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The Effect of Oil Type, Oil Quality, Vitamin E, and Phytase Supplementation on Broiler Performance, Apparent Nutrient and Energy Utilization, and the Fatty Acid Profile of Fat and Liver in Broiler Chickens

Richard A. Adefioye
University of Kentucky, richardadefioye@gmail.com
Author ORCID Identifier:
https://orcid.org/0000-0002-7824-2822
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Richard A. Adefioye, Student

Dr. Sunday Tayo Adedokun, Major Professor

Dr. David L. Harmon, Director of Graduate Studies

The Effect of Oil Type, Oil Quality, Vitamin E, and Phytase Supplementation on Broiler Performance, Apparent Nutrient and Energy Utilization, and the Fatty Acid Profile of Fat and Liver in Broiler Chickens

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THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Agriculture, Food and Environment
at the University of Kentucky

By

Richard A. Adefioye

Lexington, Kentucky

Director: Dr. Tayo Adedokun, Associate Professor of Animal and Food Sciences

Lexington, Kentucky

2021

https://orcid.org/0000-0002-7824-2822

ABSTRACT OF THESIS

The Effect of Oil Type, Oil Quality, Vitamin E, and Phytase Supplementation on Broiler Performance, Apparent Nutrient and Energy Utilization, and the Fatty Acid Profile of Fat and Liver in Broiler Chickens

Two experiments were conducted to examine the effects of oil type, oil quality, phytase, and vitamin E (VE) supplementation in broiler chickens. Experiment 1 used 378-day-old male by-product Cobb breeder chicks with 9 treatments, 7 replicates, and 6 birds per replicate, structured as a 2x2x2+1 factorial arrangement of treatments for 14 days. The first 8 treatments were based on a marginally non-phytate phosphorus (NPP) deficient diet (0.31%), while treatment 9 was a positive control (PC) diet with adequate NPP levels (0.45%). There were 2 levels of phytase (0 vs 1000 FTU/kg), 2 levels of oil quality (fresh soy oil; peroxide value (PV) = 3 meqO_2/kg and oxidized soy oil; PV =109 meqO_2/kg), and 2 levels of additional supplemental VE (basal VE vs. basal VE+150 ppm) composed of mixed tocopherols containing 55-75% γ-tocopherol. In experiment 2, 384 day-old male by-product Cobb breeder chicks were randomly assigned to 8 treatments, with 8 replicates containing 6 birds per replicate for 20 days. The treatments consisted of 2 oil types (corn vs. soy oil), 2 oil quality levels (fresh corn oil, PV = 3 meqO_2/kg, fresh soy oil; PV = 4 meqO_2/kg, oxidized corn oil; PV =104 meqO_2/kg and oxidized soy oil; PV = 109 meqO_2/kg), and 2 levels of additional supplemental VE (basal VE vs. basal VE+150 ppm) composed of mixed tocopherols containing 55-75% γ tocopherol. In the first experiment, phytase supplementation improved ($P < 0.05$) feed efficiency, calcium utilization, bone-breaking strength, tibia ash, apparent metabolizable energy (AME), and AME corrected for nitrogen (AMEn). Oxidized oil with phytase produced a higher ($P < 0.05$) AME and AMEn compared to fresh or oxidized oil without phytase supplementation. Furthermore, oxidized oil with additional supplemental VE reduced ($P < 0.05$) crude fat utilization compared to oxidized oil without additional supplemental VE. In the second experiment, Oxidized oils reduced ($P < 0.05$) feed efficiency, energy utilization, and AMEn. Moreover, birds that received oxidized soy oil with VE had the lowest ($P < 0.05$) live weight and body weight gain compared to those that received diets containing fresh corn oil or soy oil with or without VE. Also, oxidized oils alone reduced ($P < 0.05$) nitrogen utilization and this effect was more pronounced in birds fed diets containing corn oil. Surprisingly, VE supplementation reduced ($P < 0.05$) crude fat, nitrogen, and energy utilization, as well as AME and AMEn. In conclusion, oil type, oil oxidation, and phytase supplementation influenced the growth performance, and energy and nutrient utilization, while additional VE supplementation at 150 ppm above the recommended VE levels in the basal diet provided little-to-no beneficial effects on these response measures.

Keywords: oil type, oxidized oils, vitamin E, phytase, broilers.

Richard A. Adefioye

July 23, 2021

The Effect of Oil Type, Oil Quality, Vitamin E, and Phytase Supplementation on Broiler Performance, Apparent Nutrient and Energy Utilization, and the Fatty Acid Profile of Fat and Liver in Broiler Chickens

By

Richard A. Adefioye

Dr. Sunday A. Adedokun
Director of Thesis

Dr. David L. Harmon
Director of Graduate Studies

July 23, 2021

This thesis is dedicated to my wife, Mrs. Adenike Adefioye. Your prayers and endless support have made this possible.

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CHAPTER 1

Introduction

Modern lines of broiler chickens require high-nutrient diets to fulfill their full genetic potential. This is why the supplementation of poultry feed with lipids is important for raising the caloric density of the diets. Besides energy supply, dietary lipids also provide essential n-6 and n-3 fatty acids (FA), which partake in most biological processes, including immune functioning and development (Konieczka et al., 2017). Several fat sources are available for the formulation of broiler diets and these can be broadly categorized into vegetable oils, animal fats, animal-vegetable fat blends, and industrial by-products (McDonald et al., 2002; Leeson and Summers, 2005; Kellems and Church, 2010). The digestibility of dietary lipids is controlled significantly by their fatty acid profile. For instance, lipids rich in unsaturated fatty acids (UFAs) generally have better digestibility and higher metabolizable energy than those rich in saturated fats (SFAs) as a result of better intestinal absorption (Celebi and Utlu, 2006). Thus, the use of lipids rich in polyunsaturated fatty acids (PUFAs) such as corn oil (CO) and soybean oil (SO) is commonplace in augmenting broiler diets. However, PUFA is more sensitive towards oxidation especially when stored at high temperatures and humidity (Anjum et al., 2002). But when such oxidized fats are fed to birds, they have been reported to adversely affect the performance and health of broilers, as it causes oxidative stress, encephalomalacia, decreased body weight, and reduced feed efficiency. Thus, the lipid oxidation process results in severe nutritional and economic losses (Engberg et al., 1996; Anjum et al., 2002).

The go-to approach for combating lipid oxidation is the dietary supplementation of antioxidants such as vitamin E (VE), which is well known for its protective capability

against oxidative damage. Vitamin E has also been shown to restore the normal levels of lipids in the liver, lung, heart, and kidney of rats exposed to the peroxidative damage of free radicals induced by lead (Nobakht, 2012). Vitamin E also plays a role in gene expression and, for instance, it has been reported to down-regulate almost half of the genes involved in the cholesterol synthesis pathway (Landrier et al., 2010). According to the national research council (NRC), the nutritional recommendation of VE for broilers varies is 10 mg/kg (NRC, 1994). However, 80 IU/kg is recommended by the Cobb broiler management guide, while commercial diets usually contain about 50 to 300 mg/kg (Adisseo, 2002) to help buffer the stress that accompanies real-world farming conditions. The antioxidant activity of VE depends on how much α-tocopherol gets deposited in the cell membranes, where it acts as a scavenger for free radicals, and prevents the propagation of the lipid oxidation chain reaction, thereby promoting oxidative stability (Asghar et al., 1990).

Another feed additive that has been reported to influence the oxidative status of broilers but has received less attention in this regard is phytase. Phytases are popular enzymes added to animal diets to alleviate the effect of dietary phytates and increase the bioavailability of plant-based phosphorus (P). However, over the years, studies have shown that the impact of phytase is more far-reaching and may even influence the antioxidant system of animals. For instance, dietary phytase, especially at higher doses, has been reported to increase retinol and a-tocopherol concentration in the liver of chickens (Karadas et al., 2010). In another study, Gebert et al. (1999) reported that phytase supplementation reduced the oxidative stability of back fat ($P < 0.01$), which was improved by α-tocopherol supplementation when pigs were fed a low-phosphorus diet. This, therefore, suggests that

the inclusion of phytase in the diets of broilers demands an additional supplementation of VE due to a higher oxidative load caused by an increased macro and micro mineral concentration in the digesta.

While few studies have investigated the deleterious impact of oxidized diets and the ameliorative potential of dietary VE on poultry performance and meat quality (Bayraktar et al., 2011; Tavárez et al., 2011; Lu et al., 2014), a lot is yet to be understood regarding their impacts fat deposition, fatty acid composition, and nutrient utilization. Furthermore, to the best of our knowledge, there is currently no existing literature describing the impact of the dietary oil type, oil quality, phytase, and VE supplementation on broiler chickens.

So, the objectives of the studies presented in this thesis were:

1. To investigate the effect of feeding diets containing fresh or oxidized soy oil, supplemented with phytase and VE in a marginally phosphorus deficient diet on the growth performance, bone mineralization, fatty acid profile of liver and adipose tissues, relative liver and fat weight, and apparent ileal digestibility and utilization of nutrient and energy in 21-day-old broiler chickens.
2. To investigate the effects of oil type, oil quality, and VE supplementation on the growth performance, the fatty acid profile of liver and adipose tissues, relative liver and fat weight, serum and liver superoxide dismutase (SOD) activity, and the apparent utilization of energy and nutrient in 20-day-old broiler chickens.

CHAPTER 2

Literature Review

2.1 Role of Dietary Fat in Broiler Production

Fat is a term that is generally used interchangeably with lipid. Both terms describe a diverse group of compounds characterized by insolubility in water but capable of dissolving in organic solvents such as acetone, alcohol, chloroform, and diethyl ether. Lipids are very important players in the biochemical and physiological functions of plants and animals. From a nutritional perspective, the lipids of importance are phospholipids, sterols, and triglycerides (Brindley, 1984). Lipids are primarily made up of carbon hydrogen and oxygen, but they sometimes contain other elements such as nitrogen, phosphorus, sulfur, etc. The general structure of fats and oils are the same but they differ in their physical and chemical properties. Triacylglycerols, which are glycerol esters are the main component of fats and oils. Triacylglycerol comprises a glycerol molecule and 3 fatty acids (FA) and it is also called a triglyceride. In general, the word 'fat' is used to describe triglycerides that are solid at room temperature, while "oils" generally describe triglycerides that are in liquid form when at room temperature (Tisch, 2006).

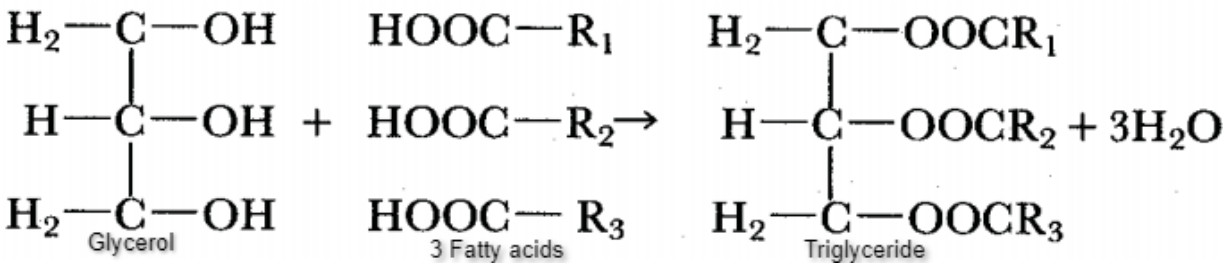


Figure 2. 1 Structure of Triglycerides. (Adapted from Tancharoenrat, 2012)

The dietary supply of fat and oils is commonplace in modern poultry production. This is done primarily to raise the energy level of the feed because fats have an energy

value that is more than twice as much as the energy in carbohydrates and proteins (NRC, 1994). But besides supplying energy, lipids also improve the absorption of fat-soluble vitamins, diminishes feed dustiness and improves its palatability, decrease feed intake, and improves feed efficiency (Firman and Kamyab, 2010). In addition, dietary fat reduces the passage rate of the digesta through the gastrointestinal tract, allowing for better nutrient digestion, absorption, and utilization (Baiao and Lara, 2005). It has also been reported that fat metabolism and deposition in poultry are affected by dietary lipid sources and quality (Pesti et al., 2002).

Dietary lipid supplementation also influences carcass characteristics (Crespo and Esteve-Garcia, 2001; Azman et al., 2004; Nayebpor et al., 2007; Febel et al., 2008). Specifically, the dietary supplementation of fats and oils impacts the fatty acid (FA) composition of the broiler carcass. For instance, when Azman et al. (2004) fed different oil sources to broilers, he observed that birds that received diets containing soy oil had a greater content of polyunsaturated fatty acids (PUFA) in their abdominal fat tissue compared to those that received diets containing beef tallow or poultry grease. Elsewhere, Crespo and Esteve-Garcia (2001) reported that broilers fed tallow-based diets had more saturated fatty acids (SFAs) in their abdominal fat, breast, and thighs than those fed diets containing sunflower oil, olive oil, and linseed oil. These observed FA changes in the carcass are believed to be due to the direct incorporation of dietary FA into the tissues.

Fatty acids have also been recognized as modulators of immune responses. Nayebpor et al. (2007) observed that when the dietary levels of soy oil were increased, the antibody titers against the infectious bursal disease also improved in broilers. These improvements in immunity were attributed to the presence of PUFA. The activity of certain

FA on gut microflora is also an area of ongoing research. Medium-chain FA, such as monocaprin (1-monoglyceride of capric acid), are particularly effective in controlling *Campylobacter jejuni* (Thormar et al., 2006). The supplementation of caprylic acid at 7 g/kg of in feed has also been shown to reduce Campylobacter counts in the caeca of broiler chickens compared to 0 and 1.4 g/kg supplementation levels (De Los Santos et al., 2008).

2.1.1 Characteristics of Different Lipids Used in Animal Feeds

2.1.1.1 FA Profiles

Different fat sources have distinct FA profiles that can be used to identify them. The FA profiles of each fat source are unique in either length of the carbon chain or its degree of saturation. The basic structure of FA is the hydrocarbon structure (containing carbon and hydrogen atoms) formed by four or more carbons attached to an acidic functional group called the carboxyl group. The higher the number of carbon atoms in the chain the higher the melting point of the FA, but the presence of a double bond lowers the melting point (Valenzuela and Valenzuela, 2013).

Based on their chain length, FAs can be classified as short-chain FA when they have four (C4) to six (C6) carbons; as medium-chain FA when they have eight (C8) to fourteen (C14) carbons; as long-chain FA with sixteen (C16) to eighteen carbons (C18); and as very-long-chain fatty acid when they have twenty (C20) or more carbon atoms. It is worthy of note that molecules with less than four carbon atoms (C2; acetic acid and C3; propionic acid) are not considered FA due to their high solubility in water (Valenzuela and Valenzuela, 2013).

Depending on the presence or absence of double bonds, FAs can be grouped into three main categories. These are saturated fatty acids (SFA) containing no double bonds, monounsaturated fatty acids (MUFAs) containing one double bond, and polyunsaturated fatty acids (PUFAs) having two to six double bonds. Furthermore, each unsaturated fatty acid (UFA) can be classified as *cis*- or *trans*- based on the configuration of the double bonds. They can also be grouped as n-3 (omega-3), n-6 (omega-6) PUFAs, or others depending on the distance of the first double bond from the FA methyl-end. Different forms of FAs function differently in animal metabolic reactions, *cis*-unsaturated FAs are potent inducers of adiposomes, while *trans*- unsaturated fatty acids are not (Orsavova et al., 2015). The names, number of carbons, and number of double bonds of the most common FAs in plant and animal tissues are shown in Table 2.1

Omega-3 (n-3) and omega-6 (n-6) PUFAs are generally considered essential fatty acids (EFAs), as they are required for proper physiological functioning and health but are not directly synthesized by the animal and thus must be provided in the feed (Kaur et al., 2014). In general, several FAs are considered essential, but as far as poultry production is concerned, there are 3 main EFAs, which are α-linolenic acid (C18:3n3), linoleic acid (C18:2), and arachidonic acid (C20:4) (Balnave, 1970). It is worthy of note, however, that the definition of EFAs may not strictly apply to arachidonic acid as it is not directly supplied in the diet like α-linolenic acid or linoleic acid. However, it can be synthesized within the body of the animal by the enzyme Δ^6-desaturase from α-linolenic acid or linoleic acid (Norris and Carr, 2013).

2.1.1.2 Dietary Fat Sources in Broiler Production

In General, the sources of the fat used in broiler production can be of vegetable or animal origin, but they can come in different forms. Commonly used fats and oils in feed formulations include rendering by-products (e.g. poultry fat, tallow), restaurant greases (e.g. processed frying oils, also called yellow grease), vegetable oils (e.g. corn oil, soybean oil, and palm oil), hydrogenated fats (fats or oils converted to SFAs by the addition of hydrogen to double bonds of UFAs), acid soapstocks (free fatty acids removed from the refining process by alkali and settled as alkali soaps) and acid oils (vegetable oil refining by-products, primarily composed of free fatty acids) (McDonald et al., 2002; Leeson and Summers, 2005; Kellems and Church, 2010)

Vegetable oils have fairly stable FA profiles, but the FA profiles of animal fats are highly affected by the dietary FA profile, thus it can vary markedly across species. Fats and oils are selected as energy sources in a diet depending on their price and quality to achieve the best economic advantage. Vegetable fats are frequently classified into pulp oil and seed oil according to their source. Pulp oil is obtained from the pulp of fruits, such as palm, olive, avocado; and seed oil is extracted from seeds, such as soybean, sesame, corn, and peanuts (Bora et al., 2001; Orsavova et al., 2015). It is worthy of note that seed oils such as corn and soil oils are commonly used in commercial poultry production.

Corn oil is a by-product of cornmeal and starch-making companies. In terms of its FA composition, crude corn oil contains a relatively high level of linoleic acid (58–62%) and it is one of the richest sources of phytosterols (8,300–25,500 ppm) and tocopherols (1,130–1,830 ppm). The main phytosterol and tocopherol in corn oil are β-sitosterol (63–70%) and γ-tocopherol (68–89%), respectively (Ghazani et al., 2016). Just like most lipids

used in the poultry diet, the inclusion range of corn oil in poultry feed may range from 3-10% depending on the energy requirements (Leeson and Summers, 2005). Speaking of energy production, corn oil is highly digestible in non-ruminants and has been reported to have digestible energy that ranges from 8,036-8,921 kcal/kg in swine and an AMEn of 6,276 – 8,072 kcal/kg in broilers depending on the amount of free fatty acids (Kerr et al., 2016).

Table 2. 1 Common fatty acids in plant and animal tissues

Chemical name	Common name	Number of carbon	Number of double bonds	Abbreviated designation
Butanoic	Butyric	4	0	C4:0
Hexanoic	Caproic	6	0	C6:0
Octanoic	Caprylic	8	0	C8:0
Decanoic	Capric	10	0	C10:0
Dodecanoic	Lauric	12	0	C12:0
Tetradecanoic	Myristic	14	0	C14:0
Pentadecanoic	-	15	0	C15:0
Hexadecanoic	Palmitic	16	0	C16:0
Hexadecenoic	Palmitoleic	16	1	C16:1
Heptadecanoic	Margaric	17	0	C17:0
Octadecanoic	Stearic	18	0	C18:0
Octadecenoic	Oleic	18	1	C18:1
Octadecadienoic	Linoleic	18	2	C18:2
Octadecadienoic	Linolenic	18	3	C18:3
Eicosanoic	Arachidic	20	0	C20:0
Eicosatetraenoic	Arachidonic	20	4	C20:4
Docosenoic	Erucic	22 `	1	C22:1
Docosapentaenoic	Clupanodonic	22	5	C22:5
Tetracosanoic	Lignoceric	24	0	C24:0

Taken from Tancharoenrat (2012)

Soy oil is composed of five main FAs: palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3). The percentage of these five FAs in soy oil averages 10, 4, 18, 55, and 13%, respectively (Clemente and Cahoon, 2009). In terms of VE composition, soy oil has one of the highest tocopherol content among plant oils with crude soybean having as high as 1,328 mg/kg tocopherol, but this value can drop by as much as 50% in refined soy oils. The main types of tocopherol in soy oil are α-tocopherol (27-42%) and γ-tocopherol (42-60%). In terms of energy, soybean oil has been reported to have an average AMEn of 8,739 kcal/kg and it may be included in broiler diets up to 20% (Brown et al., 1993).

Besides energy and tocopherol supply, however, vegetable oils are also rich sources of EFAs such as linoleic and linolenic acids, which are not synthesized by poultry. To ensure adequate levels of these EFAs, Leeson, and Summers (2005) recommended a minimum inclusion level of 10 g/kg of fat in poultry diets. The FA composition of common fats and oils used in broiler production is shown in Table 2.2.

2.1.2 Lipid Digestion, Absorption, and Metabolism

2.1.2.1 Lipid Digestion

Tancharoenrat (2012) provided an excellent review of the underlying mechanism of digestion and absorption of fats in poultry. The digestion of fats is a fairly complicated process in all species. In poultry, when lipids are ingested, it is emulsified in the intestines, digested, solubilized in micelles, permeates the cell membranes, esterified intracellularly and incorporated into lipoproteins before it is released into the intestinal fluid (Krogdahl, 1985).

Table 2. 2 Fatty acid profiles of commonly used fats and oils

	Animal fats					Vegetable oils					
Fatty acid, %	Tallow (mutton)	Tallow (beef)	Lard	Poultry fat	Herring oil	Palm oil	Soy oil	Sunflower oil	Corn oil	Rapeseed oil	safflower oil
C10:0	0.2	-	0.1	-	-	-	-	-	-	-	-
C12:0	0.3	0.1	0.1	0.1	-	0.1	-	-	-	-	-
C14:0	5.2	3.2	1.5	0.8	6.2	1	0.1	0.1	0.1	0.1	0.1
C14:1	0.3	0.9	-	0.2	-	-	-	-	-	-	-
C15:0	0.8	0.5	0.1	0.1	-	-	-	-	-	-	-
C16:0	23.6	24.3	26	25.3	12.7	44.4	10.6	10.9	7	3.8	6.7
C16:1	2.5	3.7	3.3	7.2	7.5	0.2	0.1	0.2	0.1	0.3	-
C17:0	2	1.5	0.4	0.1	-	0.1	0.1	0.1	0.1	-	0.04
C17:1	0.5	0.8	0.2	0.1	-	-	-	-	-	-	-
C18:0	24.5	18.6	13.5	6.5	1.1	4.1	4	2	4.5	1.8	2.4
C18:1	33.3	42.6	43.9	37.7	12.9	39.3	23.2	25.4	18.7	18.5	11.5
C18:2 n-6	4	2.6	9.5	20.6	1.1	10	53.7	59.6	67.5	14.5	79.0
C18:3 n-3	1.3	0.7	0.4	0.8	0.7	0.4	7.6	1.2	0.8	11	0.15
C20:0	-	0.2	0.2	0.2	-	0.3	0.3	0.4	0.4	0.7	-
C20:1	-	0.3	0.7	0.3	15.1	-	-	-	0.1	6.6	-
C20:4 n-6	-	-	-	-	0.3	-	-	-	-	-	-
C20:5 n-3	-	-	-	-	6.8	-	-	-	-	-	-
c22:0	-	-	-	-	-	0.1	0.3	0.1	0.7	0.5	-
C22:1	-	-	-	-	22	-	-	-	-	41.1	-
C22:6 n-3	-	-	-	-	5.8	-	-	-	-	-	-

Adapted from Tancharoenrat (2012) and Wang et al. (2019)

Most dietary fats occur as triglycerides, which are a combination of one glycerol molecule and three FAs. During digestion, two of these FAs are removed, and this leaves a monoglyceride (glycerol + one FA). This resulting monoglyceride and the free FAs constitute the products of triglyceride digestion and are the absorbable fat units.

Poultry have a digestive tract that is quite different from what is seen in mammals. It starts from the beak and extends to the esophagus, which widens into the crop, the lower esophagus, proventriculus, gizzard, duodenum, jejunum, and ileum. The gizzard is connected to the proventriculus by a short and narrow isthmus, and to the duodenum through a narrow pylorus. The distal end of the duodenal loop contains the opening of the bile and pancreatic and ducts, which convey enzymes and salts necessary for fat digestion (Duke, 1986). The gizzard is unique to poultry and it performs the functions of the mammalian teeth by mechanically grinding and mixing the ingested feed. The movements of the gizzard are pendular and they are followed by contractions of the proventriculus (Smulikowska, 1998). As a result of these contractions, there is shuttling of the digesta between the gizzard and proventriculus and this helps to maximize both the mechanical and enzymatic parts of the digestive process. (Klasing, 1999). In the turkey, intestinal refluxes or digesta movement, occur 2 to 3 times per hour and involve the entire duodenum and upper ileum (Duke, 1992). However, in chickens, the process is continuous, and this allows the duodenal contents to penetrate the gizzard when the gizzard contracts. This movement pattern allows for the reverse passage of intestinal digesta that contains bile, pancreatic and intestinal juice, into the gizzard and proventriculus (Sklan et al., 1978). The bile salts present in the gizzard initiate the emulsification of fat, which is a key requirement for the remaining phases of duodenal and jejunal digestion and absorption of fat.

This continuous shuttling of digesta between the gizzard and duodenum also increases the exposure time of feed to digestive enzymes and this promotes the absorption of fat in the upper parts of the small intestine (Smulikowska, 1998). The entry of digesta into the duodenum initiates fat digestion. When this happens, it stimulates the secretion of cholecystokinin which controls the secretions of bile and pancreatic enzymes (Krogdahl, 1985). The release of bile salts from the gall bladder promotes the emulsification of the fat present in the chyme. The pancreatic lipase and colipase secreted by the pancreas aid the hydrolysis of fat (Erlanson et al., 1973). However, the activity of pancreatic lipase can be inhibited by high concentrations of bile salts. Bosc-Bierne et al. (1984) reported that when a high concentration of bile salts such as sodium taurochenodeoxycholate was present in the digesta, the activity of pancreatic lipase in the chicken was reduced. However, colipase restored lipase activity. Colipase, which is made up of hydrophilic and hydrophobic amino acids, is a co-factor contained in pancreatic secretions. Colipase is important for the action of lipase on triglyceride emulsions as it keeps the lipase in an active configuration at the lipid-water interface. Colipase binds to the surface of lipid droplets and acts as an anchor for lipase allowing pancreatic lipase to digest triglycerides (Borgstrom, 1980).

Triglycerides are hydrolyzed by the action of pancreatic lipase to produce free fatty acids from the sn-1 and -3 positions, and the sn-2-monoacylglycerol. These products, which include unsaturated long-chain fatty acids (LCFAs), medium-chain fatty acids (MCFAs), monoglycerides, and phospholipids, spontaneously form mixed micelles with conjugated bile salts. The resulting micelles are then shuttled to the mucosal surface before passing through the brush border membrane (Krogdahl, 1985). Free fatty acids have been reported to inhibit the activity of lipase. Interestingly, lipase activity has been reported to

be increased by unsaturated fatty acids, decreased by long-chain saturated fatty acids, particularly stearic acid, and minimally affected by saturated free fatty acids with eight or ten carbons (Van Kuiken and Behnke, 1994). Past research has also suggested that the fatty acid-binding site in lipase may require the FA to bend at a 141° angle, but stearic acid has an angle of 180° which makes it difficult to bind with lipase. Therefore, unsaturated fatty acids, which have an angle of approximately 141°, have a greater ability to increase lipase activity compared to long-chain saturated fatty acids. It is also worthy of note that the digestibility of dietary lipids is affected by the FA profiles. For instance, studies have shown a better utilization of unsaturated fats, which results in higher metabolizable energy compared to saturated fats (Celebi and Utlu, 2004).

2.1.2.2 Lipid Absorption

It is well established that the major site of fat digestion and absorption is the small intestine (Hurwitz et al., 1973; Freeman, 1976; Krogdahl, 1985). However, there is still a lot of debate regarding the exact site of fat digestion and absorption within the small intestine. Freeman (1976) suggested that the duodenum is the preparative and absorptive site for fat in birds. Hurwitz et al. (1973) reported that the absorption of fat takes place mainly in the jejunum and continues in the ileum. Renner (1960) stated that the absorption of fat was negligible in the caeca and the large intestine. The processes involved in the digestion and absorption of fat are depicted in figure 2.2. After digestion, monoglycerides and short-chain fatty acids (SCFAs) require no emulsification, thus they are passively absorbed from the intestinal lumen via the intestinal cells to mesentery blood vessels (Pond et al., 2005). Conversely, long-chain saturated fatty acids (LCSFA), diglycerides, fat-

soluble vitamins, and cholesteryl esters must be solubilized in the hydrophobic cores of mixed micelles, before being transported to the intestinal cells.

Ockner et al. (1972) reported that a soluble intracellular protein called fatty acid-binding protein (FABP) influences the movement of fatty acids through the cytosol of absorptive cells. Interestingly, Katongole and March (1979) reported that the proximal portion of the intestine contains the highest concentration of FABP in chickens. Fatty acid-binding protein has a greater affinity for unsaturated fatty acids than saturated ones and has little to no affinity for short or medium-chain fatty acids (Ockner and Manning, 1974). This protein also protects the absorptive cells from unbound fatty acids which have the potential to be cytotoxic (Shiau, 1981).

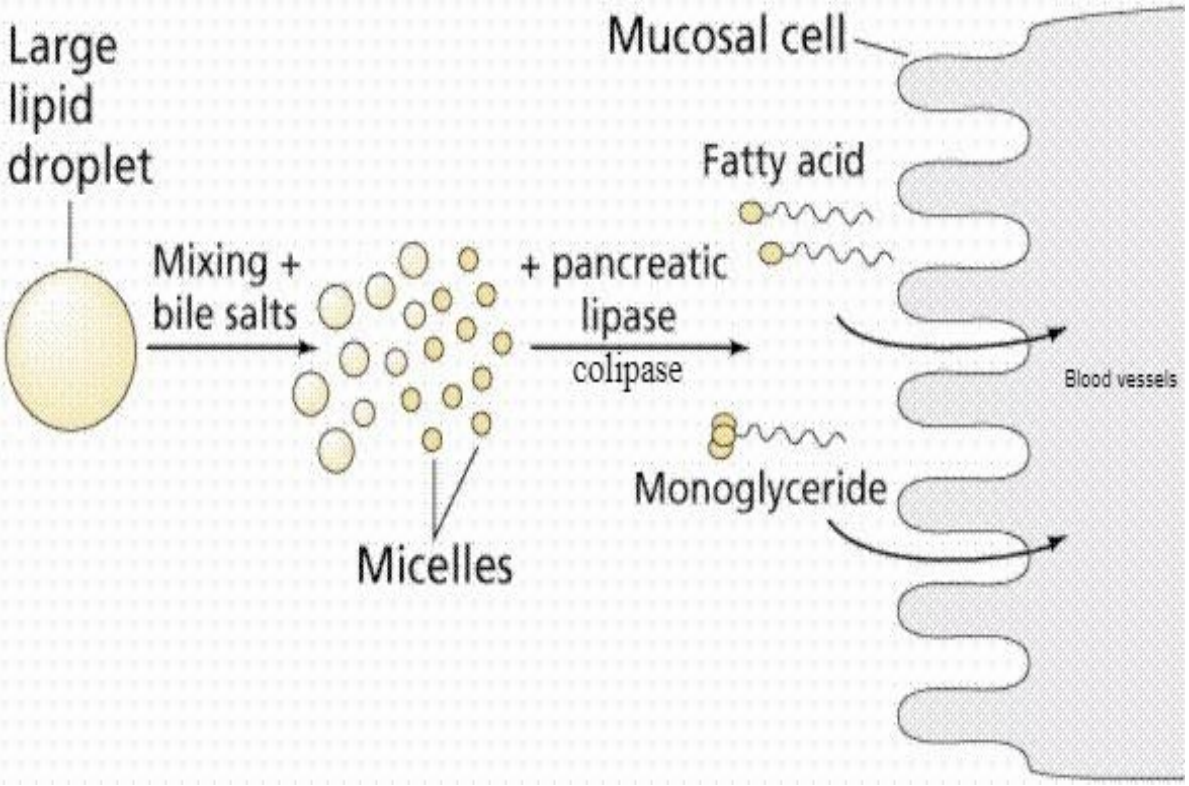


Figure 2. 2 Digestion and absorption of fat (Adapted from Tancharoenrat, 2012).

Monoglycerides and LCFAs are rebuilt into new triglycerides within the intestinal cells, after which the triglycerides are combined with phospholipids, lipoproteins, and free and esterified cholesterol to form chylomicrons, which are secreted into the lymphatic vessels. But because the lymphatic system of poultry is not well developed, the chylomicrons are directly secreted into the portal circulation and they are known as portomicrons (Hermier, 1997). These portomicrons are then shuttled to various tissues to be stored as fat deposits, metabolized as a source of energy, or used to synthesize various compounds such as phospholipids and lipoproteins in the liver (Scott et al., 1982).

2.1.2.3 Lipid Metabolism

Lindblom (2017) provided an excellent discourse on lipid metabolism. Lipids are an excellent energy source that provides 9 kcal gross energy (GE)/g in comparison to proteins and carbohydrates which only provide about 4 kcal GE/g each. When triglycerides are catabolized, adenosine triphosphate (ATP) is generated through the citric acid (TCA) cycle and the electron transport chain (ETC). The primary step to energy production from triglycerides is the cleavage of the carbon-carbon bonds, which yields 2 ATP molecules via the oxidation of flavin adenine dinucleotide ($FADH_2$) and 3 ATP molecules via NADH oxidation and acetyl CoAs are oxidized to CO_2 and water via the TCA cycle.

Fatty acids can be metabolized to generate ATP via beta-oxidation in the cells. Beta oxidation is a catabolic process that takes place within the mitochondria and peroxisomes, where FAs are used to generate coenzymes in the ETC. Beta oxidation commences when CoA ligase binds to FAs to generate a fatty acyl adenylate and an inorganic pyrophosphate. The products then react with CoA to produce a fatty acyl-CoA ester and adenosine monophosphate (AMP). The fatty acyl-CoA diffuses through the mitochondria membrane

if it has a short chain (6 carbons or less). But in the case of LCFAs, a carnitine shuttle is required to transport them from the cytosol to the mitochondria. This process involves the transfer of Acyl-CoA to the hydroxyl group of carnitine through the action of carnitine palmitoyltransferase I. As carnitine is shuttled outside, acylcarnitine is shuttled inside by carnitine-acylcarnitine translocase. The successfully transported Acyl-carnitine is then converted back into acyl-CoA through the action of palmitoyltransferase II and this occurs in the inner mitochondrial membrane, which allows the acyl-carnitine to enter into the mitochondrial matrix.

Within the mitochondrial matrix, beta-oxidation occurs by the cleavage of two carbons from the fatty acyl CoA in the form of acetyl-CoA every cycle. The first step of beta-oxidation is dehydrogenation by acyl CoA dehydrogenase to alter the configuration of the FA from cis to trans, and this uses FAD as an electron acceptor, which is consequently reduced to $FADH_2$ to generate trans-delta-2-enoyl CoA. This product is then hydrated at the trans double bond by enoyl-CoA hydratase before being dehydrogenated again. The first two carbons are liberated by the attack of CoA and the process continues until all of the carbons are turned into acetyl CoA. Acetyl CoA then enters the citric acid cycle to generate ATP. Odd-numbered carbons go through beta-oxidation until there are three carbons left in the fatty acid, which forms propionyl-CoA and succinyl-CoA. Methylmalonyl-CoA mutase catalyzes Propionyl-CoA to form succinyl-CoA which can enter into the citric acid cycle to form ATP.

2.1.2.4 Genes Related to Lipid Absorption

The deposition and mobilization of fat are regulated at several levels, including metabolites, enzymes, messenger ribonucleic acid (mRNA) expressions, and transcription

factors (Jump et al., 2005). Fat synthesis and oxidation occur within different tissues and cellular compartments and are regulated by the change in the level and activity of various enzymes, which may be regulated at transcriptional, translational, or post-translational levels (Jump et al., 2005; Duran-Montgé et al., 2009). The concentration of major lipoproteins is controlled by several genes including low-density lipoprotein receptor (LDLR), cholecystokinin receptor (CCKR), lecithin- pancreatic lipase (PNLIP), cholesterol acyltransferase (LCAT), acetyl CoA carboxylase (ACACA), fatty acid synthase (FASN), ATP binding cassette subfamily A (ABCAa), leucine-responsive regulatory protein (LRP), adipose triglyceride lipase (ATGL), peroxisome proliferator-activated receptor-alpha (PPAR-α), stearoyl CoA desaturase (SCD) and AMP-activated protein kinase gamma 1 noncatalytic subunit (PRKAG-1) (Lusis and Pajukanta, 2008).

2.1.3 Quality Characteristics of Dietary Lipids

The term lipid quality is used to describe the chemical and physical properties of lipids that are required for any given purpose. Interestingly, most animal nutrition research is focused on unraveling the factors that influence the energy value of lipids. However, lipid quality and its influence on diet acceptability and feed intake by animals is often a more pressing concern (Delles, 2013). Common measures of lipid quality, which are used to classify lipids into feed grade or human edible, only provide information on the properties of the lipids but do not provide any information regarding the feeding value of such dietary lipids. Such measures include moisture, insolubles, and unsaponifiables (MIU), color, taste, odor, free fatty acid (FFA), total fatty acids (TFA), peroxide value (PV), saponification value (SV), iodine value (IV), thiobarbituric acid reactive substances (TBARS), active oxygen method (AOM), p-anisidine value (AV), and titer (the

temperature at which the oil solidifies) according to guidelines published by National Renderers Association (2008). In some cases, aflatoxin, heavy metals (arsenic and lead), bacterial, and residuals of the organic solvents are also measured

According to a survey of lipid quality in the Midwest U.S.A reported by Shurson et al. (2015), different fats sources from local feed mills had a range in total MIU from 0.8 to 3.7%, FFA content from 5.8 to 51.6%, IV from 66.3 to 84.0 g/100 g lipid, PV from 0.4 to 7.3 mEq/kg, and AOM from 8.0 to 332 h. Although the survey was only limited to a small area, the wide range in the composition and quality of lipids being fed to livestock and poultry is very common globally.

Moisture, insolubles, and unsaponifiables (MIUs) are some of the most basic information as a reference for quality before a certain fat can be used in animal diets. Some condensation moisture and impurity materials are unavoidable during the process of fat extraction; however maximum MIU content is limited to less than 2% for most animal fats and less than 1.5% for most vegetable oils. A high content of MIU facilitates the autocatalytic hydrolysis of triglycerides and reduces the oxidative stability index of the fat sources (Shurson et al., 2015).

Total fatty acids (TFA) and FFA reflect the purity and wholesomeness of fat. Because average triglycerides contain approximately 90% fatty acids and 10% glycerol, fats with TFA levels less than 90% are normally related to a dilution of fat with other ingredients, which reduces the value of fat as an energy source (Zinn and Center, 1995). As part of the triglyceride, FFA comes from either the hydrolysis of the fat or those free fatty acids that failed to be esterified to glycerol. In the feed industry, the presence of high levels of FFA may be indicative of improper storage or handling of the fat in most cases.

Free fatty acid (FFA) has lower digestibility than triglycerides, and may also show a negative effect on the digestibility of the fat (Pesti et al., 2002; Shurson et al., 2015).

Lipid oxidation is one of the most important quality factors related to animal growth performance and health (Shurson et al., 2015). It is a complex process, which can be affected by many factors, such as the degree of saturation, temperature of storage, the presence of oxygen, transition metals (especially Cu and Fe), undissociated salts, moisture, and other non-lipidic compounds. Because of the complexity of lipid oxidation, a combination of PV and TBARS may be used to better provide a direct assessment of the extent of oxidation in a lipid at an acceptable cost. Lipid oxidation generates many secondary and tertiary oxidation products including aldehydes, ketones, alcohols, hydrocarbons, volatile organic acids, and epoxy compounds, which have detrimental effects on animal health. In a meta-analysis of 29 publications including 42 poultry and 23 swine trials, an overall reduction in ADG (5%), ADFI (3%), and feed efficiency (2%) was reported when animals were fed with isocaloric diets containing oxidized fats compared to those fed un-oxidized fats (Hung et al., 2017).

2.2. Oxidative Processes

2.2.1 Mechanism of Lipid Oxidation

Delles, (2013) provided an excellent summary of the mechanisms of lipid oxidation. Currently, lipid oxidation is considered the primary molecular mechanism involved in the oxidative damage to cell structures and in the toxicity process that leads to cell death (Repetto et al., 2012). Lipid oxidation is a free radical chain reaction that occurs in three main steps: initiation, propagation, and termination. Initiation ensues when a radical or non-radical species abstracts a labile hydrogen atom from a methylene group of

lipid (LH) to form a lipid radical (L·) (Domínguez et al., 2019). The abstraction of hydrogen atoms from fatty acid chains results in an unstable carbon radical, commonly known as an alkyl radical, which is stabilized through delocalization over the double bonds resulting in double bond shifting (Min et al., 2012). Depending on the level of molecular oxygen present within a system the formed fatty acid radical can undergo various rearrangements. In the presence of oxygen, peroxyl radicals (LOO·) are primarily generated, while under very low oxygen conditions, L· can react with other molecules such as proteins or other lipids (Min et al., 2012).

During propagation, LOO· will abstract a hydrogen atom from neighboring lipids or fatty acids to form a lipid hydroperoxide (LOOH) and a new lipid radical (L·). In food, hydroperoxides (LOOH) may be responsible for the development of off-flavors or further reactions with other constituents such as proteins. Furthermore, formed hydroperoxides may undergo scission to form additional products including ketones, aldehydes, organic acids, and hydrocarbons. Some of these products retain a double bond, which, because of the preceding bond rearrangement, makes them highly reactive α, β unsaturated aldehydes (McIntyre and Hazan, 2010). These electrophilic species readily covalently modify nucleophilic groups on target proteins and they extensively modify reduced glutathione, thereby decreasing cellular antioxidant protection (McIntyre and Hazan, 2010).

Lipid oxidation is terminated through the binding of two radical species to form a non-radical product. In the presence of oxygen, the predominant free radical is the peroxyl radical since oxygen will be added onto alkyl radicals at diffusion-limited rates (Reid and Fennema, 2008). Under atmospheric conditions, termination of lipid oxidation may occur between peroxyl and alkoxyl radicals. In low oxygen environments, such as frying oils,

termination reactions can occur between alkyl radicals to form fatty acid dimers. Furthermore, lipid oxidation products can yield polymers, which usually occur during high heating. Equation 2.1 summarizes the reactions of lipid oxidation.

Initiation: $LH + O_2 \rightarrow L^-$ ……Equation 2.1 (a)

Propagation: $L^- + O_2 \rightarrow LOO^- + LH \rightarrow LOOH + L^-$……Equation 2.1 (b)

Termination: $\rightarrow LOO^- + L^- \rightarrow$ Non-radical product. ……Equation 2.1 (c)

Taken from: Delles (2013).

2.2.1.1 Methods of Measuring Lipid Oxidation

The acceptability of a food product depends on the extent to which deterioration has occurred. Researchers are also interested in determining the effects of certain processes or antioxidants on the stability of a product. Thus, some criterion for assessing the extent of oxidation is required. Sensory analysis is one of the most sensitive techniques that provide data pertaining to practical applications, but is not useful for routine analyses and generally lacks reproducibility (Delles, 2013). Consequently, many chemical and physical methods have been developed to quantify oxidative deterioration with food products that correlate with off-flavor development. In food products abstraction reactions and rearrangements of alkoxyl and peroxyl result in the production of endoperoxides and epoxides as secondary products (Pike, 2003).

Various methods have been developed and established over the years to measure various products that are either degraded or formed during the process of lipid oxidation.

Such methods include Peroxide value, *p*-anisidine value, iodine value, volatile organic compounds, Thiobarbituric acid reactive substances (TBARS).

