowering potential. The findings of this study are consistent with those of Oskoueian *et al*. (2013), who found that feeding broilers 200 mg/kg quercetin affects the fatty acid content of their pectoralis muscles. This conclusion, however, contradicts the findings of Kyung *et al*. (2012), who found that the palmitic C16:0 was higher in the control group of broilers than in the gallic and linoleic supplemented diet supplied to the birds. When dietary SOPE supplementation was used instead of BHA the control treatment, the ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA) in the pectoralis muscles of broilers was better. The foregoing findings may be beneficial to consumers because a high PUFA/SFA ratio is associated with a lower risk of heart disease in humans (Krauss *et al*., 2001).

5.3.10 Meat quality (meat serum) of broiler chickens fed dietary graded levels of antioxidants.

The superoxide dismutase (SOD) of bird's meat on dietary SOPE 0.08 % (480.78 U/l) was higher compared to those on control diet BHA and other treatments. This result could be due to the availability of a sufficient number of antioxidant substances, which boosts the SOD enzyme's ability to prevent oxidative rancidity in broiler meat (Jiang *et al*., 2007). The findings of this study matched those of Bansal *et al*. (2005), who found a

decrease in superoxide dismutase in the liver of rats fed nitrosamine compound supplemented with vitamin E, a natural antioxidant. The blood catalase CAT (443.33 u/mg protein) and glutathione peroxidase GP_X (885.81 u/mg protein) of bird meat on the dietary control treatment BHA were likewise greater than the other treatments in this study. Because of the presence of synthetic phenolic compounds, which are thought to have a stronger antioxidant capability than natural antioxidants, BHA treatment was able to inactivate the reactive oxygen substance and regenerate oxidised antioxidants in the muscle tissue of the birds than those birds' muscle in other treatments. Daneshyar (2012) reported the influence of dietary turmeric on antioxidant properties of thigh meat in broiler chickens after slaughter, and this finding indicated a similar pattern with this study.

5.3.11 Lipid oxidation of broiler chickens fed dietary graded levels of antioxidants

The high Thiobarbituric acid reactive substance (TBARS) values observed in dietary treatment of sweet orange peel extracts SOPE 0.10 % on bird's meat both cooked (0.09 mg MDA/kg) and raw (0.06 mg MDA/kg) at storage day 0, also on both raw meat at storage days 4 (0.41 mg MDA/kg) and 6 (0.40 mg MDA/kg) indicating that there was weak oxidative protection on the meat when SOPE 0.10 % was included in the diet compared with the control treatment BHA and other treatments. The high TBARS values found on cooked (0.24 mg MDA/kg) and raw (0.17 mg MDA/kg) bird meat at storage day 2 in this study revealed that there was weak oxidative protection on the meat when SOPE 0.06 % was included in the diet compared to the control treatment BHA and other treatments. High TBARS values on cooked bird's meat (0.78 mg MDA/kg) at storage day

4 and high TBARS values on cooked bird's meat (1.88 mg MDA/kg) at storage day 6 were also observed in this study, indicating that there was weak oxidative protection on the meat when BHA and SOPE at 0.04 per cent were included in the diet compared to other treatments. These findings contrast the findings of Lau and King (2003), who found that using grape seed extracts suppressed the thiobarbituric acid reactive compound on dark fowl meat. On the other hand, it supports the findings of Tang *et al*. (2000), who found a strong antioxidant dose–response effect at catechin concentrations of 100 to 300 mg/kg of feed. The addition of dietary vitamin E to the diets of broiler chickens produced a similar response (Taulescu *et al*., 2011). This finding is consistent with the findings of Katleen *et al*. (2008), who found that various dietary antioxidants provided to broiler chickens reduced lipid oxidation to the same extent as the control treatment. Koreleski *et al*. (2003) also investigated the effects of BHA addition in feed on the oxidative stability of egg yolk. The extensively used approach for assessing lipid oxidation in meat is thiobarbituric acid reactive substance (TBARS). The amount of fat in the meat, as well as the quantity and antioxidant activity, determine the TBARS value. Delles *et al*. (2013) found a significant increase in lipid oxidation inhibition (TBARS) and protein oxidation inhibition (thiol content) in chickens fed low or highly oxidised oil diets supplemented with tocopherol/selenium-based antioxidants in the preserved breast and thigh.

5.3.12 Protein oxidation of broiler chickens fed dietary graded levels of antioxidants.

In the present study, the high carbonyl values observed in dietary treatment sweet orange peel extract SOPE 0.06 % on bird’s meat both cooked (0.15 nmol/mg protein) and raw (0.14 nmol/mg protein) at storage day 2 indicating that there was weak protein oxidative protection on the meat when SOPE 0.06 % was included in the diet compared with the

control treatment BHA and other treatments. The high carbonyl values observed on cooked (0.56 nmol/mg protein) and raw (1.70 nmol/mg protein) bird meat at storage day 4 and cooked meat at storage day 6 in this study indicated that there was weak protein oxidative protection on the meat when BHA was included in the diet when compared to other treatments. The findings of this investigation are consistent with those of Haak *et al*. (2006) and Petron *et al*. (2007), who found only a minor variation in protein oxidation between turkey and pork that had been refrigerated when the free thiol level was assessed. This is also in line with Salminen *et al*. (2006), who found that antioxidants protect lipids from oxidation and that the antioxidant supplementation's efficacy against protein oxidation in packing treatment may be attributable to the increased oxidative environment. This outcome is consistent with earlier research on meat storage in the refrigerator (Zakrys-Waliwander *et al*., 2012).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The study revealed that:

1. the data and empirical evidence obtained showed that peel extracts of sweet orange, shaddock and lemon fruits contains useful natural antioxidants that can serve as alternative to butylated hydroxyanisole (BHA), a synthetic antioxidant.