Peroxide value is one of the oldest and most commonly used methods to test for oxidative rancidity. It measures the amount of peroxides that are formed during the early phases of lipid oxidation. It is measured in milliequivalents (mEq) of active oxygen per kilogram of the sample. The chemical mechanism behind the peroxide value assay involves the reaction between excess potassium iodide and peroxides, which liberates iodine. The liberated iodine is identified via an indicator like starch before being titrated against standardized sodium thiosulfate until it yields a colorless solution.

Double bonds in lipids are changed from non-conjugated to conjugated bonds upon oxidation. Conjugated dienes give rise to an absorption peak at 230–235 nm, while conjugate trienes are measured at 270 nm. Ultraviolet detection of conjugated dienes is simple, fast, and useful for monitoring the early stages of oxidation. However, this method is less specific and sensitive compared to other methods, and the results may be affected by the presence of compounds absorbing in the same region, such as carotenoids (Shahidi and Zhong, 2005).

Primary oxidation products, such as hydroperoxides, are unstable and susceptible to decomposition. The decomposition of primary lipid oxidation products can give rise to secondary products including aldehydes, ketones, alcohols, hydrocarbons, volatile organic acids, and epoxy compounds (Shahidi and Zhong, 2005). Thiobarbituric acid reactive substances measures malondialdehyde, a compound formed during the degradation of polyunsaturated fatty acids. Malondialdehyde reacts with thiobarbituric acid to form a colored complex that can be measured spectrophotometrically.

The *p*-anisidine value (P-Anv) method estimates the amount of α- and β-unsaturated aldehydes (mainly 2-alkenes and 2,4-dienals), generated during the decomposition of hydroperoxides (Pike, 2003). The reaction with *p*-anisidine reagent with aldehydes, under acidic conditions, forms yellowish products that absorb at 350 nm.

Finally, carbonyl compounds, such as ketones and aldehydes play a major role in inducing off-flavors that stem from rancidity. Volatile products of lipid oxidation can be evaluated using gas chromatography, while colorimetric assays such as 4-dinitrophenylhydrazine (DNPH) can be used to measure total carbonyls, as it reacts with carbonyl compounds to produce yellow hydrazine.

2.2.1.2 Impact of Lipid Oxidation on Broiler Performance

The dietary supply of oxidized oils has been reported to affect feed intake and growth performance in broilers. When Tavárez et al. (2011) fed oxidized soybean oil to broilers, a decrease in weight gain of 6.06% was reported in birds fed oxidized soybean oil compared to those that received un-oxidized oil. Similarly, when Dibner et al. (1996) fed oxidized lipids to broilers, a decrease in the feed efficiency was also reported. Furthermore, a decrease in the average weight gain (4.17%) was reported by Anjum et al. (2004) when oxidized soybean oil (PV of 50 meqO_2/kg) was fed to broilers for 6 weeks, although no differences in feed intake were noted. In general, experiments in poultry and swine agree that the dietary supply of oxidized lipids has a deleterious impact on the growth performance and feed intake of broilers.

Just as increasing levels of lipid oxidation have been shown to linearly depress the growth performance, the digestibility of energy and fat has also been shown to decrease in

pigs when oxidized soybean oil is fed (Rosero et al., 2015). In contrast, Liu et al. (2014a) concluded that lipid oxidation had no significant effect on the gross energy (GE), dry matter (DM), N, and crude fat (CF) digestibility. This is supported by DeRouchey et al. (1997) who reported no significant differences in GE, DM, CF, and N digestibilities when oxidized choice white grease was fed to pigs. These conflicting reports necessitate the need for additional experiments to better understand the effects of feeding oxidized lipids on energy and nutrient utilization.

Beyond growth performance and nutrient utilization, the dietary inclusion of oxidized lipids may also affect the intestinal barrier function and morphology of pigs and broilers (Dibner et al., 1996; Liu et al., 2014a). For instance, Dibner et al. (1996) reported a reduction in the enterocyte half-life by about 24 hours when oxidized poultry fat (PV of 212.5 mEq kg oil) was fed to swine and poultry. Furthermore, Rosero et al. (2015) reported that when oxidized soybean oil was added to the diets of nursery pigs, it resulted in thinner and longer villi, and deeper crypts. Conversely, when Liu et al. (2014a) measured paracellular intestinal permeability in pigs, no differences were observed among pigs fed diets containing 10% fresh oil compared to those fed diets containing 10% oxidized oil.

Feeding oxidized lipids may also affect immune competence and disease resistance (Dibner et al., 1996; Takahashi and Akiba, 1999; Liu et al., 2014b). Furthermore, because lipid oxidation is a free radical-producing reaction, the addition of oxidized lipids serves as a good model to induce oxidative stress in broilers.

2.2.1.3. Impact of Lipid Oxidation on Meat Quality

Muscle foods are prone to lipid oxidation since they contain both unsaturated lipids and pro-oxidant components. In meat, lipids are present as either intermuscular or intramuscular fat. Intermuscular fat is generally stored in specialized connective tissues as a large deposit, while intramuscular fat is integrated into the tissue and widely dispersed. Of the muscle lipid fractions, the polar phospholipids contain the highest proportion of unsaturated fatty acids, which are primarily responsible for lipid oxidation in muscle foods.

Lipid oxidation is the main non-microbial cause of quality deterioration in meat and meat products (Min et al., 2005). Undesirable changes in color, flavor, and nutritional value occurs as lipids, present in meat, oxidize and interact with other constituents, such as pigments, proteins, carbohydrates, and vitamins. Pigment and lipid oxidation are interrelated, and ferric hemes are believed to promote lipid oxidation (Faustman et al., 2010). Iron and ascorbic acid may also function as prooxidants in meat. Sodium chloride accelerates the oxidation of triglycerides, although the mechanism of salt catalysis is not completely known.

Cooked meat undergoes rapid deterioration due to tissue lipid oxidation. Refrigerated and frozen fresh meats are also susceptible to lipid and protein oxidation, which causes quality losses due to ‘freezer-burn.’ Protein denaturation and cross-linking may result from lipid oxidation in stored freeze-dried meat. With increased consumption of prepackaged raw meat and precooked convenience meat items, control of oxidation has become increasingly important. Antioxidants, such as VE, and chelating agents, such as phosphates, are the most effective inhibitors of lipid oxidation (Mitsumoto, 2000).

2.2.2 Protein Oxidation

Compared to lipids and DNA, proteins are arguably the most affected by reactive oxygen species (ROS), because proteins are usually catalysts of oxidative damage (Dalle-Donne et al., 2003). The oxidation of proteins alters their structure and function, and the amount of protein damage depends on the protein structure, the location of the ROS relative to the protein, and its amino acid side chains (Dröge, 2002).

All amino acids are susceptible to oxidation by oxygen radicals (Berlett and Stadtman, 1997). However, different amino acids have varying degrees of susceptibility. The sulfur-containing amino acids, methionine and cysteine, are the most sensitive to ROS. Methionine is readily converted to methionine sulfoxide, while cysteine is converted to disulfides (Berlett and Stadtman, 1997). Fortunately, most biological systems contain reductases, which are capable of converting methionine sulfoxide and disulfides back to their original state (Berlett and Stadtman, 1997). Aromatic amino acids are also attacked by ROS. One notable reaction involves the oxidation of tryptophan to kynurenine and formylkynurenine. Tyrosine and phenylalanine also form several hydroxyl derivatives (Berlett and Stadtman, 1997). In general, protein oxidation leads to the formation of carbonyl groups such as ketones and aldehydes on the side chains of proteins, which modify protein function (Dalle-Donne et al., 2003).

2.3 Mechanism of Oxidative Stress

The consumption of lipid oxidation products can induce oxidative stress in animals by straining the antioxidant defense system due to the increased generation of free radicals *in vivo*. Uncontrolled leakage of electrons from the electron transport chain (ETC) in the mitochondria constitutes a major source of free radicals in the cytosol of cells. Thus, in

addition to free radicals produced by electron leakage in the mitochondria, the consumption of oxidized lipids can overwhelm the antioxidant capacity of the animal with free radicals resulting in a depletion of antioxidant storage (Lindblom, 2017).

Interestingly, free radicals are somewhat beneficial to the immune system as they influence cell signaling (Dröge, 2002; Forman and Torres, 2002). However, free radicals can also produce harmful effects that distress body functions by compromising compounds they come in contact with (Finaud et al., 2006). This is known as oxidative stress, which is a disruption in the balance between free radical production and antioxidant defenses in the body. Oxidative stress in livestock can be caused by a variety of conditions including disease, heat stress, injury, starvation, pregnancy, and consumption of oxidized lipids. Once the enzymatic antioxidant activity is overwhelmed and antioxidant stores are depleted, free radicals bind to lipids, proteins, and DNA, thereby altering their structure and function (Montuschi et al., 2004), and ultimately resulting in tissue damage if severe enough (Czerska et al., 2015).

2.3.1 Antioxidant Defense Mechanisms

The severity of oxidative stress directly depends on the balance between the antioxidant defense system and ROS. Enzymatic and non-enzymatic antioxidants are compounds that reduce the harshness or can prevent the oxidation of other molecules (Cooke et al., 2003; Poljsak et al., 2013). Antioxidants attract the free radicals that cause oxidative stress to prevent further oxidation. Antioxidants can be supplemented exogenously in the diet, but are also produced endogenously in the body.

The antioxidant system of living cells consists of three major levels of defense (Surai, 1999) and the first step prevents radical formation by removing precursors of free

radicals or by inactivating catalysts (Fig. 2.3) and this is typically accomplished by antioxidant enzymes. The second level of defense is responsible for restricting the propagation stage of the oxidation process, and this is accomplished by chain-breaking antioxidants like VE. The chain-breaking antioxidant inhibits oxidation by keeping the chain length of the propagation reaction as small as possible (Panda and Cherian, 2014). The third level of defense involves the excision and repair of damaged parts of molecules including lipolytic (lipases), proteolytic (peptidases or proteases), and other enzymes (DNA repair enzymes, polymerase, ligases, phospholipases, and nucleases). The antioxidant compounds are located in organelles, subcellular compartments, or the extracellular space to provide maximum cellular protection.

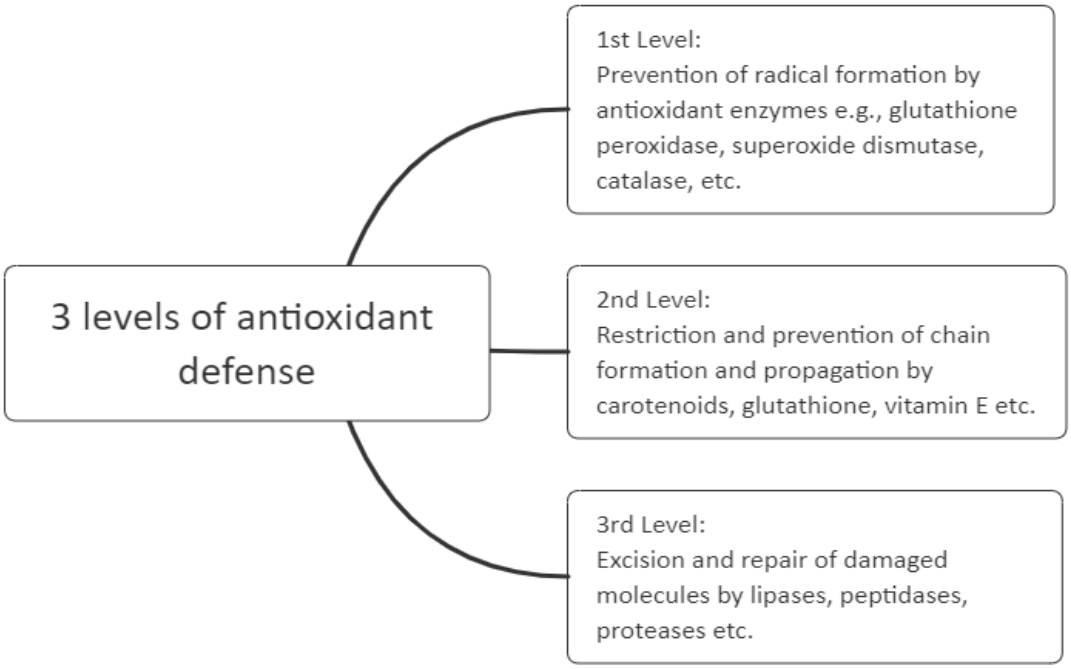


Figure 2. 3 The three levels in the antioxidant defense system (Adapted from Panda and Cherian, 2014)

Superoxide dismutase (SOD) is an important enzymatic antioxidant, which facilitates the reaction between oxygen and hydrogen-free radicals to produce hydrogen peroxide, which is a less-toxic peroxidant than free radicals. This reaction is shown in Equation 2.2. There are many known forms of SOD including manganese, zinc, and copper SOD (Kalyanaraman, 2013).

$2O_2O_2^{-\bullet} + 2H+ \text{SOD} \rightarrow H_2O_2 + O_2$ ..... Equation 2.2

Catalase (CAT) is another vital enzymatic antioxidant. It acts by converting the toxic H_2O_2 to harmless water and oxygen, as shown in Equation 2.3 (Finaud et al., 2006).

$2H_2O_2 \xrightarrow{\text{CAT}} 2H_2O + O_2$ ..... Equation 2.3

Glutathione peroxidase (GPx) is another important enzymatic antioxidant. Glutathione peroxidase is similar to CAT because it plays a role in detoxifying H_2O_2 to produce water; however, GPx is more efficient than CAT. Glutathione peroxidase acts on H_2O_2 and glutathione (GSH) to produce oxidized glutathione (GSSG) and water (see Equation 2.4). GPx is mainly present in the cytosol and mitochondria.

$H_2O_2 + 2\ \text{GSH} \xrightarrow{\text{GPx}} \text{GSSG} + 2\ H_2O$…….. Equation 2.4

These enzymatic antioxidants are crucial for the prevention of disease and oxidative stress. Other cellular defenses against oxidative damage include non-enzymatic antioxidants such as tocopherols and ascorbic acid. Vitamin E is present in the cell membrane and is arguably the most important antioxidant in the body because of its ability to readily bind to ROS, thereby breaking the chain reaction of oxidation (Finaud et al., 2006; Kalyanaraman, 2013). Vitamin C or ascorbic acid is a water-soluble vitamin that possesses antioxidant properties and acts primarily in extracellular fluids (Finaud et al., 2006; Kalyanaraman, 2013). Vitamin C can neutralize ROS by supporting the action of VE

in cells to increase antioxidant potential. Vitamin C functions primarily as a reducing agent by donating hydrogen atoms to regenerate oxidized VE (Carr and Frei, 1999). However, vitamin C can also act as a pro-oxidant when reacting with metals such as copper and iron which then form hydroxyl radicals (Gutteridge, 1995).

2.3.2 Measures of Oxidative Stress

Reactive oxygen species are known to cause lipid, protein, and DNA damage *in vivo*. Because PUFAs are more readily oxidized than saturated fatty acids, they are; therefore, more susceptible to oxidative damage (Ayala et al., 2014). Oxidative stress can be measured directly or indirectly. Measuring ROS is the only way to directly measure oxidative stress; however, ROS are very unstable and difficult to measure accurately (Poljsak et al., 2013). More commonly, indirect measurements of damage due to ROS on lipids, proteins, and DNA are adopted as accurate measures of oxidative stress because these compounds are more stable than ROS. There are many methods to measure oxidative damage as summarized in Figure 2.4.

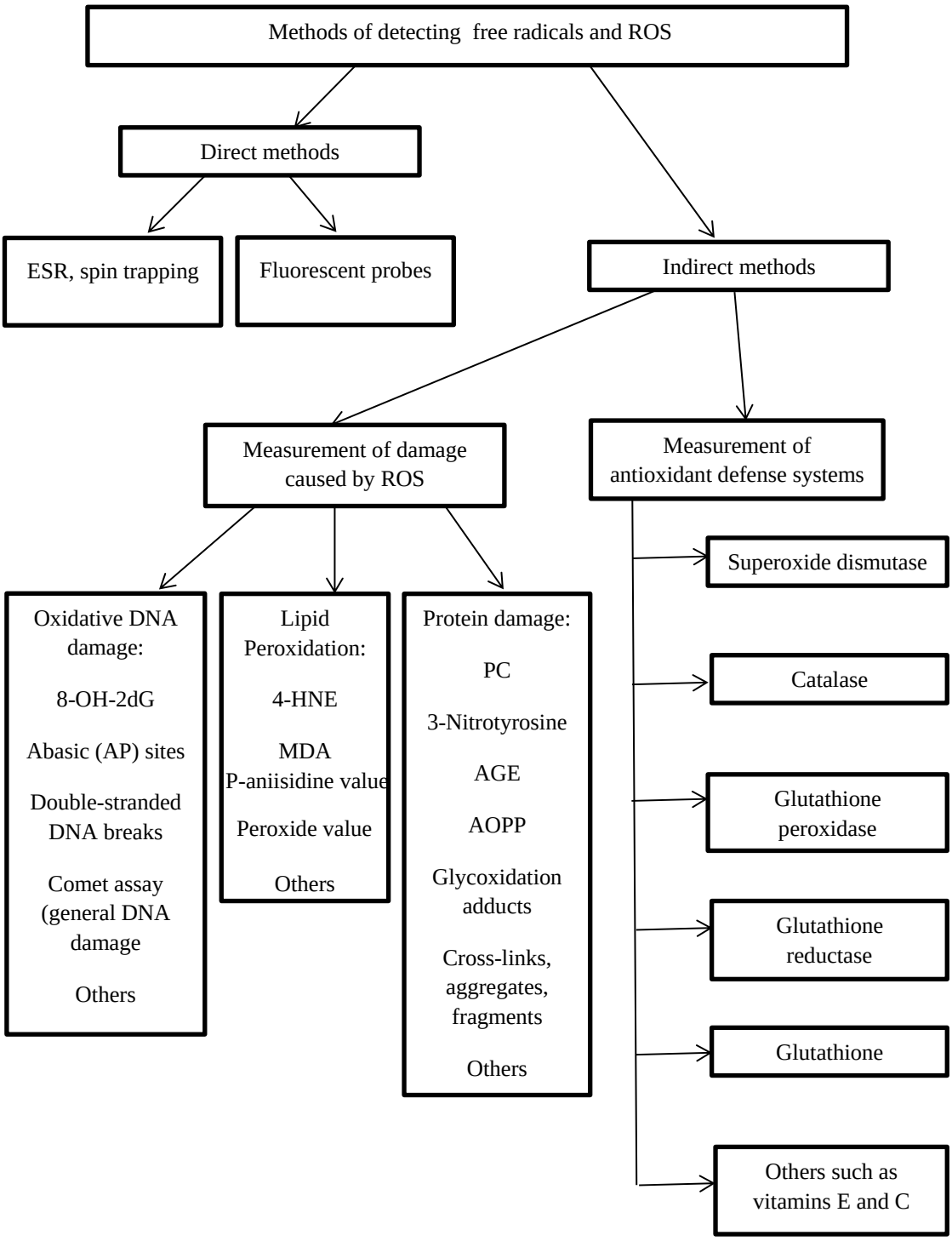


Figure 2. 4 Methods of measuring oxidative stress (Adapted from Lindblom, 2017)

ESR=electron spin resistance; 8-OH-2dG= 8-hydroxy-2deoxy-guanosine; 4-HNE= 4-Hydroxynonenal; MDA= malondialdehyde; PC= protein carbonyl concentration; AGE= advanced glycation end products; AOPP= advanced oxidation protein products.

2.4. Vitamin E

Vitamin E refers to a family of 8 structurally related fat-soluble compound isoforms, including four tocopherols (α, β, γ, and δ) and four tocotrienols (α, β, γ, and δ) (Jiang et al., 2001) as shown in Figure 2.5. Vitamin E is a vital component of biological membranes with membrane-stabilizing properties and potent antioxidant activity. Furthermore, muscle health is dependent upon an adequate supply of dietary VE, and although rare, VE deficiency in humans is associated with muscle weakness, elevated creatine kinase (CK), and myopathy (Howard et al., 2011). In animals, early studies have reported profuse myocyte necrosis and lethal muscular dystrophy due to VE deficiency.

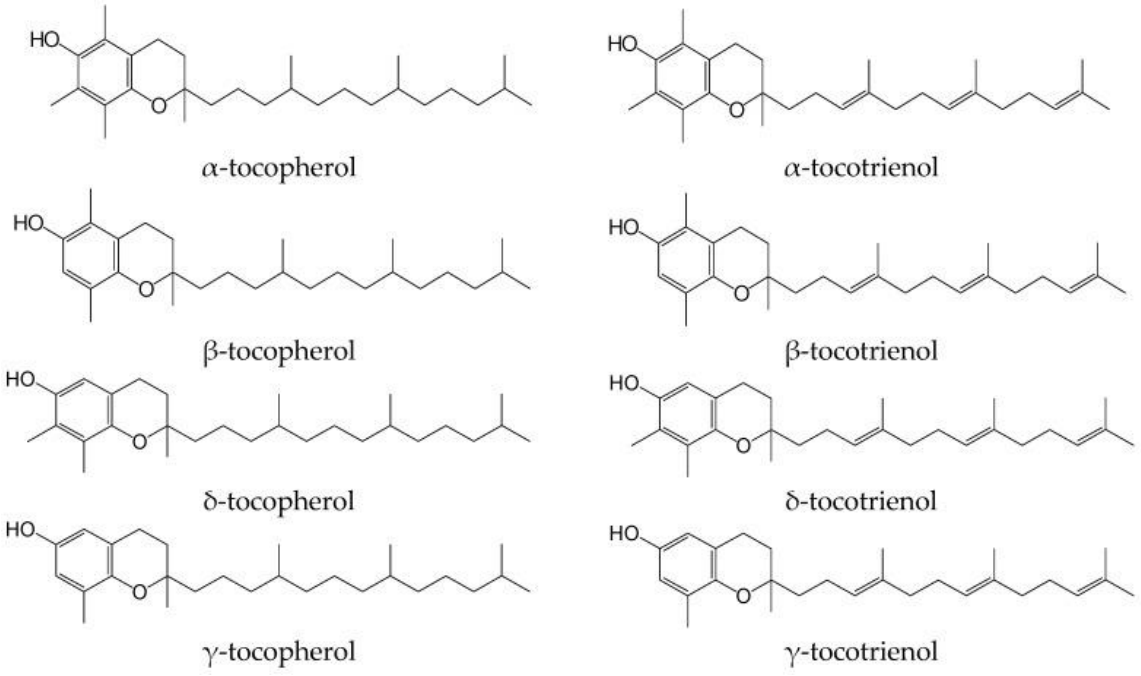


Figure 2. 5 Vitamin E isomers (Taken from Reboul, 2017)

Vitamin E cannot be synthesized by animals and has to be supplied by the diet, thus its presence in body tissues is a reflection of dietary availability. Dietary VE is commonly supplemented in the diet as α-tocopherol acetate, which is characterized by

great stability during feed processing, storage, and passage through the foregut and midgut of the animal. With dietary supplementation, the vitamin is incorporated within tissue biomembranes, where its effect is maximal, making it a more effective source than when adding VE as a postmortem supplement (Sales and Koukolová, 2011). All isoforms of VE are potent membrane-soluble antioxidants (Brigelius-Flohe and Traber, 1999). Tocopherols have a saturated phytyl side chain with three chiral centers that are R configuration at positions 2-, 4- and 8- in the naturally occurring forms. Tocopherols differ in the number of methyl groups they have at the 5- and 7- positions of the chromanol ring.

Several VE sources are available for poultry diets including both natural (D-α-tocopheryl acetate) and synthetic forms (DL-α-tocopheryl acetate). In addition to this, alcohol forms of VE such as α- and γ-tocopherols are can also be used in poultry diets, although their bioavailabilities may vary slightly (Herrera and Barbas, 2001). Despite some debates over its in vivo antioxidant activity, the beneficial effects of VE, especially α- and γ-tocopherols have been well documented in both animals and humans (Azzi et al., 2016; Galli et al., 2017). Being a fat-soluble vitamin, VE follows the same process of intestinal absorption, hepatic metabolism, and cellular uptake of other lipophilic molecules and lipids (Schmölz et al., 2016).

2.4.1 Absorption, Transport, and Metabolism of Vitamin E

Wang (2019) provided a succinct overview of the overall mechanism of absorption, transport, and metabolism of VE. The digestion process of VE starts with its dissolution in the lipid phase of the feed. This phase is then emulsified into lipid droplets at both gastric and duodenal levels. No metabolism of VE (i.e., degradation or absorption) appears to exist in the stomach (Borel et al., 2001). In the duodenum, VE is incorporated, along with lipid

digestion products, in mixed micelles, structures that are theoretically essential for its absorption by the enterocyte. Indeed, mixed micelles can solubilize hydrophobic components and diffuse into the unstirred water layer (glycocalyx) to approach the brush border membrane of the enterocytes (Reboul, 2017).

When approaching the brush border membrane, mixed micelles are supposed to dissociate due to the existing pH gradient. The released constituents can then be captured by different systems to be absorbed by the enterocyte. For more than 30 years, due to the first results obtained in rat intestinal everted sacs, VE absorption has been considered to occur by passive diffusion through the enterocyte apical membrane. However using a mice model, Reboul et al. (2006) reported that α- and γ-tocopherol absorption was mediated, at least partly, by scavenger receptor class B type I (SR-BI). They also showed that NPC1-like intracellular cholesterol transporter 1 (NPC1L1) was involved in α-tocopherol and γ-tocotrienol absorption.

The absorption rate of VE in the GIT varies between 20-80%, following intake. After being absorbed by the intestine along with dietary fat, VE is secreted into chylomicron particles, which are then shuttled to the liver. The α-tocopherol is released into the circulation from the liver in combination with a carrier protein called α-tocopherol transfer protein (α-TTP), which is then incorporated into very-low-density lipoprotein (VLDL) for delivery to tissues (Wolf, 2006). The α-TTP may also deliver a small portion of γ-tocopherol up to 9 percent (Hosomi et al., 1997).

Chylomicrons containing VE isoforms are also secreted into the intestinal lymphatic system and are infused into the systemic circulation via the thoracic duct (Hacquebard and Carpentier, 2005). The chylomicron-bound VE is transported to either

high-density lipoprotein (HDL) or extrahepatic peripheral tissues with the aid of lipoprotein lipase. The resulting chylomicron remnants and HDL are subsequently taken up by the liver (Jiang et al., 2001). Pancreatic and intestinal enzymatic digestion followed by the circulation and distribution to the liver and non-hepatic tissues is the same for all VE forms, while discrimination between different forms of VE in favor of α-tocopherol occurs mainly in the liver by α-TTP (Schmölz et al., 2016). The liver does not accumulate toxic levels of VE. Excess VE may be excreted into the bile or metabolized by side-chain degradation (ω and β oxidation) involving cytochrome P450 dependent hydroxylases (Hacquebard and Carpentier, 2005). Most α-tocopherol but a small fraction of γ-tocopherol are reincorporated into nascent VLDLs by α-TTP.

Similar to chylomicron metabolism, VLDL and LDL are very important in VE transport into peripheral tissues through LDL receptor (LDLR) and Scavenger receptor class B type 1 (SR-B1) (Hacquebard and Carpentier, 2005; Rigotti, 2007; Schmölz et al., 2016). Very-low-density-lipoprotein triacylglycerols are catabolized by LPL at the endothelium site of peripheral tissues, and the released surface remnants, which contain α-tocopherol, are transferred to HDL particles, and some α-tocopherol is delivered to adjacent tissues. High-density lipoprotein (HDL) particles uptake or remove cellular α-tocopherol via the action of transporters including SR-B1 and ATP-binding cassette transporters A1 (ABCA1), respectively (Hacquebard and Carpentier, 2005; Rigotti, 2007).

Once taken up by cells, intracellular VE content and distribution are regulated by different proteins binding specifically to α-tocopherol, such as α-TTP, tocopherol associated protein (TAP), and tocopherol binding protein (TBP). The specific roles of these

proteins and the metabolism of tocopherol at this level are still not completely clear (Hacquebard and Carpentier, 2005; Schmölz et al., 2016).

2.4.2 Bioavailability of Different Isoforms of Vitamin E

As stated earlier, humans and animals do not synthesize their VE, they primarily acquire tocopherols from plants or chemically synthesized sources. The bioavailability of VE is influenced by numerous factors including the amount of VE and intake of interfering nutrients; proteins involved in VE absorption and individual differences in the efficiency of VE absorption influenced, for example, by diseases; VE metabolism; sex, and genetic polymorphisms (Schmölz et al., 2016).

Although ATA is the most commonly used isoform in poultry and swine diets, γ-tocopherol is often the most prevalent form of VE in plant seeds oil and products derived from them (Speek et al., 1985; Grilo et al., 2014). In contrast, α-tocopherol is the predominant form of VE in most human and animal tissues, including blood plasma. In humans, plasma α-tocopherol concentrations are generally 4 – 10 times higher than those of γ-tocopherol, and γ-tocopherol concentrations in human tissues other than plasma are rare and mostly limited to adipose tissue (Jiang et al., 2001).

The biological activity of VE has traditionally been determined with a fetal resorption assay in rats, where the activity is defined as the ability to prevent embryo death in mothers depleted of VE with the supplementation of different isoforms of tocopherol or tocotrienol (Bieri and Evarts, 1974). The following bioavailability values were firstly obtained via this method using DL-α-tocopheryl acetate as 100%: D-α-tocopherol 80%; DL-α-tocopherol 59%; D-α-tocopheryl acetate 136%; D-α-tocotrienol 13%; D-β-

tocopherol 45%; D-β-tocotrienol 4%; D-γ-tocopherol 13%; D-δ-tocopherol less than 0.4% (Leth and Søndergaard, 1977). However, a later study reported different results, based on the rat bioassay work using DL- α-tocopheryl acetate as a standard (1 mg = 1 IU), the activity of 1 mg DL- α-tocopherol equal to 1.1 IU, 1 mg D- α-tocopheryl acetate equal to 1.36 IU, and 1 mg D- α-tocopherol equal to 1.49 IU VE (Ames, 1979). The values from Ames (1979) were widely accepted and extended to various animals without verification.

2.4.3 Effect of Dietary Vitamin E on Broiler Chickens

2.4.3.1 Growth Performance and Requirement of Vitamin E in Broiler Chickens

The current recommendation for VE concentrations in broiler diets ranges from 10.0 IU/kg (NRC, 1994) to 80.0 IU/kg (Aviagen, 2014) depending on the stage of growth. However, beneficial effects for performance have also been reported for up to 200 IU/kg of VE supplementation (Morrissey et al., 1997). Other factors including other antioxidants such as vitamin C and selenium, type and amount of lipids in diets, and environmental conditions also affect the recommended levels of additional supplemental VE in broiler diets (NRC, 1994). Therefore, the ideal inclusion levels of VE in broiler diets are still controversial (Kuttappan et al., 2012).

However, regardless of the specific inclusion level, many studies have reported that the inclusion of VE in broiler diets not only reduces oxidative stress but also improves the overall performance, as demonstrated by the higher body weight gain and reduced FCR of broilers fed supplemental VE (Adebiyi et al., 2011). The performance of pigs was also enhanced when the diet was supplemented with VE at the level of 100 mg/ kg of feed (Asghar et al., 1991). Furthermore, when Japanese quail were subjected to heat stress, the supplementation of VE to diets significantly alleviated heat-stress-related performance

impairment, suggesting that a high level of dietary VE supplementation is necessary under heat stress conditions (Sahin and Kucuk, 2001).

Coming specifically to broilers, Selvam et al. (2017) reported that the broilers reared at a high stocking density and fed VE presented higher BWG on day 42 compared to those that did not receive VE supplementation supplemented with VE. Hosseini-Mansoub et al. (2010) and Ismail et al. (2014) also found that dietary enrichment with VE resulted in better performance when compared to the birds fed with a standard diet without VE. Similarly, Sahin and Kucuk (2001) reported that the inclusion of VE in the diet had increased the performance in Japanese quails reared under heat stress (34 ºC). Similarly, Khattak et al. (2012) reported that VE supplementation at 300mg/kg produced a better performance in broilers under heat stress. However, other studies have reported conflicting results. For instance, Coetzee and Hoffman (2001) and Nobakht (2012), reported that there was no difference in body weight gain and FCR between the different dietary levels of VE supplementation. Moving away from animals, the supplementation of VE to broilers is also beneficial for the health of humans consuming chicken meat (Adebiyi et al., 2011) as it is known to improve meat quality by upregulating the expression of antioxidant enzyme genes in broilers (Niu et al., 2017).

2.4.3.2 Antioxidant System

Vitamin E is a potent antioxidant. The chromanol head of VE, located within the hydrophilic portion of the bilayer quenches free radicals and prevents potentially harmful phospholipid oxidation events. During strenuous exercise, skeletal muscle accumulates ROS and consequently increases lipid oxidation, which can be alleviated through VE supplementation (Sacheck et al., 2003). Similarly, the dietary supply of oxidized lipids has

been shown to subject birds to significant oxidative stress, and VE supplementation has also been shown to improve antioxidant status in animals. For instance, the addition of VE to broiler chicken diets at 200 mg/kg and was effective in improving the total antioxidant status of birds, enhancing blood antioxidant enzyme activities, and increasing VE concentrations in the liver and breast muscles (Mazur-Kusnirek et al., 2019). In a study using a tertbutyl hydroperoxide-induced lipid oxidation model in rats, lipid oxidation was reduced through the supplementation of VE of 7 to 10 ppm higher than in the control (Ham and Liebler, 1997). At the same time, the enzymatic antioxidant system including SOD, CAT, and GPx was also reported to be improved by VE supplementation over the nutritional need of 11 ppm (Lauridsen et al., 1999; Gultekin et al., 2001; Lauridsen, 2010; Cheng et al., 2017). The increased dietary VE in broilers also showed a protective effect in the lipid oxidation of broiler meat products.

Different VE isoforms function similarly on oxygen radicals. γ-tocopherol, in particular, is a powerful nucleophile that traps electrophilic mutagens in lipophilic compartments as a complementary effect of glutathione (GSH). As a result, γ-tocopherol could protect lipids, DNA, and proteins from peroxynitrite-dependent damage (Brigelius-Flohe and Traber, 1999). In a study that investigated the effect of dietary lipid sources on the oxidative stability of fresh and cooked chicken meat, dietary supplementation of VE (200 to 400 mg/kg) resulted in lower ($P < 0.05$) malonaldehyde values in both fresh and cooked chicken meat (Narciso-Gaytán et al., 2010). These findings indicate that dietary inclusion of VE can be an effective way to increase the stability of n-3 PUFA enriched broilers meat against oxidative damage.

2.5 Phytases

Phytate or Phytic acid (Myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate; PA) is the major storage form of P in plants. Phytate is an antinutritional factor capable of reducing the bioavailabilities of various mineral elements and amino acids by binding to them (Butani and Parnerkar, 2015). To alleviate the negative effect of dietary phytates, exogenous phytases (myo-inositol hexaphosphate phosphohydrolases) are added to poultry diets. Phytases are enzymes that catalyze step-wise cleavage of phosphate groups from phytic acid. Phytases can hydrolyze the ester bonds between the phosphate groups and the inositol ring in phytates and increase the availability of dietary minerals, especially P, amino acids, and energy; and their effect on performance and nutrient availability is well documented (Scott et al., 2001; Cowieson and Adeola, 2005).

The recommended phytase supplementation level in poultry diets is usually 500–1000 FTU/kg of feed (Esteve-Garcia et al., 2005). Although many studies have reported improvements in performance parameters associated with the addition of phytase to diets, only a few studies have added exogenous phytase at a concentration exceeding 1,000 (FTU/kg) phytase units and up to 10,000 FTU/kg (Augspurger and Baker, 2004) and 24,000 FTU/kg (Cowieson et al., 2006).

2.5.1 Sources of Phytases

Woyengo and Nyachoti (2011) provided an excellent review of the sources and functions of various phytases in poultry diets. Phytases are produced predominantly by plants and microorganisms. Among microorganisms, the major phytase producers are fungi, yeast, and bacteria (Pandey et al., 2001). Most phytases produced by these micro-

organisms are 3-phytases except for a few like Basidiomycete fungi (Lassen et al., 2001) and *Escherichia coli* bacteria-derived phytases, which are 6-phytases. In plants, phytases occur in seeds, where their major role appears to be the release of P from phytic acid (PA) during germination for utilization by the developing plant (Centeno et al., 2001).

Plant phytases have been shown to significantly hydrolyze PA in poultry (Paik, 2003) and pigs (Rapp et al., 2001). They are, however, not as effective as microbial phytase. For instance, PA hydrolysis in the stomach of mini pigs fed a diet supplemented with the microbial (*Aspergillus niger*) phytase at 818 FTU/kg was found to be 17% higher than in those fed a diet with supplemented plant (wheat) phytase at 1192 FTU/kg (Rapp et al., 2001). The recovery of the wheat phytase in the duodenum of the same animals was also lower than that of *Aspergillus niger* phytase (45 vs. 70%; Rapp et al., 2001).

2.5.2 Phytase and Nutrient Digestibility

Because of their ability to liberate P from PA, phytases are commercially produced and added to poultry feeds to improve nutrient digestibility and utilization. An ideal phytase for the poultry feed industry would be the one that is resistant to the acidic pH and protease enzymes in the stomach and small intestine (where P absorption takes place); is cost-effective to produce, and is resistant to high temperatures that are encountered during feed pelleting (Lei and Stahl, 2001). Because microbial phytases compared with other phytases have a greater ability to hydrolyze PA in the gastrointestinal tract, a lot of research has been focused on the identification and testing of the efficacy of the former for use in the animal feed industry. It has, however, been difficult to obtain native microbial phytases with all the above-mentioned attributes (Lei and Stahl, 2001). Thus, several microbial phytases have been modified by processes such as genetic transformation and thermo-

protective coating to achieve these desired characteristics (Lei and Stahl, 2001; Garrett et al., 2004; Barletta, 2007). Currently, most of the phytases that are commercially available to the feed industry are derived from micro-organisms, especially fungi and bacteria (Woyengo and Nyachoti, 2011).

2.5.2.1 Site of Microbial Phytase Activity

Supplemental microbial phytases hydrolyze PA in some, but not all, gastro-intestinal tract compartments in poultry. Yu et al. (2004) observed the highest activity of a fungal (*P. lycii*) phytase in the crop and ventriculus, followed by the duodenum and jejunum, and negligible activity in the ileum. Onyango et al. (2005) feeding broilers on diets supplemented with either bacterial (*E. coli*) or *P. lycii* phytase at 1,000 FTU/kg, observed the highest activity of *P. lycii* phytase in the crop (404 FTU/kg) followed by the gizzard (63 FTU/kg), and negligible activity in the jejunum (25 FTU/kg) and ileum (6 FTU/kg). For E. coli phytase, however, although the activity was highest in the crop (649 FTU/kg), it remained relatively high in the proventriculus and ventriculus combined (406 FTU/kg) and jejunum (554 FTU/kg) and was only low in the ileum (91 FTU/kg). The major sites of activity of supplemental fungal phytases are the crop and the ventriculus in poultry because they have maximal activity at the acidic pH that is within the pH range present in these gastro-intestinal tract regions and are susceptible to proteolysis that occurs in the small intestine (Simon and Igbasan, 2002). When compared with fungal phytase, E. coli phytase remains active up to the jejunal region because it is more resistant to the proteolysis that occurs in the small intestine (Onyango et al., 2005).

2.5.2.2 Effect of Phytase on Nutrient Digestibility

Phytase supplementation has been shown to improve P digestibility in poultry. Because of this improved digestibility of P due to phytase, the non-phytate P in poultry diets has been reduced by 0.10 to 0.20 percentage points without any significant effect on performance. Phytase supplementation has also been shown to increase the digestibility of other minerals, amino acid, and AME values (Woyengo and Nyachoti, 2011). However, based on the observed improvement in ileal P digestibility of 7.2% to 20.6%, phytase hydrolyzed 20 to 65% of phytate P in diet, which is incomplete and variable hydrolysis. Some of the factors that could affect the efficacy of phytase with regards to PA-P hydrolysis include dietary level of inorganic (non-phytate) P and Ca, Ca:P ratio in the diet, dietary endogenous phytase activity, and dietary non-starch polysaccharides. But the benefit of phytase extends beyond improved P utilization. Phytase has also been reported to improve performance, digestibility of proteins, and the liberation of minerals such as Ca and Zn, (Woyengo and Nyachoti, 2011).

2.5.3 Phytic Acid, Phytase, and Oxidative Stress

Dietary phytase, especially at higher doses, has been reported to increase retinol and α-tocopherol concentration in the liver of chickens (Karadas et al., 2010). In another study. Gebert et al. (1999) reported that phytase supplementation reduced the oxidative stability of back fat ($P < 0.01$), which was improved by α-tocopherol supplementation when pigs were fed a low-phosphorus diet. This, therefore, suggests that the inclusion of phytase in diets may require additional supplementation of VE due to a higher oxidative load caused by an increased macro and micro-mineral concentration.

Interestingly, phytic acid inhibits free radical generation *in vitro* but does not affect liver oxidant and antioxidant status in growing rats (Rimbach et al., 1998). However, it has been reported that intrinsic phytate in maize and soybean was protective against lipid oxidation in the colon of pigs with a moderately high level of dietary iron intake (Porres et al., 1999). It was also reported (Karadas et al., 2005) that phytase supplementation of poultry diets increased the hepatic concentration of coenzyme Q10 (ubiquinone) suggesting an improvement in the antioxidative status of phytase fed birds. The increased utilization of nutrients may enhance the antioxidant status of the birds. Conversely, the increased availability and uptake of metal ions may also increase the oxidative stress on the birds. So, further research is still required to understand the relationship between phytase and VE supplementation and its combined effect on various parameters such as performance, nutrient utilization, and antioxidant status of broiler chickens.

In summary, oxidized lipids can constitute a major challenge, not just because of its largely deleterious impact on the growth performance of birds, but also its potentially negative impact on their welfare. Furthermore, the reports of past studies have suggested that the impact of phytase far exceeds the liberation of phytate bound P, thus there is a lot yet to be understood about its underlying mechanisms. Currently, there is very little information available regarding the interactive effects of oxidized lipids of different sources, phytase, and VE supplementation on the growth performance, nutrient utilization, antioxidant status, and the fatty acid profile of adipose and liver tissues. These studies were designed to bridge this gap.