   First Feeding Trial:

2. birds on dietary lemon peel extract LMPE (0.02 %) had a better growth performance, using ranking summation index procedure (Appendix D).
3. immune performance of birds on dietary shaddock peel extract SHPE was ranked the best, as reflected in the white blood cell WBC, red blood cell RBC and haemoglobin HGB content (Appendix E).
4. serum indices assay which is the non- specific bio- marker of fat deposit and liver functions in the body were ranked the best in birds on lemon peel extract treatment, as shown by ranking summation index (Appendix F).
5. overall carcass parameters in birds on dietary lemon peel extract (0.02 %) were better as indicated by ranking summation index (Appendix G).
6. physicochemical properties (pH, water holding capacity WHC and colour coordinates) were better in bird's meat on dietary sweet orange peel extract SOPE (0.02 %), as shown in the ranking summation index (Appendix H).

7. the shear force parameters were better in bird's meat on dietary treatment shaddock peel extract (0.02 %) as indicated by the ranking summation index (Appendix I).
8. the sensory parameter perceptions were better in bird's meat on dietary lemon peel extract (0.02 %) (Appendix J).
9. fatty acids composition (saturated fatty acids SFA and mono unsaturated fatty acids MUFA) was higher in bird's meat on dietary ordinary water (negative control) as indicated by ranking summation index (Appendix K).
10. the lipid oxidative stability was better in bird's meat on dietary shaddock peel extract (0.02 %), as indicated in Appendix L.

Second Feeding Trial

11. birds on dietary with SOPE (0.10 %) had a better growth performance, using ranking summation index procedure (Appendix M).
12. the gut morphology of birds on dietary SOPE (0.10 %) were more pronounced than other treatments.
13. immune performance of birds on dietary SOPE (0.04 and 0.06 %) were ranked the best, as reflected in the WBC, RBC and HGB content (Appendix N).
14. serum biochemistry which is the non- specific bio- marker of fat deposit and liver functions in the body were ranked the best in birds on BHA and SOPE (0.04 %) treatments, as shown by ranking summation index (Appendix O).
15. overall carcass parameters in birds on dietary SOPE (0.04 and 0.10 %) were better as indicated by ranking summation index (Appendix P).

16. physicochemical properties (pH, WHC and colour coordinates) were better in bird's meat on dietary SOPE (0.06 %), as shown in the ranking summation index (Appendix Q).
17. the shear force parameters were better in bird's meat on dietary treatment SOPE (0.04 %) as indicated by the ranking summation index (Appendix R).
18. the sensory parameter perceptions were better in bird's meat on dietary SOPE (0.10 %) (Appendix S).
19. fatty acids composition (SFA and MUFA) was higher in bird's meat on dietary SOPE (0.08 %) as indicated by ranking summation index (Appendix T).
20. the lipid oxidative stability was better in bird's meat on dietary SOPE (0.08 %), as indicated in Appendix U.

6.2 Recommendations

With the completion of this research work, it is therefore recommended that:

1. the commercial utilisation of natural antioxidants, especially sweet orange peel extracts (SOPE) at 0.10 % level could be encouraged for poultry farmers for better performance of their poultry birds.
2. meat processing industries should be encouraged to use natural antioxidants like SOPE at higher dosage level of 0.08 and 0.10 % to improve the oxidative stability of broiler meat during storage as an alternative to synthetic antioxidants
3. further studies could be carried out on other natural antioxidants SHPE and LMPE at higher dosage levels to determine their efficacy on broiler chicken performance and oxidative stability in contrast to the BHA, the synthetic antioxidants

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APPENDIX A

Qualitative analysis of the phytochemicals of the extracts

Test for tannins

The approach published by Ejikeme *et al*. (2014) was used to conduct a qualitative examination of tannin. In a test tube, 0.03 g of each extract sample was weighed and cooked for 10 minutes in a water bath with 30 ml of water. After boiling, the contents were filtered through Whatman filter paper number 42 (125 mm). 1 ml of freshly made 10 % potassium hydroxide (KOH) was added to 1 ml of the extract samples. The presence of tannins is indicated by a dirty white precipitate.

Test for phlobatannins

Each extract sample was weighed into a beaker containing 30 ml of distilled water in a sample weight of 0.30 g. Each sample was measured at 10 mL and cooked in 5 ml of 1% aqueous hydrochloric acid (HCL). The presence of phlobatannins is indicated by a red precipitate (Ejikeme *et al*., 2014).

Test for saponin

n a water bath, 30 ml of distilled water was added to 0.30 g of each extract sample, which was then heated for 10 minutes before being filtered using Whatman filter paper number 42 (125 mm). A mixture of 5 ml distilled water and 10 mL filtrate was quickly agitated until froth formed. The presence of saponin is indicated by the creation of an emulsion when 3 drops of olive oil are added (Ejikeme *et al*., 2014).