CHAPTER 3

Effects of Oil Quality, Phytase, and Vitamin E Supplementation on the Growth Performance, Nutrient Utilization, Fatty Acid Profile, and Relative Weight of Adipose Tissue and Liver In 21-Day-Old Broiler Chickens

3.1 Abstract

The objective of this study was to determine the effect of oil quality, phytase, and vitamin E (VE) supplementation on the growth performance, fatty acid profile and relative weights of liver and adipose tissues, and nutrients and energy utilization in 21-day-old broiler chickens. The experiment used 378-day-old male by-product Cobb breeder chicks with 9 treatments consisting of 7 replicates of 6 birds per replicate cage. The first 8 treatments were based on a marginally non-phytate phosphorus (NPP) deficient diet (0.31%), while treatment 9 was a positive control (PC) diet with adequate NPP levels (0.45%). Specifically, the treatments included a PC, 2 oil quality levels (fresh soy oil; peroxide value (PV) = 3 meqO_2/kg and oxidized soy oil; PV =109 meqO_2/kg), 2 phytase levels (0 vs 1,000 FTU/kg), and 2 levels (0 and 150 ppm) of additional supplemental VE (mixed tocopherols containing 55-75% γ tocopherol), which was added to the basal diet that already met or exceeded the birds' requirements for VE. The objective of this study was addressed using a 2x2x2+1 factorial arrangement of treatments in a completely randomized design. All birds were fed a standard corn-soybean meal-based broiler starter diet that met or exceeded the requirements for nutrients and energy from days 0 to 7 after which the birds were placed on the experimental diets from days 7-21. The 8 marginal NPP treatments were analyzed using the GLM procedure of SAS appropriate for a factorial arrangement of treatments. The effect of marginal NPP was tested using pre-determined contrasts between the PC and the NPP-deficient diet containing fresh oil without phytase or VE supplementation (NC diet). Birds on the PC diet had lower ($P < 0.05$) total tract P

utilization but a higher ($P < 0.05$) bone breaking strength (BBS) and tibia ash content compared to birds fed the NC diet. Phytase supplementation improved ($P < 0.05$) feed efficiency (d 7-14 and 7-21), Ca utilization, BBS, tibia ash, the apparent metabolizable energy (AME), and AME corrected for nitrogen (AMEn) but decreased ($P < 0.05$) bone lipid content. Birds fed diets containing oxidized oil and phytase had a higher ($P < 0.05$) AME and AMEn compared to birds fed diets containing either fresh or oxidized oil without phytase supplementation whereas, oxidized oil without phytase supplementation resulted in higher ($P < 0.05$) utilization of crude fat compared to diets supplemented with phytase. VE supplementation at 150 ppm improved the live body weight (LBW), body weight gain (BWG), and feed intake (FI) across days 7-14. However, birds fed diets containing oxidized oil and additional VE had the lower ($P < 0.05$) crude fat utilization compared to birds fed a diet containing oxidized oil without additional supplemental VE. The results of this study showed that phytase supplementation is beneficial for improving feed efficiency, Ca utilization, BBS, tibia ash, AME, and AMEn regardless of oil quality or additional VE supplementation. Moreover, when feeding oxidized oils, phytase supplementation improved AME and AMEn, but reduced crude fat utilization. Furthermore, the supply of 150 ppm of additional supplemental VE to basal diets containing adequate levels of VE improved growth performance over days 7-14, but when oxidized oils were fed, it provided little-to-no beneficial effects as seen in the reduced crude fat utilization of birds that received diets containing oxidized oil and VE.

Keywords: oxidized oils, vitamin E, phytase, broilers.

3.2 Introduction

Dietary fats play a very important role in broiler nutrition. Apart from reducing feed dustiness, and improving palatability, dietary fats have also been reported to decrease feed intake, while improving feed efficiency and overall performance (Jeffri et al., 2010). Furthermore, dietary fats have been shown to help with vitamin A and Ca absorption (Leeson and Atteh, 1995). However, dietary lipids, especially those rich in polyunsaturated fatty acids (PUFAs), have a high potential for lipid oxidation, which results in diminished oil quality. As the amount of PUFA increases in the diet, so does the susceptibility of chicken meat to lipid oxidation (Cortinas et al., 2005; Bou et al., 2006) leading to spoilage and development of rancid flavors and off-odors (Wood et al., 2004).

Vitamin E is a powerful antioxidant that is well known for its protective capability against oxidative damage. Vitamin E has also been shown to restore the normal levels of lipids in the liver, lung, heart, and kidney of rats exposed to the peroxidative damage of free radicals induced by lead (Nobakht, 2012). Vitamin E is also involved in reproduction and gene expression, as, for instance, it has been reported to down-regulate almost half of the genes involved in the cholesterol synthesis pathway (Landrier et al., 2010). The nutritional recommendations for poultry vary from 5 to 10 mg/kg (NRC, 1994), but commercial diets usually range from 50 to 300 mg/kg (Adisseo, 2002) to account for the stress factors that accompany real-world farming conditions. The antioxidant activity of VE depends on the amount of α-tocopherol deposited in the cell membranes, where it acts as a scavenger for free radicals, inhibiting the propagation of the lipid oxidation chain reaction (Asghar et al., 1990). In poultry, the level of VE included in the diet and the length

of the feeding period determines the amount of α-tocopherol deposited in the cell membranes of muscles (Sheehy et al., 1991).

It has been reported that the addition of 3% lipid to the diet significantly increases dl-α- tocopheryl acetate bioavailability in adult cockerels. Supplementation of lipids rich in unsaturated fatty acids (UFAs) also leads to a higher dl-α-tocopheryl acetate bioavailability than lipids rich in saturated fatty acids (SFAs) (Preveraud et al., 2015). Interestingly, different authors have a wide range of propositions as touching how much supplemental dietary VE is necessary for the prevention of oxidative stress arising from the PUFA content in poultry diets. Leeson and Summers (2008) recommended 3 IU of VE for each gram of added PUFA in 1 kg of feed, which translates to 27 mg/kg at a 3% fat inclusion rate. On the other hand, it has been indicated that in broilers, approximately 200 mg/kg of α-tocopheryl acetate (ATA) during at least the first 24 days (Sheehy et al., 1991) or 4 weeks (Morrissey et al., 1997) of a feeding period is needed to reach muscle α-tocopherol plateau levels. This suggests that approximately 200 mg/kg of ATA in the diet may be needed to reach the highest antioxidant potential of VE.

Beyond oil quality and VE, phosphorus bioavailability is another issue of great significance to poultry production. Due to the finite global supply of inorganic phosphorus, researchers have had to explore alternative means of providing non-ruminants with bioavailable phosphorus. One such method is phytase supplementation. However, the implication of phytase supplementation on growth performance, nutrient utilization, and the antioxidant capacity of broilers are yet to be well understood, especially when other factors such as oil quality and VE supplementation are involved. For instance, Karadas et al. (2010) reported that phytase supplementation, especially at the higher doses (up to

12,500 FTU/kg), increased the level of coenzyme Q10, retinol-linoleate, and α-tocopherol in the liver when birds were fed a low-phosphorus diet. Elsewhere, phytase supplementation has also been reported to promote oxidative tendencies due to the release of prooxidants such as iron, copper, and zinc from the hydrolyzed phytic acid complex (Gebert et al., 1999). But this can affect the digestibility of nutrients that are very susceptible to oxidation such as unsaturated fatty acids.

This, therefore, suggests that the inclusion of phytase in diets may demand an additional supplementation of VE due to a higher oxidative load caused by an increased macro- and micro-mineral concentration in the digesta, especially when oxidized oils are involved. Thus, the objective of this study was to investigate the effects of feeding an oxidized, phosphorus-marginally-deficient diet, supplemented with phytase and VE supplementation on broiler performance, nutrient utilization, and the relative weight and fatty acid profile of the adipose and liver tissues of 21-day-old broiler chickens.

3.3 Materials and Methods

3.3.1. Lipid Oxidation Process

To oxidize the oils, aluminum pans and glass pyrexes were obtained and the vessels containing about 4 lbs oil, were heated in a convection oven at 95 °C ± 5 °C for about 12 days at an average heating duration of 7 hours per day. The oil was stirred every 2-3 hours to ensure proper oxygenation and the Peroxide value (PV), which was the primary measure of oil quality, was checked intermittently. When the PV exceeded 100 meqO_2/kg, the heating was stopped and the oxidized oil was cooled to room temperature. The oils were mixed and a sample was taken and analyzed to determine the average peroxide value of the

oils, which were then stored in a refrigerator (4 °C) before use for diet preparation. All chemicals (reagent grade) were purchased from VWR (VWR International, Radnor, PA, USA) unless specified otherwise.

3.3.2 Animals, Housing, Management, and Experimental Design

All management of birds and experimental procedures used in this study were approved by the Institutional Animal Care and Use Committee at the University of Kentucky. A total of 378 day-old male by-product Cobb breeder chicks were obtained from a commercial hatchery and fed a corn-SBM-based broiler starter diet (Table 3.1) that met or exceeded the energy and nutrient requirements of birds of this age (NRC, 1994). The birds were housed in battery cages (0.61 x 0.51 x 0.36 m) in an environmentally controlled room with 22 h of light and 2 h of darkness. On day 7, birds were weighed individually and randomly assigned to treatments. All birds had unrestricted access to feed and water throughout the experiment.

This experiment examined three main factors, which were oil quality, phytase, and additional supplemental VE. The treatments include 2 levels of oil quality (fresh soy oil; PV = 3 meqO_2/kg and oxidized soy oil; PV =109 meqO_2/kg), 2 phytase levels (0 vs 1,000 FTU/kg), and 2 levels (0 and 150 ppm) of additional supplemental VE (mixed tocopherols containing 55-75% γ-tocopherol). It is noteworthy that this "additional supplemental VE" of 150 ppm, was in addition to the already adequate levels of VE provided in the basal diet through the corn-soybean meal, and the vitamin-mineral premix (26 IU/kg). The treatments were arranged as a 2x2x2+1 factorial to yield a total of nine (9) dietary treatments consisting of seven (7) replicates of six (6) birds per replicate cage (Table 3.2). The first 8 treatments (2x2x2) were based on a marginally-non-phytate-phosphorus (NPP) deficient

diet (0.31%), while treatment 9, the PC treatment, was adequate in energy and all nutrients including the NPP (0.45%). The diet containing fresh oil, no phytase, and no additional supplemental VE (Diet A; Table 3.2) was considered the negative control (NC). All diets were mixed from the same basal diet, except the PC diet which was mixed separately. For diets supplemented with 150 ppm of VE, the VE was first dissolved in previously weighed oils to be used for each diet. The oils containing the VE were then mixed into their respective diets. The phytase used in this experiment was an enhanced *E. coli* phytase (Quantum Blue), provided by AB Vista Feed Ingredients (Marlborough, UK), with an expected activity of 5,000 FTU/g. Phytase activity was determined (ESC, Ystrad Mynach., UK) using the reference method of analysis recommended by the supplier (Basu et al., 2007) and reported in Table 3.3. The feed ingredient composition and the analyzed energy and nutrient contents of the experimental diets are presented in Table 3.3. Each diet contained 5 g/kg of titanium dioxide as an index marker for energy and nutrient digestibility and utilization. On day 7, all birds were weighed individually and randomized to cages in a completely randomized design with six birds/cage and they remained on the experimental diet until day 21.

3.3.3 Sample Collection

All birds and feed were weighed, per cage, on days 0, 7, 14, and 21 to determine the feed intake (FI), the body weight gain (BWG), and feed efficiency (FE). On d 21, all the birds were weighed in groups and one bird with a weight closest to the average cage weight was chosen for tissue collection. All the birds were euthanized by argon asphyxiation. The entire liver, abdominal fat, and subcutaneous fat were also removed and

the adhering tissues were cleaned off before weighing. The weight of these tissues was recorded and expressed relative to the final body weight (BW) of the selected sample bird

Relative organ/tissue weight, % = [((tissue weight, g)/weight of the bird, g)) × 100] ……. Equation 3. 1

The collected fat and liver tissues were immediately placed on ice before storing them at -20 ℃ for fatty acid analysis. Furthermore, the left and right tibia were removed from 1 bird per pen with a bodyweight that was close to the average bodyweight of the cage, and stored at – 20 ℃ for bone-breaking strength (BBS) and bone ash determination. The remaining birds, including the one selected for sampling (6 birds per cage), were opened up and the digesta content from the distal two-thirds of the ileum was collected by flushing with nanopure water into clean pre-labeled plastic containers. Digesta samples from all birds in a cage were pooled in the same plastic container and frozen at -20 ℃ until processed. The ileal digesta samples were freeze-dried afterward and ground using a coffee grinder before storing in airtight bags until they were analyzed for titanium, Ca, P, N, dry matter, and energy content.

Excreta samples were collected per cage on days 19 and 20 and the contents were pooled together before drying at 55 ℃ in a forced-air oven for 5 days. The dried samples were ground using a Wiley Mill Laboratory Standard (Model No. 3, Arthur H. Thomas Co., Philadelphia, PA, USA) fitted with a 1 mm screen and then stored in airtight plastic bags before being analyzed for titanium, crude fat (CF), Ca, P, N, dry matter, fatty acid, and energy determination.

3.3.4 Measurement of Lipid Oxidation

Soybean oil was acquired from the University of Kentucky's feed mill unit of C. Oran Little Research Center. The p-anisidine value was analyzed at Barrow-Agee Laboratories (Memphis, TN, USA, 38116) using the AOCS Cd 18-90 method. The peroxide value (PV), was determined using the AOCS (2007) method. Briefly, 5 g of the oil was weighed in duplicates into an Erlenmeyer flask before dissolving it in a 30 ml, 3:2 acetic acid-chloroform mix. Saturated potassium iodide solution (0.5 mL) was added and the mixture was shaken intermittently for 1 minute before 30 mL deionized water and a magnetic stirrer was added. A starch indicator solution (0.5 mL) was added before titrating with 0.1M sodium thiosulfate solution until the point at which the blue color disappeared. A sample blank was analyzed similarly and used to calculate the peroxide value (Equation 3.2). The full details of the PV determination method is provided in Appendix 1.

$$PV = \frac{(S - B) \times M \times 1000}{W} \quad \text{Equation 3. 2}$$

where S = titre of sample (mL), B = titre of blank (mL); M = molarity of the sodium thiosulfate solution; W = weight of sample (g).

3.3.5 Bone Breaking Strength (BBS) and Bone Ash

The frozen tibias were thawed before the surrounding soft tissues, flesh, and bone cap were removed manually. The BBS was subsequently measured using an Instron Materials tester (model 4301, Instron Corp., Canton, MA) at a loading rate of 40 mm/min. Before ash content determination, the bones were first dried at 105 ^{o}C for 24 hours (Precision Scientific Co., Chicago, IL), after which they were weighed. The lipid content of the bones was then determined via lipid extraction with petroleum ether for 3 extraction periods that lasted 72 h. Bones were completely soaked in a glass jar containing petroleum

ether. The ether was drained and replaced with fresh ether every 24 hours until no change in the color of the ether after 24 h was observed. After the final extraction, the bones were dried under the hood at room temperature for 6 h before drying at 100 ℃ to remove all remaining moisture. After drying, the bones were weighed individually for lipid content determination and then placed in a porcelain crucible for ashing in a muffle furnace overnight at 600 ℃. The bone weight was taken again after ashing to determine the percentage of the remaining ash relative to the dry weight of the bones before ashing.

3.3.6 Chemical Analysis

All analyses were conducted in duplicates and wherever the coefficient of variation was greater than 5%, the analysis was repeated for such pen. The dry matter (DM) contents of the nine diets, digesta, and excreta samples were determined in duplicates by drying the samples at 110 °C for 16 h (AOAC International, 2006). The nitrogen contents of the diets, digesta, and excreta samples were analyzed at the Agricultural Experiment Station Chemical Laboratories, University of Missouri-Columbia (Columbia, MO) by the combustion method (model FP2000, Leco Corp., St. Joseph, MI; AOAC International, 2000; method 990.03), with EDTA as the internal standard. The gross energy (GE) of the feed ingredients, diets, digesta, and excreta samples was analyzed using a bomb calorimeter (Parr adiabatic bomb calorimeter, model 6200, Parr Instruments, Moline, IL, USA) with benzoic acid as a calibration standard.

The titanium content of the diets and excreta was determined in duplicates using the method of Short et al. (1996). Briefly, the diets, digesta, and excreta samples were ashed at 580 ℃ overnight, after which they were digested at 250 ℃ in 7.4 M sulfuric acid for about one hour. Five milliliters of 30% hydrogen peroxide was added before bringing the

solution to volume with nanopure water. Titanium content was determined after 24 hours via a UV-visible spectrophotometer (UV-1800, Shimadzu Scientific, Kyoto, Japan) at a wavelength of 410 nm using a standard curve. Full details of this method are provided in Appendix 5.

The crude fat was determined in duplicates using the ether extraction method. Briefly, one gram of diet or excreta samples was weighed into a filter paper, which was placed into a cotton thimble and placed in the fat extraction machine (Velp Scientifica, Bohemia, NY, USA). The fat was extracted using petroleum ether. The weight of the resulting fat was expressed as a percentage of the starting sample weight.

Calcium (Ca) and phosphorus (P) contents were determined in duplicates by ashing 0.5 g of diet or excreta at 580 ℃ overnight before digesting the ash in diluted (1:3) hydrochloric acid for 15 mins. The digest was transferred into a 100 ml volumetric flask where it was shaken thoroughly before dispensing it in 50-ml tubes left overnight to settle before using it for Ca and P analysis. For Ca analysis, a 1:100 dilution of the digest was done using a diluting machine (Hamilton, Reno, NV, USA). Six standards ranging from 0-3 ppm were created to form the determination curve. The Ca concentration of the samples was determined using the atomic absorption spectrophotometer (AAnalyst 200, PerkinElmer, Waltham, MA, USA) using a nitrous oxide-acetylene gas mixture.

Phosphorus was determined in duplicates using the gravimetric quimociac method. Details of this method are provided in Appendix 2. Summarily, 15 ml of the digest was boiled with water at 500 °C before adding the quimociac reagent while swirling gently. The solution is reheated for about 5 minutes until a yellow precipitate was formed. This precipitate is then filtered using a fiber-glass filter paper fitted into a gooch crucible and

the resulting precipitate was dried overnight at 110 °C. The crucible containing the precipitate is then cooled in a desiccator and weighed to the nearest 0.1 mg (Shaver, 2008). The percent P is determined using equation 3.3.

$$\% \text{ P} = \frac{weight\ of\ precipitate * 100\ ml * 0.013997 * 100}{ml\ of\ aliquot * sample\ weight} \quad \text{..... Equation 3. 3}$$

Apparent ileal digestibility (AID) and energy and nutrient utilization (TTU) of DM, energy, N, Ca, P and crude fat were calculated using the method of Kong and Adeola (2014) (see Equation 3.4).

$$\text{AID or TTU (\%)} = 100 - \left[100\ x \left(\frac{Ti}{To}\right) x \left(\frac{No}{Ni}\right)\right] \text{..... Equation 3. 4}$$

where Ti is the initial concentration of the titanium marker in the feed, To is the concentration of the titanium marker in the excreta, No is the concentration of energy or nutrients the excreta, and Ni is the concentration of energy or nutrients in the feed.

The apparent metabolizable energy (AME) and ileal digestible energy (DE) were calculated using the following formula below (Equation 3.5). The caloric value of 8.22 kcal/g was used to correct AME for N to give the apparent metabolizable energy corrected for nitrogen (AMEn) (Hill and Anderson, 1958).

AME or $DE, kcal/kg = Calculated$ energy utilization or ileal energy digestibility (%) × $GE\ of$ the $diet\ (kcal/kg)$ ….Equation 3. 5

The fatty acid analyses of the diet, liver, abdominal fat, and subcutaneous fat tissues were done at the University of Georgia using a procedure modified from Park and Goins (1994). A summary of this procedure is provided in Appendix 6.

Total VE analysis was determined at DSM research laboratory (DSM Nutritional Products, Belvidere, NJ), using a modified version of AOAC official method 971.30.

Briefly, vitamin E is dispersed in deionized water and 3A alcohol and extracted with petroleum ether. The extract is then analyzed by a normal phase HPLC system using fluorescence detection.

3.3.7 Statistical Analysis

Prior to statistical analysis, outliers were removed from the data set by removing any data that falls outside Mean±3SD. Where outliers were found, the remaining number of replicates per treatment were indicated in the results tables. For the growth performance, ileal digestibility, and nutrient utilization data, the cage was considered the experimental unit, while one bird/cage constituted the experimental unit for all other response measurements. The 8 marginally deficient NPP treatments were subjected to a three-way ANOVA using the GLM procedure of SAS 9.4 (SAS Institute Inc., Cary, NC) as a completely randomized design with a 2 x 2 x 2 factorial arrangement of treatments. The effect of marginally deficient NPP was tested using a contrast between the PC and the NC (NPP-deficient diet containing fresh oil without phytase or VE supplementation). The significance of the main effects (oil quality, phytase, and VE), as well as the two-way and three-way interactions, were determined. A summary of the locational deposition of selected fatty acids was created by choosing the most abundant FAs (C16:0, C18:0, and C18:1) and the essential FAs (C18:2, C18:3n-3, C20:4) across all cages within the abdominal fat, subcutaneous fat, and liver. These FAs were then compared across the abdominal fat, subcutaneous fat, and liver regardless of the dietary treatments using the GLM procedure of SAS. This comparison was repeated across the abdominal fat and subcutaneous fat regardless of dietary treatments, using the GLM procedure of SAS. Wherever significant 2- or 3-way interactions were observed, treatment means were

separated by Tukey's Honest Significant Difference and the level of significance was set at $P < 0.05$.

3.4 Results

3.4.1 Diet Composition and Lipid Oxidation

The ingredients and analyzed nutrient composition of the experimental diets including DM, Ca, P, GE, crude fat (CF), crude protein (CP =N x 6.25), and analyzed phytase activity are shown in Table 3.3. All diets had similar gross energy, while the PC diets have a slightly lower dry matter (g/kg), crude protein (g/kg), and crude fat (g/kg). The analyzed Ca were exactly as expected but the total P values were slightly higher than expected in both the PC (7.2 vs 7.0) and the other diets (5.8 vs 5.6). The phytase activity was close to the desired levels, with <50 FTU/kg in the diets without phytase supplementation while the average phytase activity in the phytase-containing diets was 1,148 FTU/kg (115% of expected). Apart from the PC diet, all other diets were formulated from the same basal diet. Diets A-D (shown in Table 3.3) have the same ingredient composition as diets E-H (shown in Table 3.3), except that diets A-D had no additional supplemental VE of 150 ppm while diets E-H contained additional supplemental VE of 150 ppm. The diets without the additional supplemental VE had analyzed total VE levels that ranged from 79 IU/kg to 107 IU/kg in the diets. Conversely, for diets with additional supplemental VE, the analyzed VE levels ranged from186 IU/kg to 246 IU/kg. Peroxide value and p-anisidine value (P-Anv) were used to measure the degree of lipid oxidation. For the fresh soy oil, the PV and P-Anv were 4 meqO_2/kg and 0.5%, respectively, while the oxidized soy oil had PV and P-Anv of 109 meqO_2/kg and 35.2%, respectively.

3.4.2 Growth Performance

Table 3.4 shows the effects of the factors investigated in this study and their interaction on the BWG, FI, and FE of the broiler chickens over days 7-14 and 7-21, respectively. There was no significant difference in the growth performance of birds fed the PC and NC diets. Phytase supplementation significantly ($P < 0.05$) improved feed efficiency for day 7-14 and this benefit persisted till day 21 (Table 3.4). However, phytase did not affect the FI or BWG, neither did it interact with the other factors. Additional supplemental VE at 150 ppm improved ($P < 0.05$) the live weight, BWG, and FI through day 14, and although this effect was not considered statistically significant by day 21, there was still a numerical increase in the groups that received additional supplemental VE at 150 ppm. The results of this study also show that there were no simple or main effects of feeding oxidized oils on all growth performance response measures examined across all time periods.

3.4.3 Relative Liver and Fat Percentages

Table 3.5 shows the effect of the factors on the relative weights of the liver, as well as the abdominal and subcutaneous fats. From the results, there were no significant simple or main effects of all the factors on fat percentages. However, there was a main effect of oil quality on liver percentage as the birds fed oxidized oils had a higher ($P < 0.05$) relative liver weights compared to those fed fresh oils (~5.5%).

3.4.4 Bone Breaking Strength, Tibia Ash, and Bone Lipid

The simple and main effects of phytase, oil quality, and VE on BBS, tibia ash, and bone lipid content are reported in Table 3.6. The PC diet resulted in a higher ($P < 0.05$)

bone BBS and tibia ash content compared to the NC diet with deficient NPP level without phytase or VE supplementation. Phytase supplementation improved ($P < 0.05$) BBS and tibia ash content but decreased ($P < 0.05$) bone lipid content.

3.4.5 Apparent Ileal Dry Matter and Nutrient Digestibility

Table 3.7 shows the simple and main effects of phytase, oil quality, and VE on apparent ileal dry matter and nutrient digestibility. The PC diet reduced ($P < 0.05$) apparent ileal digestibility of Ca but did not affect apparent ileal digestibility of DM, N, and P. There was a significant 2-way interaction between phytase and oil quality for apparent ileal N digestibility where birds that received fresh oil with phytase had a higher ($P < 0.05$) apparent ileal N digestibility compared to those that received fresh oil with no phytase and those fed diets containing oxidized oil with no phytase. Phytase supplementation also increased ($P < 0.05$) apparent ileal DM (3.4%), N (2.1%), and P (63%) digestibility.

3.4.6 Nutrient and Energy Utilization

Table 3.8 shows the simple and main effects of phytase, oil quality, and VE on apparent dry matter and nutrient utilization. The PC diet resulted in lower ($P < 0.05$) P utilization compared to the NC diet, but no difference was observed for DM, CF, N, Ca, and energy utilization. Phytase supplementation affected ($P < 0.05$) the retention of Ca (increased) and crude fat (reduced) but no significant improvement was observed for P and N utilization, although P utilization trended towards significance when phytase was supplemented. Phytase supplementation also improved ($P < 0.05$) the AME and AMEn regardless of oil quality or VE supplementation. There was an interaction between oil quality and VE on crude fat utilization. Birds that were fed diets containing oxidized oil with no VE had higher ($P < 0.05$) utilization of crude fat compared to those fed diets

containing oxidized oil with VE. There was also a significant interaction between phytase and oil quality, as birds that received diets containing oxidized oil with phytase or fresh oil with no phytase had a higher ($P < 0.05$) P utilization compared to those fed diets containing oxidized oil with no phytase. Similarly, AME and AMEn were also greater ($P < 0.05$) in birds fed diets containing oxidized oil with phytase compared to birds fed diets containing either fresh or oxidized oil without phytase supplementation. For this same interaction, crude fat utilization was highest ($P < 0.05$) in birds that were fed diets containing oxidized oil but without phytase supplementation compared to birds that received oxidized oil with phytase and those that received fresh oil with phytase (Table 3.8).

A 3-way interaction was observed for P utilization, where birds that received diets containing fresh or oxidized oil with phytase and VE, as well as those that received oxidized oil with phytase but no VE, had a greater ($P < 0.05$) P-utilization compared to those that received oxidized oil with no phytase and no VE or fresh oil with phytase but no VE.

3.4.7 Fatty Acid Profiles

3.4.7.1 Fatty Acid Profile of Oil and Diet

In the current study, the percent concentrations of linoleic (C18:2) and linolenic acid (C18:3n-3) in the oils decreased from 52.9 to 50.8 (~4%) and from 7.34 to 6.43 (12%), respectively after heating. There was also an increase in the total SFA and MUFA content and a decrease in the total PUFA and UFA content as the oils were oxidized (Table 3.9). This is also reflected in the slightly higher SFA:UFA ratio of oxidized oils. Caprylic acid (C8:0) was present in the oxidized oil but not in the fresh oil. Regardless of oxidation, the

most abundant FA in the oils was C18:2 (51.8%), followed by C18:1 (23.1%), C16:0 (11.2%), and C18:3n-3 (6.89%).

As expected, the FA profile of the diet (Table 3.10) follows the FA profile of the oils added to it although the concentrations of C16:0, C16:1, C18:2, and C22:2 FAs were slightly higher in the diet (Table 3.10). In order of magnitude, C18:2 (54.5%) was the most abundant, followed by C18:1 (23.4%), C16:0 (12.2%), and C18:3n-3 (5.0%). Diets formulated with fresh oil (diets A, B, E, and F and PC) had a higher PUFA content and a lower SFA content compared to those formulated with oxidized oils (diets C, D, G, and H). Interestingly, C8 (caprylic acid) did not show up in the FA profile of the diets formulated with oxidized oils. The SFA:UFA ratio of the diets also mimics that the oils used in formulating them.

3.4.7.2 Fatty Acid Profile in the Adipose Tissues

Table 3.11 and 3.12 show the effect of the treatments on the selected FAs in the abdominal and subcutaneous fats, respectively. The full FA composition of the abdominal and subcutaneous fats are provided in the appendix Tables A.3.1and A.3.2, respectively. There were no simple or main effects of oil quality, phytase, and VE supplementation on the content of SFA, UFA, and SFA:UFA in the abdominal fat tissue. In both fat depots, oxidized lipids increased ($P < 0.05$) the total content of MUFA but decreased the total PUFA content of the abdominal fat. Interestingly, the PC diet reduced ($P < 0.05$) SFA:UFA in the abdominal fat compared to the NC diet. In the subcutaneous fat, VE supplementation increased ($P < 0.05$) the total PUFA and UFA content but reduced ($P < 0.05$) the SFA:UFA. Two interactions were observed in the same fat depot, as birds fed diets containing fresh oil but no VE had a much higher ($P < 0.05$) SFA content compared to other groups (Table

3.12). Also, the birds that received phytase but no VE supplementation had a higher ($P < 0.05$) total SFA content than those fed both phytase and VE.

In both fat depots, the most abundant FA is C18:1 (oleic acid), followed by C18:2 (linoleic acid) and C16:0 (palmitic acid). Conversely, in the diets, C18:2 was the most abundant, followed by C18:1 and C16:0. In the abdominal fat, the PC diet resulted in greater ($P < 0.05$) deposition of C20:1 and a decrease ($P < 0.05$) in the amount of C17:1. Phytase supplementation increased ($P < 0.05$) the concentration of C20:0 in the abdominal fat but not in the subcutaneous fat. Lipid oxidation increased ($P < 0.05$) the concentration of C18:1 and decreased the amount of (C18:3n-3) in both abdominal and subcutaneous fats. In the abdominal fat, lipid oxidation decreased ($P < 0.05$) the concentration of C17:0 and C20:2 but increased ($P < 0.05$) the amount of C18:3n-6. Conversely, lipid oxidation also increased the content of C20:0 in the subcutaneous fat but not in the abdominal fat. The percent concentration of linoleic acid (C18:2) was not affected by oil quality, phytase, or VE supplementation in both fat depots. In both adipose tissues, VE supplementation decreased ($P < 0.05$) the content of C14:0 (myristic acid). VE also decreased ($P < 0.05$) the concentration of C16:0 in the subcutaneous fat tissue, but not in the abdominal fat tissue.

A few two-way interactions were observed in the abdominal and subcutaneous fat tissues. In the abdominal fat, birds that received diets containing oxidized oils with no phytase supplementation and those that received diets containing fresh oil with phytase supplementation had a higher ($P < 0.05$) content of C14:1 compared to birds fed diets containing fresh oil with no phytase supplementation. Also, the birds that received diets containing oxidized oil with phytase had a higher ($P < 0.05$) deposition of C18:0 in their abdominal fat pads compared to those fed diet containing fresh oil with phytase

supplementation. In the subcutaneous fat tissue, a phytase x oil quality interaction was also observed for C14:1, where the birds that were fed diets containing oxidized oil with no phytase had higher ($P < 0.05$) levels of C14:1 compared to birds fed diets containing fresh oil with no phytase. In the same fat depot, the supply of fresh oil with phytase produced a higher ($P < 0.05$) content of C14:0 compared to diets fresh oil with no phytase and oxidized oil with phytase. .

Two 3-way interactions were observed in the subcutaneous fat tissue for C18:0 and C22:0. The highest ($P < 0.05$) content of C18:0 was found in birds fed diets containing oxidized oils with phytase and VE compared to those fed either diet containing fresh oil with phytase and VE or those fed diets containing oxidized oil with VE but no phytase. Conversely, the highest content of C22:0 was observed in birds fed diets containing oxidized oil with phytase but no VE and oxidized oil with VE but no phytase, compared to those that received diets containing oxidized oil with both phytase and VE.

3.4.7.3 Fatty Acid Profile in the Liver

Table 3.13 shows the effect of phytase, oil quality, and additional supplemental VE on selected FAs in the liver. The full FA composition of the abdominal and subcutaneous fats are provided in the appendix Tables A.3.3. Vitamin E supplementation reduced ($P < 0.05$) the SFA:UFA ratio. The total content of PUFA and UFA was reduced ($P < 0.05$) in birds fed phytase. Also, the total PUFA content of birds fed oxidized oils was lower ($P < 0.05$) than those that received fresh oils. Furthermore, birds that were fed either the oxidized oils with no phytase supplementation or fresh oil with phytase supplementation had higher ($P < 0.05$) content of SFA and SFA:UFA compared to those fed fresh oil with no phytase supplementation. Furthermore, the total UFA content was higher ($P < 0.05$) in

birds fed fresh oil with no phytase supplementation compared to birds that received fresh oil with phytase supplementation.

Phytase and VE supplementation reduced the concentration of C17:0 independently, but neither phytase nor VE independently affected the composition of any other FA. An interaction between phytase and VE produced a significantly higher C17:1 and C22:4 content in birds fed no phytase and no VE compared to all other groups. Oxidized oils decreased ($P < 0.05$) the percent deposition of C18:2, C18:3n-3, C20:2, C22:0, and C24:1. There was an interaction between oil quality and VE in the concentration of C17:0, as the birds fed fresh oil with VE had a greater percentage of C17:0 compared to those fed oxidized oil with VE. Furthermore, birds that were fed diets containing oxidized oil with VE had a greater content of C20:0 compared to those fed oxidized oil with no VE. Also, the dietary supply of oxidized oil with phytase produced a greater content of C20:1 compared to those fed diet containing fresh oil with phytase. Moreover, birds that received oxidized oil with no phytase and fresh oil with phytase had a higher concentration of C22:4 compared to those fed oxidized oil with phytase. Also, the highest content of C24:0 was observed in birds fed no phytase and no VE compared to those fed either VE with no phytase or those fed or phytase with no VE. A three-way interaction was observed in C14:1 where the dietary treatment containing fresh oil with phytase and no VE had a significantly greater content of the FA than the treatment containing fresh oil with no phytase and no VE.

3.4.7.4 Comparison of the Mean Fatty Acid Profile of the Adipose and Liver Tissues

The mean composition of select FAs in the adipose and liver tissues is presented in Table 3.14. The liver had the greatest ($P < 0.05$) content of C16:0, C18:0 and C20:4

compared to the subcutaneous and abdominal fats. Conversely, the content of C18:1 and C18:2 were greater in the abdominal and subcutaneous fats compared to the liver. The content of C18:3n-3 was greatest in the abdominal fat compared to the subcutaneous fat and the liver. In general, the liver had a greater level of saturation as shown in the higher SFA and SFA:UFA, compared to the abdominal and subcutaneous fat tissues. However, a greater content of MUFA and UFA was observed in the abdominal and subcutaneous fats compared to the liver.

3.4.7.5 Comparison of the Mean Fatty Acid Profile of the Abdominal and Subcutaneous Fat Tissues

The mean composition of select FAs in the abdominal and subcutaneous fat tissues is presented in Table 3.15. The content of C16:0 and C18:0 were greater ($P < 0.05$) in the subcutaneous fat than the abdominal fat. Similarly, a greater ($P < 0.05$) content of total SFA and SFA:UFA was observed in the subcutaneous fat than the abdominal fat. Conversely, a greater content of C20:4 and total UFA was greater in the abdominal fat than the subcutaneous fat.

Table 3. 1 Ingredient composition and calculated energy and nutrient contents of the broiler starter diet fed from day 0-7 (on as-fed basis)

Ingredients	g/kg
Corn	583.6
Soybean meal (47% CP)	348.0
Soy oil	30.0
L-Lysine HCl	1.5
DL-Methionine	2.0
Tryptophan	1.0
Salt (NaCl)	3.6
Limestone	10.3
Dicalcium phosphate	18.0
Vitamin-mineral premix[1]	2.0
Total	1000
Calculated nutrients and energy	
Crude protein, g/kg	215
MEn, kcal/kg	3110
Calcium, g/kg	9.6
Phosphorus, g/kg	7.4
Non-phytate phosphorus, g/kg	4.8

[1]Provided the following quantities of vitamins and micro minerals per kilogram of complete diet: iron, 32 mg; copper, 8 mg; manganese, 51 mg; zinc, 60 mg; iodine, 1.48 mg; selenium, 0.24 mg; vitamin A (retinyl acetate), 8,820 IU; vitamin D3 (cholecalciferol), 2,822 IU; vitamin E (dl-α-tocopheryl acetate), 26 IU; vitamin K activity, 0.73 mg; thiamine, 1.76 mg; riboflavin, 6.17 mg; pantothenic acid, 14 mg; niacin, 44 mg; pyridoxine, 4 mg; folic acid, 0.88 mg; biotin, 0.18 mg; vitamin B-12, 0.02 mg; choline, 383 mg.

Table 3. 2 Details of the nine dietary treatments fed to broiler chickens in this study.

Diet/Treatment	Details	NPP[5] level (%)
A[1]	Fresh oil + no phytase + no additional vitamin E	0.31
B	Fresh oil + phytase + no additional vitamin E	0.31
C	Oxidized oil + no phytase + no additional vitamin E	0.31
D	Oxidized oil + phytase[3]+ no additional vitamin E	0.31
E	Fresh oil + no phytase + 150 ppm additional vitamin E[4]	0.31
F	Fresh oil + phytase + 150 ppm additional vitamin E	0.31
G	Oxidized oil + no phytase + 150 ppm additional vitamin E	0.31
H	Oxidized oil + phytase + 150 ppm additional vitamin E	0.31
PC[2]	Fresh oil + no phytase + no additional vitamin E	0.45

[1]This is the negative control (NC) containing 0.31 % NPP
[2]Same as NC except for a higher NPP of 0.45%
[3]Supplemental phytase was provided at 1,000 FTU/kg of diet
[4]Supplemental vitamin E was added in addition to the 26 IU/kg of supplemental vitamin E content of the basal diet.
[5]Non-phytate phosphorus

Table 3. 3 Ingredients composition and analyzed energy and nutrient contents of the experimental diets fed to broiler chickens from day 7-21[1] (on as-fed basis).

Diet ID[2]	PC	A (E)	B (F)	C (G)	D (H)
Oil quality	Fresh	Fresh	Fresh	Oxidized	Oxidized
Phytase	No	No	Yes	No	Yes
Vitamin E[3]	No	No	No	No	No
Ingredients, g/kg					
Corn	618	623	623	623	623
Soybean meal (47%CP)	300	300	300	300	300
Soyoil normal	30	30	30	0	0
Soyoil oxidized	0	0	0	30	30
Limestone	9.6	11.9	11.9	11.9	11.9
Dicalcium phosphate	17.0	9.6	9.6	9.6	9.6
L-Lysine HCl	1.5	1.5	1.5	1.5	1.5
DL-Methionine	2.0	2.0	2.0	2.0	2.0
Tryptophan	1.0	1.0	1.0	1.0	1.0
Salt (NaCl)	3.6	3.6	3.6	3.6	3.6
Vitamin mineral premix[4]	2.0	2.0	2.0	2.0	2.0
Titanium dioxide	5.0	5.0	5.0	5.0	5.0
Phytase premix[5]	0	0	10	0	10
Total	1,000	1,000	1,000	1,000	1,000
Analyzed nutrients and energy[6]					
Dry matter g/kg	870		880		
Gross energy, kcal/kg	4,150		4,149		
Crude protein, g/kg	199		207		
Calcium g/kg	9.0		7.9		
Phosphorus, g/kg	7.2		5.8		
Non-phytate P (npp), g/kg[7]	4.5		3.1		
Crude fat, g/kg	57.0	59.0	60.3	60.4	59.6
Phytase activity, FTU/kg	<50	<50	902	<50	1,380
Basal vitamin E (IU/kg)[8]	105	107	105	86	79
Total vitamin E (IU/kg)[9]	--	192	246	186	192

[1]All birds were fed the PC diet from days 0-7 before they were switched to experimental diets.
[2]PC=positive control diet with 0.45% NPP. Diets ABCD = Diets EFGH, except that ABCD has no supplemental Vitamin E, while EFGH does.
[3]Represents supplemental vitamin E at 150 mg/kg. This is in addition to the vitamin E supplied by the vitamin-mineral premix.
[4]Provided the following quantities of vitamins and micro minerals per kilogram of complete diet: iron, 32 mg; copper, 8 mg; manganese, 51 mg; zinc, 60 mg; iodine, 1.48 mg; selenium, 0.24 mg; vitamin A (retinyl acetate), 8,820 IU; vitamin D3 (cholecalciferol), 2,822 IU; vitamin E (dl-α-tocopheryl acetate), 26 IU; vitamin K activity, 0.73 mg; thiamine, 1.76 mg; riboflavin, 6.17 mg; pantothenic acid, 14 mg; niacin, 44 mg; pyridoxine, 4 mg; folic acid, 0.88 mg; biotin, 0.18 mg; vitamin B-12, 0.02 mg; choline, 383 mg.
[5]Phytase premix was added to diets B and D at the expense of corn to supply phytase at 1000 FTU/kg of diet.
[6]Since the same basal diet was used in the study, the average values of the analyzed nutrients (except crude fat) were used to determine the utilization values but the analyzed value for the PC diet was used for the PC utilization calculations.
[7]Calculated value.
[8]Analyzed total vitamin E level in diets without supplemental vitamin E
[9] Analyzed total vitamin E level in diets containing supplemental vitamin E at 150 mg/kg

Table 3. 4 Main and simple effects of oil quality, phytase, and vitamin E supplementation on the performance of 21 day-old broiler chickens

			7-14 d				7-21 d			
Oil quality	Phytase	Vitamin E	LBW, g/bird	Gain, g/bird	FI, g/bird	G:F ratio, g/kg	LBW,[1] g/bird	Gain, g/bird	FI,[2] g/bird	G:F ratio,[3] g/kg
	Main effect									
Fresh			484	295	375	786	960	769	1052	731
Oxidized			482	292	371	787	959	764	1050	728
	No		480	290	372	779[b]	957	763	1053	724[b]
	Yes		486	297	374	794[a]	963	771	1049	734[a]
		No	476[b]	288[b]	368[b]	781	953	760	1044	727
		Yes	490[a]	299[a]	377[a]	792	966	774	1058	731
	Dietary treatments									
Fresh	No	No[4]	473[r]	284	368	771	943	753	1044	721
Fresh	No	Yes	490[p]	298	377	791	966	774	1055	733
Oxidized	No	No	473[r]	284	366	775	953	757	1047	723
Oxidized	No	Yes	486[q]	293	376	778	964	768	1065	721
Fresh	Yes	No	482[q]	297	375	792	967	779	1058	737
Fresh	Yes	Yes	490[p]	299	379	789	965	772	1053	733
Oxidized	Yes	No	477[s]	287	365	785	949	752	1028	730
Oxidized	Yes	Yes	494	305	377	809	969	781	1059	737
	SEM[5]		6.307	5.993	5.337	8.933	13.068	14.505	14.089	6.422
	PC[6]		481	293	378	777	954	766	1049	731
	SEM		5.518	4.911	4.934	7.383	4.076	16.793	16.854	6.347
			----------Probability----------							
Oil quality			0.771	0.568	0.323	0.876	0.881	0.618	0.807	0.491
Phytase			0.222	0.086	0.553	0.021	0.506	0.446	0.717	0.037
Vitamin E			0.003	0.013	0.026	0.078	0.163	0.200	0.176	0.422
Oil quality x vitamin E			0.755	0.497	0.479	0.701	0.793	0.518	0.300	0.887
Phytase x oil quality			0.883	0.903	0.539	0.355	0.553	0.682	0.358	0.672

Phytase x vitamin E	0.808	0.847	0.833	0.945	0.653	0.800	0.914	0.719
Phytase x oil quality x vitamin E	0.468	0.190	0.578	0.091	0.338	0.261	0.471	0.161
PC vs. NC	0.313	0.190	0.192	0.570	0.574	0.579	0.831	0.296

[a–b]Means with different superscripts within the same column differ significantly based on Tukey HSD ($P < 0.05$).

[1]Live body weight at day 21. The average live weight at day 7 was 189 g

[2]Feed Intake

[3]G:F=gain to feed ratio or feed efficiency

[4]Negative control (NC) diet

[5]Standard error of the mean for the 2x2x2 factorial arrangement of treatments

[6]PC=positive control

[p-s]Represents the number or mortalities per dietary treatment, where p, q, r and s represents 1, 2, 3, and 4 mortalities respectively.

Table 3. 5 Main and simple effects of oil quality, phytase, and vitamin E supplementation on the relative adipose tissue and liver weights of 21-day-old broiler chickens

			21 d			
Oil quality	Phytase	Vitamin E	Abdominal fat, %	Subcutaneous fat, %	Total fat,[1] %	Liver, %
Main effect						
Fresh			1.112	1.030	2.143	2.800[b]
Oxidized			1.206	1.129	2.334	2.955[a]
	No		1.188	1.043	2.230	2.819
	Yes		1.130	1.117	2.247	2.937
		No	1.174	1.059	2.231	2.916
		Yes	1.144	1.101	2.246	2.839
Dietary treatments						
Fresh	No	No[2]	1.210	1.063	2.272	2.779
Fresh	No	Yes	1.067	0.954	2.023	2.674
Oxidized	No	No	1.174	1.091	2.263	2.870
Oxidized	No	Yes	1.299	1.061	2.362	2.951
Fresh	Yes	No	1.113	1.030	2.144	3.016
Fresh	Yes	Yes	1.059	1.074	2.131	2.733
Oxidized	Yes	No	1.197	1.050	2.246	3.000
Oxidized	Yes	Yes	1.153	1.313	2.466	2.999
	SEM[3]		0.119	0.131	0.223	0.098
	PC[4]		1.14	1.21	2.35	2.85
	SEM[5]		0.093	0.092	0.153	0.068
			----------Probability----------			
Oil quality			0.272	0.293	0.232	0.030
Phytase			0.500	0.427	0.918	0.094
Vitamin E			0.729	0.651	0.928	0.272
Oil quality x vitamin E			0.414	0.427	0.868	0.670
Phytase x oil quality			0.960	0.742	0.575	0.349
Phytase x vitamin E			0.813	0.235	0.361	0.098
Phytase x oil quality x vitamin E			0.449	0.707	0.854	0.731
PC vs. NC			0.613	0.293	0.733	0.454

[1]Values represent the sum of the abdominal and subcutaneous fat percentages.
[a–b]Means with different superscripts within the same column differ significantly based on Tukey HSD ($P < 0.05$).
[2]Negative control (NC) diet
[3]Standard error of the mean for the 2x2x2 factorial arrangement of treatments
[4]PC=positive control
[5]Standard error of the mean for the comparison between the PC and NC treatments

Table 3. 6 Main and simple effects of oil quality, phytase, and vitamin E supplementation on Bone breaking strength (BBS), tibia ash, and tibia lipid.

			21 d		
Oil quality	Phytase	Vitamin E	BBS,[1] kg/f	Ash, %	Lipid, %
	Main effect				
Fresh			22.1	51.0	0.137
Oxidized			22.5	51.2	0.128
	No		20.8[b]	49.2[b]	0.147[a]
	Yes		23.8[a]	53.0[a]	0.118[b]
		No	22.9	50.9	0.134
		Yes	21.7	51.3	0.131
	Dietary treatments				
Fresh	No	No[2]	21.8	49.0	0.136
Fresh	No	Yes	20.1	49.1	0.160
Oxidized	No	No	19.9	48.6	0.164
Oxidized	No	Yes	21.4	50.2	0.130
Fresh	Yes	No	23.5	54.0	0.124
Fresh	Yes	Yes	23.1	51.9	0.128
Oxidized	Yes	No	26.3[x]	52.1	0.113
Oxidized	Yes	Yes	22.2	53.9	0.107
	Pooled SD[3]		4.350	0.032	0.042
	PC[4]		26.0	52.9	0.111
	SEM		0.782	0.003	0.016
			----------Probability----------		
Oil quality			0.824	0.836	0.424
Phytase			0.017	<.001	0.012
Vitamin E			0.646	0.892	0.501
Oil quality x vitamin E			0.350	0.679	0.797
Phytase x oil quality			0.384	0.585	0.852
Phytase x vitamin E			0.927	0.118	0.135
Phytase x oil quality x vitamin E			0.147	0.484	0.291
PC vs NC			0.003	<.001	0.300

[1]Bone breaking strength

[a–b]Means with different superscripts within the same column differ significantly based on Tukey HSD ($P < 0.05$).

[2]Negative control (NC) diet

[3]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

[4]PC = positive control diet

[x]Values represent the mean of 7 replicate cages (pooled excreta of 6 birds per replicate) except for mean values with x where the number of replicates (n) was 6.

Table 3. 7 Main and simple effects of oil quality, phytase, and vitamin E supplementation on apparent ileal dry matter, nitrogen, calcium, and phosphorus digestibility in 21-day-old broiler chickens

Oil quality	Phytase	Vitamin E	DM, %	N, %	Ca, %	P, %
	Main effect					
Fresh			68.8	83.2	46.5	48.3
Oxidized			68.1	82.7	42.1	47.8
	No		67.3[b]	82.1[b]	46.0	36.6[b]
	Yes		69.6[a]	83.8[a]	42.6	59.6[a]
		No	68.4	83.0	44.9	48.0
		Yes	68.5	82.9	43.7	48.1
	2-way interaction effect					
	Phytase x oil quality					
Fresh	No	-	67.5	81.9[b]	49.4	37.7
Fresh	Yes	-	70.1	84.5[a]	43.6	59.0
Oxidized	No	-	67.0	82.3[b]	42.7	35.5
Oxidized	Yes	-	69.1	83.1[ab]	41.5	60.2
	Dietary treatments					
Fresh	No	No[1]	67.6	82.1	48.9[x]	38.7[x]
Fresh	No	Yes	67.4	81.6	50.0	36.6[x]
Oxidized	No	No	67.2[x]	82.0	45.7[x]	36.5[x]
Oxidized	No	Yes	66.9	82.7[x]	39.6[x]	34.5[x]
Fresh	Yes	No	69.6	84.5[x]	42.0	56.6
Fresh	Yes	Yes	70.6	84.4	45.2[x]	61.4[x]
Oxidized	Yes	No	69.1	83.3	43.0	60.3
Oxidized	Yes	Yes	69.0[x]	82.9	40.0[x]	60.0
	Pooled SD		3.123	1.514	8.650	6.230
	PC[2]		69.8[x]	82.1	37.52	42.9
	Pooled SD[3]		2.083	2.115	7.733	7.021
			----------Probability----------			

Oil quality	0.366	0.277	0.069	0.782
Phytase	0.009	<.001	0.153	<.001
Vitamin E	0.757	0.825	0.337	0.345
Oil quality x vitamin E	0.744	0.617	0.165	0.470
Phytase x oil quality	0.898	0.034	0.613	0.966
Phytase x vitamin E	0.685	0.714	0.591	0.221
Phytase x oil quality x vitamin E	0.776	0.359	0.918	0.451
PC vs. NC	0.092	1.000	0.023	0.314

[a–b]Means with different superscripts within the same column differ significantly based on Tukey HSD ($P < 0.05$).

[1]Negative control (NC) diet

[2]PC=positive control

[3]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

[x]Values represent the mean of 7 replicate cages (pooled excreta of 6 birds per replicate) except for mean values with x where the number of replicates (n) was 6.

Table 3. 8 Main and simple effects of oil quality, phytase, and vitamin E supplementation on dry matter, energy and nutrient utilization, apparent metabolizable energy (AME), and AME corrected for nitrogen (AMEn) in 21-day-old broiler[1]

Oil quality	Phytase	Vitamin E	DM, %	CF, %	N, %	Ca, %	P, %	Energy, %	AME, kcal/kg	AMEn, kcal/kg
Main effect										
Fresh			73.9	83.5	66.2	54.9	53.0	76.9	3182^{b}	3080
Oxidized			73.6	83.7	65.7	55.8	51.7	76.6	3200^{a}	3086
	No		73.5	84.3^{a}	65.4	53.4^{b}	51.0	76.6	3174^{b}	3063^{b}
	Yes		74.0	83.0^{b}	66.5	57.3^{a}	53.7	76.9	3208^{a}	3103^{a}
		No	73.9	84.1	66.0	54.3	50.4^{b}	76.9	3189	3080
		Yes	73.6	83.2	65.9	56.5	54.3^{a}	76.7	3193	3086
2-way interaction effect										
Oil quality x vitamin E										
Fresh	-	No	74.2	83.7^{ab}	66.2	54.0	50.6	77.2	3186	3082
Fresh	-	Yes	73.6	83.4^{ab}	66.2	55.8	55.4	76.7	3178	3077
Oxidized	-	No	73.6	84.5^{a}	65.8	54.5	50.3	76.5	3192	3077
Oxidized	-	Yes	73.6	83.0^{b}	65.7	57.1	53.2	76.7	3209	3095
Phytase x oil quality										
Fresh	No	-	73.8	84.2^{ab}	65.7	53.0	53.6^{a}	76.8	3178^{b}	3071^{b}
Fresh	Yes	-	74.0	82.9^{c}	66.6	56.9	52.4^{ab}	77.1	3186^{b}	3088^{ab}
Oxidized	No	-	73.2	84.4^{a}	65.2	53.9	48.4^{b}	76.4	3170^{b}	3054^{b}
Oxidized	Yes	-	73.9	83.1^{bc}	66.3	57.7	55.1^{a}	76.8	3231^{a}	3118^{a}
Dietary treatments										
Fresh	No	No[2]	73.4	84.6^{x}	66.0^{x}	51.9	^{x}52.9^{ab}	77.1	3180^{x}	3075^{x}
Fresh	No	Yes	73.3	83.8	65.4	54.1	^{x}54.4^{ab}	76.4	3176	3067
Oxidized	No	No	73.2	85.2	65.0	53.0	45.2^{c}	76.3	3164	3057
Oxidized	No	Yes	73.2	83.6^{x}	65.4^{x}	54.9	^{x}51.5^{abc}	76.4	3176^{x}	3051
Fresh	Yes	No	74.1	82.7^{x}	66.3	56.1	48.2^{bc}	77.3^{y}	3193	3089
Fresh	Yes	Yes	73.9	83.1	66.9	57.6	^{x}56.5^{a}	76.9	3179^{x}	3087
Oxidized	Yes	No	74.0	83.8	66.7^{x}	56.1	^{x}55.4^{a}	76.7	3219^{y}	3097^{x}

Oxidized	Yes	Yes	73.9 [x]	82.4[x]	65.9[x]	59.3	[x]54.9[a]	76.9	3242[x]	3139[x]
	Pooled SD		0.890	1.070	2.031	4.681	5.800	0.902	30.810	40.531
	PC[3]		74.22[x]	84.5[x]	66.3	52.7	45.1	77.4	3182[x]	3083[x]
	Pooled SD[4]		0.764	1.161	2.210	5.795	3.961	0.873	34.510	36.791
						----------Probability----------				
Oil quality			0.179	0.509	0.467	0.476	0.452	0.170	0.041	0.582
Phytase			0.089	<.001	0.074	0.004	0.101	0.172	<.001	0.001
Vitamin E			0.165	0.964	0.864	0.084	0.023	0.442	0.616	0.569
Oil quality x vitamin E			0.217	0.042	0.868	0.771	0.566	0.151	0.141	0.300
Phytase x oil quality			0.266	0.005	0.893	0.941	0.019	0.782	0.004	0.044
Phytase x vitamin E			0.354	0.237	0.985	0.909	0.997	0.608	0.985	0.230
Phytase x oil quality x vitamin E			0.309	0.459	0.274	0.694	0.045	0.894	0.543	0.351
PC vs. NC			0.689	0.893	0.775	0.787	0.005	0.765	0.935	0.720

[a–b]Means with different superscripts within the same column differ significantly (P < 0.05).

[1]DM= dry matter, CF= crude fat, N= nitrogen, Ca=calcium, P= phosphorus

[2]Negative control (NC) diet

[3]PC=positive control

[4]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

[x-y]Values represent the mean of 7 replicate cages (pooled excreta of 6 birds per replicate) except for mean values with x and y where the number of replicates (n) was 6 and 5 , respectively.

Table 3. 9 Fatty acid compositions and peroxide value of the oils used in the experimental diets

Fatty acid composition, %	Fresh soy oil	Oxidized soy oil
C8:0 (Caprylic)	0.00	0.05
C14:0 (Myristic)	0.07	0.08
C15:0 (Pentadecanoic)	0.00	0.03
C16:0 (Palmitic)	10.85	11.59
C16:1 (Palmitoleic)	0.11	0.12
C17:0 (Margaric)	0.10	0.11
C17:1 (cis-10-Heptadecenoic acid)	0.06	0.07
C18:0 (Stearic)	4.41	4.68
C18:1 (Oleic)	22.54	23.72
C18:2 (Linoleic)	52.88	50.84
C18:3n-3 (alpha linolenic acid)	7.34	6.44
C20:0 (Arachidic)	0.35	0.36
C20:1 (Eicosenoic acid)	0.27	0.33
C20:2 (Eicosadienoic acid)	0.04	0.07
C22:0 (Behenic **a**cid)	0.31	0.39
C22:1 (Erucic)	0.04	0.36
C22:2 (Docosadienoic acid)	0.03	0.01
C24:0 (Lignoceric)	0.08	0.06
Others	0.51	0.70
∑SFA	16.17	17.34
∑MUFA	23.03	24.59
∑PUFA	60.29	57.37
∑UFA	83.32	81.96
SFA:UFA	0.19	0.21
Peroxide value, meqO_2/kg	4	109
p-anisidine value, %	0.5	35.2

Table 3. 10 Analyzed fatty acid contents of the experimental diets (%)

Diet ID	PC	A	B	C	D	E	F	G	H
Oil quality	Fresh	Fresh	Fresh	Oxidized	Oxidized	Fresh	Fresh	Oxidized	Oxidized
Phytase	No	No	Yes	No	Yes	No	Yes	No	Yes
Vitamin E[1]	No	No	No	No	No	Yes	Yes	Yes	Yes
Lipid, %	5.26	5.40	5.89	5.20	5.39	5.25	5.54	5.25	5.10
Fatty acid profile, %									
C14:0 (Myristic)	0.06	0.07	0.07	0.07	0.07	0.06	0.07	0.07	0.07
C16:0 (Palmitic)	11.97	12.00	11.93	12.49	12.50	11.80	11.90	12.46	12.42
C16:1 (Palmitoleic)	0.14	0.14	0.14	0.15	0.15	0.14	0.14	0.13	0.15
C17:0 (Margaric)	0.08	0.09	0.09	0.10	0.09	0.08	0.09	0.09	0.09
C17:1 (cis-10-Heptadecenoic acid)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
C18:0 (Stearic)	3.41	3.40	3.47	3.62	3.59	3.27	3.38	3.69	3.51
C18:1 (Oleic)	23.00	23.18	23.21	23.71	23.35	23.33	23.49	23.54	23.96
C18:2 (Linoleic)	54.99	54.76	54.60	53.91	54.24	55.34	54.75	53.81	53.89
C18:3n3 (alpha linolenic acid)	5.20	5.21	5.36	4.82	4.80	4.88	5.09	5.00	4.59
C20:0 (Arachidic)	0.35	0.35	0.34	0.35	0.36	0.34	0.35	0.36	0.36
C20:1 (Eicosenoic acid)	0.24	0.24	0.24	0.24	0.23	0.24	0.24	0.23	0.24
C22:0 (Behenic **a**cid)	0.27	0.29	0.29	0.29	0.33	0.27	0.27	0.34	0.32
C22:2 (Docosadienoic acid)	0.23	0.23	0.22	0.22	0.23	0.19	0.19	0.23	0.36
∑SFA	16.15	16.19	16.19	16.91	16.94	15.82	16.05	17.01	16.76
∑MUFA	23.43	23.61	23.63	24.15	23.79	23.77	23.92	23.96	24.40
∑PUFA	60.43	60.19	60.18	58.95	59.27	60.41	60.03	59.04	58.84
∑UFA	83.85	83.81	83.81	83.09	83.06	84.18	83.95	82.99	83.24
SFA:UFA	0.19	0.19	0.19	0.20	0.20	0.19	0.19	0.20	0.20

[1]Represents supplemental vitamin E at 150 g/kg. This is in addition to the vitamin E supplied by the vitamin-mineral premix.

Table 3. 11 Main and simple effects of oil quality, phytase, and vitamin E supplementation on selected fatty acids in the abdominal fat of 21 day-old broiler chickens (%)

Oil quality	Phytase	Vitamin E	C14:0	C14:1	C16:0	C17:0	C17:1	C18:0	C18:1	C18:2	C18:3n-6	C18:3 n-3	C20:0
Main effect													
Fresh			0.459	0.142	21.5	0.234[a]	0.113	5.4	35.3[b]	26.7	0.267[b]	2.5[a]	0.089
Oxidized			0.466	0.163	21.8	0.196[b]	0.109	5.5	36.5[a]	25.4	0.292[a]	2.2[b]	0.096
	No		0.464	0.147	21.6	0.223	0.113	5.4	35.9	26.1	0.277	2.4	0.088[b]
	Yes		0.461	0.159	21.6	0.207	0.109	5.5	35.9	26.0	0.282	2.3	0.098[a]
		No	0.472[a]	0.157	21.8	0.213	0.112	5.3	36.0	25.8	0.289	2.3	0.095
		Yes	0.451[b]	0.148	21.5	0.217	0.111	5.5	35.8	26.3	0.271	2.4	0.090
2-way interaction effect													
Phytase x oil quality													
Fresh	No	-	0.453	0.119[b]	21.4	0.244	0.117	5.4[ab]	35.1	26.9	0.269	2.6	0.087
Fresh	Yes	-	0.465	0.165[a]	21.5	0.224	0.110	5.3[b]	35.4	26.5	0.265	2.5	0.092
Oxidized	No	-	0.474	0.175[a]	21.9	0.201	0.111	5.3[ab]	36.6	25.2	0.286	2.2	0.089
Oxidized	Yes	-	0.458	0.152[ab]	21.7	0.190	0.108	5.7[a]	36.5	25.6	0.298	2.2	0.104
Dietary treatments													
Fresh	No	No[1]	0.468	0.122[x]	21.8	0.251	0.120	5.2	35.3	26.8	0.279[x]	2.6	0.088
Fresh	No	Yes	0.438[x]	0.117	21.1	0.238	0.114	5.5[x]	34.9	27.0[x]	0.260	2.6[x]	0.086
Oxidized	No	No	0.471	0.171	21.7[x]	0.192	0.107	5.3[x]	36.7	24.9	0.298[x]	2.1	0.096
Oxidized	No	Yes	0.478	0.179[x]	22.0	0.211	0.114	5.4	36.5[x]	25.6	0.274[x]	2.2[x]	0.081[x]
Fresh	Yes	No	0.480	0.172[x]	21.7	0.221[x]	0.111	5.3	35.3[x]	26.3	0.269	2.5	0.095
Fresh	Yes	Yes	0.449	0.159[x]	21.3	0.227[x]	0.109	5.3[x]	35.6	26.6	0.262[x]	2.5	0.089
Oxidized	Yes	No	0.474[x]	0.164[x]	21.9	0.188	0.109[x]	5.4[x]	36.6	25.1	0.308	2.2	0.102
Oxidized	Yes	Yes	0.442	0.140	21.6	0.193	0.107	5.9	36.3	26.2	0.289[x]	2.2	0.106
Pooled SD			0.034	0.039	1.254	0.030	0.017	0.365	1.294	2.348	0.041	0.306	0.017
PC[2]			0.443	0.151[x]	21.4	0.204[x]	0.101[x]	5.3	36.0[x]	27.0	0.245	2.5[x]	0.099
Pooled SD[3]			0.040	0.051	0.909	0.026	0.014	0.456	1.100	1.982	0.043	0.240	0.019

	----------Probability----------										
Oil quality	0.449	0.066	0.991	<.001	0.378	0.144	0.001	0.976	0.039	<.001	0.116
Phytase	0.786	0.316	0.333	0.061	0.301	0.295	0.830	0.053	0.705	0.715	0.029
Vitamin E	0.026	0.424	0.431	0.605	0.878	0.072	0.673	0.367	0.134	0.681	0.287
Oil quality x vitamin E	0.355	0.967	0.709	0.339	0.557	0.383	0.717	0.521	0.715	0.536	0.863
Phytase x oil quality	0.136	0.003	0.824	0.557	0.349	0.026	0.550	0.853	0.471	0.595	0.268
Phytase x vitamin E	0.284	0.380	0.393	0.878	0.785	0.731	0.663	0.606	0.741	0.666	0.411
Phytase x oil quality x vitamin E	0.318	0.597	0.532	0.320	0.482	0.079	0.538	0.889	0.882	0.580	0.227
PC vs. NC	0.280	0.340	0.429	0.008	0.042	0.997	0.316	0.815	0.194	0.693	0.305

[a–b]Means with different superscripts within the same column differ significantly ($P < 0.05$).

[1]Negative control (NC) diet

[2]PC=positive control

[3]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

[x]Values represent the mean of 7 replicate cages (pooled excreta of 6 birds per replicate) except for mean values with x and y where the number of replicates (n) was 6 and 5, respectively.

Table 3. 11 continued. Main and simple effects of oil quality, phytase, and vitamin E supplementation on selected fatty acids in the abdominal fat of 21 day-old broiler chickens (%)*

Oil quality	Phytase	Vitamin E	C20:1	C20:2	C20:3	C20:4	C22:0	∑SFA	∑MUFA	∑PUFA	∑UFA	SFA:UFA
	Main effect											
Fresh			0.333	0.116[a]	0.165	0.234	0.047	28.0	41.6[b]	30.1[a]	71.7	0.392
Oxidized			0.324	0.101[b]	0.156	0.219	0.050	28.1	43.6[a]	28.1[b]	71.6	0.396
	No		0.321	0.113	0.161	0.232	0.051	28.2	42.5	29.0	71.6	0.396
	Yes		0.336	0.104	0.160	0.211	0.046	28.0	42.7	29.2	71.7	0.392
		No	0.334	0.106	0.159	0.219	0.047	28.0	42.8	28.8	71.6	0.393
		Yes	0.323	0.110	0.162	0.224	0.050	28.1	42.4	29.4	71.7	0.395
	2-way interaction effect											
	Phytase x oil quality											
Fresh	No	-	0.323	0.121	0.165	0.234	0.049	28.2	41.2	29.9	71.5	0.399
Fresh	Yes	-	0.339	0.111	0.166	0.214	0.045	28.1	43.8	28.0	71.7	0.393
Oxidized	No	-	0.314	0.105	0.158	0.229	0.052	27.8	42.1	30.2	72.0	0.385
Oxidized	Yes	-	0.334	0.096	0.154	0.209	0.047	28.1	43.3	28.2	71.5	0.400
	Dietary treatments											
Fresh	No	No[1]	0.330	0.119[x]	0.156[x]	0.241	0.048	28.4[x]	41.5[x]	29.4[x]	71.1[y]	0.407[y]
Fresh	No	Yes	0.324[x]	0.122[x]	0.174[x]	0.228	0.050	28.0[x]	40.8[x]	30.4[x]	71.9[x]	0.391[x]
Oxidized	No	No	0.322[x]	0.105	0.153	0.190[x]	0.046	27.9[x]	43.7	27.8[y]	71.9[x]	0.389[x]
Oxidized	No	Yes	0.307	0.105	0.163	0.268	0.058[x]	28.3	43.9[x]	28.3	71.5[x]	0.398[x]
Fresh	Yes	No	0.339	0.112[x]	0.165	0.221	0.047	27.9	42.1[x]	30.1[x]	72.2[x]	0.384[x]
Fresh	Yes	Yes	0.338	0.110	0.167	0.206[x]	0.043[x]	27.6[x]	42.1[x]	30.4[x]	71.8[x]	0.385[x]
Oxidized	Yes	No	0.346	0.090[x]	0.161[x]	0.223[x]	0.046	27.9[x]	44.0[x]	28.0	71.5[x]	0.392[x]
Oxidized	Yes	Yes	0.322[x]	0.103[x]	0.146	0.194[x]	0.049	28.4[y]	42.7[x]	28.4[x]	71.5[x]	0.408[x]
	Pooled SD		0.033	0.022	0.034	0.069	0.016	1.236	1.542	2.400	1.253	0.023
	PC[2]		0.371	0.104	0.159[x]	0.224	0.050	27.5	41.5[x]	29.4[x]	71.4[x]	0.387[x]
	Pooled SD[3]		0.034	0.043	0.026	0.078	0.014	1.163	2.369	1.652	1.421	0.013

						Probability				
Oil quality	0.348	0.018	0.325	0.784	0.507	0.778	<.000	0.006	0.690	0.485
Phytase	0.087	0.158	0.870	0.285	0.335	0.585	0.574	0.725	0.655	0.592
Vitamin E	0.211	0.590	0.723	0.785	0.484	0.878	0.320	0.448	0.944	0.718
Oil quality x vitamin E	0.368	0.669	0.530	0.317	0.335	0.301	0.807	0.920	0.590	0.136
Phytase x oil quality	0.667	0.934	0.765	0.989	0.906	0.534	0.125	0.875	0.325	0.130
Phytase x vitamin E	0.933	0.730	0.289	0.151	0.388	0.903	0.680	0.786	0.597	0.374
Phytase x oil quality x vitamin E	0.727	0.497	0.831	0.177	0.881	0.944	0.195	0.814	0.266	0.690
PC vs. NC	0.040	0.551	0.834	0.699	0.748	0.197	0.984	0.987	0.677	0.031

[a–b]Means with different superscripts within the same column differ significantly ($P < 0.05$).

[1]Negative control (NC) diet

[2]PC=positive control

[3]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

[x-y]Values represent the mean of 7 replicate cages (pooled excreta of 6 birds per replicate) except for mean values with x and y where the number of replicates (n) was 6 and 5, respectively.

*∑represents the sum of: SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; UFA = unsaturated fatty acids. SFA:UFA = ratio of saturated fatty acids to unsaturated fatty acids

Table 3. 12 Main and simple effects of oil quality, phytase, and vitamin E supplementation on selected fatty acids in the subcutaneous fat of 21 day-old broiler chickens (%)

Oil quality	Phytase	Vitamin E	C14:0	C14:1	C16:0	C18:0	C18:1	C18:2	C18:3 n-3
	Main effect								
Fresh			0.473	0.152	22.3	5.70	35.2^{b}	26.1	2.35^{a}
Oxidized			0.470	0.155	22.3	5.76	36.1^{a}	25.5	2.10^{b}
	No		0.470	0.153	22.4	5.67	35.6	25.7	2.24
	Yes		0.472	0.155	22.2	5.78	35.7	25.8	2.22
		No	0.481^{a}	0.158	22.6^{a}	5.74	35.7	25.4	2.17
		Yes	0.461^{b}	0.150	21.9^{b}	5.71	35.5	26.2	2.29
	2-way interaction effect								
	Phytase x oil quality								
Fresh	No	-	0.458^{b}	0.145^{b}	22.2	5.75	35.2	26.3	2.40
Fresh	Yes	-	0.487^{a}	0.160^{ab}	22.3	5.64	35.2	25.9	2.30
Oxidized	No	-	0.482^{ab}	0.161^{a}	22.6	5.59	35.9	25.2	2.08
Oxidized	Yes	-	0.457^{b}	0.149^{ab}	22.0	5.92	36.2	25.7	2.13
	Dietary treatments								
Fresh	No	No[1]	0.466	0.154^{x}	22.4	^{x}5.67^{ab}	35.5	26.2	2.40
Fresh	No	Yes	0.450^{x}	0.135	21.9^{x}	5.82^{ab}	35.0^{x}	26.3	2.40
Oxidized	No	No	0.484^{x}	0.16^{x}	22.8	^{x}5.65^{ab}	36.1^{x}	24.9	2.00
Oxidized	No	Yes	0.481	0.162	22.5	^{x}5.54^{b}	35.7	25.5	2.14
Fresh	Yes	No	0.506	0.159	23.1	5.87^{ab}	34.9^{x}	25.4	2.16^{x}
Fresh	Yes	Yes	0.468	0.161	21.5^{x}	5.41^{b}	35.6	26.5	2.43
Oxidized	Yes	No	0.470	0.157	22.3	5.79^{ab}	36.5	24.9	2.09
Oxidized	Yes	Yes	0.444^{x}	0.141^{x}	21.7	6.06^{a}	35.9	26.5	2.18
	Pooled SD		0.037	0.019	1.066	0.425	1.155	2.159	0.244
	PC[2]		0.450	0.142	21.9	5.7	35.4	26.4^{x}	2.48
	Pooled SD[3]		0.035	0.016	0.856	0.285	1.250	0.077	0.253
			…..……Probability……..						

Oil quality	0.757	0.549	0.953	0.534	0.015	0.297	< 0.001
Phytase	0.795	0.676	0.407	0.365	0.534	0.899	0.783
Vitamin E	0.043	0.165	0.020	0.762	0.508	0.147	0.071
Oil quality x vitamin E	0.524	0.873	0.337	0.268	0.346	0.692	0.909
Phytase x oil quality	0.012	0.014	0.173	0.065	0.572	0.455	0.262
Phytase x vitamin E	0.252	0.849	0.253	0.631	0.487	0.421	0.432
Phytase x oil quality x vitamin E	0.999	0.083	0.470	0.038	0.267	0.977	0.229
PC vs. NC	0.419	0.183	0.280	0.947	0.909	0.874	0.564

[a–b]Means with different superscripts within the same column differ significantly ($P < 0.05$).

[1]Negative control (NC) diet

[2]PC=positive control

[3]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

[x]Values represent the mean of 7 replicate cages (pooled excreta of 6 birds per replicate) except for mean values with x and y where the number of replicates (n) was 6 and 5, respectively.

Table 3.12 continued. Main and simple effects of oil quality, phytase, and vitamin E supplementation on selected fatty acids in the subcutaneous fat of 21 day-old broiler chickens (%)

Oil quality	Phytase	Vitamin E	C20:0	C22:0	∑SFA	∑MUFA	∑PUFA	∑UFA	SFA:UFA
	Main effect								
Fresh			0.095[b]	0.041	28.7	41.4[b]	29.1	70.6	0.409
Oxidized			0.111[a]	0.043	28.4	42.7[a]	28.5	71.2	0.399
	No		0.106	0.044	28.6	42.1	28.9	70.9	0.403
	Yes		0.099	0.040	28.5	42.0	28.7	70.9	0.405
		No	0.106	0.042	28.8	42.2	28.2[b]	70.6[b]	0.413[a]
		Yes	0.099	0.041	28.3	41.9	29.5[a]	71.3[a]	0.395[b]
	2-Way interaction effect								
	Oil quality x vitamin E								
Fresh	-	No	0.099	0.042	29.3[a]	41.8	28.4	70.0	0.422
Fresh	-	Yes	0.091	0.040	28.1[b]	41.0	29.8	71.2	0.396
Oxidized	-	No	0.113	0.043	28.4[b]	42.7	27.9	71.1	0.404
Oxidized	-	Yes	0.108	0.042	28.4[b]	42.7	29.2	71.3	0.395
	Phytase x vitamin E								
-	No	No	0.102	0.041	28.5[ab]	42.4	28.6	70.8	0.410
-	No	Yes	0.109	0.046	28.6[ab]	41.9	29.2	71.1	0.396
-	Yes	No	0.11	0.043	29.1[a]	42.1	27.7	70.4	0.416
-	Yes	Yes	0.089	0.036	27.9[b]	41.8	29.8	71.4	0.395
	Dietary treatments								
Fresh	No	No[1]	0.100	[x]0.045[ab]	29.1[x]	42.2[x]	28.7[x]	70.5[x]	0.416[x]
Fresh	No	Yes	0.100	[x]0.042[ab]	28.6[x]	40.8[x]	29.4	70.9[x]	0.401[x]
Oxidized	No	No	0.104	0.037[ab]	28.0	42.6	28.5	71.1	0.405
Oxidized	No	Yes	0.118	[x]0.051[a]	28.6[x]	42.9[x]	29.0[x]	71.3[x]	0.390[x]
Fresh	Yes	No	0.097	0.039[ab]	29.5[x]	41.4[x]	28.2[x]	69.6[x]	0.428[x]
Fresh	Yes	Yes	0.082[x]	[x]0.039[ab]	27.6[x]	41.2[y]	30.2[x]	71.5[x]	0.390[x]
Oxidized	Yes	No	0.122[x]	[x]0.048[a]	28.7[x]	42.8[x]	27.3[x]	71.2[x]	0.403[x]
Oxidized	Yes	Yes	0.098[x]	0.034[b]	28.2	42.5	29.4	71.3	0.399

Pooled SD	0.026	0.012[ab]	0.740	1.292	1.734	0.954	0.018
PC[2]	0.091[x]	0.044	28.4[y]	42.0[x]	29.5[x]	71.5[x]	0.398[x]
Pooled SD[3]	0.015	0.007	1.023	1.387	2.086	1.099	0.020
				Probability			
Oil quality	0.038	0.641	0.284	0.003	0.326	0.065	0.111
Phytase	0.387	0.254	0.931	0.657	0.779	0.921	0.690
Vitamin E	0.445	0.802	0.065	0.367	0.031	0.043	0.004
Oil quality x vitamin E	0.819	0.852	0.040	0.332	0.955	0.128	0.170
Phytase x oil quality	0.570	0.850	0.450	0.850	0.648	0.729	0.781
Phytase x vitamin E	0.067	0.079	0.039	0.734	0.244	0.265	0.600
Phytase x oil quality x vitamin E	0.419	0.032	0.812	0.242	0.871	0.168	0.141
PC vs. NC	0.329	0.893	0.149	0.780	0.461	0.127	0.119

[a–b]Means with different superscripts within the same column differ significantly ($P < 0.05$).

[1]Negative control (NC) diet

[2]PC=positive control

[3]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

[x-y]Values represent the mean of 7 replicate cages (pooled excreta of 6 birds per replicate) except for mean values with x and y where the number of replicates (n) was 6 and 5, respectively.

*$\sum$represents the sum of: SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; UFA = unsaturated fatty acids. SFA:UFA = ratio of saturated fatty acids to unsaturated fatty acids

Table 3. 13 Main and simple effects of oil quality, phytase, and vitamin E supplementation on selected fatty acids in the liver of 21-day-old broiler chickens (%)

Oil quality	Phytase	Vitamin E	C14:1	C16:0	C17:0	C17:1	C18:1	C18:2	C18:3 n-3	C20:0	C20:1
Main effect											
Fresh			0.159	23.0	0.088	0.091	23.6	17.0^{a}	0.384^{a}	0.070	0.215
Oxidized			0.157	23.2	0.075	0.076	25.1	15.8^{b}	0.312^{b}	0.076	0.227
	No		0.159	23.0	0.085	0.108^{a}	24.5	16.7	0.354	0.071	0.221
	Yes		0.157	23.2	0.077	0.060^{b}	24.2	16.2	0.343	0.075	0.221
		No	0.156	23.0	0.085	0.103^{a}	23.4	16.6	0.339	0.068	0.238
		Yes	0.160	23.2	0.077	0.065^{b}	25.3	16.2	0.358	0.078	0.204
2-way interaction effect											
Oil quality x vitamin E											
Fresh	-	No	0.166	23.1	0.084^{ab}	0.105	22.4	17.0	0.382	0.080^{ab}	0.232
Fresh	-	Yes	0.151	23.0	0.091^{a}	0.077	24.9	17.1	0.387	0.059^{ab}	0.199
Oxidized	-	No	0.146	23.0	0.086^{ab}	0.100	24.5	16.3	0.296	0.057^{b}	0.245
Oxidized	-	Yes	0.169	23.4	0.063^{b}	0.053	25.8	15.4	0.329	0.095^{a}	0.209
Phytase x oil quality											
Fresh	No	-	0.148^{b}	22.6	0.089	0.110	24.5	17.2	0.396	0.077	0.239^{ab}
Fresh	Yes	-	0.169^{a}	23.4	0.082	0.106	24.6	16.1	0.312	0.066	0.203^{ab}
Oxidized	No	-	0.169^{a}	23.5	0.086	0.073	22.7	16.9	0.373	0.063	0.192^{b}
Oxidized	Yes	-	0.146^{b}	23.0	0.067	0.047	25.7	15.6	0.312	0.086	0.251^{a}
Phytase x vitamin E											
-	No	No	0.145^{b}	22.9	0.095	0.141^{a}	23.1	16.9	0.335	0.072	0.239
-	No	Yes	0.172^{a}	23.1	0.076	0.074^{b}	26.0	16.4	0.373	0.070	0.203
-	Yes	No	0.167^{ab}	23.2	0.075	0.064^{b}	23.7	16.4	0.343	0.064	0.238
-	Yes	Yes	0.147^{ab}	23.3	0.079	0.056^{b}	24.7	16.1	0.342	0.085	0.205
Dietary treatments											
Fresh	No	No[1]	0.134^{b}	22.7	0.092	0.139^{x}	22.3	17.4^{x}	0.392^{x}	0.094^{x}	0.240^{x}
Fresh	No	Yes	0.162^{ab}	22.4	0.086	0.080	26.8^{x}	17.0	0.400	0.059^{x}	0.238
Oxidized	No	No	^{x}0.156^{ab}	23.0	0.097	0.143^{x}	24.0	16.4	0.277^{x}	0.051^{x}	0.238

Oxidized	No	Yes	0.182[ab]	23.8	0.066	0.068[x]	25.1	15.8	0.347	0.081	0.168
Fresh	Yes	No	[x]0.199[a]	23.5	0.075	0.072	22.4[x]	16.6	0.372[x]	0.066[x]	0.223
Fresh	Yes	Yes	0.139[ab]	23.5[x]	0.097	0.074	23.0	17.1[x]	0.374[x]	0.061[x]	0.161
Oxidized	Yes	No	[x]0.136[ab]	23.0	0.074[x]	0.056	25.0	16.2	0.314	0.062[x]	0.253[x]
Oxidized	Yes	Yes	0.155[ab]	23.0[x]	0.061	0.038[x]	26.4	15.0[x]	0.310[x]	0.110	0.250
	Pooled SD		0.035	1.452	0.026	0.046	3.459	1.655	0.059	0.046	0.081
	PC[2]		0.168	22.7[x]	0.090	0.083	24.0	17.2	0.441	0.073[x]	0.216
	Pooled SD[3]		0.053	0.704	0.023	0.058	3.871	1.224	0.060	0.044	0.065
					----------Probability----------						
Oil quality			0.894	0.657	0.067	0.259	0.116	0.011	<.001	0.647	0.597
Phytase			0.910	0.511	0.218	0.001	0.713	0.341	0.500	0.792	0.982
Vitamin E			0.707	0.740	0.303	0.006	0.051	0.383	0.252	0.473	0.129
Oil quality x vitamin E			0.058	0.500	0.037	0.484	0.509	0.305	0.396	0.029	0.933
Phytase x oil quality			0.026	0.090	0.442	0.401	0.118	0.886	0.491	0.202	0.037
Phytase x vitamin E			0.020	0.849	0.102	0.029	0.333	0.852	0.234	0.369	0.937
Phytase x oil quality x vitamin E			0.046	0.437	0.747	0.928	0.294	0.386	0.299	0.828	0.162
PC vs. NC			0.254	0.987	0.823	0.110	0.423	0.766	0.175	0.440	0.523

[a–b]Means with different superscripts within the same column differ significantly ($P < 0.05$).

[1]Negative control (NC) diet

[2]PC=positive control

[3]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

[x]Values represent the mean of 7 replicate cages (pooled excreta of 6 birds per replicate) except for mean values with x and y where the number of replicates (n) was 6 and 5, respectively.

Table 3.13 continued. Main and simple effects of oil quality, phytase, and vitamin E supplementation on selected fatty acids in the liver of 21-day-old broiler chickens (%)*

Oil quality	Phytase	Vitamin E	C20:2	C22:0	C22:4	C24:0	C24:1	∑SFA	∑MUFA	∑PUFA	∑UFA	SFA:UFA
	Main effect											
Fresh			0.334[a]	0.429[a]	0.200	0.114	0.447[a]	40.2	28.6	29.4[a]	58.0	0.694
Oxidized			0.290[b]	0.380[b]	0.160	0.102	0.244[b]	40.4	30.0	27.6[b]	58.0	0.707
	No		0.303	0.385	0.204	0.111	0.314	40.2	29.5	29.4[a]	59.0[a]	0.682
	Yes		0.321	0.424	0.157	0.105	0.377	40.5	29.0	27.6[b]	56.9[b]	0.720
		No	0.308	0.419	0.216	0.111	0.415	40.5	28.2	28.6	57.2	0.721[a]
		Yes	0.316	0.390	0.144	0.105	0.276	40.2	30.3	28.4	58.9	0.680[b]
	2-way interaction effect											
	Oil quality x vitamin E											
Fresh	-	No	0.318	0.437	0.198	0.108	0.447	40.7	27.5	29.0	57.0	0.722
Fresh	-	Yes	0.351	0.421	0.203	0.120	0.180	39.8	29.6	29.9	59.1	0.667
Oxidized	-	No	0.299	0.401	0.234	0.114	0.383	40.4	29.0	28.3	57.3	0.721
Oxidized	-	Yes	0.281	0.360	0.086	0.090	0.372	40.5	30.9	26.9	58.7	0.694
	Phytase x oil quality											
Fresh	No	-	0.336	0.418	0.170[ab]	0.108	0.400	39.3[b]	29.7	30.7	59.9[a]	0.645[b]
Fresh	Yes	-	0.270	0.352	0.238[a]	0.113	0.227	41.1[a]	29.4	28.1	58.1[ab]	0.718[a]
Oxidized	No	-	0.333	0.440	0.231[a]	0.120	0.494	41.2[a]	27.5	28.2	56.1[b]	0.744[a]
Oxidized	Yes	-	0.309	0.408	0.082[b]	0.091	0.261	39.8[ab]	30.5	27.1	57.9[ab]	0.696[ab]
	Phytase x vitamin E											
-	No	No	0.286	0.403	0.315[a]	0.128[a]	0.447	40.6	28.0	29.9	58.8	0.698
-	No	Yes	0.320	0.367	0.092[b]	0.093[b]	0.180	39.9	31.1	28.9	59.3	0.666
-	Yes	No	0.330	0.435	0.117[b]	0.094[b]	0.383	40.5	28.5	27.4	55.5	0.745
-	Yes	Yes	0.312	0.414	0.196[ab]	0.116[ab]	0.372	40.5	29.5	27.9	58.5	0.695
	Dietary Treatments											
Fresh	No	No[1]	0.303[x]	0.432	0.251[x]	0.125[x]	0.581	40.1	27.2	31.2[x]	58.9[x]	0.679[x]
Fresh	No	Yes	0.368	0.404	0.088[x]	0.092	0.219	38.5[x]	32.2[x]	30.2[x]	61.1[x]	0.611[x]
Oxidized	No	No	0.270[x]	0.375[x]	0.379	0.131[x]	0.313	41.0	28.9	28.7	58.7[x]	0.717

Oxidized	No	Yes	0.271	0.329	0.096[x]	0.095	0.141[x]	41.2	30.0	27.5	57.5	0.720
Fresh	Yes	No	0.333[x]	0.443	0.145[x]	0.092[x]	0.502	41.2	27.8[x]	26.8	55.1[x]	0.765[x]
Fresh	Yes	Yes	0.333[x]	0.437[x]	0.318	0.149	0.486	41.2[x]	27.1	29.5[x]	57.1[x]	0.723[x]
Oxidized	Yes	No	0.328	0.427	0.090[x]	0.097	0.265[x]	39.8[x]	29.1	27.9[x]	55.9	0.725
Oxidized	Yes	Yes	0.291[x]	0.390[x]	0.075[x]	0.084	0.258	39.8[x]	31.9	26.4[x]	59.9[x]	0.667[x]
	Pooled SD		0.071	0.082	0.151	0.041	0.291	4.502	4.501	2.328	3.820	0.074
	PC[2]		0.318	0.442	0.095[x]	0.095[x]	0.180[x]	36.4[x]	31.0[x]	29.1	58.2	0.626[x]
	Pooled SD[3]		0.059	0.059	0.128	0.044	0.351	2.133	4.153	2.864	3.107	0.067
			----------Probability----------									
Oil quality			0.032	0.036	0.350	0.279	0.015	0.743	0.218	0.029	0.941	0.492
Phytase			0.373	0.089	0.279	0.639	0.439	0.632	0.633	0.034	0.026	0.051
Vitamin E			0.708	0.210	0.100	0.589	0.081	0.536	0.076	0.762	0.057	0.034
Oil quality x vitamin E			0.219	0.600	0.079	0.116	0.531	0.425	0.920	0.174	0.687	0.477
Phytase x oil quality			0.304	0.451	0.015	0.128	0.676	0.009	0.148	0.347	0.044	0.002
Phytase x vitamin E			0.204	0.728	0.001	0.015	0.109	0.582	0.386	0.320	0.164	0.639
Phytase x oil quality x vitamin E			0.743	0.878	0.693	0.141	0.569	0.424	0.113	0.232	0.138	0.256
PC vs. NC			0.660	0.767	0.061	0.274	0.065	0.164	0.151	0.133	0.744	0.244

[a–b]Means with different superscripts within the same column differ significantly ($P < 0.05$).