Test for steroid

Test of Salkowski. Each extract sample was weighed into a beaker and diluted with 2 ml acetic anhydride before being diluted with 5 drops of concentrated sulphuric acid

(H_2SO_4). The presence of steroids is shown by a red colour shift in the sample(s) (Ejikeme *et al*., 2014).

Test for terpenoids

In a test tube, 5 ml of the samples' aqueous extract was combined with 2 ml of HCl_3, and 3 ml of concentrated sulphuric acid (H_2SO_4) was carefully added to produce a layer. The presence of terpenoids is indicated by a reddish-brown coloured interface (Ejikeme *et al*., 2014).

Test for flavonoids

To each of the aqueous extract samples, 1 ml of 10 % sodium hydroxide (NaOH) was added to 3 ml of water. When a few drops of dilute acid were added, a bright yellow colour developed, which faded to colourless, indicating the presence of flavonoids (Muhammad and Amzad, (2014).

Test for alkaloids

In a test tube, 3 ml of each extract sample was mixed with 1 ml of 1 percent hydrochloric acid (HCl). The mixtures were then heated for 20 minutes, cooled, and filtered through Whatman number 42 (125 mm) filter paper. The filtrates were then treated with 2 drops of Wagner's reagent in 1 ml of water. The presence of alkaloids is indicated by a reddish-brown precipitate (Rufai *et al*., 2016).

Test for glycoside

To 1 ml of extract samples, 10 ml of 50 percent tetraoxosulphate (VI) (H_2SO_4) was added.

The combinations were then cooked for 15 minutes in boiling water. The mixture was then added 10 ml of Fehling's solution and heated for another 10 minutes. The presence of glycosides is indicated by a brick red precipitate (Rufai *et al*., 2016).

Test for phenolics

In each test tube containing extract samples, 2 drops of 5 percent iron chloride ($FeCl_3$) were added to 1 ml. The presence of phenols is indicated by a greenish precipitate (Kunze *et al*., 2018).

Test for coumarins

1mole sodium hydroxide (NaOH) + 1ml sodium hydroxide (NaOH) = 1mole extract = 1mole extract = 1mole extract = 1mole extract = 1mole extract = 1mole extract = 1mole extract = 1mole extract After cooling the sample, 0.75 ml of 5N sulphuric acid was added and properly mixed. Anhydrous sodium bicarbonate, 0.25 g, was added and stirred. The presence of coumarins is indicated by the formation of a yellow colour (Huang *et al*., 2017).

Test for titerpenes

To 1 ml of extract samples, five drops of acetic anhydride were applied. After that, a drop of concentrated sulphuric acid (H_2SO_4) was added, and the mixture was steamed for 1 hour before being neutralized with sodium hydroxide (NaOH) and chloroform. The presence of triterpenes is indicated by a blue green colour (Katritzky, 1995).

APPENDIX B

Quantitative analysis of the phytochemicals of the extracts

Total tannin content determination

A total of 0.20 g of extract samples were placed in a 50 ml beaker containing 20 ml of 50 % methanol, which was then covered with paraffin and heated for an hour in a water bath at 77 – 80 ^{0}C. The contents were carefully mixed and filtered into a 100 ml volumetric flask using a double-layered Whatman number 41 filter paper. Following that, 20 ml of water, 2.5 ml of Folin-Denis's reagent, and 10 ml of 17 percent sodium carbonate (Na_2CO_3) were added to the volumetric flask's contents and thoroughly mixed. A bluish-green colour was noted after adding more water to the mixture in the volumetric flask up to the mark and allowing it to stand for 20 minutes. As a 1 ml blank sample, about 10 ppm was created using the aforesaid approach. The absorbance readings of the tannic acid standard solution together with the samples were read after colour development on a spectronic 21D spectrophotometer at a wavelength of 760 nm (Amadi *et al.,* 2004). Total tannin was calculated using the formula:

$$\text{Total tannin} = \frac{\text{absorbance of sample} \times \text{average gradient} \times \text{dilution factor}}{\text{Weight of sample}}$$

Total saponin content determination

In a 250 ml beaker, a gram of extract samples was weighed, and 100 ml of isobutyl alcohol was added. To ensure consistent mixing, the contents were shaken for 5 hours on a UDY shaker. After that, the contents were filtered through a Whatman number 1 filter paper into a 100 ml beaker containing 20 ml of a 40 % saturated magnesium carbonate solution ($MgCO_3$). The resulting combinations were filtered once more to obtain a clear,

colourless solution. One millilitre of the filtrates was pipetted into a 50 ml volumetric flask containing 2 ml of 5 % iron chloride ($FeCl_3$) solution, with distilled water added to fill the flask to the mark level. The contents of the flask were allowed to sit for 30 minutes to produce a blood red colour. Then, using the same process as before, a 10 ppm saponin standard was made and treated with 2 ml of 5 % iron chloride ($FeCl_3$). After colour development, the absorbance of the samples and standard saponin solution was measured with a Jenway V6300 spectrophotometer at 380nm (Ejikeme *et al.,* 2014). Quantity of saponin was calculated as follows:

$$\text{Total saponin} = \frac{\text{absorbance of sample} \times \text{average gradient} \times \text{dilution factor}}{\text{Weight of sample} \times 10{,}000}$$