[1]Negative control (NC) diet

[2]PC=positive control

[3]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

[x]Values represent the mean of 7 replicate cages (pooled excreta of 6 birds per replicate) except for mean values with x and y where the number of replicates (n) was 6 and 5, respectively.

*∑represents the sum of: SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; UFA = unsaturated fatty acids. SFA:UFA = ratio of saturated fatty acids to unsaturated fatty acids

Table 3. 14 Comparison of selected fatty acids in the adipose and liver tissues regardless of dietary treatments[1]

Fatty acid composition, %	Abdominal fat	Subcutaneous fat	Liver	Pooled SD	P-value
C16:0 (Palmitic acid)	21.6[c]	22.3[b]	23.1[a]	1.210	<.001
C18:0 (Stearic acid)	5.4[b]	5.7[b]	16.2[a]	1.212	<.001
C18:1 (Oleic acid)	35.9[a]	35.6[a]	24.3[b]	2.373	<.001
C18:2 (Linoleic acid)	26.2[a]	25.8[a]	16.5[b]	2.014	<.001
C18:3n-3 (Alpha-linoleic acid)	2.364[a]	2.257[b]	0.360[c]	0.257	<.001
C20:4 (Arachidonic acid)	0.222[b]	0.197[b]	7.935[a]	0.846	<.001
ΣSFA	28.0[b]	28.5[b]	39.9[a]	2.030	<.001
ΣMUFA	42.5[a]	42.0[a]	29.4[b]	2.931	<.001
ΣPUFA	29.1	28.9	28.5	2.484	0.461
ΣUFA	71.6[a]	70.9[a]	57.9[b]	2.334	<.001
SFA:UFA	0.393[b]	0.404[b]	0.694[a]	0.051	<.001

[1]The deposition of the most abundant FAs (C16:0, C18:0, and C18:1) and the essential FAs (C18:2, C18:3n-3, and C20:4) were compared across the abdominal fat, subcutaneous fat and the liver regardless of dietary treatments, using the GLM procedure of SAS.

*∑represents the sum of: SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; UFA = unsaturated fatty acids. SFA:UFA = ratio of saturated fatty acids to unsaturated fatty acids

Table 3. 15 Comparison of selected fatty acids in the abdominal and subcutaneous tissues regardless of dietary treatments[1]

Fatty acid composition, %	Abdominal fat	Subcutaneous fat	Pooled SD	P-value
C16:0 (Palmitic acid)	21.6[b]	22.3[a]	1.125	0.002
C18:0 (Stearic acid)	5.4[b]	5.7[a]	0.408	<.001
C18:1 (Oleic acid)	35.9	35.6	1.254	0.200
C18:2 (Linoleic acid)	26.2	25.8	2.158	0.416
C18:3n-3 (Alpha-linoleic acid)	2.364	2.257	0.308	0.058
C20:4 (Arachidonic acid)	0.222[a]	0.197[b]	0.062	0.024
ΣSFA	27.9[b]	28.5[a]	1.112	0.011
ΣMUFA	42.5	42.1	1.738	0.198
ΣPUFA	29.1	28.9	2.188	0.639
ΣUFA	71.6[a]	71.0[b]	1.225	0.008
SFA:UFA	0.393[b]	0.404[a]	0.022	0.016

[1]The deposition of the most abundant FAs (C16:0, C18:0, and C18:1) and the essential FAs (C18:2, C18:3n-3, and C20:4) were compared across the abdominal and subcutaneous fats regardless of dietary treatments, using the GLM procedure of SAS.

*∑represents the sum of: SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; UFA = unsaturated fatty acids. SFA:UFA = ratio of saturated fatty acids to unsaturated fatty acids

3.5 Discussion

3.5.1 Lipid Oxidation, Diet Composition, and Growth Performance

Lipid sources rich in (PUFA) are prone to oxidation that can occur both at low and high temperatures. At low temperatures, for instance during the storage of unsaturated fats at ambient temperature, lipid hydroperoxides (LHPs), which are the primary products of oxidation, increase until they reach a plateau, and then decompose into secondary oxidation compounds (Tres et al., 2010). At high temperatures, the decomposition of LHPs is faster and the isomerization of FAs is also favored, leading to the appearance of both geometric and positional FA isomers. A simple and easy way of measuring LHP content in fats and oils is the peroxide value (PV). In the current study, the PV rose from 3 meqO_2/kg in the fresh oil to 109 meqO_2/kg in the oxidized oil after exposure to thermal treatment at 95 ^oC for about 84 cumulative hours. Delles et al. (2013) achieved a PV of 121 meqO_2/kg in soy oil after heating the fresh oil for 7 days at 95 ^oC.

The use of phytase in broiler diets has been well researched (Dilger et al., 2004; Panda et al., 2007; Walters et al., 2019; Broch et al., 2020). For this study, the target phytase activity level in the phytase-supplemented diets was 1,000 FTU/kg of diet but the analyzed values ranged from 902 to 1380 FTU/kg, which was still within acceptable standards based on the typically high degree of variability associated with phytase analysis (Gizzi et al., 2008). In the current study, phytase supplementation significantly improved feed efficiency on day 14 and this benefit persisted till day 21. This is supported by Dos Santos et al. (2012) who reported an improvement (reduction) in the feed conversion ratio of chicks given a marginally P-deficient diet supplemented with 1,000 and 1,500 FTU/kg of phytase. These

results agree with the fact that the hydrolysis of phytate by phytase can reduce the anti-nutritional effects of phytate, thereby improving performance, especially the feed efficiency. However, in the current study, phytase did not affect the FI or BWG, neither did it interact with the other factors. While the beneficial effects of phytase for growth performance have been well documented (Ravindran et al., 2006; Panda et al., 2007), a few studies have also reported no benefit. For instance, Atker et al. (2019) reported no benefit for BWG or FI when phytase was supplied at 500 FTU/g to broiler chickens for 35 days.

In the current study, there was no significant difference in the growth performance of birds fed the PC and NC diets, suggesting that there could have been an excessive accumulation of P in the body and bones of chicks during the first seven days when all birds were fed a corn-SBM-based broiler starter diet containing the adequate level of NPP. For future studies, we would avoid feeding a pre-experimental diet and consider increasing the length of time during which birds are on the marginally deficient NPP experimental diets. Moreover, the NPP inclusion level for this study was based on NRC recommendation of 0.45% for birds of this age (NRC, 1994), but various studies have suggested that the actual P requirement of broiler chickens may be lower than the NRC recommendation (NRC, 1994) depending on factors such as age and phytase supplementation (Waldroup et al., 2000; Yan et al., 2001). The available P requirement of chicks from days 0 to 21 has been determined to be 0.39% (Waldroup et al., 2000), while birds aged 21 to 42 days require 0.33% available phosphorus (Yan et al., 2001). The NC diet in the current study was formulated with a calculated NPP of 0.31%, which may be too close to the required NPP level of 0.39% required for birds of this age as reported by Waldroup et al. (2000).

The results of this study also show that there were no simple or main effects of feeding oxidized oils on all growth performance parameters across both periods considered. This was quite unexpected as we had hypothesized that oxidized oils would depress performance (Gray et al., 1996; Tavárez et al., 2011). This hypothesis was based on many past studies that have reported depression in growth performance for broiler chickens due to the presence of various oxidation products that negatively impact the chickens (Takahashi and Akiba, 1999; Anjum et al., 2004; Tavárez et al., 2011). However, based on other studies that reported no negative effect, we suspect that the deleterious impacts of oxidized oils may not be immediately noticed in the short term depending on factors such as the degree of lipid oxidation of the oils and the length of the feeding trial. This is because some studies have reported that when heated oils are added to feeds, it may not lead to toxic effects in animals when the lipids are added to such feeds at the usual amounts, and if the lipid contains less than 25% of polar compounds (Marquez-Ruiz and Dobarganes, 1996; Billek, 2000).

The results of this study showed VE supplementation at 150 ppm improved the live weight, BWG, and FI over days 7-14, although these benefits were not observed by day 21. This beneficial effect of VE, although short-lived was very much expected because as an antioxidant, VE inhibits the propagation of lipid oxidation, thereby protecting nutrients from degradation and improving the efficiency of intestinal absorption by reducing oxidative load within the gut. The short-lived benefit of VE supplementation suggests that there may be a limit to the degree to which antioxidants such as VE can help mitigate the deleterious effects of oxidized oils especially when added at supra-nutritional levels.

Further investigation involving graded levels of mixed tocopherols on broiler performance may be required in this regard.

3.5.2 Relative Liver and Fat Percentages

The results of this study showed that there were no significant simple or main effects of all the factors on fat percentages. However, there was a main effect of oil quality on liver percentage. The size of the liver relative to body weight is known as the relative liver weight or hepatosomatic index and it serves as an indicator of toxicity in biological systems (Juberg et al., 2006). In the current study, oxidized oils alone significantly increased the relative liver percentage and this agrees with many past studies that have shown that feeding oxidized lipids causes an increased liver size in broilers (Anjum et al., 2004), rodents (Huang et al., 1988; Eder, 1999) and swine (Liu, 2012). Although the specific mechanism behind this is not entirely clear, the increases in liver size may be due to increased enzyme synthesis to ameliorate toxicity (Huang et al., 1988) or greater hepatocyte proliferation (Dibner et al., 1996). However, it is still unclear whether these changes in liver size affect hepatic metabolism and the efficiency of nutrient utilization.

3.5.3 Bone Breaking Strength, Bone Ash, and Bone Lipid

In this study, the PC diet resulted in a higher BBS and ash content compared to the NC diet containing a marginally low level of NPP without phytase or VE supplementation. This is in agreement with the results of Lan et al. (2012), who fed 2.1 g/kg NPP to broiler chicks for 21 days and found a significantly reduced tibia ash, Ca, and P content, compared to those fed adequate NPP levels. Similarly, Panda et al. (2007) also reported an increased incidence of leg abnormality and a significant decrease in BBS and tibia ash when birds

were fed diets containing 0.3% and 0.35% NPP for 21 days. In the current study, phytase supplementation improved BBS and ash content. This was expected because phytase hydrolyzes phytic acid to make more phytate-bound phosphorus available to the birds. Studies have also reported improvement in BBS and bone ash when phytase was added to low-NPP diets (Panda et al., 2007; Lalpanmawia et al., 2014).

3.5.4 Nutrient and Energy Digestibility and Utilization

Although the PC diet did not affect ileal P digestibility, it produced a significantly lower total tract P utilization compared to the NC diet, but it did not affect the total tract utilization of DM, CF, N, Ca, and energy. It has been reported that birds fed higher NPP diets tend to excrete more P (Rousseau et al., 2012). We suspect that this is the case here. Interestingly, the PC diet reduced the ileal digestibility of Ca but did not affect DM, N, and P digestibilities. While the reason for this is not entirely clear, Driver et al. (2005) reported that 0.9% Ca (which was supplied in the PC diet) may be excessive for optimum bird performance and this may be a possible explanation for this effect.

The utilization of Ca was significantly improved, while that of CF was reduced by phytase supplementation, but no statistically significant benefit of phytase was observed for P and N utilizations. However, there was a numerical improvement in P utilization when phytase was supplemented and this improvement approached significance.. Although the improvement in Ca utilization as a result of phytase supplementation was very much expected, the reduction in CF utilization was not. Theoretically, phytate is a strong acid that can form various salts with essential minerals, thereby reducing their solubility and ultimately their absorption (Sandberg and Svanberg, 1991). But when phytate is hydrolyzed by phytase, it releases all constituent minerals, myo-inositol and

inorganic phosphates, thereby making these nutrients available for absorption, resulting in the significant improvement in Ca utilization and the numerical increase in P utilization.

The seemingly negative effect of phytase on CF observed in this study can be traced to a 2-way interaction between oil quality and phytase where CF utilization was highest in birds that were fed diets containing oxidized oil but no phytase, compared to those that received diets containing oxidized or fresh oil with phytase. This result was surprising for two reasons. Firstly, oxidized oils have a reputation of depressing CF utilization, rather than improving them (Inoue et al., 1984; Liu and Huang, 1995), secondly, the theoretical operational mechanism of phytase supports improved CF utilization, as it frees phytate-bound lipids, thereby making them available for absorption. (Rao et al., 1999; Ravindran et al., 2000). This negative effect of phytase could be due to the various unfavorable oxidation products present in the oxidized oils. Further analysis of the various oxidation products found in the oxidized oil may help understand the underlying mechanism driving this negative effect.

Surprisingly, phytase supplementation did not improve N and P utilization and this is contrary to various studies that have reported improvements in N and P utilization when phytase was fed. For instance, Panda et al. (2007) reported an improvement in P and N utilization when Phytase was added to low- NPP diets. Similarly, Sebastian et al. (1996) reported that supplementation of 600 FTU of microbial phytase/kg diet to 0.31% NPP diet enhanced the retention of N, Ca, and P. A possible reason for this lack of effect could be the short experimental period, as the birds were only on the experimental diets for 14 days. As expected, supplemental phytase improved the apparent ileal digestibility of DM, N, and P and this is supported by various past studies (Dilger et al., 2004; Onyango et al., 2005).

Phytase supplementation also improved the AME and AMEn regardless of oil quality or VE supplementation. The positive effects of phytase on the AME of corn-soy diets have been previously reported by various authors (Ravindran et al., 2006; Ledoux et al., 1999; Namkung and Leeson, 1999; Camden et al., 2001). In the present study, an improvement of 34 kcal/kg was observed in the AME when phytase was supplemental at 1,000 FTU/kg. This is comparable to an improvement of 43 kcal/kg observed in the low-phytate, corn-soy diets supplemented with 500 FTU of phytase/kg reported by Ravindran et al. (2006). Similarly, Camden et al. (2001) reported an improvement of 45 kcal/kg of DM while Namkung and Leeson (1999) reported a value of 65 kcal/kg. However, Dilger et al. (2004) found no effect of phytase on the apparent retention of energy in broiler chicks fed corn-soy diets. The mode of action underlying the effect of phytase on energy utilization is not fully understood, but it has been suggested that this effect is possibly multi-faceted, resulting from small, and possibly additive, improvements in the digestion of protein, fat, and starch (Camden et al., 2001). Furthermore, phytase products tend not to be entirely pure, as they contain small amounts of other enzymes which may also play a role in improving the utilization of other nutrients and eventually the AME and AMEn.

There was a significant interaction between phytase and oil quality, as the birds that received oxidized oil and phytase had a higher AME and AMEn compared to birds fed diets containing either fresh or oxidized oil without phytase supplementation. While the role of phytase in producing this effect was expected, that of oxidized oil is unclear. This is because past studies have associated a negative effect with oxidized oils regarding AME (Inoue et al., 1984).

This interaction was also observed in the ileal digestibility of N where birds that received fresh oil with phytase had a better N digestibility compared to those that received diets with fresh oil but no phytase supplementation and those fed oxidized oils with no phytase. To the best of our knowledge, there is no existing literature with data regarding the interactive effect of phytase and oil quality on N digestibility, but this effect was expected. This is because fresh oil has been reported to produce better N utilization than oxidized oils in pigs (Yuan et al., 2007); and phytase (Dilger et al., 2004; Onyango et al., 2005) has also been reported to promote the utilization of nutrients including N, so a beneficial interaction between these factors was expected.

There was also an interaction between oil quality and VE on CF utilization. Birds that were fed oxidized oil with no VE had significantly improved CF utilization compared to those fed oxidized oil with VE. This result completely contradicted the study hypothesis and is somewhat difficult to explain because most past studies have reported a negative impact of oxidized oil on CF utilization and VE supplementation either had a positive effect or no effect, but not a negative effect. One possible explanation could be that the VE had become completely oxidized and turned into VE radicals by the various oxidation products within the oxidized oil before its addition to the feed. This is possible because the VE was first mixed with the oils before the oils were mixed into the feed. Because VE is a radical-scavenging antioxidant, when it donates a proton to stabilize peroxyl/lipid radicals, it is converted into vitamin E radical, which may be further oxidized into α-tocopheryl quinone or reduced by vitamin C or other reducing compounds to regenerate vitamin E (Niki, 2015). But in the absence of these reducing agents, VE can act as a radical capable of oxidizing lipids or completely binding to lipid radicals (Rizvi et al., 2014), in which case, its effect

would be somewhat negative. Another possible reason could be the presence of certain harmful oxidation products which probably inhibited the antioxidant activity of VE. Further analysis of the oxidized oil regarding the types and level of the oxidation products may help in addressing this.

Furthermore, in the current study, a 3-way interaction was observed for P utilization, where birds that received diets containing fresh or oxidized oil with phytase and VE, as well as those that received oxidized oil with phytase but no VE, had a greater ($P < 0.05$) P-utilization compared to those that received oxidized oil with no phytase and no VE or fresh oil with phytase but no VE. This general improvement in the P utilization as a result of phytase supplementation was expected due to the greater bioavailability of P that results when phytic acid is hydrolyzed by phytase. Furthermore, just as observed with AME and AMEn, the results of this study show that phytase supplementation helps ameliorate the negative effects of oxidized oils as observed in the improved P utilization.

3.5.5 Fatty Acid Profiles

3.5.5.1 Fatty Acid Profile of Oil and Diet

Polyunsaturated FAs are very susceptible to oxidation (Belitz et al., 2009). During oxidation, lipids are ultimately degraded into a variety of primary, secondary, and tertiary products. Oils rich in PUFA can be oxidized through thermal processing in the presence of oxygen and this results in a decrease in the total percentage of unsaturated FAs, and an increase in the SFA content due to the oxidation of double bonds into fully saturated states (Yin et al., 2011). This process is accompanied by an increase in the production of various lipid oxidation products (Shurson et al., 2015). Interestingly, various volatile compounds

are produced by the oxidation of linoleic acid (Belitz et al., 2009). So, in the current study, the concentrations of linoleic (C18:2) and linolenic acid (C18:3) decreased by about 4% (from 52.9 to 50.8) and 12% (from 7.34 to 6.43), respectively after heating. There was also an increase in the total SFA and MUFA content and a decrease in the total PUFA and UFA content as the oils were oxidized. This is also reflected in the greater SFA:UFA ratio of oxidized oils. Other authors have also reported a decline in PUFA after oil oxidation (DeRouchey et al., 2004; Liu, 2012). The data obtained in the present study also agrees with the fact that PUFAs are degraded preferentially compared to other FAs during oxidation because SFA concentration changed minimally and the concentration of MUFA increased after heating.

As expected, the FA profile of the diet followed the FA profile of the oils added to it. Diets formulated with fresh oil (Diets A, B, E F, and PC) had a higher PUFA content and a lower SFA content compared to those formulated with oxidized oils (Diets C, D, G, and H). Interestingly, The SFA:UFA ratio of the diets also mimiced the oils used in formulating them.

3.5.5.2 Fatty Acid Profile of the Abdominal and Subcutaneous Fat

To the best of our knowledge, very few studies have examined the main or interactive effects of oil quality, phytase, and VE supplementation on the FA composition of the different fat depots in broiler chickens. The few studies that have investigated this focused either on FA digestibility or the FA profile of the muscle products such as the breast meat, but not of the adipose tissues or liver. Interestingly, the FA composition of various tissues in chickens differs markedly, and the most pronounced differences occur between adipose and non-adipose tissues (Marion and Woodroof, 1966). Thus it is difficult

to make comparisons between the FA profiles of muscle products and those of adipose or hepatic tissues.

It is well established that the FA profile of adipose tissues is influenced by the FA profile of the diet (Hwong et al., 1988) and this is mainly because dietarily non-essential FAs are directly incorporated into adipose tissues with little to no modification (Corino et al., 2002; Rentfrow et al., 2003; King et al., 2004). In the current study, there were no simple or main effects of oil quality, phytase, and VE supplementation on the content of SFA, UFA, and SFA:UFA in the abdominal fat tissue. But in both fat depots, oxidized lipids increased the total content of MUFA but decreased the total PUFA content of the abdominal fat. This trend can be traced back to the dietary oils where the total content of MUFA increased and PUFA content decreased as a result of lipid oxidation. Thus it is believed that the dietary levels of these groups of FAs played a significant role in influencing their levels in the adipose tissues. Interestingly, VE supplementation increased the total PUFA and UFA content but reduced the SFA:UFA in the subcutaneous fat. Furthermore, birds fed diets containing fresh oil but no VE had a much higher SFA content compared to other groups. Also, the birds that received phytase but no VE had a higher SFA content than those fed diets containing both phytase and VE supplementation. These results suggest that VE plays a role in reducing FA saturation in subcutaneous adipose tissues. This result was unexpected because as FA unsaturation increases, the peroxidizability of the lipids rises as well (Valk and Hornstra, 2000). So, as an antioxidant, we expected that VE supplementation would encourage biochemical processes that make for a reduced oxidative load and not the other way around.

Of all types of FAs, MUFA was the most abundant (>36%) in both fat depots and this dominance may be related to the difference between structural and depot lipids (Hrdinka et al., 1996). In both fat depots, regardless of dietary treatments, the most dominant FA is C18:1 (35.5%), followed by C18:2 (25.8%) and C16:0 (21.9%). This is in stark contrast to the diets where C18:2 (54.5%) was the most abundant, followed by C18:1 (23.4%) and C16:0 (12.2%). In swine studies, various authors have also reported that oleic acid (C18:1) is the most abundant FA in adipose tissues. For instance, Wang (2019) reported that in both the belly and back fat of pigs, C18:1 was the most abundant FA regardless of dietary treatment. This dominance may be related to the fact that the oleic acid content in tissue lipids depends not only on the oleic acid intake but also on *de novo* synthesis (Skřivan et al., 2018). This also suggests a relatively high metabolic activity of oleic acid in swine and poultry.

Interestingly, the FA profile of the adipose tissues of swine and poultry varies considerably, especially in the order of the most abundant FAs. For instance, Wang (2019) reported that the 3 most abundant FAs in the belly and back fat of pigs are C18:1 (~42%), C16:0 (~24%), and C18:2 (~11.5%). Conversely, from the results of this study, the 3 most abundant FAs in the abdominal and subcutaneous fats of the broiler chickens were C18:1 (~35%), C18:2 (~26%), and C16:0 (~22%). These variations in the locational deposition/synthesis of FAs between swine and poultry suggests that the biological importance of different FAs can differ markedly between mammalian and avian species.

Moving on, lipid oxidation increased the concentration of C18:1 and decreased the amount of C18:3n-3 in both abdominal and subcutaneous fats. This shows that there is a preferential degradation/synthesis of FAs when lipids are oxidized. Phytase

supplementation increased the concentration of C20:0 in the abdominal fat but not in the subcutaneous fat. This may be due to the improved FA digestibility that results from the hydrolysis of phytate, which reduces the amount of insoluble Ca-phytate soaps present in the gut of broiler chickens. But the further investigation will be required to understand the mechanism behind this preferential deposition.

3.5.5.3 Fatty Acid Profile of the Liver

As stated earlier, the FA composition of many tissues, particularly the liver reflects the FA profile of dietary fats used to formulate the diets (Hwong et al., 1988). In this study, the total PUFA content of birds fed oxidized oils was lower than those that received fresh oils. This was the same trend observed in the diets where PUFA content dropped by 4.9% when the oils were oxidized. This dietary PUFA level is believed to have significantly influenced the liver PUFA content. This effect of lipid oxidation on PUFA content can be seen in C18:2, where oxidized oils significantly decreased its amount in the liver. However, irrespective of the specific dietary treatment, the FA profile of the liver was somewhat different from those of adipose tissues. For instance, although the content of C18:1 was highest in the liver and both adipose tissues, the liver had a higher percentage of C16:0, C18:0, and C20:4 compared to the adipose tissues. The higher concentration of C20:4 (arachidonic acid) in the liver, which corresponds with a decrease in the percent content of C18:2 and C18:3 is indicative of active metabolism and the modification of essential FA within the liver (Jump et al., 2005; Kloareg et al., 2007). Although individual differences exist among the FA concentration across the various treatments, the liver FA content of non-essential FAs was related to the dietary FA levels, but not entirely decided by the dietary levels. For instance, although the dietary level of C16:0 was just around 12%, its

concentration in the liver was well above 22%, which further reiterates the fact that active modification such as elongation and desaturation occurs in the liver (Duran-Montgé et al., 2009; Kloareg et al., 2007).

3.5.5.4 Comparison of the Mean Fatty Acid Profile of the Adipose and Liver Tissues

While it is generally accepted that the FA composition of tissues is greatly influenced by the diet, the distribution of FAs can vary markedly across tissues (Marion and Woodroof, 1966). Despite feeding a PUFA-rich diet, the greater deposition of SFAs in the liver was not surprising. However, this higher degree of saturation may pose some health concerns to humans who consume lots of chicken liver, as saturated fats have been closely linked to cardiovascular diseases (Briggs et al., 2017). It was quite interesting to see from the results of this study that the adipose tissues had a greater degree of unsaturation than the liver. Among other things, this suggests a high sensitivity of adipose tissues to dietary FA levels, and this is a trend that has been corroborated by previous studies (Long et al., 2020). But beyond a general sensitivity to dietary FA levels, the results of this comparison also revealed a preferential deposition of essential FAs such as linoleic acid and alpha-linolenic acid in the abdominal fat, which is believed to be a conservative strategy for these highly important FAs in the event of inadequate dietary supply. Conversely, arachidonic acid was preferentially secreted in the liver, where it is synthesized from linoleic acid (C18:2) and α-linolenic acid (C18:3n-3) by the enzyme Δ^6-desaturase (Norris and Carr, 2013). It was also interesting to see a greater degree of saturation in the subcutaneous fat compared to the abdominal fat. Again this higher level of SFAs is generally unfavorable for human health. Thus, when consuming poultry products such as wings and thighs, which contain the skin, it might be a good idea to

remove the skin before consumption. However, it is worthy of note that the absolute proportion of SFAs in the subcutaneous fat (28.5%) of the current study can be considered moderate.

3.6 Conclusion

The results of this study showed that phytase supplementation was beneficial for feed efficiency, Ca utilization, BBS, tibia ash, AME, and AMEn regardless of oil quality or VE supplementation. When oxidized oils were supplied in diets, phytase supplementation improved AME and AMEn, but reduced crude fat utilization. Furthermore, the addition of 150 ppm of additional supplemental VE to basal diets containing adequate levels of VE improved growth performance over days 7-14, but when oxidized oils were fed, it provided little-to-no ameliorative effects as seen in the reduced crude fat utilization of birds that received diets containing oxidized oil and VE. The FA profile of the liver and adipose tissues were significantly affected by all the factors examined in this study. The results of this study further reiterates the fact that the FA profile of the adipose and liver tissues are considerably influenced by the dietary FA levels. Further investigation into graded supplemental levels of mixed tocopherols when feeding oxidized oils or phytase may help to better understand the optimum additional supplemental VE level needed to provide maximum protection for the broiler chickens, especially when fed diets containing oxidized oils. Future studies should also focus on identifying and understanding the various oxidation products formed during the oxidation of soy oil and how they impact feed additives such as phytase and VE in the diets of broilers.

CHAPTER 4

Effects of Oil Type, Oil Quality, and Vitamin E Supplementation on the Growth Performance, Nutrient Utilization, and the Relative Weight and Fatty Acid Profile of the Fat and Liver in 20-Day-Old Broiler Chickens

4.1 Abstract

The objective of this study was to determine the effect of oil quality, oil type, and vitamin E (VE) supplementation on the growth performance, the fatty acid profile of liver and adipose tissues, relative liver and fat weight, and the apparent total tract utilization of nutrients in broiler chickens. Three-hundred and eighty-four (384) day-old male by-product Cobb breeder chicks were randomly assigned to eight (8) treatments with eight (8) replicates containing six (6) birds per replicate for 20 days. The treatments consisted of two oil types (corn vs. soy oil), two oil quality levels (fresh corn oil; peroxide value [PV] = 3 meqO_2/kg, fresh soy oil; PV = 4 meqO_2/kg, oxidized corn oil; PV =104 meqO_2/kg and oxidized soy oil; PV = 109 meqO_2/kg), and two levels (0 and 150 ppm) of additional supplemental VE (mixed tocopherols containing about 55-75% γ-tocopherol) in a 2x2x2 factorial arrangement of treatments. This additional supplemental VE was in addition to the VE already in the basal diet, supplied through the corn-soybean meal and vitamin-mineral premix, which provided adequate VE amounts to all diets. The results showed that the dietary supply of oxidized oils produced a lower ($P < 0.05$) feed efficiency across days 0-20. Interestingly, birds that received oxidized soy oil with additional VE had the lowest ($P < 0.05$) live weight and BWG across both days 0-14 and 0-20 compared to those that received fresh corn oil with or without additional VE and oxidized or fresh soy oil with or without additional VE. Also, oxidized oils alone reduced nitrogen utilization ($P < 0.05$)

and this effect was more pronounced in birds fed oxidized corn oil ($P < 0.05$). Surprisingly, additional VE supplementation reduced ($P < 0.05$) crude fat, nitrogen, and energy utilization, as well as AME and AMEn. Birds that received a diet containing oxidized corn oil had a lower ($P < 0.05$) nitrogen utilization, AME, and AMEn compared to birds that received fresh corn oil. There was an increase ($P < 0.05$) in the total saturated fatty acid (SFA) content of abdominal fat tissues when birds were fed soy oil. Lipid oxidation decreased ($P < 0.05$) the concentration of C12:0, C14:0, and C18:0, but increased ($P < 0.05$) that of C18:1, C18:3n-6, C20:3, and C22:0 in the abdominal and subcutaneous fat tissues. Birds that received diet with fresh soy oil with additional VE had the highest ($P < 0.05$) content of C18:3n-3, while those that received oxidized soy oil with additional VE had the highest ($P < 0.05$) content of C24:0. From the results of this study, oxidized lipids significantly alter the FA profile of the liver and adipose tissues and also have a deleterious impact on the nutrient and energy utilization in 20-day-old broilers. Also, the additional supplementation of VE (mainly composed of γ-tocopherols) at 150 ppm exerted no beneficial effect on nutrient utilization of 20-day old broiler chickens, when added to basal diets containing adequate levels of VE, suggesting that the basal VE level was sufficient to handle the oxidative load.

Keywords: oxidized oils, vitamin E, soy oil, corn oil, oil source, oil type, broilers.

4.2 Introduction

Lipids are cheap sources of energy in broiler diets as they yield 2.25 more calories than proteins and carbohydrates (Baião and Lara, 2005), thus their use in the formulation of broiler diets have become commonplace. Several fat sources are available for the formulation of broiler diets and these can be broadly classified into animal fats, vegetable oils, animal-vegetable fat blends, and industrial by-products (McDonald et al., 2002; Leeson and Summers, 2005; Kellems and Church, 2010). The digestibility of dietary lipids is controlled significantly by their FA profile. Lipids rich in unsaturated fatty acids (UFAs) generally have better digestibility and higher metabolizable energy than those rich in saturated fatty acids (SFAs) as a result of better intestinal absorption (Celebi and Utlu, 2006). Common oil sources for broiler diets include corn oil (CO) and soybean oil (SO), which are very rich in PUFAs. Although both oils are rich sources of PUFAs, past studies have shown their effect are not necessarily the same. In a study that compared the digestibility and economics of SO and CO, it was reported corn oil resulted in higher apparent metabolizable energy (AME) and apparent metabolizable energy corrected for nitrogen (AMEn) in broilers, while SO had greater economic viability (Antunes et al., 2016). Another study reported that the inclusion of 1.5 and 3% CO compared to animal fat improved the carcass yield (breast, back, and wings) of broilers at 43 days of age. In contrast, diets containing soybean oil produced a better ($P < 0.05$) feed conversion ratio in comparison to those supplemented with fish oil (Fernandes et al., 2018). So, there's a lot yet to be understood regarding the effects of oils of different sources on broiler performance and other parameters, especially when those oils are oxidized.

Oils rich in PUFAs are highly susceptible to oxidative deterioration due to the presence of several double bonds which can be easily oxidized especially when exposed to high oxygen and temperature or metallic catalysts (Jakobsen, 1999). This oxidative deterioration of lipids has been linked to various deleterious impacts such as poor performance and oxidative stress in broiler chickens (Tavárez et al., 2011). Besides, the free radicals and other oxidation products such as malondialdehyde can constitute a potential health risk to consumers (Jakobsen, 1999). The ingestion of oxidized oils has also been reported to reduce tocopherol levels of tissues and plasma (Bayraktar et al., 2011). Tocopherol is the chemical name for a common group of compounds known as vitamin E. Vitamin E (VE) is a powerful antioxidant commonly used in broiler production as a biological chain-breaking compound that protects cells and tissues from free-radical induced oxidative damage (Panda and Cherian, 2014). Although the NRC recommendations of VE for poultry vary from 5 to 10 mg/kg (NRC, 1994), the inclusion levels in commercial diets usually range from 50 to 300 mg/kg (Adisseo, 2002) to account for the stress factors that accompany production conditions. Currently, very little information is available regarding the main and interactive effect of oil sources, oil quality, and VE supplementation on broiler performance, nutrient utilization, fat properties, and antioxidant status of broiler chickens. This study was designed to address this need.

4.3 Materials and Methods

4.3.1 Animals, Housing, Management, and Experimental Design

A total of 384 day-old male by-product Cobb breeder chicks were obtained from a commercial hatchery and fed a corn-SBM-based broiler starter diet that met or exceeded

the energy and nutrient requirements of birds of this age (NRC 1994). The birds were raised in battery cages (0.61 x 0.51 x 0.36 m) placed in an environmentally controlled room, with a lighting regimen of 22 h of light and 2 h of darkness. Six birds were placed in each cage and the birds were afforded *ad libitum* feeding and watering throughout the experiment.

The three main factors examined in this experiment were oil type, oil quality, and VE supplementation. Specifically, the treatments involved 2 oil types (CO vs, SO), which was subdivided into 2 levels of oil quality (fresh CO; PV= 3 meqO_2/kg and fresh SO; PV = 4 meqO_2/kg vs. oxidized CO; PV =104 meqO_2/kg and oxidized SO; PV =109 meqO_2/kg), with or without additional supplemental VE (0 vs. 150 ppm; mixed tocopherols containing 55-75% γ-tocopherol). It is noteworthy, however, that this additional supplemental VE is in addition to the VE already in the basal diet, supplied through the corn-soybean meal and the vitamin-mineral premix, which was supplied in adequate amounts to all diets. These resulted in a 2x2x2 factorial arrangement of treatments to yield a total of eight (8) dietary treatments consisting of eight (8) replicates of six (6) birds per replicate cage (Table 4.1). All diets were mixed from a single basal diet and all birds were placed on their respective experimental diets from day 0 till the end of the experiment (day 20). For diets supplemented with 150 ppm of VE, the VE was first dissolved in previously weighed oils to be used for each diet. The oils containing the VE were then mixed into their respective diets. The ingredient composition and analyzed energy and nutrient composition of these diets are presented in Table 4.2. Each diet contained 5 g/kg of titanium dioxide as an index marker for energy and nutrient digestibility and utilization calculation. All the animal care and procedures used in this study were approved by the Institutional Animal Care and Use Committee at the University of Kentucky.

4.3.2 Sample Collection

All birds and feed were weighed per cage on days 0, 7, 14, and 20 to determine the body weight gain (BWG), feed intake (FI), and feed efficiency (FE). On d 20, all the birds were weighed in groups (per cage) and one bird with weight closest to the average cage weight was selected for blood and tissue collection. All the birds were euthanized by argon asphyxiation. Blood (serum) was collected from the selected bird, into serum tubes via the jugular vein immediately after the birds had been euthanized. The collected blood was allowed to sit at room temperature for an hour to allow it to clot, after which it was centrifuged at 1500 x g for 10 min. The resulting serum was then aliquoted into pre-labelled microcentrifuge tubes and stored at -80 ^{o}C until they were analyzed for antioxidant enzymes, specifically superoxide dismutase (SOD) activity.

A tissue section was removed from the mid-jejunum and immediately flushed with phosphate-buffered saline (PBS), before slicing it open longitudinally to collect mucosal samples by scraping with sterile glass microscope slides. The collected mucosal samples were immediately transferred into microcentrifuge tubes and instantly snap-frozen in liquid nitrogen before storing at -80 ℃ for the gene expression analysis. The entire liver, abdominal fat, and subcutaneous fat were also removed and the adhering tissues were cleaned off before weighing. The weight of these tissues was recorded and expressed relative to the final body weight (BW) of the selected sample bird [(tissue weight/final BW) × 100]. The collected fat tissues were immediately placed on ice before storing at -20 ℃ for FA analysis. A small piece of the liver was sub-sectioned into microtubes, which were then snap-frozen in liquid nitrogen and stored at -80 ℃ until SOD analysis. The rest of the liver was also placed on ice immediately before storing them at -20 ℃ for FA analysis.

Excreta samples were collected per cage on days 19 and 20 and the content was pooled together before drying at 55 ℃ in a forced-air oven for 5 days. The dried samples were ground using a Wiley Mill Laboratory Standard (Model No. 3, Arthur H. Thomas Co., Philadelphia, PA, USA) fitted with a 1 mm screen and then stored in airtight plastic bags before being analyzed for titanium, N, dry matter, and energy determination.

4.3.3 Lipid oxidation

Soybean oil and corn oil were acquired from the feed mill unit of C. Oran Little Research Center. To oxidize the soy oils, glass pyrexes and aluminum pans were purchased and filled with about 4 lbs of oil, before placing them in a convection oven and heated at 95 °C ± 5 °C for about 12 days, at an average heating duration of 7 hours per day. The corn oil was oxidized similarly but spent a longer period of about 15-17 days in the oven to attain a similar peroxide value (PV). The oil was stirred every 2-4 hours to ensure proper oxygenation and the PV of the oxidized oil was checked intermittently. When the PV reached the target level of >100 meq O_2/kg, the heating was stopped and the oxidized oil was cooled to room temperature. The oils were then mixed and a sample was taken to determine the final peroxide value. The oils were subsequently stored in a refrigerator (4 °C) before use for diet preparation. All chemicals (reagent grade) were purchased from VWR (VWR International, Radnor, PA, USA) unless specified otherwise.

4.3.4 Chemical Analysis

All analyses were conducted in duplicates and wherever the coefficient of variation was greater than 5%, the analysis was repeated for that pen. The p-anisidine value was analyzed at Barrow-Agee Laboratories (Memphis, TN, USA, 38116) using the AOCS Cd 18-90 method. The initial and final PV were analyzed using the AOCS (2007) method.

Briefly, 5 g of the oil was weighed into an Erlenmeyer flask and dissolved in a 30 ml, 3:2 acetic acid-chloroform mix. Saturated potassium iodide solution (0.5 mL) was then added to the mixture and shaken intermittently for 1 minute before a magnetic stirrer and 30 mL deionized water was added. A starch indicator solution (0.5 mL) was added before titrating with 0.1M sodium thiosulfate solution until the blue color just disappears. A sample blank was analyzed similarly and used to calculate the PV (see Equation 3.1). The full details of the PV determination method is provided in Appendix 1.

$$\text{POV} = \frac{(\text{S} - \text{B}) \times \text{M} \times 1000}{W} \quad \text{Equation 3.1}$$

Where S = titer of sample (mL), B = titer of blank (mL); M = molarity of the sodium thiosulfate solution; W = weight of sample (g).

The DM content of the eight diets and excreta samples were determined in duplicates by drying the samples at 110 °C for 16 h (AOAC International, 2006). The N contents of the diets and excreta samples were analyzed at the Agricultural Experiment Station Chemical Laboratories, University of Missouri-Columbia (Columbia, MO) by the combustion method (model FP2000, Leco Corp., St. Joseph, MI; AOAC International, 2000; method 990.03), with EDTA as the internal standard. The gross energy (GE) of the feed ingredients, diets, and excreta samples was analyzed using a bomb calorimeter (Parr adiabatic bomb calorimeter, model 6200, Parr Instruments, Moline, IL, USA) with benzoic acid as a calibration standard.

The titanium content of the diets and excreta was determined in duplicates using the method of Short et al. (1996). Briefly, the diets and excreta samples were ashed at 580 °C overnight, after which they were digested at 250 °C in 7.4 M sulfuric acid for about one

hour. The digest was transferred into a 100 ml volumetric flask by rinsing the glass crucible with nano-pure water. Five milliliters of 30% hydrogen peroxide was added and the solution was brought to the 100-ml mark with the nano-pure water and inverted four times to mix. Titanium content was determined after 24 hours via a UV-visible spectrophotometer (UV-1800, Shimadzu Scientific, Kyoto, Japan) at a wavelength of 410 nm using a standard curve. Full details of this method are provided in Appendix 5.

The crude fat was determined in duplicates using the ether extraction method. Briefly, one gram of diet and excreta samples were weighed into a filter paper, which was placed into a cotton thimble and placed in the fat extraction machine (Velp Scientifica, Bohemia, NY, USA). The fat was extracted using petroleum ether. The weight of the resulting fat was expressed as a percentage of the sample weight.

The total tract nutrient utilization (TTU) of DM, energy, N, and crude fat were calculated using the method of Kong and Adeola (2014) (Equation 3.3). The apparent metabolizable energy (AME) and was calculated using the following formula; AME, $kcal/kg = Calculated$ energy digestibility (%) × $GE\ of$ the $diet$ $(kcal/kg)$.

The caloric value of 8.22 kcal/g was used to correct AME for N to give the apparent metabolizable energy corrected for nitrogen (AMEn) (Hill and Anderson, 1958).

AID or TTU (%) = $100 - \left[100 * \left(\frac{Ti}{To}\right) * \left(\frac{No}{Ni}\right)\right]$……..Equation 3.3

where *Ti* is the initial concentration of the titanium marker in the feed, *To* is the concentration of the titanium marker in the excreta, *No* is the concentration of energy or nutrients the excreta, and *Ni* is the concentration of energy or nutrients in the feed.

The fatty acid analyses of the diet, liver, abdominal fat, and subcutaneous fat tissues were done at the University of Georgia using a procedure modified from Park and Goins (1994). A summary of this procedure is provided in Appendix 6.

Total VE analysis was determined at DSM research laboratory (DSM Nutritional Products, Belvidere, NJ), using a modified version of AOAC official method 971.30. Briefly, vitamin E is dispersed in deionized water and 3A alcohol and extracted with petroleum ether. The extract is then analyzed by a normal phase HPLC system using fluorescence detection.

4.3.5 Antioxidant Enzymes

The collected serum and liver samples were analyzed for SOD activity using commercially available kits obtained from Cayman Chemicals (Ann Arbor, MI). Briefly, the liver samples were homogenized in a 50mM phosphate buffer containing 1mM EDTA (pH 7.3) using an Omni tissue homogenizer with 5 mm plastic disposable probes (Omni International, GA, USA). The homogenate was aliquoted into two 1.5 ml microtubes and centrifuged at 1000 x g for 15 minutes at 4 ^{o}C. The resulting supernatant was aliquoted into three microcentrifuge tubes and stored at -80 ^{o}C until analysis. This supernatant and serum samples were used to determine the SOD activity.

Total SOD activity was determined using a commercial SOD kit (Catalog Number 706002, Cayman, Ann Arbor, MI, USA). Briefly, a tetrazolium salt was used to detect the superoxide radicals generated by xanthine oxidase and hypoxanthine. The analysis was performed according to the manufacturer's instructions using a 96-well plate and read at a wavelength of 450 nm on a UV-visible spectrophotometer (Bio-Rad, Hercules, CA, USA). One unit is defined as the amount of enzyme required to achieve a 50% dismutation of the

produced superoxide radical. The final SOD activity for each standard curve was 0.005 to 0.050 U/mL SOD.

4.2.6 Statistical Analysis

Prior to statistical analysis, outliers (data outside mean ±3 standard deviation) were removed from each set of the data set. Where outliers were found, the remaining number of replicates per treatment were indicated in the results tables. For performance and nutrient utilization data, the cage was considered the experimental unit, while the sampled bird (one bird/cage) constituted the experimental unit for all other analyses. All calculations for the nutrient and gross energy data were done using the average value of the GE or nutrients in the diet, except for crude fat where the individual diet values were utilized. All data were subjected to a three-way ANOVA using the GLM procedure of SAS (SAS 9.4, SAS Institute Inc., Cary, NC) appropriate for a factorial arrangement of treatments. The significance of the main effects (oil type, oil quality, and VE), as well as the two-way and three-way interactions, were determined. A summary of the locational deposition of selected fatty acids was created by choosing the most abundant FAs (C16:0, C18:0 and C18:1) and the essential FAs (C18:2, C18:3n-3, C20:4) across all cages within the abdominal fat, subcutaneous fat and liver. These FAs were then compared across the abdominal fat, subcutaneous fat and liver regardless of the dietary treatments using the GLM procedure of SAS. This comparison was repeated across the abdominal fat and subcutaneous fat regardless of dietary treatments, using the GLM procedure of SAS. Wherever significant 2- or 3-way interactions were observed, treatment means were separated by Tukey's Honest Significant Difference and the level of significance was set at $P < 0.05$.

4.4 Results

4.4.1 Diet Composition and Lipid Oxidation

The ingredients and analyzed nutrient composition of the experimental diets including DM, GE, CF, and CP are shown in Table 4.2. Diet A had a slightly lower gross energy and DM, compared to the other diets. The crude protein level was similar for all diets. All diets were formulated from the same basal diet. The average values of the analyzed energy and nutrient levels in the diets were used for nutrient utilization calculations. Although a calculated VE level of 26 IU/kg was added to the basal diet, through the vitamin-mineral premix, the results of the VE analysis, showed that the VE levels ranged from 83 IU/kg to 117 IU/kg for diets that did not receive additional VE supplementation at 150 ppm, while the analyzed VE content of diets that received additional supplemental VE at 150 ppm ranged from 168 IU/kg to 227 IU/kg.

Peroxide value and p-anisidine value were used to measure the degree of lipid oxidation. Fresh CO and SO had a PV of 3 meqO_2/kg and 4 meqO_2/kg, respectively while the oxidized corn and soy oils had a PV of 104 meqO_2/kg and 109 meqO_2/kg, respectively. Similarly, the p-anisidine value for the fresh CO and SO were 5.06% and 0.5% respectively, while the oxidized corn and soy oils had p-anisidine values of 35.15% and 35.2% respectively.

4.4.2 Growth Performance

Table 4.3 shows the effects of the various factors and their interaction on the LBW, BWG, FI, and FE of the broiler chickens over days 0-14, and 0-20 respectively. The dietary supply of oxidized oils resulted in a lower ($P < 0.05$) feed efficiency over days 0-20 but

did not affect LBW, BWG, and feed intake in both periods. For both days 0-14 and 0-20, there was an interaction between oil quality and VE where birds fed diets containing oxidized oil with VE had a lower ($P < 0.05$) LBW and BWG compared to other groups. For the same interaction, over days 0-20, birds that received oxidized oil with VE also had lower ($P < 0.05$) FI compared to those that received either oxidized oil with no VE or fresh oil with VE. A few interesting 3-way interactions were observed across both periods. Notably, birds that received oxidized soy oil with VE had the lowest ($P < 0.05$) LBW and BWG across both days 0-14 and 0-20 compared to those that received fresh corn oil with or without VE and oxidized or fresh soy oil with or without VE. Furthermore, across days 0-14, birds that received oxidized soy oil with VE had a worse ($P < 0.05$) FI compared to those fed diets containing either fresh corn oil with no VE or those fed diets containing fresh soy oil with or without VE. Over days 0-21, birds that received oxidized soy oil with VE had the lowest ($P < 0.05$) feed efficiency compared to those fed fresh corn oil with or without VE.

4.4.3 Relative Fat and Liver Percentages

Table 4.4 shows the effects of the various factors on the fat and liver percentages in 21-day-old broiler chickens. There were no significant main and interactive effects of oil type, oil quality, or VE supplementation on the relative percentages of the subcutaneous fat, total fat, and liver percentages. However, an interaction between oil type and VE was observed for the relative abdominal fat weight, where the birds that received soy oil and no VE had a higher ($P < 0.05$) relative abdominal fat weight compared to those fed soy oil with VE.

4.4.4 Nutrient and Energy Utilization

Table 4.5 shows the main and simple effects of oil type, oil quality, and vitamin E supplementation on the total tract utilization of nutrients and energy in 21-day-old broiler chickens. The dietary supply of SO improved ($P < 0.05$) N utilization but did not affect DM or CF utilizations. Additional VE supplementation decreased ($P < 0.05$) the utilization of CF, N, energy, AME, and AMEn. An interaction between oil type and oil quality was observed, where oxidized CO resulted in reduced ($P < 0.05$) N retention compared to the fresh CO, fresh SO, and oxidized SO. For this same interaction, birds that received oxidized CO had a lower ($P < 0.05$) AME and AMEn compared to those fed fresh CO. Another 2-way interaction was observed between oil type and VE, where birds that received SO with additional VE had a lower ($P < 0.05$) energy utilization compared to those that received CO with no additional VE supplementation or SO with no additional VE supplementation.

4.4.5 Fatty Acid Profiles

4.4.5.1 Fatty Acid Profile of the Oils and Diet

Tables 4.6 and 4.7 show the FA composition of the oils that were mixed into the diets and the diets fed to birds in this study. In both CO and SO, the percent concentrations of linoleic (C18:2) decreased by 8.1% and 4.4%, respectively as the oils were thermally oxidized. Both oils had a fairly similar composition of FA before and after heating but CO had higher levels of C18:1 in both the fresh (28.94 vs. 21.82%) and oxidized oils (30.94 vs. 23.25) compared to SO. Conversely, SO had a much higher content of C18:3n-3 in both the fresh (7.28 vs. 1.36) and oxidized oils (6.21 vs. 1.11) compared to CO. There was also an increase in the total SFA and MUFA content and a decrease in the total PUFA and UFA

content as both oils were oxidized (Table 4.7). This is also reflected in the slightly higher SFA:UFA ratio of oxidized oils. Trace levels (< 0.09%) of caprylic acid (C8:0) was present in both oxidized oils but not in the fresh oil. Regardless of oxidation, the most abundant FAs in both oils were C18:2, followed by C18:1 and C16:0.

As expected, the FA profile of the diet followed the FA profile of the oils added to it although the concentrations of C16:0, C18:2, and C22:2 FAs were slightly higher in the diets. In order of magnitude, C18:2 was the most abundant, followed by C18:1, and C16:0. Interestingly, C8 (caprylic acid) was not picked up in the FA profile of the diets formulated with oxidized oils. The SFA:UFA ratio of the diets is also close to that of the oils used in formulating them (e.g. Diet A, 0.18 vs. 0.17; Diet B, 0.19 vs. 0.19).

4.4.5.2 Fatty Acid Profile of the Abdominal and Subcutaneous Fat

Tables 4.8 and 4.9 show the effect of the treatments on selected fatty acids within the abdominal and subcutaneous fats, respectively. The full FA composition of the abdominal and subcutaneous fats are provided in the appendix Tables A.4.1 and A.4.2, respectively. There were no interactive or main effects of oil type, oil quality, and VE supplementation on the total content of PUFA in the abdominal fat tissue. There was, however, an increase ($P < 0.05$) in the total SFA content of abdominal fat tissue when birds were fed SO. Also, in the abdominal fat, the SFA:UFA was higher ($P < 0.05$) in birds fed SO. Furthermore, birds that received CO but no VE had the lowest ($P < 0.05$) SFA:UFA, compared to the other groups. An interaction between oil type and oil quality was observed for total MUFA content in both abdominal and subcutaneous fats. In the abdominal and subcutaneous fat tissues, the birds that received oxidized CO had a greater ($P < 0.05$) MUFA content than the other groups. Interactions were also observed between oil type and

VE in both abdominal and subcutaneous fat tissues. In the abdominal fat, the birds fed SO with no VE had a greater ($P < 0.05$) SFA content than those fed CO with or without VE. Similarly, birds that received CO with no VE had the lowest ($P < 0.05$) SFA content in the subcutaneous fat. Although no significant interactions were observed for PUFA in the abdominal fat, two significant interactions were observed for it in the subcutaneous fat. Firstly, birds that received fresh oil with VE had the lowest ($P < 0.05$) PUFA content among all the groups. Secondly, the birds that were fed diets containing fresh SO had lower ($P < 0.05$) PUFA content compared to those fed diets containing fresh CO and oxidized SO. In the abdominal fat, the total SFA content was greatest ($P < 0.05$) in birds fed fresh SO compared to the other groups. In the same fat depot, the birds that received oxidized oils with no VE had a greater ($P < 0.05$) MUFA content compared to those fed either fresh oil with no VE or oxidized oil with VE. Still within the abdominal fat, the total UFA content was higher ($P < 0.05$) in birds fed CO with no VE compared to those fed SO with no VE. Consequently, the SFA:UFA was lowest ($P < 0.05$) in the abdominal fat of birds fed CO with no VE compared to the other groups.

In both fat depots, the most abundant FAs were C18:1 (35.8%), followed by C18:2 (26.8%) and C16:0 (21.5%). This is in contrast to the diets where C18:2 (54.6%) was the most abundant, followed by C18:1 (25.12%) and C16:0 (12.5%). Furthermore, lipid oxidation decreased ($P < 0.05$) the concentration of C12:0, C14:0 and C18:0, but increased ($P < 0.05$) that of C18:3n-6, C20:3, and C22:0, in both fat depots. The oxidized lipids also decreased ($P < 0.05$) the content of C17:0 and increased C18:1 content in the abdominal fat but not in the subcutaneous fat. Conversely, oxidized lipids increased ($P < 0.05$) the concentration of C17:1, C20:0, and C20:4 in the subcutaneous but not abdominal fat. The

effect of oil type was observed as the concentration of C18:0, C18:3n-3, and C22:0 decreased ($P < 0.05$), while that of C18:1, C18:3n-6, C20:3, and C20:4 increased ($P < 0.05$) in both adipose tissue types when the birds were fed diets containing CO. Interestingly, the concentration of C17:0 increased ($P < 0.05$) in the abdominal fat but decreased ($P < 0.05$) in the subcutaneous fat when the birds were fed CO. The dietary supply of SO increased ($P < 0.05$) the amount of C12:0, C14:0, and C20:0 in the abdominal fat but this effect was not observed in the subcutaneous fat. Similarly, SO increased ($P < 0.05$) the amount of C17:1 in the subcutaneous fat but not in the abdominal adipose tissue. The main effect of VE was observed in the abdominal fat tissues where the concentration of C14:0, C18:0, and C20:0 increased ($P < 0.05$) when diets containing additional supplemental VE was fed. In the subcutaneous fat, the concentration of C17:0 was increased by VE supplementation but this effect was not observed in the abdominal fat.

A few interesting interactions were also observed in the adipose tissues. For instance, in both abdominal and subcutaneous fat, the concentration of C18:1 was higher in the birds that received oxidized CO compared to birds on the other treatments. In the abdominal fat, SO with no VE supplementation resulted in a higher ($P < 0.05$) content of C16:0 compared to CO with no VE supplementation. For the same interaction and in the same fat depot, diets containing CO with no VE supplementation resulted in a lower ($P < 0.05$) content of C18:0 compared to the other groups. Also, the concentration of C14:1 was higher in birds that received oxidized oil with no VE compared to those fed fresh oil with no VE. The content of C18:2 was higher in birds fed fresh CO compared to those fed either oxidized CO or fresh SO. Still, in the abdominal fat depot, C20:2 was greater when birds were fed fresh CO compared to when they were fed oxidized CO.

In the subcutaneous fat, a lot more interactions were observed. For instance, the amount of C17:0 and C17:1 was higher ($P < 0.05$) in the birds that received oxidized oil with VE compared to those fed diets containing fresh oil with VE. For this same oil quality x VE interaction in the subcutaneous fat, birds fed oxidized oil with no VE had the highest content of C18:1 compared to others. Also, C15:0 was greater in birds fed fresh oil with no VE compared to those fed fresh oil with VE. The interaction between oil type and oil quality was also observed for several FAs in the subcutaneous fat. The amount of C16:1 was higher in birds fed oxidized CO compared to those fed fresh CO. The concentration of C17:0 was higher ($P < 0.05$) when diets containing oxidized SO was fed, compared to feeding diets containing oxidized CO or fresh SO. The content of 18:2 was higher (P 0.05) in the birds that received fresh CO compared to those that received fresh SO. Oxidized SO increased the concentration of C20:2 in the subcutaneous fat, compared to fresh SO. Similarly, the amount of C20:3 was lowest ($P < 0.05$) when birds were fed fresh SO.

4.4.5.3 Fatty Acid Profile of the Liver

Table 4.10 shows the effect of oil type, oil quality, and additional supplemental VE on selected FAs in the liver. There were no significant main or interactive effects on the total PUFA content. The full FA composition of the liver is provided in the appendix Table A.4.3. However, the total SFA content and SFA:UFA was greater ($P < 0.05$) in birds fed oxidized oils compared to those that received fresh oils. Also, the birds that received fresh SO had the greater content of MUFA and total UFA compared to those fed fresh CO. An interaction between oil quality and VE was also observed for the total liver UFA content, as the birds fed fresh oil with no VE had a significantly lower UFA content compared to those fed either fresh oil with VE or oxidized oil with no VE.

Oxidized lipids alone decreased ($P < 0.05$) the concentration of C18:2 and C20:0. The dietary supply of SO increased ($P < 0.05$) the concentration of C18:2, C18:3n-3, C20:0, C22:0 and C24:0, but decreased ($P < 0.05$) the concentration of C22:4 (Table 4.10). An interaction between oil type and oil quality was observed, as the birds fed diets containing fresh SO had the lowest ($P < 0.05$) amount of C18:0 in their livers compared to the other groups. Also, birds that received fresh SO had a greater ($P < 0.05$) content of C18:1 and C22:2 compared to those that received fresh CO and oxidized SO. For the same interaction, the content of C16:1 was higher ($P < 0.05$) in birds fed fresh SO compared to those fed fresh CO. Although no significant interaction between oil type and VE was observed, several FAs were affected by the interactive effect of oil quality and VE. For instance, when birds were fed diets containing oxidized oil with VE supplementation, it resulted in a higher ($P < 0.05$) content of C20:4 and C24:0, compared to those that received fresh oil with VE. Also, the concentration of C18:1 was highest ($P < 0.05$) when birds were fed diets containing fresh oil with VE supplementation compared to when they were fed diets containing oxidized oil with VE. The concentration of C16:1 was greatest ($P < 0.05$) either when birds received fresh oil with VE or oxidized oil with no VE.

Three-way interactions were observed in C18:3n-3 and C24:0. The content of C18:3n-3 was highest ($P < 0.05$) when birds received diets containing fresh SO with VE supplementation compared to the other dietary treatments. Conversely, the content of C24:0 was highest ($P < 0.05$) in birds fed diets containing oxidized SO with VE supplementation.

4.4.5.4 Comparison of the Mean Fatty Acid Profile of the Adipose and Liver Tissues

The mean composition of select FAs in the abdominal and subcutaneous fat tissues is presented in Table 4.11. A greater ($P < 0.05$) content of C18:0, C20:4, total SFA, PUFA, and SFA:UFA was observed in the liver compared to the abdominal and subcutaneous fats. Conversely, the content of C18:1, C18:2, C18:3n-3, and total MUFA was greater ($P < 0.05$) in the abdominal and subcutaneous fats compared to the liver. The subcutaneous fat had the greatest ($P < 0.05$) content of C16:0 compared to the abdominal fat and liver. On the other side, the abdominal fat had the greatest ($P < 0.05$) content of total UFA, compared to the subcutaneous fat and liver.

4.4.5.4 Comparison of the Mean Fatty Acid Profile of the Abdominal and Subcutaneous Fat Tissues

The mean composition of select FAs in the abdominal and subcutaneous fat tissues is presented in Table 4.12. A greater ($P < 0.05$) content of C16:0, C18:0, C20:4, and total SFA was observed in the subcutaneous fat compared to the abdominal fat. Conversely, a greater ($P < 0.05$) content of total PUFA and UFA was observed in the abdominal fat compared to the subcutaneous fat.

4.4.6 Superoxide Dismutase (SOD) Activity

Table 4.13 shows the effect of oil type, oil quality, and VE supplementation on liver and serum SOD activity. From the results of this study, there were no main or interactive effects of oil type, lipid oxidation, or VE supplementation on the activity of SOD in both the serum and liver.

Table 4. 1 Details of the eight dietary treatments fed to broiler chickens in this study.

Diet/Treatment	Details
A	Corn oil (fresh) + 0 ppm of additional vitamin E
B	Corn oil (oxidized) + 0 ppm of additional vitamin E
C	Soybean oil (fresh) + 0 ppm of additional vitamin E
D	Soybean oil (oxidized) + 0 ppm of additional vitamin E
E	Corn oil (fresh) + 150 ppm of additional vitamin E[1]
F	Corn oil (oxidized) + 150 ppm of additional vitamin E
G	Soybean oil (fresh) + 150 ppm of additional vitamin E
H	Soybean oil (oxidized) + 150 ppm of additional vitamin E

[1]Supplemental vitamin E was added on top of the 26 IU/kg of supplemental vitamin E from the vitamin-mineral premix added to the basal diet.

Table 4. 2 Ingredients and nutrient composition of the diets fed to broiler chickens (as-fed basis).

Diet type	A	B	C	D	E	F	G	H
Oil type	Corn oil	Corn oil	Soy oil	Soy oil	Corn oil	Corn oil	Soy oil	Soy oil
Oil quality	Fresh	Oxidized	Fresh	Oxidized	Fresh	Oxidized	Fresh	Oxidized
Vitamin E[1]	No	No	No	No	Yes	Yes	Yes	Yes
Ingredients, g/kg								
Corn	593.2	593.2	593.2	593.2	593.2	593.2	593.2	593.2
Soybean meal (47% CP)	335	335	335	335	335	335	335	335
Oil	30	30	30	30	30	30	30	30
L-Lysine HCl	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
DL-Methionine	2	2	2	2	2	2	2	2
Tryptophan	1	1	1	1	1	1	1	1
Salt (NaCl)	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
Limestone	10.2	10.2	10.2	10.2	10.2	10.2	10.2	10.2
Dicalcium phosphate	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5
Vitamin-mineral[2] premix	2	2	2	2	2	2	2	2
Titanium dioxide	5	5	5	5	5	5	5	5
Total	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Analyzed nutrients and energy[3]								
Dry matter g/kg	897	906	907	909	911	917	911	914
Gross energy, kcal/kg	4,081	4,110	4,117	4,109	4,103	4,120	4,113	4,133
Crude Protein, g/kg	219	219	220	215	217	219	217	218
Crude fat, g/kg	58.0	61.2	59.5	59.6	60.4	60.9	55.7	56.2
Total vitamin E (IU/kg)[4]	112	86	117	83	227	168	202	178

[1]Represents supplemental vitamin E at 150 g/kg. This is in addition to the vitamin E supplied by the vitamin-mineral premix.

[2]Provided the following quantities of vitamins and micro minerals per kilogram of complete diet: iron, 32 mg; copper, 8 mg; manganese, 51 mg; zinc, 60 mg; iodine, 1.48 mg; selenium, 0.24 mg; vitamin A (retinyl acetate), 8,820 IU; vitamin D3 (cholecalciferol), 2,822 IU; vitamin E (dl-α-tocopheryl acetate), 26 IU; vitamin K activity, 0.73 mg; thiamine, 1.76 mg; riboflavin, 6.17 mg; pantothenic acid, 14 mg; niacin, 44 mg; pyridoxine, 4 mg; folic acid, 0.88 mg; biotin, 0.18 mg; vitamin B-12, 0.02 mg; choline, 383 mg.

[3]Since the same basal diet was used in the study, the average values of the analyzed nutrients (except crude fat) were used to determine the utilization values but the analyzed value for the PC diet was used for the PC utilization calculations.

[4]Analyzed total vitamin E value.

Table 4. 3 Main and simple effect of oil type, oil quality, and vitamin E supplementation on the growth performance of 20-day-old broiler chickens

Oil quality	Oil type	Vitamin E	0-14 d				0-20 d			
			LBW, g/bird	Gain, g/bird	FI, g/bird	G:F ratio, g/kg	LBW [1] g/bird	Gain, g/bird	FI, [2] g/bird	G:F[3] ratio, g/kg
Main effect										
Fresh			475	429	501	857	885	839	1073	782[a]
Oxidized			462	417	493	844	855	809	1060	762[b]
	Corn oil		472	426	499	854	877	830	1069	776
	Soy oil		465	420	496	847	863	818	1064	768
		No	475	430	502	855	880	834	1074	776
		Yes	462	416	492	846	860	814	1059	768
2-way interaction effect										
Oil quality x vitamin E										
Fresh	-	No	474[a]	429[a]	501	856	877[a]	831[a]	1062[ab]	783
Fresh	-	Yes	475[a]	430[a]	501	859	892[a]	847[a]	1084[a]	781
Oxidized	-	No	477[a]	431[a]	504	855	883[a]	837[a]	1087[a]	770
Oxidized	-	Yes	448[b]	402[b]	482	833	828[b]	781[b]	1034[b]	755
Dietary treatments										
Fresh	Corn oil	No	[s]486[a]	440[a]	510[a]	863	897[a]	850[a]	1073	793[a]
Fresh	Corn oil	Yes	[p]476[a]	430[a]	497[ab]	866	896[a]	851[a]	1082	786[a]
Oxidized	Corn oil	No	[r]465[ab]	420[ab]	497[ab]	844	855[ab]	810[ab]	1068	758[bc]
Oxidized	Corn oil	Yes	[r]460[ab]	414[ab]	490[ab]	845	858[ab]	811[ab]	1054	768[ab]
Fresh	Soy oil	No	[r]462[ab]	417[ab]	491[a]	848	858[ab]	812[ab]	1050	773[ab]
Fresh	Soy oil	Yes	475[a]	430[a]	505[a]	851	889[a]	843[a]	1086	776[ab]
Oxidized	Soy oil	No	488[a]	443[a]	511[ab]	866	910[a]	864[a]	1106	781[ab]
Oxidized	Soy oil	Yes	[q]436[b]	390[b]	475[b]	822	798[b]	752[b]	1014	742[c]
SEM			10.486	10.486	9.488	9.813	21.949	21.945	22.610	8.877
----------Probability----------										
Oil quality			0.102	0.094	0.263	0.064	0.060	0.059	0.447	0.003
Oil type			0.385	0.417	0.660	0.279	0.404	0.431	0.738	0.189
Vitamin E			0.074	0.067	0.116	0.195	0.204	0.203	0.335	0.203
Oil quality x vitamin E			0.047	0.042	0.104	0.087	0.029	0.026	0.023	0.303
Oil type x oil quality			0.427	0.422	0.680	0.290	0.509	0.507	0.794	0.298
Oil type x vitamin E			0.432	0.432	0.930	0.120	0.189	0.191	0.424	0.120
Oil type x oil quality x vitamin E			0.019	0.023	0.040	0.120	0.022	0.024	0.102	0.021

[a–b]Means with different superscripts within the same column differ significantly based on Tukey HSD ($P < 0.05$).

[1]Live body weight at day 20. The average live weight at day 0 was 45.0

[2]Feed Intake

[3]G:F=gain to feed ratio or feed efficiency

[p-s]Represents the number or mortalities per dietary treatment, where p, q, r, and s represent 1, 2, 3, and 4 mortalities respectively.

Table 4. 4 Main and simple effect of oil quality, oil type, and vitamin E supplementation on the relative tissue weights of 20-day-old broilers

Treatment effect			20 d			
Oil quality	Oil type	Vitamin E	Abdominal fat, %	Subcutaneous fat, %	Total fat,[1] %	Liver, %
Main effect						
Fresh			0.909	0.731	1.638	2.522
Oxidized			0.900	0.725	1.634	2.569
	Corn oil		0.872	0.688	1.553	2.538
	Soy oil		0.938	0.769	1.719	2.553
		No	0.941	0.750	1.691	2.544
		Yes	0.869	0.706	1.581	2.547
Oil type x vitamin E						
-	Corn oil	No	0.838[b]	0.700	1.531	2.500
-	Corn oil	Yes	0.906[ab]	0.675	1.575	2.575
-	Soy oil	No	1.044[a]	0.800	1.850	2.588
-	Soy oil	Yes	0.831[b]	0.738	1.588	2.519
Dietary Treatments						
Fresh	Corn oil	No	0.750	0.625	1.350	2.525
Fresh	Corn oil	Yes	0.938	0.713	1.625	2.388
Oxidized	Corn oil	No	0.925	0.775	1.713	2.475
Oxidized	Corn oil	Yes	0.875	0.638	1.525	2.763
Fresh	Soy oil	No	1.100	0.813	1.913	2.650
Fresh	Soy oil	Yes	0.850	0.775	1.663	2.525
Oxidized	Soy oil	No	0.988	0.788	1.788	2.525
Oxidized	Soy oil	Yes	0.813	0.700	1.513	2.513
	SEM		0.091	0.098	0.157	0.118
			----------Probability----------			
Oil type			0.311	0.245	0.142	0.852
Oil quality			0.884	0.928	0.978	0.576
Vitamin E			0.267	0.530	0.330	0.970
Oil quality x vitamin E			0.529	0.325	0.278	0.113
Oil type x oil quality			0.311	0.530	0.232	0.171
Oil type x vitamin E			0.033	0.787	0.174	0.393
Oil type x oil quality x vitamin E			0.228	0.530	0.330	0.353

[a–b]Means with different superscripts within the same column differ significantly based on Tukey HSD ($P < 0.05$).
[1]Values represent the sum of the abdominal and subcutaneous fat percentages.

Table 4. 5 Main and simple effects of oil quality, oil type, and vitamin E supplementation on the apparent dry matter, nutrient, and energy utilization in 20-day-old broiler chickens

Treatment effect								
Oil quality	Oil type	Vitamin E	Dry matter, %	Nitrogen, %	Crude fat, %	Energy, %	AME, kcal/kg	AMEn, kcal/kg
Main effect								
Fresh			71.4	63.2[a]	81.3	75.5[a]	2987	2873[a]
Oxidized			71.3	59.5[b]	80.8	74.1[b]	2965	2838[b]
	Corn oil		71.2	60.4[b]	79.7	75.1	2975	2853
	Soy oil		71.6	62.3[a]	82.3	74.6	2976	2858
		No	71.3	62.4[a]	83.0[a]	75.3[a]	2992[a]	2874[a]
		Yes	71.5	60.3[b]	79.0[b]	74.4[b]	2960[b]	2837[b]
2-way interaction effect								
Oil type x oil quality								
Fresh	Corn oil	-	71.1	63.5[a]	79.4	75.9	3004[a]	2890[a]
Fresh	Soy oil	-	71.8	63.0[a]	83.2	75.2	2970[ab]	2855[ab]
Oxidized	Corn oil	-	71.3	57.4[b]	80.1	74.2	2947[b]	2815[b]
Oxidized	Soy oil	-	71.4	61.5[a]	81.5	74.0	2983[ab]	2861[ab]
Oil type x vitamin E								
-	Corn oil	No	71.0	61.1	81.2	75.2[a]	2981	2860
-	Corn oil	Yes	71.3	59.8	78.3	74.9[ab]	2970	2845
-	Soy oil	No	71.5	63.8	84.9	75.3[a]	3002	2887
-	Soy oil	Yes	71.6	60.7	79.8	73.9[b]	2951	2829
Dietary treatments								
Fresh	Corn oil	No	71.4	65.5	82.0	76.2[x]	3010	2903
Fresh	Corn oil	Yes	70.8	61.5	76.8	75.6	2998	2878
Oxidized	Corn oil	No	70.6	56.7	80.4	74.2	2952	2817
Oxidized	Corn oil	Yes	71.9	58.1	79.8[x]	74.3[x]	2942	2812
Fresh	Soy oil	No	71.3	64.2	84.4	75.7	2987	2873
Fresh	Soy oil	Yes	71.3	61.8[y]	82.0	74.7	2953[x]	2838
Oxidized	Soy oil	No	71.8	63.3	85.4	74.9	3018	2902

Oxidized	Soy oil	Yes	71.0	59.7	77.5	73.1	2948	2820
	Pooled SD[1]		1.855	3.597	1.855	1.161	50.735	59.120
					----------Probability----------			
Oil quality			0.841	0.001	0.715	<.001	0.092	0.022
Oil type			0.385	0.049	0.076	0.132	0.939	0.705
Vitamin E			0.639	0.020	0.007	0.005	0.018	0.017
Oil type x oil quality			0.547	0.014	0.399	0.441	0.009	0.008
Oil type x vitamin E			0.841	0.349	0.449	0.041	0.120	0.149
Oil quality x vitamin E			0.947	0.254	0.856	0.882	0.505	0.652
Oil type x oil quality x vitamin E			0.056	0.078	0.085	0.223	0.475	0.265

[a–b]Means with different superscripts within the same column differ significantly based on Tukey-Kramer comparison (P < 0.05).

[x-y]Values represent the mean of 8 replicates cages (pooled excreta of 6 birds per replicate) except for mean values with x and y where the number of replicates (n) was 7 and 6, respectively.

[1]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

Table 4. 6 Analyzed fatty acid compositions and quality measures of the oils added to the experimental diets (%)

Fatty Acid composition, %	Fresh corn oil	Oxidized corn oil	Fresh soy oil	Oxidized soy oil
C8:0 (Caprylic)	0.00	0.08	0.00	0.06
C14:0 (Myristic)	0.03	0.04	0.07	0.07
C15:0 (Pentadecanoic)	0.00	0.04	0.01	0.03
C16:0 (Palmitic)	11.95	13.16	10.54	11.42
C16:1 (Palmitoleic)	0.15	0.17	0.11	0.12
C17:0 (Margaric)	0.07	0.08	0.10	0.11
C17:1 (cis-10-Heptadecenoic acid)	0.04	0.05	0.06	0.07
C18:0 (Stearic)	1.79	1.98	4.02	4.36
C18:1 (Oleic)	28.94	30.94	21.82	23.25
C18:2 (Linoleic)	54.56	50.13	54.47	52.06
C18:3 n3 (alpha linolenic acid)	1.36	1.11	7.28	6.21
C20:0 (Arachidic)	0.36	0.39	0.32	0.32
C20:1 (Eicosenoic acid)	0.35	0.53	0.26	0.35
C20:2 (Eicosadienoic acid)	0.02	0.08	0.04	0.09
C22:0 (Behenic acid)	0.11	0.19	0.29	0.36
C22:1 (Erucic)	0.06	0.45	0.04	0.36
C22:2 (Docosadienoic acid)	0.00	0.01	0.04	0.01
C24:0 (Lignoceric)	0.06	0.12	0.04	0.05
Others	0.13	0.48	0.48	0.69
∑SFA	14.38	16.08	15.40	16.78
∑MUFA	29.54	32.12	22.29	24.15
∑PUFA	55.95	51.33	61.83	58.37
∑UFA	85.49	83.45	84.12	82.52
SFA:UFA	0.17	0.19	0.18	0.20
Peroxide value, meqO_2/kg	3	104	4	109
p-anisidine value, %	5.06	35.15	0.5	35.2

Table 4. 7 Analyzed fatty acid compositions of the experimental diets (%)

Diet ID	A	B	C	D	E	F	G	H
Oil type	Corn oil	Corn oil	Soy oil	Soy oil	Corn oil	Corn oil	Soy oil	Soy oil
Oil quality	Fresh	Oxidized	Fresh	Oxidized	Fresh	Oxidized	Fresh	Oxidized
Vitamin E[1]	No	No	No	No	Yes	Yes	Yes	Yes
C14:0 (Myristic)	0.05	0.05	0.07	0.07	0.04	0.05	0.07	0.07
C16:0 (Palmitic)	12.55	13.00	11.83	12.45	12.46	13.24	11.80	12.26
C16:1 (Palmitoleic)	0.16	0.17	0.15	0.15	0.17	0.17	0.14	0.14
C17:0 (Margaric)	0.09	0.08	0.09	0.10	0.09	0.08	0.09	0.09
C17:1 (cis-10-Heptadecenoic acid)	0.04	0.04	0.05	0.05	0.04	0.04	0.05	0.05
C18:0 (Stearic)	2.12	2.23	3.37	3.66	2.08	2.25	3.46	3.27
C18:1 (Oleic)	26.33	27.04	23.53	23.80	26.26	26.89	23.78	23.37
C18:2 (Linoleic)	55.49	54.29	54.84	53.58	55.34	54.08	54.47	54.97
C18:3n3 (alpha linolenic acid)	2.19	2.07	5.00	4.84	2.18	2.10	5.13	4.61
C20:0 (Arachidic)	0.36	0.38	0.36	0.35	0.38	0.37	0.34	0.32
C20:1 (Eicosenoic acid)	0.29	0.27	0.23	0.23	0.28	0.29	0.24	0.23
C22:0 (Behenic acid)	0.19	0.19	0.26	0.34	0.17	0.22	0.27	0.26
∑SFA	15.35	15.93	15.97	16.97	15.23	16.22	16.02	16.26
∑MUFA	26.83	27.52	23.97	24.24	26.74	27.39	24.21	23.79
∑PUFA	57.82	56.55	60.06	58.80	58.03	56.39	59.77	59.95
∑UFA	84.65	84.07	84.03	83.03	84.77	83.78	83.98	83.74
SFA:UFA	0.18	0.19	0.19	0.20	0.18	0.19	0.19	0.19

[1]supplemental vitamin E added at 150 mg/kg

Table 4. 8 Main and simple effects of oil quality, oil type, and vitamin E supplementation on selected fatty acids in the abdominal fat of 20-day old broiler chickens (%)

Oil quality	Oil type	Vitamin E	C12:0	C14:0	C14:1	C16:0	C16:1	C17:0
	Main effect							
Fresh			0.022[a]	0.446[a]	0.137	20.9	5.8	0.249[a]
Oxidized			0.019[b]	0.412[b]	0.147	20.6	6.2	0.201[b]
	Corn oil		0.020[b]	0.419[b]	0.138	20.6	5.9	0.234[a]
	Soy oil		0.022[a]	0.439[a]	0.146	21.1	6.0	0.216[b]
		No	0.021	0.421[b]	0.142	20.7	6.1	0.225
		Yes	0.021	0.438[a]	0.142	20.9	5.9	0.225
	Oil quality x vitamin E							
Fresh	-	No	0.022	0.440	0.131[b]	20.6	5.6	0.251
Fresh	-	Yes	0.022	0.452	0.144[ab]	21.3	5.9	0.248
Oxidized	-	No	0.019	0.401	0.154[a]	20.8	6.5	0.200
Oxidized	-	Yes	0.020	0.423	0.140[ab]	20.5	5.9	0.201
	Oil type x vitamin E							
-	Corn oil	No	0.019	0.406	0.133	20.1[b]	5.9	0.237
-	Corn oil	Yes	0.020	0.432	0.144	20.9[ab]	5.8	0.230
-	Soy oil	No	0.022	0.435	0.152	21.3[a]	6.1	0.213
-	Soy oil	Yes	0.022	0.444	0.141	20.8[ab]	5.8	0.219
	Dietary treatments							
Fresh	Corn oil	No	0.021	0.430[x]	0.118[x]	19.8[x]	5.3	0.261
Fresh	Corn oil	Yes	0.021[x]	0.450[x]	0.139	21.1[x]	5.7	0.257
Oxidized	Corn oil	No	0.018[x]	0.383[x]	0.147	20.4[x]	6.5	0.213[x]
Oxidized	Corn oil	Yes	0.020	0.414[x]	0.149	20.7[x]	6.1[x]	0.202[x]
Fresh	Soy oil	No	0.024	0.451	0.144[x]	21.4	5.9[x]	0.240[x]
Fresh	Soy oil	Yes	0.023[x]	0.455	0.150[x]	21.4	6.1	0.239
Oxidized	Soy oil	No	0.020[x]	0.419[x]	0.160	21.2	6.5[x]	0.186[x]
Oxidized	Soy oil	Yes	0.020	0.433[x]	0.132[x]	20.1	5.6	0.200[x]

Pooled SD[1]	0.002	0.032	0.024	1.162	0.918	0.022
		----------Probability----------				
Oil quality	<.001	0.000	0.131	0.287	0.086	<.001
Oil type	0.008	0.020	0.210	0.096	0.619	0.004
Vitamin E	0.454	0.045	0.984	0.644	0.470	0.888
Oil quality x vitamin E	0.382	0.548	0.034	0.092	0.051	0.708
Oil type x vitamin E	0.370	0.311	0.072	0.025	0.551	0.234
Oil type x oil quality	0.110	0.396	0.097	0.165	0.132	0.656
Oil type x oil quality x vitamin E	0.861	0.987	0.532	0.838	0.828	0.323

[a–b]Means with different superscripts within the same column differ significantly based on Tukey-Kramer comparison ($P < 0.05$).

[1]SEM can be calculated from pooled SD: $SEM = \frac{SD}{\sqrt{n}}$

[x]Values represent the mean of 8 replicates cages (pooled excreta of 6 birds per replicate) except for mean values with x and y where the number of replicates (n) was 7 and 6, respectively.

Table 4.8 continued. Main and simple effects of oil quality, oil type, and vitamin E supplementation on selected fatty acids in the abdominal fat of 20-day old broiler chickens (%)

Oil quality	Oil type	Vitamin E	C18:0	C18:1	C18:2	C18:3 n-6	C18:3 n-3	C20:0	C20:2
	Main effect								
Fresh			5.2[a]	35.9[b]	28.4	0.284[b]	1.756	0.093[b]	0.134
Oxidized			4.9[b]	36.8[a]	27.4	0.320[a]	1.652	0.111[a]	0.128
	Corn oil		4.9[b]	37.0[a]	28.3	0.323[a]	1.048[b]	0.099	0.132
	Soy oil		5.3[a]	35.7[b]	27.5	0.281[b]	2.360[a]	0.105	0.130
		No	5.0[b]	36.4	28.0	0.296	1.694	0.098[b]	0.130
		Yes	5.2[a]	36.3	27.8	0.308	1.714	0.106[a]	0.133
	2-way interaction effect								
	Oil type x oil quality								
Fresh	Corn oil	-	5.0	36.0[b]	29.6[a]	0.302	1.074	0.089	0.142[a]
Oxidized	Corn oil	-	4.7	37.9[a]	27.0[b]	0.343	1.021	0.108	0.122[b]
Fresh	Soy oil	-	5.5	35.7[b]	27.2[b]	0.266	2.438	0.097	0.126[ab]
Oxidized	Soy oil	-	5.2	35.6[b]	27.8[ab]	0.297	2.283	0.113	0.135[ab]
	Oil type x vitamin E								
-	Corn oil	No	4.6[b]	36.9	28.9	0.315	1.076	0.097	0.133
-	Corn oil	Yes	5.1[a]	37.1	27.7	0.330	1.020	0.100	0.131
-	Soy oil	No	5.3[a]	35.9	27.1	0.276	2.312	0.098	0.127
-	Soy oil	Yes	5.3[a]	35.5	27.9	0.287	2.409	0.112	0.134
	Dietary treatments								
Fresh	Corn oil	No	4.7[x]	35.4	30.5	0.292[x]	1.117	0.085[x]	0.147[x]
Fresh	Corn oil	Yes	5.3[x]	36.6[x]	28.7	0.312	1.030[x]	0.093[x]	0.137
Oxidized	Corn oil	No	4.5	38.3	27.3[x]	0.337	1.034[x]	0.109	0.118
Oxidized	Corn oil	Yes	4.9	37.5	26.7	0.349	1.009	0.107	0.126[x]
Fresh	Soy oil	No	5.6	35.6	27.6	0.267	2.489	0.087[x]	0.131
Fresh	Soy oil	Yes	5.4[x]	35.9[x]	26.8[x]	0.266	2.387[x]	0.107[x]	0.121[x]
Oxidized	Soy oil	No	5.0	36.1[x]	26.7[x]	0.286[x]	2.134[x]	0.109	0.123[x]
Oxidized	Soy oil	Yes	5.3[x]	35.1	29.0	0.307[x]	2.432	0.117[x]	0.146

Pooled SD[1]	0.453	1.667	2.723	0.051	0.225	0.016	0.026
		----------Probability----------					
Oil quality	0.014	0.043	0.172	0.009	0.082	<.000	0.397
Oil type	<.001	0.004	0.259	0.003	<.000	0.134	0.803
Vitamin E	0.036	0.879	0.771	0.329	0.723	0.045	0.704
Oil quality x vitamin E	0.816	0.064	0.125	0.773	0.053	0.164	0.060
Oil type x oil quality	0.997	0.025	0.026	0.679	0.383	0.766	0.040
Oil type x vitamin E	0.046	0.519	0.152	0.833	0.193	0.206	0.566
Oil type x oil quality x vitamin E	0.101	0.715	0.510	0.563	0.155	0.943	0.551

[a–b]Means with different superscripts within the same column differ significantly based on Tukey-Kramer comparison ($P < 0.05$).

[1]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

[x]Values represent the mean of 8 replicates cages (pooled excreta of 6 birds per replicate) except for mean values with x and y where the number of replicates (n) was 7 and 6, respectively.