Total steroid content determination

A 0.05 g extract sample was weighed into a 100 ml beaker, 20 ml chloroform – methanol in a 2:1 ratio was added, and the contents were agitated for 30 minutes to dissolve the extract samples. Following that, 1 ml of the contents was pipette into a 30 ml test tube containing 5 ml of alcoholic potassium hydroxide (KOH) and thoroughly shaken until a homogeneous mixture was achieved. After that, the mixture was placed in a water bath at 37 °C to 40 °C for 1 hour and 30 minutes. The ingredients were brought to room temperature before adding 10 ml petroleum ether and 5 ml distilled water. On the water bath, the content was later evaporated to dryness. A total of 6 ml of Liebermann Buchard reagent was added to the residue in the dry bottle, and the absorbance was measured at 620nm using a spectronic 21 D digital spectrophotometer. A standard solution of 0–4 mg/ml was made using the same process as previously described by (Chukwuma and Chigozie, 2016).

Total steroid content was calculated as follow:

$$\text{Total steroid} = \frac{\text{absorbance of sample} \times \text{average gradient} \times \text{dilution factor}}{\text{Weight of sample} \times 10{,}000}$$

Total terpenoid content determination

The approach described by (Feng *et al*., 2013) was employed, which involved liquid chromatography, electrospray ionisation, and mass spectrometry. In 45 minutes, a sample of 0.5 g extracts was combined with 0.03 percent formic acid aqueous solution. The chromatographic separations were carried out on an Agilent Poroshell SB-C18 column (150x 4.6mm, 3.5m) with acetonitrile gradient elution. The precursor-extract combination was monitored in the positive ionisation mode for detection.

Total flavonoid content determination

Calabro *et al*. (2004) and Ebrahimzadeh *et al*. (2008) used an aluminum chloride colorimetric assay method to evaluate total flavonoids in peel extract samples. In a 10 ml volumetric flask containing 4 ml distilled water, a ml of extract samples and a 500 g/ml standard solution of quarcetin were added. After that, 0.3 ml of sodium nitrite ($NaNO_2$) at a concentration of 5 % was added. 5 minutes later, 0.3 ml of 10 % aluminum chloride ($AlCl_3$) was added, followed by 2 ml of 1 M sodium hydroxide (NaOH) 6 minutes later. To produce the entire volume of the content 10 ml, distilled water was added. The contents were properly mixed, and the colorimeter's absorbance measurement (CS-200/210/220/260, CHN- Spec, China) was compared to a manufactured reagent blank at 510 nm. The samples' total flavonoid content was calculated as mg of quarcetin equivalent per 100 g of fresh content.

Total alkaloid content determination

The approach described by (Hikino *et al*., 1984) was used to determine the quantitative content of alkaloids. Two grams of extract samples were weighed into a 100 ml beaker, followed by 20 ml of 80 % alcohol to make a smooth paste. The mixture was placed into a 250 ml round bottom flask, then 30 ml of alcohol and 1 g of magnesium oxide were added. The contents were then digested for 1 hr 30 min in a boiling water bath (Model number DK-420, LIAM Medical England) with a reflux air condenser and occasionally shook. While the mixture was still hot, it was filtered through a Buchner funnel. The residue was transferred to the flask and digested again for 30 minutes with 50 ml alcohol, which was then evaporated. Three drops of 10 % hydrochloric acid (HCl) were added to the mixture.

After that, the entire contents were put into a 250 ml volumetric flask. In the flask containing the solution, 5 ml zinc acetate and 5 ml potassium ferricyanide solution were added and thoroughly mixed to produce a homogeneous solution. The contents of the flask were left to stand for a few minutes before being filtered through a dry filter paper. Ten milliliters of the filtrate were placed into a separating funnel, and the alkaloids in the contents were extracted by vigorous shaking with parts of chloroform. The recovered residue was diluted in 10 ml hot distilled water and placed into a Kjedahl tube with a 0.2 g selenium solution for digestion, resulting in a colourless solution. Kjedahl distillation apparatus was used to determine the nitrogen content of the clear colourless solution. The distillate was later back titrated using 0.01 mole hydrochloric acid (HCl) and the titre value obtained was used to calculate the percentage nitrogen as follow:

$$\% N = \frac{\text{titre value} \times \text{atomic mass of nitrogen} \times \text{normality of HCL} \times 100}{\text{Weight of sample (mg)}}$$

$$\%\ Alkaloid = \%\ Nitrogen\ x\ 3.26\ where\ 3.26\ is\ a\ constant$$

Total glycoside content determination

10 ml extract samples were pipette into a 250 ml conical flask, 50 ml chloroform was added, and the mixture was thoroughly stirred for an hour on a Vortex mixer. The sample mixtures were filtered into a conical flask, which was then filled with 10 ml pyridine and 2 ml 2 percent sodium nitropruside and agitated for 10 minutes. After that, 3 ml of 20 % sodium hydroxide (NaOH) was added to give the mixture a brownish yellow colour. Following the process previously described, glycoside standards ranging from 0 to 5 mg/ml were generated from 100 mg/ml stock glycoside. A spectronic 21 D digital spectrophotometer was used to measure the absorbance of the samples and the standard at a wavelength of 510 nm (Amadi *et al*., 2004). Total glycoside of each of the extract samples were calculated as follow:

$$\text{Total glycoside} = \frac{\text{absorbance of sample} \times \text{average gradient} \times \text{dilution factor}}{\text{Weight of sample} \times 10{,}000}$$

Total phenols content determination

The total phenolic content of the samples was determined using the method described by Talari *et al.* (2012). A total of 0.5 ml of the aliquots extract samples was placed into a test tube, which was then filled with distilled water to make it 1 ml, followed by 0.5 ml Folin-Ciocalteu reagent (1:1 with water) and 2.5 ml 20 percent sodium carbonate solution. The test tubes were placed in the dark cabinet for 40 minutes after the reaction mixture was vortexed. The absorbance of the solutions was measured using a spectrophotometer at a

wavelength of 725 nm against a blank reagent. A standard curve was created using Gallic acid monohydrate.

The linearity obtained was in the 1–10 g/ml range. Using the standard curve, the total phenolic content was calculated and expressed as Gallic acid equivalent in mg/g of the extracts.

Total coumarin content determination

The total coumarin content was measured using the procedure described by Isabella *et al*. (2016). To 1 ml of extracts, 0.5 ml of 5 M sodium hydroxide (NaOH) was added and heated at 80 ^{0}C for 5 minutes. In the cooled content, 0.75 ml of 5M sulphuric acid (H_2SO_4) was added and thoroughly mixed with 0.25 g of anhydrous $NaHCO_3$, which was then transferred to the extractor. The content was then removed using the Soxhlet extraction method for 3 hours with petroleum ether. In a water bath set at 50 - 55 ^{0}C, 20 ml of water was added to the petroleum ether extract, and the petroleum ether was carefully evaporated.

The aqueous solution was then transferred to a volumetric flask and mixed continuously until it reached the 50 ml level. After that, 25 ml of the solution was pipetted into a flask with 2 drops of 1 % sodium carbonate (Na_2CO_3) solution and heated for 15 minutes in a water bath at 85 ^{0}C before cooling. 5 ml diazonium solution was added and allowed to sit for 2 hours. At 540 nm, the absorbance was measured against a blank reagent. The total coumarin was calculated from the standard curve derived.

Total triterpenes content determination

Total triterpenes content was measured using the procedure given by Malik *et al*. (2017).

In a 50 ml conical flask, 0.5 g of extract samples were weighed, and 20 ml of a 2:1 chloroform – methanol mixture was added, thoroughly agitated, and allowed to stand for 15 minutes. The supernatant was discarded, and the precipitate was centrifuged after being rinsed with 20 ml of a 2:1 chloroform – methanol combination. The precipitate was then dissolved in 40 ml of sodium dodecyl sulphate (SDS) solution at 10 %. For 30 seconds, a milliliter of 0.01 M ferric chloride ($FeCl_2$) solution was added to the content. The items were vigorously shaken before being set aside for 30 minutes. From a 100 mg/l stock solution purchased from Sigma-Aldrich chemicals, Germany, standard triterpenes with concentrations ranging from 0 to 5 mg/ml were produced. A digital spectrophotometer with a wavelength of 510 nm was used to measure the absorbance of the samples and the standard amounts of triterpenes. The total amount of triterpenes was determined using the formula:

$$\text{Total triterpene} = \frac{\text{absorbance of sample} \times \text{average gradient} \times \text{dilution factor}}{\text{Weight of sample} \times 10{,}000}$$

APPENDIX C

Determination of chemical composition of diet

Moisture content- In a sterile aluminum dish, 5.0 g of feed sample (starting and finisher diets) were weighed. The dish was weighed, and feed samples were taken. This was then placed in an oven at 80 ^{0}C for 2 hours. This was taken out and placed in desiccators to cool. A measuring scale balance was then used to determine the weight. The feed sample in the crucible dish was then reweighed after being returned to the oven for another hour. The procedure was repeated until a steady weight was achieved. The difference in weight between the initial weight and the constant weight gained represents the moisture content.

$$Moisture\ content = loss\ in\ weight \left[\frac{W2 - W3}{W2 - W1}\right] X100$$

Where W1- initial weight of crucible dish, W2 is weight of crucible +feed before drying and W3 is final weight of crucible + feed after drying.

Ash content - 20 g of the feed samples were loaded into a dried platinum crucible and placed in a 550 ^{0}C furnace for 3 hours of blasting. It was then taken out and placed in desiccators to cool before being weighed again.

Ash content = weight of ash / weight of original feed sample used x 100

Lipid content – Weighing 15 g of feed samples and gently placing them inside a fat-free thimble. To prevent sample loss, this was wrapped in cotton wool and placed in the Soxhlet extractor. A weighted fat-free Soxhlet flask was filled with 200 ml of petroleum ether, and the flask was linked to the extractor. The petroleum ether in the flask was

refluxed by placing it on a heated mantle. The extractor was cooled for at least 6 hours with running tap water, after which the solvent was entirely siphoned into the flask. The solvent was evaporated using a rotary vacuum evaporator, leaving the extracted lipids in the Soxhlet. The flask was taken out of the evaporator and dried in the oven at 60 ^{0}C to a constant weight. The flask was then weighed after cooling in the desiccators. The amount of fat extracted was calculated by difference.