Table 4.8 continued. Main and simple effects of oil quality, oil type, and vitamin E supplementation on selected fatty acids in the abdominal fat of 20-day old broiler chickens (%)*

Oil quality	Oil type	Vitamin E	C20:3	C20:4	C22:0	∑SFA	∑MUFA	∑PUFA	∑UFA	SFA:UFA
Main effect										
Fresh			0.179[b]	0.268	0.031[b]	26.9	42.3	31.0	72.8	0.370
Oxidized			0.200[a]	0.300	0.044[a]	26.4	43.4	30.0	73.5	0.360
	Corn oil		0.196[a]	0.315[a]	0.029[b]	25.8[b]	43.3	30.3	73.5	0.353[b]
	Soy oil		0.183[b]	0.253[b]	0.046[a]	27.4[a]	42.4	30.7	72.9	0.374[a]
		No	0.189	0.277	0.038	26.5	43.1	30.6	73.3	0.360
		Yes	0.191	0.291	0.037	26.8	42.6	30.4	73.1	0.370
Oil quality x vitamin E										
Fresh	-	No	0.177	0.270	0.031	26.5	41.9[b]	31.7	73.0	0.365
Fresh	-	Yes	0.181	0.266	0.031	27.2	42.7[ab]	30.3	72.6	0.367
Oxidized	-	No	0.201	0.284	0.045	26.4	44.3[a]	29.5	73.5	0.360
Oxidized	-	Yes	0.200	0.316	0.043	26.4	42.4[b]	30.6	73.5	0.363
Oil type x oil quality										
Fresh	Corn oil	-	0.189	0.305	0.022	25.6[b]	42.1[b]	31.5	73.2	0.349
Oxidized	Corn oil	-	0.204	0.324	0.036	26.1[b]	44.5[a]	29.0	73.7	0.357
Fresh	Soy oil	-	0.169	0.230	0.040	28.1[a]	42.5[b]	30.4	72.4	0.383
Oxidized	Soy oil	-	0.196	0.276	0.052	26.7[b]	42.3[b]	31.0	73.3	0.366
Oil type x vitamin E										
-	Corn oil	No	0.200	0.308	0.031	25.2[c]	43.4	30.9	74.0[a]	0.339[b]
-	Corn oil	Yes	0.193	0.322	0.027	26.5[b]	43.1	29.6	72.9[ab]	0.366[a]
-	Soy oil	No	0.178	0.246	0.045	27.8[a]	42.8	30.3	72.6[b]	0.384[a]
-	Soy oil	Yes	0.188	0.260	0.047	27.1[ab]	42.0	31.2	73.2[ab]	0.364[a]
Dietary treatments										
Fresh	Corn oil	No	0.196	0.312[x]	0.026[x]	22.3[x]	41.4	32.5	73.9	0.332[x]
Fresh	Corn oil	Yes	0.182	0.299	0.019	24.0[x]	38.4[x]	30.5	68.9[x]	0.366[x]
Oxidized	Corn oil	No	0.204[x]	0.304[x]	0.037	23.1[x]	45.4	25.7[x]	71.2[x]	0.348[x]
Oxidized	Corn oil	Yes	0.205	0.345	0.036	23.8[x]	43.5	28.8	72.2[x]	0.366[x]

Fresh	Soy oil	No	0.158	0.227	0.037	27.9 [x]	41.3 [x]	30.8	72.2	0.397 [x]
Fresh	Soy oil	Yes	0.180	0.232	0.043	27.0 [x]	38.2 [x]	26.4 [x]	64.5 [x]	0.369 [x]
Oxidized	Soy oil	No	0.198 [x]	0.265	0.053	27.1	37.9 [x]	26.0 [x]	63.9 [x]	0.371 [x]
Oxidized	Soy oil	Yes	0.195	0.287	0.051	25.5 [x]	41.4	32.3	73.8	0.360 [x]
	Pooled SD[1]		0.027	0.086	0.012	1.677	2.294	2.964	1.513	0.030
						----------Probability----------				
Oil quality			0.003	0.141	<.001	0.315	0.064	0.218	0.095	0.553
Oil type			0.050	0.006	<.001	0.001	0.145	0.549	0.143	0.009
Vitamin E			0.801	0.525	0.803	0.486	0.362	0.821	0.595	0.704
Oil quality x vitamin E			0.705	0.413	0.879	0.488	0.026	0.112	0.614	0.975
Oil type x oil quality			0.381	0.538	0.774	0.039	0.038	0.050	0.498	0.118
Oil type x vitamin E			0.235	0.993	0.245	0.031	0.651	0.154	0.040	0.005
Oil type x oil quality x vitamin E			0.149	0.670	0.249	0.457	0.577	0.501	0.970	0.293

[a–b]Means with different superscripts within the same column differ significantly based on Tukey-Kramer comparison (P < 0.05).

[1]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

[x]Values represent the mean of 8 replicates cages (pooled excreta of 6 birds per replicate) except for mean values with x and y where the number of replicates (n) was 7 and 6, respectively.

*∑represents the sum of: SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; UFA = unsaturated fatty acids. SFA:UFA = ratio of saturated fatty acids to unsaturated fatty acids

Table 4. 9 Main and simple effects of oil quality, oil type, and vitamin E supplementation on selected fatty acids in the subcutaneous fat of 20-day old broiler chickens (%)

Oil quality	Oil type	Vitamin E	C12:0	C14:0	C15:0	C16:0	C16:1	C17:0	C17:1
	Main effect								
Fresh			0.021[a]	0.457[a]	0.058	22.1	5.4	0.121	0.074[b]
Oxidized			0.018[b]	0.431[b]	0.059	21.6	5.8	0.124	0.077[a]
	Corn oil		0.019	0.440	0.058	21.7	5.6	0.119[b]	0.073[b]
	Soy oil		0.020	0.448	0.058	22.0	5.7	0.126[a]	0.079[a]
		No	0.019	0.436	0.059	21.7	5.6	0.119[b]	0.076
		Yes	0.020	0.452	0.058	22.0	5.6	0.126[a]	0.076
	2-way interaction effect								
	Oil quality x vitamin E								
Fresh	-	No	0.021	0.445	0.060[a]	21.8	5.3	0.124[ab]	0.076[ab]
Fresh	-	Yes	0.021	0.470	0.055[c]	22.4	5.6	0.118[b]	0.073[b]
Oxidized	-	No	0.018	0.426	0.057[abc]	21.5	6.0	0.114[b]	0.076[ab]
Oxidized	-	Yes	0.019	0.435	0.061[ab]	21.7	5.6	0.134[a]	0.079[a]
	Oil type x oil quality								
Fresh	Corn oil	-	0.020	0.454	0.057	21.8	5.2[b]	0.123[ab]	0.073
Oxidized	Corn oil	-	0.018	0.425	0.059	21.6	6.0[a]	0.114[b]	0.073
Fresh	Soy oil	-	0.021	0.460	0.058	22.4	5.7[ab]	0.119[b]	0.076
Oxidized	Soy oil	-	0.019	0.436	0.059	21.6	5.6[ab]	0.133[a]	0.081
	Dietary treatment								
Fresh	Corn oil	No	0.021[x]	0.441	0.060	21.4	4.9	0.127[x]	0.074[x]
Fresh	Corn oil	Yes	0.020	0.468	0.055[x]	22.2	5.4	0.119[x]	0.071
Oxidized	Corn oil	No	0.017[x]	0.420[x]	0.059	21.2[x]	6.0	0.108[x]	0.073
Oxidized	Corn oil	Yes	0.019[x]	0.431[x]	0.059[x]	22.0	5.9	0.120[x]	0.073[x]
Fresh	Soy oil	No	0.020	0.449	0.060[x]	22.1	5.6	0.122[x]	0.077[x]
Fresh	Soy oil	Yes	0.023[x]	0.471	0.056[x]	22.6	5.8	0.117[x]	0.074
Oxidized	Soy oil	No	0.018	0.433[x]	0.055	21.9[x]	6.0[x]	0.120[x]	0.078[x]
Oxidized	Soy oil	Yes	0.020[x]	0.439	0.062	21.3	5.3	0.147[x]	0.085

Pooled SD[1]	0.003	0.036	0.008	1.137	0.878	0.012	0.006
		----------Probability----------					
Oil quality	0.003	0.005	0.556	0.111	0.100	0.443	0.049
Oil type	0.155	0.379	0.923	0.384	0.672	0.020	0.001
Vitamin E	0.123	0.072	0.819	0.223	0.901	0.033	0.956
Oil quality x vitamin E	0.486	0.385	0.039	0.412	0.114	<.001	0.041
Oil type x oil quality	0.836	0.796	0.788	0.264	0.048	0.001	0.121
Oil type x vitamin E	0.341	0.793	0.367	0.150	0.315	0.179	0.194
Oil type x oil quality x vitamin E	0.373	0.990	0.490	0.345	0.684	0.375	0.375

[a–b]Means with different superscripts within the same column differ significantly based on Tukey-Kramer comparison ($P < 0.05$).

[1]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

[x]Values represent the mean of 8 replicates cages (pooled excreta of 6 birds per replicate) except for mean values with x and y where the number of replicates (n) was 7 and 6, respectively.

Table 4.9 continued. Main and simple effects of oil quality, oil type, and vitamin E supplementation on selected fatty acids in the subcutaneous fat of 20-day old broiler chickens (%)

Oil quality	Oil type	Vitamin E	C18:0	C18:1	C18:2	C18:3 n-6	C18:3 n-3	C20:0	C20:1	C20:2
	Main effect									
Fresh			5.6[a]	35.8	27.4	0.284[b]	1.574	0.092[b]	0.374	0.129
Oxidized			5.3[b]	36.5	27.0	0.322[a]	1.572	0.106[a]	0.375	0.136
	Corn oil		5.3[b]	36.7[a]	27.7	0.322[a]	0.969[b]	0.098	0.394[a]	0.133
	Soy oil		5.6[a]	35.6[b]	26.8	0.284[b]	2.176[a]	0.099	0.355[b]	0.132
		No	5.4	36.1	27.6	0.297	1.589	0.099	0.367	0.131
		Yes	5.5	36.2	26.9	0.309	1.557	0.099	0.382	0.134
	2-way interaction effect									
	Oil quality x vitamin E									
Fresh	-	No	5.6	35.3[b]	28.3	0.287	1.647	0.092	0.365	0.130
Fresh	-	Yes	5.6	36.3[ab]	26.5	0.280	1.500	0.091	0.384	0.127
Oxidized	-	No	5.2	36.9[a]	26.9	0.306	1.531	0.105	0.369	0.131
Oxidized	-	Yes	5.4	36.1[ab]	27.2	0.338	1.614	0.106	0.381	0.140
	Oil type x oil quality									
Fresh	Corn oil	-	5.4	35.8[b]	28.7[a]	0.300	0.972	0.092	0.397	0.140[ab]
Oxidized	Corn oil	-	5.1	37.6[a]	26.7[ab]	0.344	0.967	0.103	0.392	0.125[ab]
Fresh	Soy oil	-	5.7	35.8[b]	26.2[b]	0.267	2.175	0.091	0.352	0.117[b]
Oxidized	Soy oil	-	5.5	35.4[b]	27.4[ab]	0.300	2.178	0.108	0.358	0.146[a]
	Oil type x vitamin E									
-	Corn oil	No	5.1	36.6	28.5	0.320	1.001	0.099	0.394	0.138
-	Corn oil	Yes	5.4	36.9	26.8	0.325	0.938	0.097	0.394	0.128
-	Soy oil	No	5.6	35.6	26.6	0.274	2.177	0.098	0.340	0.123
-	Soy oil	Yes	5.7	35.5	26.9	0.293	2.176	0.100	0.370	0.140
	Dietary treatments									
Fresh	Corn oil	No	5.3	35.3	29.8	0.305[x]	1.009[x]	0.094	0.399	0.147
Fresh	Corn oil	Yes	5.6[x]	36.4	27.5	0.296	0.936[x]	0.091	0.394	0.134
Oxidized	Corn oil	No	4.9	37.9	27.2[x]	0.335	0.994[x]	0.104	0.389[x]	0.129[x]

Oxidized	Corn oil	Yes	5.3	37.4	26.1	0.353	0.939	0.103	0.395	0.121 [x]
Fresh	Soy oil	No	5.8	35.3	26.8	0.270	2.286	0.090	0.331 [x]	0.113 [x]
Fresh	Soy oil	Yes	5.7	36.2	25.6 [x]	0.264	2.064	0.091	0.373	0.121
Oxidized	Soy oil	No	5.4	35.9 [x]	26.5 [x]	0.278 [x]	2.067 [x]	0.107	0.349 [x]	0.134 [x]
Oxidized	Soy oil	Yes	5.6 [x]	34.8	28.3	0.323 [x]	2.288	0.109	0.367	0.158
	Pooled SD[1]		0.409	1.579	2.495	0.049	0.233	0.019	0.058	0.024
					----------Probability----------					
Oil quality			0.009	0.079	0.521	0.004	0.979	0.005	0.978	0.286
Oil type			0.001	0.005	0.189	0.004	<.001	0.734	0.011	0.829
Vitamin E			0.098	0.810	0.271	0.344	0.595	0.999	0.313	0.666
Oil quality x vitamin E			0.326	0.029	0.113	0.133	0.061	0.873	0.815	0.380
Oil type x oil quality			0.513	0.007	0.018	0.673	0.945	0.512	0.733	0.001
Oil type x vitamin E			0.259	0.643	0.127	0.553	0.603	0.676	0.331	0.037
Oil type x oil quality x vitamin E			0.710	0.866	0.461	0.630	0.083	0.968	0.569	0.670

[a–b]Means with different superscripts within the same column differ significantly based on Tukey-Kramer comparison (P < 0.05).

[1]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

[x]Values represent the mean of 8 replicates cages (pooled excreta of 6 birds per replicate) except for mean values with x and y where the number of replicates (n) was 7 and 6, respectively.

Table 4.9 continued. Main and simple effects of oil quality, oil type, and vitamin E supplementation on selected fatty acids in the subcutaneous fat of 20-day old broiler chickens (%)

Oil quality	Oil type	Vitamin E	C20:3	C20:4	C22:0	∑SFA	∑MUFA	∑PUFA	∑UFA	SFA:UFA
Main effect										
Fresh			0.161[b]	0.202[b]	0.019[b]	28.3	41.8	28.1	70.7	0.409
Oxidized			0.197[a]	0.275[a]	0.037[a]	27.8	42.8	29.5	72.1	0.535
	Corn oil		0.187[a]	0.266[a]	0.019[b]	27.7	42.9	29.4	72.0	0.385
	Soy oil		0.171[b]	0.210[b]	0.037[a]	28.3	41.8	28.1	70.8	0.559
		No	0.178	0.234	0.027	27.6[b]	42.2	29.9	72.1	0.531
		Yes	0.180	0.242	0.028	28.5[a]	42.4	27.6	70.7	0.413
2-way interaction effect										
Oil quality x vitamin E										
Fresh	-	No	0.162	0.212	0.019	27.7	41.1	30.6[a]	72.0	0.384
Fresh	-	Yes	0.160	0.192	0.020	28.9	42.4	25.5[b]	69.5	0.435
Oxidized	-	No	0.194	0.256	0.036	27.5	43.4	29.2[a]	72.3	0.678
Oxidized	-	Yes	0.200	0.293	0.037	28.1	42.3	29.7[a]	71.8	0.391
Oil type x oil quality										
Fresh	Corn oil	-	0.178[a]	0.233	0.012	27.9	41.5[b]	30.3[a]	71.8	0.389
Oxidized	Corn oil	-	0.195[a]	0.299	0.026	27.6	44.2[a]	28.5[ab]	72.2	0.382
Fresh	Soy oil	-	0.143[b]	0.171	0.026	28.6	42.0[b]	25.9[b]	69.7	0.430
Oxidized	Soy oil	-	0.198[a]	0.250	0.047	28.0	41.5[b]	30.4[a]	71.9	0.688
Oil type x vitamin E										
-	Corn oil	No	0.186	0.270	0.017	26.9[b]	42.6	30.3	72.6	0.371
-	Corn oil	Yes	0.188	0.262	0.021	28.5[a]	43.1	28.5	71.5	0.399
-	Soy oil	No	0.170	0.198	0.038	28.1[a]	41.9	29.6	71.7	0.691
-	Soy oil	Yes	0.172	0.223	0.036	28.4[a]	41.6	26.7	69.9	0.427
Dietary treatment										
Fresh	Corn oil	No	0.181	0.248[x]	0.009[x]	27.5[x]	40.8	31.4	72.2	0.381[x]
Fresh	Corn oil	Yes	0.175[x]	0.218[x]	0.015	27.7[x]	42.3	29.1	71.4	0.389[x]
Oxidized	Corn oil	No	0.190[x]	0.293[x]	0.025	24.1[x]	44.4	25.6[x]	70.0[x]	0.335[x]

Oxidized	Corn oil	Yes	0.200	0.306	0.027	27.9 [x]	43.9	28.0	71.9 [x]	0.390 [x]
Fresh	Soy oil	No	0.142	0.176	0.028	28.7 [x]	41.4	29.8	71.2 [x]	0.403 [x]
Fresh	Soy oil	Yes	0.144 [x]	0.165	0.025	29.0	42.6	21.9	64.5 [x]	0.466
Oxidized	Soy oil	No	0.197 [x]	0.220 [x]	0.048 [x]	25.3 [x]	37.1 [x]	25.7 [x]	62.8 [x]	0.989
Oxidized	Soy oil	Yes	0.199	0.280	0.047	27.0 [x]	40.7	31.5	72.2	0.375 [x]
Pooled SD[1]			0.026	0.079	0.013	4.304	5.673	7.065	10.255	0.599
			----------Probability----------							
Oil quality			<.001	0.001	<.001	0.057	0.056	0.322	0.133	0.303
Oil type			0.023	0.009	<.001	0.100	0.061	0.278	0.106	0.453
Vitamin E			0.744	0.694	0.753	0.002	0.822	0.079	0.080	0.481
Oil quality x vitamin E			0.551	0.171	0.950	0.036	0.496	0.666	0.651	0.384
Oil type x oil quality			0.006	0.746	0.249	0.287	0.032	0.032	0.222	0.315
Oil type x vitamin E			0.998	0.433	0.346	0.600	0.008	0.018	0.257	0.430
Oil type x oil quality x vitamin E			0.536	0.740	0.644	0.363	0.746	0.089	0.112	0.316

[a–b]Means with different superscripts within the same column differ significantly based on Tukey-Kramer comparison (P < 0.05).

[1]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

[x]Values represent the mean of 8 replicates cages (pooled excreta of 6 birds per replicate) except for mean values with x and y where the number of replicates (n) was 7 and 6, respectively.

*∑represents the sum of: SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; UFA = unsaturated fatty acids. SFA:UFA = ratio of saturated fatty acids to unsaturated fatty acids

Table 4. 10 Main and simple effects of oil quality, oil type, and vitamin E supplementation on selected fatty acids in the liver of 20-day old broiler chickens (%)

Oil quality	Oil type	Vitamin E	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3 n-3	C20:0	C20:1	C20:4
Main effect											
Fresh			18.5	2.2	17.5^{b}	17.2	22.3^{a}	0.633^{a}	0.056^{a}	0.200	12.4
Oxidized			18.8	2.2	18.4^{a}	16.7	20.8^{b}	0.507^{b}	0.044^{b}	0.197	13.5
	Corn oil		18.7	2.1	18.4	17.1	20.8^{b}	0.424^{b}	0.043^{b}	0.211	13.5
	Soy oil		18.7	2.3	17.5	16.8	22.4^{a}	0.716^{a}	0.056^{a}	0.186	12.4
		No	18.4	2.2	17.9	16.9	21.5	0.595	0.048	0.195	13.0
		Yes	19.0	2.2	18.0	16.9	21.7	0.544	0.052	0.202	12.9
2-way interaction effect											
Oil quality x vitamin E											
Fresh	-	No	17.9	1.8^{b}	18.4^{ab}	16.4^{bc}	22.4	0.615	0.050	0.172^{b}	13.2^{ab}
Fresh	-	Yes	19.2	2.5^{a}	16.6^{b}	17.7^{a}	22.3	0.650	0.061	0.229^{a}	11.6^{b}
Oxidized	-	No	18.9	2.6^{a}	17.3^{bc}	17.5^{ab}	20.5	0.576	0.045	0.218^{ab}	12.8^{ab}
Oxidized	-	Yes	18.8	1.8^{b}	19.4^{a}	16.2^{c}	21.2	0.438	0.042	0.176^{ab}	14.2^{a}
Oil type x oil quality											
Fresh	Corn oil	-	18.2	1.9^{b}	18.5^{a}	15.9^{bc}	21.8	0.454	0.045	0.200	13.5
Oxidized	Corn oil	-	19.1	2.3^{ab}	18.2^{a}	18.3^{ab}	19.8	0.394	0.041	0.222	13.5
Fresh	Soy oil	-	18.8	2.4^{a}	16.5^{b}	18.5^{a}	22.9	0.811	0.066	0.200	11.3
Oxidized	Soy oil	-	18.6	2.1^{ab}	18.5^{a}	15.2^{c}	21.9	0.620	0.046	0.172	13.5
Oil type x vitamin E											
-	Corn oil	No	18.1	2.1	18.6	16.4	20.8	0.482	0.043	0.193	13.7
-	Corn oil	Yes	19.2	2.2	18.1	17.7	20.8	0.367	0.043	0.229	13.2
-	Soy oil	No	18.6	2.4	17.2	17.5	22.1	0.709	0.052	0.197	12.3
-	Soy oil	Yes	18.7	2.2	17.8	16.2	22.7	0.722	0.061	0.176	12.5
Dietary treatments											
Fresh	Corn oil	No	17.6^{x}	1.5	19.3^{x}	14.1^{x}	21.4^{x}	0.533^{b}	0.045	0.166^{x}	14.1
Fresh	Corn oil	Yes	18.8	2.2	17.7^{x}	17.7	22.2	0.376^{b}	0.045^{x}	0.235	12.8
Oxidized	Corn oil	No	18.6	2.6	17.8^{x}	18.8	20.1	0.431^{b}	0.042^{x}	0.221	13.4

Oxidized	Corn oil	Yes	19.6	2.1	18.6	17.7	19.4	0.357[b]	0.040 [x]	0.224	13.6
Fresh	Soy oil	No	18.1 [x]	2.1 [x]	17.5 [x]	16.8 [x]	23.3 [x]	0.698[ab]	0.055	0.177 [x]	12.3 [x]
Fresh	Soy oil	Yes	19.5	2.8	15.5	20.2 [x]	22.5	0.924[a]	0.077	0.223	10.4
Oxidized	Soy oil	No	19.1	2.7	16.8 [x]	18.1	21.0	[x] 0.721[ab]	0.049	0.216	12.3
Oxidized	Soy oil	Yes	18.0	1.5	20.2 [x]	12.2 [x]	22.9	[x] 0.520[b]	0.044	0.128 [x]	14.7
Pooled SD[1]			2.088	0.743	1.625	3.448	2.653	0.225	0.022	0.077	2.636
			----------Probability----------								
Oil quality			0.533	0.729	0.047	0.594	0.031	0.033	0.046	0.886	0.104
Oil type			0.988	0.384	0.053	0.786	0.019	<.001	0.025	0.206	0.116
Vitamin E			0.259	0.739	0.781	0.995	0.674	0.376	0.491	0.711	0.837
Oil quality x vitamin E			0.200	0.000	<.001	0.000	0.627	0.138	0.219	0.015	0.032
Oil type x oil quality			0.330	0.040	0.010	0.002	0.420	0.263	0.166	0.211	0.118
Oil type x vitamin E			0.389	0.438	0.209	0.162	0.682	0.269	0.418	0.152	0.569
Oil type x oil quality x vitamin E			0.280	0.421	0.086	0.181	0.134	0.030	0.302	0.393	0.291

[a–b]Means with different superscripts within the same column differ significantly based on Tukey-Kramer comparison (P < 0.05).

[1]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

[x]Values represent the mean of 8 replicates cages (pooled excreta of 6 birds per replicate) except for mean values with x and y where the number of replicates (n) was 7 and 6, respectively.

Table 4.10 continued. Main and simple effects of oil quality, oil type, and vitamin E supplementation on selected fatty acids in the liver of 20-day old broiler chickens (%)*

Oil quality	Oil type	Vitamin E	C22:0	C22:2	C22:4	C24:0	∑SFA	∑MUFA	∑PUFA	∑UFA	SFA:UFA
Main effect											
Fresh			0.331	1.165	1.192	1.064	36.1^{b}	20.9	40.6	60.2	0.596^{b}
Oxidized			0.361	1.101	1.251	1.142	38.5^{a}	20.5	39.7	60.5	0.643^{a}
	Corn oil		0.194^{b}	1.082	1.582^{a}	0.759^{b}	36.9	20.4	40.2	59.4	0.622
	Soy oil		0.498^{a}	1.184	0.861^{b}	1.447^{a}	37.7	21.0	40.1	61.3	0.616
		No	0.351	1.132	1.206	1.060	36.4	20.7	40.3	59.8	0.606
		Yes	0.340	1.134	1.237	1.146	38.2	20.7	40.0	60.9	0.632
2-way interaction effect											
Oil quality x vitamin E											
Fresh	-	No	0.341	1.087	1.238	1.217^{ab}	35.2	18.9^{b}	41.6	57.8^{b}	0.596
Fresh	-	Yes	0.322	1.243	1.146	0.912^{b}	37.1	22.9^{a}	39.6	62.6^{a}	0.595
Oxidized	-	No	0.362	1.177	1.174	0.903^{b}	37.5	22.5^{a}	39.0	61.8^{a}	0.616
Oxidized	-	Yes	0.359	1.024	1.329	1.381^{a}	39.4	18.5^{b}	40.4	59.1^{ab}	0.670
Oil type x oil quality											
Fresh	Corn oil	-	0.177	1.032^{b}	1.666	0.793	35.5	18.9^{c}	41.4	57.6^{b}	0.608
Oxidized	Corn oil	-	0.212	1.140^{ab}	1.498	0.724	38.2	21.8^{ab}	38.9	61.1^{ab}	0.637
Fresh	Soy oil	-	0.486	1.302^{a}	0.718	1.336	36.8	22.8^{a}	39.8	62.7^{a}	0.583
Oxidized	Soy oil	-	0.509	1.061^{b}	1.004	1.559	38.7	19.2^{bc}	40.4	59.8^{ab}	0.649
Oil type x vitamin E											
-	Corn oil	No	0.193	1.080	1.638	0.774	35.3	19.6	40.8	58.1	0.602
-	Corn oil	Yes	0.196	1.084	1.525	0.743	38.4	21.1	39.6	60.7	0.643
-	Soy oil	No	0.510	1.185	0.773	1.345	37.4	21.7	39.8	61.5	0.610
-	Soy oil	Yes	0.485	1.184	0.949	1.549	38.1	20.3	40.5	61.1	0.622
Dietary treatments											
Fresh	Corn oil	No	0.165	0.994^{x}	1.773	0.859^{bc}	33.3	16.8^{x}	42.2^{x}	53.5	0.590^{x}
Fresh	Corn oil	Yes	0.188	1.058^{x}	1.559	0.727^{c}	37.7^{x}	21.1	40.6	61.7	0.625^{x}
Oxidized	Corn oil	No	0.220	1.165^{x}	1.504	0.689^{c}	37.4^{x}	22.5	39.3	62.6^{x}	0.613^{x}

Oxidized	Corn oil	Yes	0.204	1.109^{x}	1.492	0.760^{c}	39.1	21.1	38.5	59.6	0.661
Fresh	Soy oil	No	0.517	1.180^{x}	0.703	1.575^{ab}	37.2^{x}	21.0^{x}	41.0^{x}	62.0^{x}	0.602^{x}
Fresh	Soy oil	Yes	0.455	1.427^{x}	0.733^{x}	1.096^{bc}	36.4	24.7^{x}	38.6	63.5^{x}	0.565^{x}
Oxidized	Soy oil	No	0.504	1.189^{x}	0.843	1.116^{bc}	37.7^{x}	22.4	38.6	61.0	0.619^{x}
Oxidized	Soy oil	Yes	0.515^{x}	0.940^{x}	1.166	2.002^{a}	39.7^{x}	16.0^{x}	42.3	58.7^{x}	0.678^{x}
Pooled SD[1]			0.089	0.289	0.497	0.483	4.361	3.679	4.381	5.313	0.074
			----------Probability----------								
Oil quality			0.197	0.409	0.637	0.524	0.047	0.675	0.394	0.840	0.020
Oil type			<.001	0.190	<.001	<.001	0.445	0.490	0.962	0.170	0.744
Vitamin E			0.626	0.986	0.801	0.476	0.107	0.965	0.809	0.437	0.183
Oil quality x vitamin E			0.715	0.052	0.329	0.002	0.963	0.001	0.129	0.009	0.170
Oil type x oil quality			0.780	0.028	0.076	0.231	0.713	0.001	0.169	0.025	0.365
Oil type x vitamin E			0.519	0.972	0.254	0.336	0.278	0.151	0.404	0.282	0.445
Oil type x oil quality x vitamin E			0.215	0.229	0.857	0.020	0.224	0.261	0.236	0.175	0.307

[a–b]Means with different superscripts within the same column differ significantly based on Tukey-Kramer comparison (P < 0.05).

[1]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

[x]Values represent the mean of 8 replicates cages (pooled excreta of 6 birds per replicate) except for mean values with x and y where the number of replicates (n) was 7 and 6, respectively.

*∑represents the sum of: SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; UFA = unsaturated fatty acids. SFA:UFA = ratio of saturated fatty acids to unsaturated fatty acids

Table 4. 11 Comparison of the mean fatty acid profile of the adipose and liver tissues[1]

Fatty acid composition, %	Abdominal fat	Subcutaneous fat	Liver	Pooled SD	P-value
C16:0 (Palmitic acid)	20.8[b]	21.8[a]	8.7[c]	1.55	<.001
C18:0 (Stearic acid)	5.1[b]	5.4[b]	17.9[a]	1.243	<.001
C18:1 (Oleic acid)	36.3[a]	36.1[a]	17.0[b]	2.752	<.001
C18:2 (Linoleic acid)	27.9[a]	27.3[a]	21.6[b]	2.783	<.001
C18:3n-3 (Alpha-linoleic acid)	1.708[a]	1.594[a]	0.568[b]	0.576	<.001
C20:4 (Arachidonic acid)	0.283[c]	1.594[b]	12.9[a]	1.671	<.001
ΣSFA	26.6[c]	28.0[b]	37.2[a]	2.938	<.001
ΣMUFA	42.8[a]	42.3[a]	20.8[b]	3.230	<.001
ΣPUFA	30.5[b]	28.8[c]	40.1[a]	4.454	<.001
ΣUFA	73.2[a]	71.4[b]	60.2[c]	3.975	<.001
SFA:UFA	0.364[b]	0.481[b]	0.620[a]	0.3686	0.001

[1]The deposition of the most abundant FAs (C16:0, C18:0, and C18:1) and the essential FAs (C18:2, C18:3n-3, and C20:4) were compared across the abdominal fat, subcutaneous fat and the liver regardless of dietary treatments, using the GLM procedure of SAS.

*∑represents the sum of: SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; UFA = unsaturated fatty acids. SFA:UFA = ratio of saturated fatty acids to unsaturated fatty acids

Table 4. 12 Comparison of the mean fatty acid profile of the abdominal and subcutaneous fat tissues[1]

Fatty acid composition, %	Abdominal fat	Subcutaneous fat	Pooled SD	P-value
C16:0 (Palmitic)	20.8[b]	21.8[a]	1.199	<.001
C18:0 (Stearic)	5.1[b]	5.4[a]	0.509	0.001
C18:1 (Oleic)	36.3	36.1	1.842	0.575
C18:2n-6 (Linoleic)	27.9	27.3	2.770	0.180
C18:3n-3(Alpha linolenic acid)	1.708	1.594	0.679	0.362
C20:4 (Arachidonic acid)	0.283[b]	1.594[a]	0.462	<.001
∑SFA	26.6[b]	28.0[a]	1.610	<.001
∑MUFA	42.8	42.3	2.487	0.239
∑PUFA	30.5[a]	28.8[b]	4.487	0.029
∑UFA	73.2[a]	71.4[b]	2.564	0.001
SFA:UFA	0.364	0.481	0.448	0.165

[1]The deposition of the most abundant FAs (C16:0, C18:0, and C18:1) and the essential FAs (C18:2, C18:3n-3, and C20:4) were compared across the abdominal and subcutaneous fats regardless of dietary treatments, using the GLM procedure of SAS.

*∑represents the sum of: SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; UFA = unsaturated fatty acids. SFA:UFA = ratio of saturated fatty acids to unsaturated fatty acids

Table 4. 13 The effect of oil quality, oil type, and vitamin E supplementation on antioxidant enzymes

Oil quality	Oil type	Vitamin E	Serum SOD[1] (U/mL)	Liver SOD (U/mL)
	Main effect			
Fresh			5.920	3.420
Oxidized			6.010	3.410
	Corn oil		5.670	3.450
	Soy oil		6.270	3.380
		No	5.540	2.780
		Yes	6.400	4.050
	Dietary treatments			
Fresh	Corn oil	No	5.420[x]	2.860[x]
Fresh	Corn oil	Yes	6.470[x]	4.050
Oxidized	Corn oil	No	4.980[x]	3.220[x]
Oxidized	Corn oil	Yes	5.800[x]	3.670[x]
Fresh	Soy oil	No	6.310[x]	2.240[x]
Fresh	Soy oil	Yes	5.500[x]	4.540
Oxidized	Soy oil	No	5.460[x]	2.800[x]
Oxidized	Soy oil	Yes	7.810	2.940
	Pooled SD[2]		2.160	2.820
……Probability……..				
Oil quality			0.879	0.983
Oil type			0.297	0.920
Vitamin E			0.142	0.091
Oil quality x vitamin E			0.207	0.519
Oil type x oil quality			0.268	0.997
Oil type x vitamin E			0.883	0.544
Oil type x oil quality x vitamin E			0.146	0.887

[1]Superoxide dismutase

[2]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

[x]Values represent the mean of 8 replicates cages (pooled excreta of 6 birds per replicate) except for mean values with x where the number of replicates (n) was 7

4.5 Discussion

4.5.1 Lipid Oxidation, Diet Composition, and Growth Performance.

A variety of oil types such as palm oil, corn oil, soy oil, and linseed oil are used in the formulation of poultry and swine diets, in an attempt to raise the energy levels of feed. Interestingly, most of these commonly used dietary oils have a high PUFA content. Lipid sources that are rich in PUFA are highly prone to oxidation both at low and high temperatures. During storage of unsaturated fats at ambient temperature, lipid hydroperoxides (LHPs), which are the primary products of oxidation, increase until they reach a plateau, and then decompose into secondary oxidation products (Tres et al., 2010). At high temperatures, however, the decomposition of LHP is faster and this favors the isomerization of FA, which leads to the appearance of both geometric and positional FA isomers. Thus, lipid oxidation can be accomplished by thermal processing of PUFA-rich oils and this results in an increase in the total SFA content, a decrease in the total UFA content, and a greater production of lipid oxidation products (Shurson et al., 2015). A simple and easy way of measuring LHP content in fats and oils is the peroxide value (PV). In the current study, the PV rose from 3 meqO_2/kg to 104 meqO_2/kg in corn oil and from 4 meqO_2/kg to 109 meqO_2/kg in soy oil after the fresh oils were exposed to thermal treatment at 95 ^oC for about 84 cumulative hours. Delles et al. (2013) also achieved a PV of 121 meqO_2/kg in soy oil after heating the fresh oil for 7 days at 95 ^oC.

In the current study, the dietary supply of oxidized oils resulted in significantly lower feed efficiency over days 0-20 but did not significantly affect LBW, BWG, or FI. The lack of effect of oxidized oils on the LBW, BWG, and FI was somewhat surprising

because many past studies have reported a depression in various growth performance parameters in broiler chickens fed oxidized oils, which is due to the presence of various oxidation products that negatively impact the chickens (Takahashi and Akiba, 1999; Anjum et al., 2004; Tavárez et al., 2011). Other authors such as Dibner et al. (1996) and Takahashi and Akiba (1999) have also reported a decrease in the feed efficiency of broilers fed oxidized lipids, as observed in the current study.

Furthermore, the results of the current study showed an interaction between oil quality and VE across days 0-14 and 0-20, where birds fed diets containing oxidized oil with VE had a lower LBW and BWG compared to other groups. Interestingly, this result can be traced to various 3-way interactions where the birds that received diets containing oxidized soy oil with VE performed significantly worse than most of the other treatment groups in terms of their LBW, BWG, and FI across days 0-14 and their LBW, BWG and FE across days 0-20. This result was particularly strange because the whole purpose of providing additional supplemental VE at 150 ppm was to help ameliorate the potentially deleterious impacts of oxidized oils. But rather than ameliorating this effect, additional supplemental VE at 150 ppm worsened the negative impacts of the oxidized oils, particularly the oxidized soy oil, on the growth performance. While the reason for this is not entirely clear at the moment, one possible explanation could be that the VE had become completely oxidized and turned into VE radicals by the various oxidation products within the oxidized oil before its addition to the feed. This is possible because the VE was first mixed with the oils before the oils were mixed into the feed. Because VE is a radical-scavenging antioxidant, when it donates a proton to stabilize peroxyl/lipid radicals, it is converted into vitamin E radical, which may be further oxidized into α-tocopheryl quinone

or reduced by vitamin C or other reducing compounds to regenerate vitamin E (Niki, 2015). But in the absence of reducing agents, VE can act as a radical capable of oxidizing lipids or completely binding to lipid radicals (Rizvi et al., 2014), in which case, its effect could be somewhat negative. However, it is still unclear why this effect was more pronounced with oxidized soy oil. Further analysis of the various oxidation products within the oxidized oils might provide more insight in this regard.

4.5.2 Relative Fat and Liver Percentages

In this study, an interaction between oil type and VE was observed for the relative abdominal fat weight, where the birds that received soy oil and no VE had a higher relative abdominal fat weight compared to those fed diets containing soy oil and VE. Although the role of VE as an antioxidant has been established by many studies, very little information is available about its role in adipogenesis. However, VE has been reported to reduce the accumulation of lipids in the liver of mice (Alcalá et al., 2015), which suggests that oxidative stress may play a role in adipogenesis and tissue lipid distribution. Thus the dietary supplementation of VE at the right dose could help reduce abdominal fat deposition. Further investigation is required in this regard.

No simple or main effects of oil type, oil quality, or VE supplementation was observed on liver percentages. This was surprising because, at the very least, it was expected that oxidized oils would increase the size of the liver and thus, its relative weight. The relative liver weight or hepatosomatic index serves as an indicator of toxicity in biological systems (Juberg et al., 2006). A possible reason for this lack of effect could be that the degree of oxidation was not high enough or that the length of the feeding trial (20

days) was not sufficient to trigger greater hepatocyte proliferation (Dibner et al., 1996) and increased enzyme synthesis (Huang et al., 1988) that accompanies significant toxicity.

4.5.3 Nutrient and Energy Utilization

The products of lipid oxidation can contribute significantly to oxidative stress and reduce the efficiency of nutrient utilization as animals attempt to deal with the damage occurring at the cellular and systemic levels (Lykkesfeldt and Svendsen, 2007). In the current study, oxidized oils reduced Nitrogen retention and this effect was more pronounced in birds fed corn oil. While it has been reported that fresh oil produces a better N utilization than oxidized oils (Yuan et al., 2007), the reason behind the significantly worse impact of oxidized corn oil compared to oxidized soy oil is not entirely clear at this time. But a possible explanation may be the lower total tocopherol content of corn oil, which possibly aggravated the progression of lipid oxidation and its deleterious impacts on nutrient utilization.

Oxidized oils also reduced energy utilization as well as AMEn, but did not affect AME, crude fat, and dry matter utilizations. Some studies have suggested that the energy value of lipids is decreased by oxidation (Dibner et al., 2011). Furthermore, the digestibility of certain nutrients such as crude protein (Yuan et al., 2007), and energy (Inoue et al., 1984; Engberg et al., 1996) have been reported to decrease when oxidized lipids are fed. However, this effect may depend on the level of oxidation products present, as some other studies have reported no differences in the utilization of nutrients and energy when oxidized lipids are fed (DeRouchey et al., 2004; Liu, 2012). The effect of oxidized lipids seen in AMEn and not in AME in the current study is likely due to the deleterious impact

of lipid oxidation products on N retention, which is believed to result from the damage of amino acids by free radicals, as well as an alteration in the gastrointestinal physiology.

Surprisingly, in this study, additional VE supplementation reduced crude fat, nitrogen, and energy utilization, as well as AME and AMEn. Similarly, the birds fed the diet containing soy oil with additional VE supplementation produced a lower energy utilization compared to those that received diet containing corn oil with no additional VE or diet containing soy oil with no additional VE supplementation. These seemingly negative effects of additional VE supplementation beyond the level of the VE in the basal diet (which already exceeded the requirements for birds of this age) were unexpected because VE is considered a powerful antioxidant that reduces oxidative stress in broilers and improves the efficiency of nutrient utilization. To the best of our knowledge, past studies that have investigated the impact of VE supplementation on nutrient and energy utilization in broilers have either reported a positive effect or no effect (Pompeu et al., 2018), but not a negative effect. It is noteworthy, however, that the additional supplemental VE added at 150 ppm was added to basal diets containing VE levels which were between 83-117 IU/kg and had already exceeded the Cobb recommendation (80 IU/kg). In VE supplemented groups, the analyzed VE content ranged from 168-227 IU/kg. Although Morrissey et al. (1997) reported that birds can obtain beneficial performance effects from dietary VE up to 200 mg/kg, it is possible that the birds in the current study could not tolerate such a high level of dietary total VE and this may have played a role in the negative VE effects observed. Furthermore, VE forms a radical after donating a hydrogen atom to interrupt a free radical chain reaction. But if there are not sufficient reducing agents such

as vitamin C in the system, it can also function as a pro-oxidant, in which case it could have a somewhat negative effect.

4.5.4 Fatty Acid Profiles

4.5.4.1 Fatty Acid Profile of Oil and Diet

The FA profile of oils is highly influenced by its source. For instance, palm oil generally contains more SFAs than corn or soy oils which are typically rich in PUFAs. In the current study, C16:0 was the major SFA for both corn and soy oils and this is consistent with the reports of past studies (Zambiazi et al., 2007). Both oils had a fairly similar composition before and after heating but corn oil had higher levels of C18:1 in both the fresh (28.94 vs. 21.82) and oxidized oils (30.94 vs. 23.25). Conversely, soy oil had a much higher content of C18:3n-3 in both the fresh (7.28 vs. 1.36) and oxidized oils (6.21 vs. 1.11). Zambiazi et al. (2007) also reported similar findings of C18:1 (24.23 vs. 21.35) and C18:3 (0.99 vs.7.15) in corn and soy oils, respectively. In the current study, the percent concentrations of linoleic (C18:2) decreased by 8.1% and 4.4%, in both corn and soy oils, respectively, as the oils were thermally oxidized. This was expected because PUFAs are very susceptible to oxidation (Belitz et al., 2009), which results in their degradation and ultimately decreases their concentration. There was also an increase in the total SFA and MUFA content and a decrease in the total PUFA and UFA content as both oils were oxidized. This result agrees with the report of other authors who have also declared a decline in PUFA and a rise in SFA after oil oxidation (DeRouchey et al., 2004; Liu, 2012). The results obtained in this study also support the idea that PUFAs are degraded preferentially compared to other FA during oxidation because SFA concentration changed

minimally and the concentration of MUFA increased after heating. As expected, the FA profile of the diet follows the FA profile of the oils added to it although the concentrations of C16:0, C18:2, and C22:2 FAs were slightly higher in the diet.

4.5.4.2 Fatty Acid Profile of the Abdominal Fat and Subcutaneous Fat

To a reasonable extent, the FA profile of adipose tissues is influenced by the FA profile of the diet (Hwong et al., 1988) and this is mainly because dietarily non-essential FAs are directly incorporated into adipose tissues with little to no modification (Corino et al., 2002; Rentfrow et al., 2003; King et al., 2004). For instance, in the current study, the percent concentration of C17:0 and C17:1 were both individually below 0.1% in the diet and in the adipose tissues, the percent composition of the same FAs was still below 0.47%. Although there is a rise in the concentration of these FAs in the adipose tissues, which may be accounted for by *de novo* lipogenesis, the lower levels of these FAs in the adipose tissues are still relatively closer to dietary levels compared to essential FAs. In both fat depots, the most abundant FA is C18:1 (oleic acid), followed by C18:2 (Linoleic acid) and C16:0 (palmitic acid). This is clearly a deviation from the FA levels in the diet, where C18:2 was the most abundant, followed by C18:1 and C16:0.

Various studies have also reported the dominance of oleic acid (C18:1) in adipose tissues (Zollitsch et al., 1997; Bavelaar and Beynen, 2003). For instance, when Zollitsch et al. (1997) fed different lipid sources, oleic acid was the most abundant FA in the abdominal fat tissue regardless of dietary treatment. Elsewhere, Wang et al. (2019) reported that in both the belly and back fat of pigs, oleic acid was the most abundant FA regardless of dietary treatment. In another swine study, Bavelaar and Beynen (2003) also corroborated these findings and reported that oleic acid had the highest concentration in the backfat of

pigs regardless of dietary treatments. This dominance of oleic acid regardless of dietary treatments in different monogastric species, therefore, suggests that it plays an important role especially in terms of metabolic activity. The high concentration of oleic acid may also be related to the fact that its tissue concentration depends not only on its intake but also on *de novo* synthesis (Skřivan et al., 2018). Interestingly, in the current study, in both abdominal and subcutaneous fat depots, the concentration of C18:1 was higher in the birds that were fed oxidized corn oil compared to others. This may be because oxidized corn oil had the highest content of C18:1, which may have influenced the higher adipose tissue concentration of oleic acid. This result suggests that although oleic acid tissue concentration is not entirely dictated by the FA composition of the diet, it is still influenced by it.