Ether extracts (100 g) dry matter = weight of extracted lipids / weight of dry feed sample) x 100.

Protein content – The Kjeldahl method was used to determine total protein. 20 g of feed samples were weighed and placed in a Kjeldahl flask with filter paper. 10 tablets of Na_2SO_4 and 1 g of $CuSO_4$ were used in the experiment. 20 ml concentrated H_2SO_4 was added, then the solution was digested in a fume cupboard until it became colourless. The solution was allowed to cool overnight before being transferred to a 500 ml flat bottom flask containing 200 ml of water. It was then cooled down with the use of ice packs. Using 3 days of screened methyl red indicator, 60–70 ml of 40 % NaOH and 50 ml of 4 % boric acid were poured into the conical flask. The ammonia gas was then distilled until it completely evaporated in the conical flask. In the receiver conical flask, 0.01 M HCl was titrated until the solution became colourless.

The percentage protein was calculated as follows: Vs – Vb x 0.01401 x N acid (6.25) x 100 where Vs is volume of acid required to titrate the sample, Vb is volume of acid required to titrate blank, N acid is normality of acid

Crude fibre content- 20 g of feed samples were defatted for 8 hours with diethyl ether and then boiled for exactly 30 minutes with 200 ml of 1.25 percent H_2SO_4 under reflux. On a flutter funnel, it was then filtered through cheese cloth. The acid was then totally removed by washing it with boiling water. The residue was then heated for another 30 minutes in a round bottom flask with 200 ml of 1.25 percent sodium hydroxide (NaOH) before being filtered through a couch crucible that had been previously weighed. The crucible was then dried with samples in a 100 °C oven, cooled in desiccators, and weighed later. This was then cremated for 2 to 3 hours at 600 °C in a muffle furnace, then allowed to cool in desiccators before being weighed.

Carbohydrate determination- available carbohydrate (%) = 100 – (protein %+ moisture % + ash % + fibre % + fat %)

APPENDIX D (Ranking summation Index for First Feeding Growth Performance)

Factor	IBW	rank	FBW	Rank	WG	rank	FI	rank	WI	rank	FCR	rank	Sum	over all rank
BHA (0.02%)	41.08	5	3337	2	58.86	2	89.86	1	0.24	1	1.53	2	11	2
LMPE (0.02%)	41.1	3	3390	1	59.8	1	90.48	2	0.25	3	1.51	1	10	1
OW	41.12	1	2878.5	5	50.67	5	93.27	5	0.25	3	1.84	5	19	5
SHPE (0.02%)	41.12	1	3093.5	3	54.51	3	90.93	3	0.24	1	1.67	3	11	2
SOPE (0.02%)	41.1	3	2888.5	4	50.85	4	92.34	4	0.25	3	1.82	4	18	4

APPENDIX E (Ranking summation Index for First Feeding Haematology)

Factor	WBC	rank	RBC	Rank	HGB	rank	Sum	over all rank
BHA	14.6	3	3.32	5	10.6	5	13	5
LMPE	39.9	4	8.4	2	16.1	2	8	2
OW	53.5	5	6.98	3	11.6	4	12	4
SHPE	7.3	1	8.87	1	16.8	1	3	1
SOPE	10.4	2	6.93	4	11.8	3	9	3

APPENDIX F (Ranking summation Index for First Feeding Blood Serum)

Factor	TP	rank	TCHL	rank	TRYG	rank	SOD	Rank	GPx	rank	CAT	rank	Sum	over all rank
BHA	8.18	3	366.91	1	116.6	3	29.85	5	156.38	1	545.62	5	18	4
LMPE	7.54	5	426.47	3	104.15	1	74.62	3	19.05	3	718.39	1	16	1
OW	8.87	1	433.62	4	130.1	4	89.55	2	38.13	2	596.31	4	17	2
SHPE	8.47	2	383.58	2	133.9	5	119.4	1	16.84	5	688.87	2	17	2
SOPE	7.71	4	468.16	5	112.45	2	59.7	4	18.54	4	621.17	3	22	5

APPENDIX G (Ranking summation Index for First Feeding Carcass Traits)

Factor	LW	rank	CW	rank	D%	rank	AF	rank	Sum	over all ranking
BHA	3337	2	2387	2	71.53	2	1.77	5	11	3
LMPE	3390	1	2466	1	72.74	4	0.89	2	8	1
OW	2878	5	2178	4	75.68	5	1.76	4	18	5
SHPE	3093	3	2193	3	70.9	1	1.46	3	10	2
SOPE	2888	4	2088	5	72.3	3	0.86	1	13	4

APPENDIX H (Ranking summation Index for First Feeding Physicochemical Properties)

Factor	pH	rank	WHC	rank	L*	rank	a*	rank	b*	rank	Sum	over all ranking
BHA	5.6	1	25.1	4	20.03	2	2.82	2	5.33	5	14	2
LMPE	5.05	5	24.2	5	22.52	3	1.95	4	2.33	1	18	4
OW	5.4	2	26.2	3	38.23	5	1.22	5	4.67	3	18	4
SHPE	5.2	4	26.8	1	34.62	4	1.97	3	5.11	4	16	3
SOPE	5.35	3	26.8	1	18.47	1	8.19	1	3.12	2	8	1

APPENDIX I (Ranking summation Index for First Feeding Shear Force Analysis)

Factor	FP	rank	FY	Rank	Sum	over all rank
BHA	15.9	5	3.2	3	8	4
LMPE	10.4	3	3.9	4	7	3
OW	12	4	11.6	5	9	5
SHPE	3.5	1	1.2	1	2	1
SOPE	7.2	2	1.5	2	4	2

APPENDIX J (Ranking summation Index for First Feeding Sensory Evaluation)

Factor	AP	rank	Taste	rank	Text.	rank	Aroma	rank	Sum	over all ranking
BHA	7.5	3	7.4	3	7.8	1	7.35	3	10	2
LMPE	7.55	2	7.75	2	7.6	2	7.45	2	8	1
OW	7.95	1	7	4	7.25	3	6.5	4	12	3
SHPE	7.2	4	6.5	5	6.55	4	6.3	5	18	5
SOPE	7.05	5	8	1	6.5	5	7.8	1	12	3

APPENDIX K (Ranking summation Index for First Feeding Fatty Acid Profiles)

Factor	Caproic	rank	Capric	rank	Palmitic	rank	Valeric	rank	caprylic	rank	caproleic	rank	palmitoleic	rank
BHA	1.72	2	25.28	3	0.79	4	2.96	5	12.71	3	1.07	1	0	1
LMPE	0	1	20.19	2	0	1	0	1	15.5	4	4.86	4	3.5	5
OW	7.71	5	39.66	5	0	1	0	1	5.72	1	1.23	2	1.69	4
SHPE	6.94	4	30.99	4	4.25	5	0	1	12.33	2	2.01	3	0	1
SOPE	2.41	3	12.1	1	0	1	0	1	15.96	5	9.17	5	1.29	3

maristoleic	rank	oleic	rank	linoleic	rank	DHA	rank	acetic	rank	ECA	rank	ARACH	rank	sum	over all rank
1.84	5	1.07	3	7.98	3	0	2	0.93	2	0	2	0	2	38	4
0	1	2.07	4	14.62	1	0	2	0	3	0	2	0	2	33	3
0	1	0	1	3.58	4	0.84	1	1.47	1	0	2	0	2	31	1
0	1	6	5	0	5	0	2	0	3	5.55	1	2.13	1	38	4
0	1	0	1	9.08	2	0	2	0	3	0	2	0	2	32	2

APPENDIX L (Ranking summation Index for First Feeding Lipid Oxidation)

Factor	Tbars C0	rank	Tbars R0	rank	Tbars C2	rank	Tbars R2	Rank	Tbars C4	rank
BHA	0.37	4	0.11	4	0.31	2	0.28	1	0.56	2
LMPE	0.3	3	0.25	5	0.32	3	0.3	4	0.63	5
OW	0.28	2	0.05	1	0.5	5	0.46	5	0.56	2
SHPE	0.01	1	0.08	3	0.28	1	0.28	1	0.5	1
SOPE	0.46	5	0.05	1	0.46	4	0.28	1	0.58	4

Tbars R4	rank	Tbars C6	rank	Tbars R6	rank	sum	over all rank
0.32	2	1.98	1	1.95	5	21	2
0.34	3	1.98	1	1.91	2	26	5
0.22	1	1.98	1	1.94	4	21	2
0.48	4	1.98	1	1.91	2	14	1
0.92	5	1.98	1	1.88	1	22	4

APPENDIX M (Ranking summation Index for Second Feeding Growth Performance)

Factor	IBW	rank	FBW	rank	WG	rank	FI	rank	WI	rank	FCR	rank	Sum	over all rank
BHA	41.1	5	3300	3	58.19	3	96.74	2	0.25	1	1.66	3	17	3
SOPE 0.04%	41.12	4	3500	1	61.77	1	101.73	5	0.27	3	1.65	2	16	2
SOPE 0.06%	41.14	1	3200	5	56.41	5	96.32	1	0.26	2	1.71	4	18	4
SOPE 0.08%	41.13	2	3250	4	57.3	4	99.17	4	0.27	3	1.73	5	22	5
SOPE 0.10%	41.13	2	3400	2	59.98	2	98.18	3	0.27	3	1.64	1	13	1

APPENDIX N (Ranking summation Index for Second Feeding Growth Haematology Performance)

Factor	WBC	rank	RBC	rank	HGB	Rank	Sum	over all rank	
BHA	8.2	3	0.96	5	8.13	2	10		4
SOPE 0.04%	7.4	2	1.68	3	5.1	3	8		1
SOPE 0.06%	6.6	1	1.99	2	2.8	5	8		1
SOPE 0.08%	10.5	4	2.4	1	3	4	9		3
SOPE 0.10%	19.67	5	1.5	4	11.12	1	10		4

APPENDIX O (Ranking summation Index for Second Feeding Blood Serum)

Factor	Tchl	rank	Trigly	rank	SOD	rank	GPx	rank	CAT	rank	Sum	over all rank
BHA	169.5	3	101.28	4	391.79	2	3108.1	2	4433.33	1	12	1
SOPE 0.04%	118.21	1	38.98	1	369.32	4	1175.7	4	4353.3	2	12	1
SOPE 0.06%	279.01	5	65.5	3	433.25	1	1250.6	3	1506.6	5	17	4
SOPE 0.08%	221.04	4	50.48	2	348.26	5	3143.8	1	3066.67	3	15	3
SOPE 0.10%	165.32	2	102.87	5	373.13	3	1020	5	1806.67	4	19	5

APPENDIX P (Ranking summation Index for Second Feeding Carcass Traits)

Factor	LW	rank	CW	rank	D%	Rank	AF	rank	Sum	over all rank
BHA	3300	3	2800	4	84.85	2	0.69	3	12	3
SOPE 0.04%	3500	1	3160	1	90.29	5	0.54	1	8	1
SOPE 0.06%	3200	5	2830	3	88.44	4	0.72	5	17	5
SOPE 0.08%	3250	4	2800	4	86.15	3	0.6	2	13	4
SOPE 0.10%	3400	2	2870	2	84.41	1	0.69	3	8	1

APPENDIX Q (Ranking summation Index for Second Feeding Physicochemical Properties)

Factor	pH	rank	WHC	rank	L*	rank	a*	rank	b*	rank	sum	over all rank
BHA	5.85	5	64.04	1	20	2	2.82	2	5.33	5	15	2
SOPE 0.04%	6.15	2	58.74	2	38.23	5	1.22	5	4.67	3	17	5
SOPE 0.06%	5.95	4	44.99	3	18.47	1	1.97	3	3.12	2	13	1
SOPE 0.08%	6.18	1	27.56	5	34.62	4	8.19	1	5.11	4	15	2
SOPE 0.10%	6.05	3	34.33	4	22.52	3	1.95	4	2.32	1	15	2

APPENDIX R (Ranking summation Index for Second Feeding Shear Force Analysis)

Factor	FP	rank	FY	Rank	sum	over all rank
BHA	17.8	3	4	2	5	2
SOPE 0.04%	5.3	1	1.3	1	2	1
SOPE 0.06%	17.9	4	4.1	3	7	4
SOPE 0.08%	24.5	5	10.66	5	10	5
SOPE 0.10%	8.3	2	5.2	4	6	3

APPENDIX S (Ranking summation Index for Second Feeding Sensory Evaluation)

Factor	APP	rank	Taste	rank	Text.	rank	Aroma	rank	Ov accept	rank	Sum	over all rank
BHA	7.5	2	7.4	3	7.8	1	7.35	2	7.4	3	11	2
SOPE 0.04%	7.05	4	7.35	4	7.2	4	7.15	4	7.25	4	20	4
SOPE 0.06%	7	5	7	5	6.75	5	6.65	5	7.25	4	24	5
SOPE 0.08%	7.3	3	7.45	1	7.45	3	7.2	3	7.5	2	12	3
SOPE 0.10%	7.6	1	7.45	1	7.55	2	7.45	1	7.95	1	6	1

APPENDIX T (Ranking summation Index for Second Feeding Fatty Acid Profiles)

Factor	Palm.	Rank	Capric	rank	Myristic	rank	Lauric	Rank	Palmitoleic	rank	Oleic	rank
BHA	20.09	2	3.02	4	15.64	4	0	1	1.43	4	100.65	5
SOPE 0.04%	9.93	1	0	1	0	1	2.28	5	0	1	84.35	4
SOPE 0.06%	35.73	4	3.02	4	15.66	5	0	1	1.43	4	62.94	3

SOPE 0.08%	28.26	3	0	1	9.15	3	0	1	0	1	17.18	1
SOPE 0.10%	98.09	5	0	1	0	1	0	1	0	1	53.78	2

Caproleic	rank	Linoleic	rank	DHA	rank	Sum	over all rank
0	1	0	2	0	2	25	4
2.28	4	40.82	1	0	2	20	2
0	1	0	2	0	2	26	5
0	1	0	2	5.3	1	14	1
56.24	5	0	2	0	2	20	2

APPENDIX U (Ranking summation Index for Second Feeding Lipid Oxidation)

Factor	Tbars C0	rank	Tbars R0	rank	Tbars C2	rank	Tbars R2	rank	Tbars C4	rank
BHA	0.08	2	0.02	1	0.18	2	0.13	4	0.78	5
SOPE 0.04%	0.07	1	0.04	3	0.18	2	0.06	2	0.74	4
SOPE 0.06%	0.08	2	0.06	4	0.24	5	0.17	5	0.49	2
SOPE 0.08%	0.08	2	0.02	1	0.01	1	0.06	3	0.63	3
SOPE 0.10%	0.09	5	0.06	4	0.19	4	0.03	1	0.16	1

Tbars R4	rank	Tbars C6	rank	Tbars R6	rank	Sum	over all rank
0.32	1	0.83	2	0.65	5	22	3
0.35	2	1.88	5	0.44	2	21	2
0.36	3	1.05	4	0.59	4	29	5

0.38	4	0.5	1	0.47	3	18	1
0.41	5	0.88	3	0.4	1	24	4