Linoleic acid (C18:2) is an essential FA that occurs in the greatest proportion in both corn and soy oils. The level of C18:2 in the adipose tissue is highly influenced by dietary fat intake in poultry. At low lipid intake levels, diet-supplied C18:2 will likely be more diluted by FAs generated by de novo synthesis, compared to high intake levels (Beynen et al., 1980). In the current study, C18:2 was the second most abundant FA in the adipose tissues and although its deposition in the abdominal fat was uninfluenced by oil type, oil quality, or VE, an interaction between oil type and VE affected its concentration in the subcutaneous fat. Specifically, birds that received fresh corn oil had significantly higher levels than those that received fresh soy oil. Although the reason for this selective deposition is not entirely clear, the dietary levels of the FA may have influenced this result, as fresh corn oil had a slightly higher content of C18:2 than fresh soy oil.

Alpha-linolenic acid (C18:3n-3) is a major essential FA that must be supplied in the diets of poultry to ensure proper physiological functions. Among other benefits, C18:3n-3 elongates in the tissues to produce long-chain n-3 PUFA, which are highly beneficial for immune response and the overall health of the cardiovascular system (Wood and Enser, 1997). In the current study, the dietary supply of soy oil increased the concentration of C18:3n-3 in both the abdominal and subcutaneous adipose tissues. This increase is mainly due to the higher levels of C18:3n-3 in the diets formulated with soy oil (Kloareg et al., 2007), thereby suggesting that soy oil may be more beneficial for broilers as far as increasing the tissue levels of this FA is concerned.

4.5.4.3 Fatty Acid Profile of the Liver

In general, the FA composition of the liver reflects the FA profile of dietary fats used to formulate the diets (Hwong et al., 1988), especially for non-essential FAs. In the current study, the FA profile of the liver was considerably different from that of the adipose tissues. For instance, just like the diets, the most abundant FA in the liver is C18:2, much unlike the adipose tissue where the concentration of C18:1 was the highest. The liver also had the highest content of C18:0 (stearic acid) and C20:4 (arachidonic acid). The higher concentration of C20:4 in the liver corresponds with a decrease in the percent concentration of C18:2 and C18:3 and this indicates an active metabolism and the modification of essential FAs within the liver (Jump et al., 2005; Kloareg et al., 2007).

The content of 18:3n-3 in the liver is important because it is the substrate of elongases and desaturases (Yan et al., 2015), which give rise to longer chain FAs. Interestingly, in the current study, the dietary treatment containing fresh soy oil with VE had the highest content of C18:3n-3 compared to the other dietary treatments. This is

believed to be influenced by dietary levels of this FA, as fresh soy oil had the highest concentration of C18:3n-3.

In summary, although individual differences exist in the liver among the FA concentrations across the various treatments, the liver FA content of non-essential FAs was influenced the dietary FA intake levels in this study, but not entirely controlled by the dietary FA profile. For instance, although the dietary level of C16 was just above 11%, its concentration in the liver was greater than 18%. This further reiterates the fact that active modification such as elongation and desaturation of FAs occurs in the liver (Duran-Montgé et al., 2009; Kloareg et al., 2007).

4.5.4.4 Comparison of the Mean Fatty Acid Profile of the Adipose and Liver Tissues

As expected, the average composition of the essential FAs (C18:2, C18:3n-3 and C20:4) and the most abundant FAs (C16:0, C18:0 and C18:1), across the abdominal fat, subcutaneous fat, and liver were very similar to that of the previous experiment. As expected, the general composition of the selected FAs varied significantly across the tissues. Again the liver had the greatest amount of SFAs compared to the subcutaneous and abdominal fats. In general, a greater SFAs content in the liver reduces its susceptibility to lipid oxidation. However, from a human consumption standpoint, a higher SFA:UFA content is generally considered unfavorable as saturated fats have been closely linked to cardiovascular diseases (Briggs et al., 2017). The results of this comparison also showed a greater content of MUFAs and UFAs in the abdominal fat compared to the liver, while the liver had a greater content of PUFA. The high PUFA content in the liver was expected because PUFAs such as C18:2 and C18:3n-3 are major substrates of elongases and desaturases which are responsible for synthesizing longer chain FAs. This explanation is

corroborated by the higher content of arachidonic acid (C20:4) in the liver, which indicates an active metabolism and the modification of essential FAs such as C18:2 and C18:3n-3 within the liver (Jump et al., 2005; Kloareg et al., 2007).

4.5.5 Superoxide Dismutase (SOD) Activity

Superoxide dismutase (SOD) is an important antioxidant enzyme that reduces oxidative load in biological systems by converting oxygen and hydrogen-free radicals into hydrogen peroxide, which is less toxic than free radicals (Kalyanaraman, 2013). In the current study, there were no significant differences in the SOD activity in both the liver and serum. Although we expected an increase in SOD activity due to the potentially greater oxidative load oxidized oils could cause, the lack of effect was not completely surprising because other studies have also reported similar findings. For instance, Lindblom (2017) observed no significant differences in SOD activity when pigs were fed oxidized oils. Similarly, Lin et al. (2006) also reported no changes in liver SOD after inducing oxidative stress in broilers via heat exposure. However, this result is in contrast to authors such as Altan et al. (2003) who reported a 47.4% increase in plasma SOD in heat-stressed broilers. The lack of differences in SOD activity observed in this study could be due to the limited degree of oxidation in the oils (average of 106 meqO_2/kg) as well as the short length of the feeding trial.

4.6 Conclusion

The results of this study showed that oxidized lipids significantly altered the FA profile of the liver and adipose tissues and also have a deleterious impact on weight gain, feed efficiency, and nutrient utilization in 20-day-old broilers. Also, the additional

supplementation of VE (mainly composed of γ-tocopherols) at 150 ppm exerted no beneficial effects on the growth performance and nutrient utilization of 20-day old broilers chickens when added to basal diets containing adequate levels of vitamin E. Furthermore, VE supplementation at 150 ppm negatively affected growth performance when oxidized soy oil with at peroxide value of 109 meqO_2/kg was fed. So, if a poultry producer intends to use oxidized soy oil in their broiler feed, a dietary supplementation of mixed tocopherols (mainly composed of gamma-tocopherol) at 150 ppm above basal diet VE level is strongly discouraged.

CHAPTER 5

Summary and Conclusion

Poultry diets are routinely supplemented with different types of vegetable oils for various reasons, such as raising the energy level of feeds, reducing feed dustiness, improving feed palatability, and providing fat-soluble vitamins. Different oil types have a unique fatty acid profile although certain oils can be grouped together if they have similar fatty acid profiles. Speaking of fatty acids profiles, most vegetable oils used in poultry diets are rich in polyunsaturated fatty acids (PUFA), which are highly susceptible to degradation through lipid oxidation. Lipid oxidation is easily induced by various factors such as heat, light, and certain pro-oxidant metals such as copper and iron. It is a chain reaction that leads to the formation of various undesired products which can induce stress, affect nutrient utilization, and reduce performance, and negatively impacts the welfare of the animals. Unfortunately, most poultry producers do not pay close attention to the quality of the oils used in formulating animal diets, which may end up affecting the welfare of the animals. One of the commonly proposed ways of combating the negative effects of oxidized oils is the use of antioxidants such as VE. However, the extent of the proposed beneficial effect of VE is still unclear especially when birds are fed oxidized lipids.

Phytase is another commonly used feed additive that is supplemented to poultry diets primarily to improve the availability of phytate-bound phosphorus present in plant feed ingredients. However, phytase does not just release phosphorus when it hydrolyses phytate, the hydrolysis also releases some amino acids and minerals which exert an "extra-phosphoric effect" on the bird. Interestingly, poultry producers routinely utilize the feed ingredients and additives discussed above without paying much attention to the possible

interactive effects they may have on the birds. Currently, there is little to no information available on the potentially interactive effects that oil type, oil quality, phytase and VE supplementation may have on the growth performance, nutrient utilization, relative fat and liver percentages, and the fatty acid profiles of the liver and adipose tissues of broiler chickens. Thus we conducted two studies to address this need.

In the first experiment, we examined the effects of oil quality, phytase, and VE supplementation on the performance, fatty acid profile and relative weights of liver and fat, and apparent utilization of energy and nutrients in 21-day-old broiler chickens. The results of this study revealed that phytase supplementation in broiler diets comes with various benefits including improvements in feed efficiency, calcium utilization, bone-breaking strength, tibia ash, AME, and AMEn. Phytase supplementation is also beneficial when feeding oxidized oils as it improved AME and AMEn. Oxidized oils had a minimally negative effect on performance as they only increased the relative liver weight (as an indicator of toxicity) but did not affect growth performance and nutrient utilization. This suggests straightaway that the previously reported negative effects of oxidized oils may not be noticeable in the performance of the birds in the short term depending on the degree of oxidation and how long the birds stay on the oxidized diet. The danger here is that poultry producers may be convinced to keep feeding oxidized oils since no noticeable reduction in performance takes place in the short term. But this would be bad for the welfare of the animal.

In the end, VE supplementation at 150 ppm above adequate basal levels had a moderately beneficial effect, as it improved the live weight, body weight gain, and feed intake of the birds, although this effect only lasted till day 14. Also, the results of this study

revealed that the fatty acid profiles of adipose tissues and liver are influenced either independently or interactively by oil quality, phytase, and VE supplementation. For instance, the total content of polyunsaturated fatty acids reduced when birds were fed oxidized lipids. It was quite interesting to see that VE supplementation at 150 ppm promoted FA unsaturation, which increased the chances of lipid oxidation. This was unexpected because as an antioxidant, it was hypothesized that VE would slow down the processes that foster lipid oxidation. But this also suggests that high doses of VE may have a more unfavorable impact on broiler chickens. Further investigation is required to fully understand the underlying mechanism behind the impacts of these factors on FA synthesis, degradation, and deposition in the adipose and hepatic tissues of broiler chickens.

In the second experiment, we examined the effects of oil type, oil quality, and VE supplementation on the performance, the fatty acid profile of liver and adipose tissues, relative liver and fat weight, and the apparent total tract utilization of nutrients in 20-day-old broiler chickens. The results of this study showed the potentially deleterious impact that diets containing oxidized oils can have on broiler chickens. Such negative impacts included a reduction in weight gain, feed efficiency, nitrogen, and energy utilization, and AMEn. We speculate that the more profound negative impacts of oxidized oils in experiment 2 are likely due to the greater length of exposure of the birds to the experimental diets (20 days) compared to experiment 1 (14 days). Also, in the current study, VE supplementation at 150 ppm to basal diets with adequate VE levels had a largely negative effect on the birds, as it reduced crude fat, nitrogen, and energy utilization, as well as AME and AMEn. We suspect that these negative effects of VE are likely because the supplied dose was too high for the birds. A strong influence of dietary FA profile was observed on

the FA composition of the adipose and hepatic tissues. Furthermore, the concentration of essential fatty acids such as linoleic and linolenic acids in the liver was independently affected by oil type and oil quality. This buttresses the importance of paying attention to the source and quality of lipids fed to broilers as far as the fatty acid composition of tissues is concerned.

In both studies, one rather strange trend that stood out and defied our hypothesis was that the birds that received oxidized oils generally performed better without additional supplemental VE at 150 ppm. This was quite interesting because it suggests that greater antioxidant supplementation is not necessarily the key to ameliorating the deleterious impacts of oxidized oils. From the comparison of the main fatty acids in the liver, abdominal and subcutaneous fats, it was interesting to see that the essential fatty acids including linoleic acid (C18:2) and alpha-linolenic acid (C18:3n-3) are primarily deposited in the adipose tissues and not in the liver. Also from the overall fatty acid profile of the liver, abdominal fat, and subcutaneous fat, it was clear that the liver and subcutaneous fat had more saturated fatty acids, which are generally considered unhealthy. So, when consuming poultry products, it would be a good practice to cut down on liver consumption and remove the skin before consuming chicken meat.

Looking ahead, further investigation into graded supplemental levels of mixed tocopherols when feeding oxidized oils or phytase is required to better understand the optimal additional supplemental VE level needed to provide maximum protection for the broiler chickens under these dietary conditions. Future studies should also focus on identifying and understanding the various oxidation products formed during the oxidation of soy oil and how they impact feed additives such as phytase and VE in the diets of broilers

APPENDICES

Appendix 1. Peroxide value determination protocol (AOAC)

Reagents required:

- Acetic acid : Chloroform solution (3:2)

Under the fume hood, while using nitrile gloves, use a glass graduated cylinder to measure 400 ml of chloroform and transfer to a glass amber bottle. Using a glass graduated cylinder, measure 600 ml of acetic acid and transfer to the bottle. Mix. Store in the cabinet under the fume hood. This is enough for approximately 30 tests.

- Saturated Potassium Iodide (KI) solution

Add 14 g potassium iodide to 10 ml of freshly boiled and cooled deionized water. Use within the same day. This is enough for 20 tests. Prepare only the amount you will need as KI quickly crystalizes after a while.

- 1% (w/v) Starch solution

Weigh 0.5 g of soluble starch into a 100 ml beaker. Add a few drops of cold deionized water to suspend. Add 50 ml of boiling deionized water. Boil 1 minute while stirring. Cool before use.

- 0.1N Sodium thiosulfate solution ($Na_2S_2O_3$)

Transfer 5 g of sodium thiosulfate pentahydrate to a 200 ml volumetric flask and dilute to volume with freshly boiled and cooled deionized water. Mix. Store in an amber bottle in the refrigerator. More dilute solutions are less stable and should be prepared just before use. For 0.01N sodium thiosulfate, transfer 50.0 ml with a volumetric pipet to a 500 ml

volumetric flask and dilute to volume with freshly boiled and cooled deionized water. To standardize the solution, accurately weigh 0.20 – 0.23 g to the nearest 0.0001 g of potassium dichromate ($K_2Cr_2O_7$) (that has been previously finely ground, dried at 100°C for 2 hours, and cooled in a desiccator) into a 250 ml screw-cap Erlenmeyer flask. Dissolve in 80 ml of deionized water containing 2 g potassium iodide. While stirring, add 20 ml 1N hydrochloric acid. Place in the dark immediately for 10 minutes. Titrate with sodium thiosulfate until the yellow color is pale but not gone. Add 0.5 ml 1% starch solution. Mix and titrate until the blue / purple color just disappears.

$$\text{Normality} = \frac{\text{g } K_2Cr_2O_7 \text{ x } 1000}{\text{ml } Na_2S_2O_3 \text{ x } 49.032}$$

Procedure for peroxide value determination in oils:

1. Run the blank (reagents only) and samples in duplicate. Weigh approximately 5 g of sample into a 250 ml screw-cap Erlenmeyer flask. Record the weight. The remaining steps should be carried out under the fume hood while using nitrile gloves. Using a glass graduated cylinder, add 30 mL of acetic acid : chloroform solution and swirl to dissolve.

2. Add 0.5 ml freshly prepared saturated KI solution and swirl to mix. Let stand with occasional shaking for 1 minute (fats and oils). Allow meat samples to mix by stirring or swirling in a dark room for 20 minutes.

3. Under the fume hood, use a bottle-top dispenser to add 30 ml of deionized water and mix.

4. While vigorously mixing on a stir plate under the fume hood, add 0.5 mL 1% starch solution and mix.

5. Continue vigorous mixing on the stir plate and very slowly titrate with the sodium thiosulfate (typically 0.01 N but may require more or less concentration; if less than 0.5 ml of 0.1 N $Na_2S_2O_3$ is used, repeat determination with 0.01 N $Na_2S_2O_3$) until the blue / purple color just disappears.

6. Record the amount of sodium thiosulfate solution used. All waste must be collected and disposed of as hazardous.

Calculation:

$$PV\ (meq/kg) = \frac{(Sample - Blank) \times N \times 1000}{Weight\ of\ sample}$$

N = Normality of the sodium thiosulfate solution used to titrate

Appendix 2. Phosphorus determination method

Phosphorus was determined in duplicates using the gravimetric quimociac method. The quimociac reagent is prepared one or two days in advance. This was done by dissolving ammonium molybdate tetrahydrate in nano-pure water. In a different beaker, citric acid monohydrate was dissolved in dilute nitric acid. Once cooled, the molybdate solution was added to the citric–nitric acid mixture while stirring via a magnetic stir bar. In another beaker, synthetic quinoline is added to nano-pure water. Then the quinoline solution is added to the molybdate–citric–nitric acid solution and left to sit overnight. The solution was filtered after which acetone and nano-pure water were added. The solution was then mixed and stored in an amber bottle prior to use for P analysis. For the actual P determination, 15 ml of the digest was transferred via a pipet to a 250 ml Erlenmeyer flask before 50 ml of nano-pure water was added. The solution was brought to a boil on a hot plate at about 500 °C before adding the quimociac reagent while swirling gently. The solution was heated for about 5 minutes, during which a yellow precipitate was formed. The mixture is then allowed to cool to room temperature before filtering the precipitate with the aid of a vacuum using a dry, clean, tared, medium-porosity fiber-glass filter fitted into a gooch crucible. The content of the Erlenmeyer flask was washed into the gooch crucible using nano-pure water and the resulting precipitate was dried overnight at 110 °C. The crucible containing the precipitate is cooled in a desiccator and weighed to the nearest 0.1 mg (Shaver, 2008). The percent P is determined using the following equation:

$$\% \text{ P} = \frac{weight\ of\ precipitate * 100\ ml * 0.013997 * 100}{ml\ of\ aliquot * sample\ weight}$$

Appendix 3. Analyzed fatty acids profile of adipose and liver tissues for chapter 3

Table A.3. 1 The effects of oil quality, phytase, vitamin E supplementation on the Fatty acid profile of the abdominal fat (Chapter 3)

Oil quality	Phytase	Vitamin E	C12:0	C14:0	C14:1	C15:0	C16:0	C16:1	C17:0	C17:1
Main effect										
Fresh			0.024	0.459	0.142	0.056	21.5	6.079	0.234[a]	0.113
Oxidized			0.023	0.466	0.163	0.057	21.8	6.365	0.195[b]	0.109
	No		0.023	0.464	0.147	0.056	21.6	6.208	0.223	0.113
	Yes		0.023	0.461	0.159	0.057	21.6	6.236	0.207	0.109
		No	0.024	0.472[a]	0.157	0.056	21.8	6.304	0.213	0.112
		Yes	0.023	0.451[b]	0.148	0.057	21.5	6.139	0.217	0.111
2-way interaction effect										
Oil quality x vitamin E										
Fresh	-	No	0.024	0.474	0.147	0.055	21.8	6.101	0.236	0.115
Fresh	-	Yes	0.023	0.444	0.138	0.057	21.2	6.057	0.232	0.112
Oxidized	-	No	0.023	0.472	0.168	0.057	21.8	6.508	0.190	0.108
Oxidized	-	Yes	0.023	0.460	0.159	0.057	21.8	6.222	0.202	0.110
Phytase x oil quality										
Fresh	No	-	0.023	0.453	0.119[b]	0.056	21.4	5.878	0.244	0.117
Fresh	Yes	-	0.024	0.465	0.165[a]	0.056	21.5	6.279	0.224	0.110
Oxidized	No	-	0.024	0.474	0.175[a]	0.055	21.9	6.538	0.201	0.111
Oxidized	Yes	-	0.022	0.458	0.152[ab]	0.058	21.7	6.192	0.190	0.108
Phytase x vitamin E										
-	No	No	0.024	0.469	0.147	0.055	21.7	6.188	0.221	0.114
-	No	Yes	0.023	0.458	0.148	0.056	21.5	6.227	0.224	0.114
-	Yes	No	0.024	0.477	0.168	0.056	21.8	6.419	0.204	0.110
-	Yes	Yes	0.022	0.445	0.149	0.058	21.5	6.051	0.210	0.108
Dietary treatments										
Fresh	No	No[1]	0.024	0.468	0.122[x]	0.056	21.8	5.949	0.251	0.120
Fresh	No	Yes	0.022	0.438[x]	0.117	0.056	21.1	5.808	0.238	0.114

Oxidized	No	No	0.023	0.471	0.171	0.055	21.7[x]	6.428	0.192	0.107
Oxidized	No	Yes	0.024	0.478	0.179[x]	0.056	22.0	6.648	0.211	0.114
Fresh	Yes	No	0.024	0.480	0.172[x]	0.055	21.7	6.252	0.221[x]	0.111
Fresh	Yes	Yes	0.024	0.449	0.159[x]	0.058	21.3	6.306	0.227[x]	0.109
Oxidized	Yes	No	0.023	0.474[x]	0.164[x]	0.058	21.9	6.587	0.188	0.109[x]
Oxidized	Yes	Yes	0.021	0.442	0.140	0.057	21.6	5.796	0.193	0.107
	Pooled SD		0.003	0.034	0.039	0.007	1.254	0.700	0.030	0.017
	PC[2]		0.022	0.443	0.151[x]	0.055	21.4	5.960	0.204	0.101
	Pooled SD[3]		0.003	0.040	0.051	0.005	0.909	0.343	0.026	0.014
			----------Probability----------							
Oil quality			0.428	0.449	0.066	0.717	0.333	0.141	<.001	0.378
Phytase			0.711	0.786	0.316	0.454	0.991	0.887	0.061	0.301
Vitamin E			0.272	0.026	0.424	0.664	0.431	0.393	0.605	0.878
Oil quality x vitamin E			0.644	0.355	0.967	0.666	0.393	0.530	0.339	0.557
Phytase x oil quality			0.105	0.136	0.003	0.607	0.709	0.057	0.557	0.349
Phytase x vitamin E			0.482	0.284	0.380	0.882	0.824	0.292	0.878	0.785
Phytase x oil quality x vitamin E			0.162	0.318	0.597	0.629	0.532	0.121	0.320	0.482
PC vs. NC			0.284	0.280	0.340	0.678	0.429	0.923	0.008	0.042

[a–b]Means with different superscripts within the same column differ significantly based on Tukey HSD ($P < 0.05$).

[1]Negative control (NC) diet

[2]PC=positive control

[3]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

Table A.3.1 continued. The effects of oil quality, phytase, vitamin E supplementation on the Fatty acid profile of the abdominal fat (Chapter 3)

Oil quality	Phytase	Vitamin E	C18:0	C18:1	C18:2	C18:3 n-6	C18:3 n-3	C20:0	C20:1	C20:2
Main effect										
Fresh			5.4	35.3[b]	26.7	0.267[b]	2.5[a]	0.089	0.333	0.116[a]
Oxidized			5.5	36.5[a]	25.4	0.292[a]	2.2[b]	0.096	0.324	0.101[b]
	No		5.4	35.9	26.1	0.277	2.4	0.088[b]	0.321	0.113
	Yes		5.5	35.9	26.0	0.282	2.3	0.098[a]	0.336	0.104
		No	5.3	36.0	25.8	0.289	2.3	0.095	0.334	0.106
		Yes	5.5	35.8	26.3	0.271	2.4	0.090	0.323	0.110
2-way interaction effect										
Oil quality x vitamin E										
Fresh	-	No	5.3	35.3	26.6	0.274	2.5	0.091	0.334	0.115
Fresh	-	Yes	5.4	35.3	26.8	0.261	2.5	0.087	0.331	0.116
Oxidized	-	No	5.4	36.7	25.0	0.303	2.1	0.099	0.334	0.097
Oxidized	-	Yes	5.6	36.4	25.9	0.281	2.2	0.094	0.314	0.104
Phytase x oil quality										
Fresh	No	-	5.4[ab]	35.1	26.9	0.269	2.6	0.087	0.323	0.121
Fresh	Yes	-	5.3[b]	35.4	26.5	0.265	2.5	0.092	0.339	0.111
Oxidized	No	-	5.3[ab]	36.6	25.2	0.286	2.2	0.089	0.314	0.105
Oxidized	Yes	-	5.7[a]	36.5	25.6	0.298	2.2	0.104	0.334	0.096
Phytase x vitamin E										
-	No	No	5.3	36.0	25.8	0.288	2.3	0.092	0.326	0.112
-	No	Yes	5.5	35.7	26.3	0.267	2.4	0.083	0.315	0.113
-	Yes	No	5.4	35.9	25.7	0.289	2.3	0.098	0.342	0.101
-	Yes	Yes	5.6	36.0	26.4	0.275	2.3	0.097	0.330	0.106
Dietary treatments										
Fresh	No	No[1]	5.3	35.3	26.8	0.279[x]	2.6	0.088	0.330	0.119[x]
Fresh	No	Yes	5.5	34.9	27.0[x]	0.260	2.6[x]	0.086	0.324[x]	0.122[x]
Oxidized	No	No	5.3	36.7	24.9	0.298[x]	2.1	0.096	0.322[x]	0.105

Oxidized	No	Yes	5.4	36.5	25.6	0.274[x]	2.2[x]	0.081[x]	0.307	0.105
Fresh	Yes	No	5.3	35.3	26.3	0.269	2.5	0.095	0.339	0.112[x]
Fresh	Yes	Yes	5.3	35.6	26.6	0.262[x]	2.5	0.089	0.338	0.110
Oxidized	Yes	No	5.4	36.6	25.1	0.308	2.2	0.102	0.346	0.090[x]
Oxidized	Yes	Yes	5.9	36.3	26.2	0.289[x]	2.2	0.106	0.322[x]	0.103[x]
Poled SD			0.365	1.294	2.348	0.041	0.306	0.017	0.033	0.022
PC[2]			5.3	36.0	27.0	0.245	2.5 [x]	0.099	0.371	0.104
Pooled SD[3]			0.456	1.094	1.982	0.043	0.240	0.019	0.034	0.043
			----------Probability----------							
Oil quality			0.144	0.001	0.053	0.039	<.0001	0.116	0.348	0.018
Phytase			0.295	0.830	0.976	0.705	0.715	0.029	0.087	0.158
Vitamin E			0.072	0.673	0.367	0.134	0.681	0.287	0.211	0.590
Oil quality x vitamin E			0.383	0.717	0.606	0.715	0.536	0.863	0.368	0.669
Phytase x oil quality			0.026	0.550	0.521	0.471	0.595	0.268	0.667	0.934
Phytase x vitamin E			0.731	0.663	0.853	0.741	0.666	0.411	0.933	0.730
Phytase x oil quality x vitamin E			0.079	0.538	0.889	0.882	0.580	0.227	0.727	0.497
PC vs. NC			0.997	0.316	0.815	0.194	0.693	0.305	0.040	0.551

[a–b]Means with different superscripts within the same column differ significantly based on Tukey HSD (P < 0.05).

[1]Negative control (NC) diet

[2]PC=positive control

[3]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

Table A.3.1 continued. The effects of oil quality, phytase, vitamin E supplementation on the fatty acid profile of the abdominal fat (Chapter 3)

`Oil quality	Phytase	Vitamin E	C20:3	C20:4	C22:0	∑SFA	∑MUFA	∑PUFA	∑UFA	SFA:UFA
Main effect										
Fresh			0.165	0.234	0.047	28.0	41.6[b]	30.1[a]	71.7	0.392
Oxidized			0.156	0.219	0.050	28.1	43.6[a]	28.1[b]	71.6	0.396
	No		0.161	0.232	0.051	28.2	42.5	29.0	71.6	0.396
	Yes		0.160	0.211	0.046	28.0	42.7	29.2	71.7	0.392
		No	0.159	0.219	0.047	28.0	42.8	28.8	71.6	0.393
		Yes	0.162	0.224	0.050	28.1	42.4	29.4	71.7	0.395
2-way interaction effect										
Oil quality x vitamin E										
Fresh	-	No	0.161	0.231	0.047	28.2	41.8	29.8	71.6	0.396
Fresh	-	Yes	0.170	0.217	0.046	27.8	41.5	30.4	71.8	0.388
Oxidized	-	No	0.157	0.207	0.046	27.9	43.8	27.9	71.7	0.390
Oxidized	-	Yes	0.155	0.231	0.054	28.3	43.3	28.3	71.5	0.403
Phytase x oil quality										
Fresh	No	-	0.165	0.234	0.049	28.2	41.2	29.9	71.5	0.399
Fresh	Yes	-	0.166	0.214	0.045	28.1	43.8	28.0	71.7	0.393
Oxidized	No	-	0.158	0.229	0.052	27.8	42.1	30.2	72.0	0.385
Oxidized	Yes	-	0.154	0.209	0.047	28.1	43.3	28.2	71.5	0.400
Phytase x vitamin E										
-	No	No	0.155	0.215	0.047	28.1	42.6	28.6	71.5	0.398
-	No	Yes	0.168	0.248	0.054	28.2	42.3	29.3	71.7	0.394
-	Yes	No	0.163	0.222	0.047	27.9	43.0	29.0	71.8	0.388
-	Yes	Yes	0.156	0.200	0.046	28.0	42.4	29.4	71.7	0.397
Dietary treatments										
Fresh	No	No[1]	0.156[x]	0.241	0.048	28.4[x]	41.5[x]	29.4[x]	71.1[y]	0.407[y]
Fresh	No	Yes	0.174[x]	0.228	0.050	28.0[x]	40.8[x]	30.4[x]	71.9[x]	0.391[x]
Oxidized	No	No	0.153	0.190[x]	0.046	27.9[x]	43.7	27.8[y]	71.9[x]	0.389[x]

Oxidized	No	Yes	0.163	0.268	0.058^{x}	28.3	43.9^{x}	28.3	71.5^{x}	0.398^{x}
Fresh	Yes	No	0.165	0.221	0.047	27.9	42.1^{x}	30.1^{x}	72.2^{x}	0.384^{x}
Fresh	Yes	Yes	0.167	0.206^{x}	0.043^{x}	27.6^{x}	42.1^{x}	30.4^{x}	71.8^{x}	0.385^{x}
Oxidized	Yes	No	0.161^{x}	0.223^{x}	0.046	27.9^{x}	44.0^{x}	28.0	71.5^{x}	0.392^{x}
Oxidized	Yes	Yes	0.146	0.194^{x}	0.049	28.4^{y}	42.7^{x}	28.4^{x}	71.5^{x}	0.408^{x}
Poled SD			0.034	0.069	0.016	1.236	1.542	2.400	1.253	0.023
PC[2]			0.159^{x}	0.224	0.050	27.5	41.5^{x}	29.4^{x}	71.4^{x}	0.387^{x}
Pooled SD[3]			0.026	0.078	0.014	1.163	2.369	1.652	1.421	0.013
			----------Probability----------							
Oil quality			0.325	0.784	0.507	0.779	<.001	0.007	0.690	0.485
Phytase			0.870	0.285	0.335	0.586	0.575	0.725	0.656	0.593
Vitamin E			0.723	0.785	0.484	0.535	0.125	0.876	0.326	0.131
Oil quality x vitamin E			0.530	0.317	0.335	0.301	0.808	0.921	0.591	0.137
Phytase x oil quality			0.765	0.989	0.906	0.878	0.320	0.449	0.945	0.718
Phytase x vitamin E			0.289	0.151	0.388	0.904	0.680	0.787	0.597	0.374
Phytase x oil quality x vitamin E			0.831	0.177	0.881	0.945	0.195	0.815	0.267	0.690
PC vs. NC			0.834	0.699	0.748	0.198	0.984	0.987	0.677	0.032

[a–b]Means with different superscripts within the same column differ significantly based on Tukey HSD ($P < 0.05$).

[1]Negative control (NC) diet

[2]PC=positive control

[3]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

Table A.3. 2 The effects of oil quality, phytase, vitamin E supplementation on the Fatty acid profile of the subcutaneous fat (Chapter 3)

Oil quality	Phytase	Vitamin E	C12:0	C14:0	C14:1	C15:0	C16:0	C16:1	C17:0	C17:1
Main effect										
Fresh			0.025	0.473	0.152	0.058	22.3	5.82	0.135	0.080
Oxidized			0.023	0.470	0.155	0.058	22.3	6.02	0.138	0.082
	No		0.025	0.470	0.153	0.057	22.4	5.94	0.144	0.082
	Yes		0.024	0.472	0.155	0.058	22.2	5.90	0.129	0.080
		No	0.024	0.481^{a}	0.158	0.058	22.6^{a}	6.01	0.138	0.079
		Yes	0.024	0.461^{b}	0.150	0.058	21.9^{b}	5.83	0.135	0.083
2-way interaction effect										
Oil quality x vitamin E										
Fresh	-	No	0.025	0.486	0.157	0.058	22.8	5.96	0.126	0.075
Fresh	-	Yes	0.025	0.459	0.148	0.058	21.8	5.68	0.144	0.085
Oxidized	-	No	0.024	0.477	0.159	0.058	22.5	6.06	0.149	0.084
Oxidized	-	Yes	0.023	0.463	0.152	0.058	22.1	5.97	0.126	0.080
Phytase x oil quality										
Fresh	No	-	0.025	0.458^{b}	0.145^{b}	0.058	22.2	5.75	0.143	0.079
Fresh	Yes	-	0.026	0.487^{a}	0.160^{ab}	0.057	22.3	5.89	0.128	0.086
Oxidized	No	-	0.025	0.482^{ab}	0.161^{a}	0.057	22.6	6.12	0.146	0.081
Oxidized	Yes	-	0.022	0.457^{b}	0.149^{ab}	0.059	22.0	5.91	0.129	0.078
Phytase x vitamin E										
-	No	No	0.024	0.475	0.157	0.057	22.6	5.97	0.139	0.081
-	No	Yes	0.025	0.465	0.149	0.058	22.2	5.90	0.150	0.084
-	Yes	No	0.024	0.488	0.158	0.058	22.7	6.05	0.136	0.077
-	Yes	Yes	0.023	0.456	0.151	0.058	21.6	5.75	0.121	0.082
Dietary treatments										
Fresh	No	No[1]	0.024	0.466	0.154^{x}	0.058	22.4	5.95	0.128	0.077
Fresh	No	Yes	0.025	0.450^{x}	0.135	0.058	21.9^{x}	5.55	0.157	0.081
Oxidized	No	No	0.024	0.484^{x}	0.16^{x}	0.056	22.8	5.99	0.149	0.085

Oxidized	No	Yes	0.024	0.481	0.162	0.057	22.5	6.25	0.142	0.087
Fresh	Yes	No	0.026	0.506	0.159	0.057	23.1	5.98	0.124	0.072
Fresh	Yes	Yes	0.026	0.468	0.161	0.058	21.5[x]	5.81	0.131	0.090
Oxidized	Yes	No	0.023	0.470	0.157	0.059	22.3	6.13	0.148	0.083
Oxidized	Yes	Yes	0.022	0.444[x]	0.141[x]	0.059	21.7	5.68	0.111	0.074
Poled SD			0.003	0.037	0.019	0.007	1.066	0.686	0.046	0.017
PC[2]			0.023	0.45	0.142	0.059	21.9	5.79	0.137	0.073
Pooled SD[3]			0.002	0.035	0.016	0.007	0.856	0.298	0.044	0.007
			----------Probability----------							
Oil quality			0.062	0.757	0.549	0.947	0.953	0.279	0.845	0.709
Phytase			0.632	0.795	0.676	0.737	0.407	0.906	0.237	0.667
Vitamin E			0.889	0.043	0.165	0.878	0.020	0.354	0.841	0.467
Oil quality x vitamin E			0.516	0.524	0.873	0.951	0.337	0.607	0.122	0.144
Phytase x oil quality			0.058	0.012	0.014	0.382	0.173	0.345	0.952	0.328
Phytase x vitamin E			0.661	0.252	0.849	0.999	0.253	0.522	0.309	0.859
Phytase x oil quality x vitamin E			0.961	0.999	0.083	0.689	0.470	0.224	0.862	0.197
PC vs. NC			0.343	0.419	0.183	0.946	0.280	0.364	0.731	0.340

[a–b]Means with different superscripts within the same column differ significantly based on Tukey HSD ($P < 0.05$).

[1]Negative control (NC) diet

[2]PC=positive control

[3]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

Table A.3.2 continued. The effects of oil quality, phytase, vitamin E supplementation on the Fatty acid profile of the subcutaneous fat (Chapter 3)

Oil quality	Phytase	Vitamin E	C18:0	C18:1	C18:2	C18:3 n-6	C18:3 n-3	C20:0	C20:1	C20:2
Main effect										
Fresh			5.7	35.2[b]	26.1	0.282	2.35[a]	0.095[b]	0.352	0.116
Oxidized			5.8	36.1[a]	25.5	0.290	2.10[b]	0.111[a]	0.357	0.107
	No		5.7	35.6	25.7	0.283	2.24	0.106	0.358	0.117
	Yes		5.8	35.7	25.8	0.288	2.22	0.099	0.350	0.106
		No	5.7	35.7	25.4	0.287	2.17	0.106	0.362	0.110
		Yes	5.7	35.5	26.2	0.285	2.29	0.099	0.460	0.112
2-way interaction effect										
Oil quality x vitamin E										
Fresh	-	No	5.8	35.2	25.8	0.276	2.28	0.099	0.365	0.112
Fresh	-	Yes	5.6	35.3	26.4	0.286	2.42	0.091	0.337	0.119
Oxidized	-	No	5.7	36.3	24.9	0.980	2.05	0.113	0.359	0.109
Oxidized	-	Yes	5.8	35.8	26.0	0.283	2.16	0.108	0.354	0.104
Phytase x oil quality										
Fresh	No	-	5.8	35.2	26.3	0.277	2.40	0.100	0.359	0.125
Fresh	Yes	-	5.6	35.2	25.9	0.285	2.30	0.089	0.344	0.107
Oxidized	No	-	5.6	35.9	25.2	0.288	2.08	0.111	0.357	0.109
Oxidized	Yes	-	5.9	36.2	25.7	0.292	2.13	0.109	0.356	0.104
Phytase x vitamin E										
-	No	No	5.7	35.8	25.6	0.283	2.20	0.102	0.363	0.116
-	No	Yes	5.7	35.3	25.9	0.283	2.27	0.109	0.353	0.117
-	Yes	No	5.8	35.7	25.2	0.292	2.13	0.110	0.362	0.104
-	Yes	Yes	5.7	35.7	26.5	0.286	2.31	0.089	0.339	0.107
Dietary treatments										
Fresh	No	No[1]	[x]5.67[ab]	35.5	26.2	0.277	2.40	0.100	0.371	0.123
Fresh	No	Yes	5.82[ab]	35.0[x]	26.3	0.278	2.40	0.100	0.347	0.126
Oxidized	No	No	[x]5.65[ab]	36.1[x]	24.9	0.289	2.00	0.104	0.355	0.109

Oxidized	No	Yes	$^{x}5.54^{b}$	35.7	25.5	0.288	2.14	0.118	0.359	0.108
Fresh	Yes	No	5.87^{ab}	34.9^{x}	25.4	0.276	2.16^{x}	0.097	0.360	0.100
Fresh	Yes	Yes	5.41^{b}	35.6	26.5	0.294	2.43	0.082^{x}	0.328	0.114
Oxidized	Yes	No	5.79^{ab}	36.5	24.9	0.307	2.09	0.122^{x}	0.363	0.108
Oxidized	Yes	Yes	6.06^{a}	35.9	26.5	0.277	2.18	0.098^{x}	0.349	0.101
Poled SD			0.425	1.155	2.159	0.036	0.244	0.026	0.038	0.021
PC[2]			5.7	35.4	26.4^{x}	0.258	2.48	0.091^{x}	0.366	0.132
Pooled SD[3]			0.285	1.25	0.077	0.025	0.253	0.015	0.037	0.016
----------Probability----------										
Oil quality			0.534	0.015	0.297	0.368	< .001	0.038	0.579	0.130
Phytase			0.365	0.534	0.899	0.609	0.783	0.387	0.441	0.087
Vitamin E			0.762	0.508	0.147	0.729	0.071	0.445	0.146	0.744
Oil quality x vitamin E			0.268	0.346	0.692	0.216	0.909	0.819	0.281	0.322
Phytase x oil quality			0.065	0.572	0.455	0.849	0.262	0.570	0.467	0.277
Phytase x vitamin E			0.631	0.487	0.421	0.719	0.432	0.067	0.494	0.863
Phytase x oil quality x vitamin E			0.038	0.267	0.977	0.274	0.229	0.419	0.813	0.499
PC vs. NC			0.947	0.909	0.8743	0.213	0.564	0.329	0.816	0.346

[a–b]Means with different superscripts within the same column differ significantly based on Tukey HSD ($P < 0.05$).

[1]Negative control (NC) diet

[2]PC=positive control

[3]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

Table A.3.2 continued. The effects of oil quality, phytase, vitamin E supplementation on the Fatty acid profile of the subcutaneous fat (Chapter 3)

Oil quality	Phytase	Vitamin E	C20:3	C20:4	C22:0	∑SFA	∑MUFA	∑PUFA	∑UFA	SFA:UFA
Main effect										
Fresh			0.161	0.192	0.041	28.7	41.4[b]	29.1	70.6	0.409
Oxidized			0.157	0.199	0.043	28.4	42.7[a]	28.5	71.2	0.399
	No		0.163	0.202	0.044	28.6	42.1	28.9	70.9	0.403
	Yes		0.155	0.188	0.040	28.5	42.0	28.7	70.9	0.405
		No	0.160	0.183	0.042	28.8	42.2	28.2[b]	70.6[b]	0.413[a]
		Yes	0.159	0.207	0.041	28.3	41.9	29.5[a]	71.3[a]	0.395[b]
2-way interaction effect										
Oil quality x vitamin E										
Fresh	-	No	0.159	0.182	0.042	29.3[a]	41.8	28.4	70.0	0.422
Fresh	-	Yes	0.164	0.201	0.040	28.1[b]	41.0	29.8	71.2	0.396
Oxidized	-	No	0.161	0.184	0.043	28.4[b]	42.7	27.9	71.1	0.404
Oxidized	-	Yes	0.153	0.213	0.042	28.4[b]	42.7	29.2	71.3	0.395
Phytase x oil quality										
Fresh	No	-	0.166	0.201	0.043	28.8	41.5	29.1	70.7	0.409
Fresh	Yes	-	0.157	0.182	0.039	28.3	42.7	28.8	71.2	0.397
Oxidized	No	-	0.160	0.203	0.044	28.6	41.3	29.2	70.6	0.409
Oxidized	Yes	-	0.154	0.195	0.041	28.5	42.6	28.3	71.3	0.401
Phytase x vitamin E										
-	No	No	0.159	0.189	0.041	28.5[ab]	42.4	28.6	70.8	0.410
-	No	Yes	0.166	0.215	0.046	28.6[ab]	41.9	29.2	71.1	0.396
-	Yes	No	0.159	0.178	0.043	29.1[a]	42.1	27.7	70.4	0.416
-	Yes	Yes	0.151	0.199	0.036	27.9[b]	41.8	29.8	71.4	0.395
Dietary treatments										
Fresh	No	No[1]	0.162	0.203	[x]0.045[ab]	29.1[x]	42.2[x]	28.7[x]	70.5[x]	0.416[x]
Fresh	No	Yes	0.170	0.199	[x]0.042[ab]	28.6[x]	40.8[x]	29.4	70.9[x]	0.401[x]
Oxidized	No	No	0.158	0.175	0.037[ab]	28.0	42.6	28.5	71.1	0.405

Oxidized	No	Yes	0.163	0.231	$^{x}0.051^{a}$	28.6^{x}	42.9^{x}	29.0^{x}	71.3^{x}	0.390^{x}
Fresh	Yes	No	0.155	0.162	0.039^{ab}	29.5^{x}	41.4^{x}	28.2^{x}	69.6^{x}	0.428^{x}
Fresh	Yes	Yes	0.158	0.202	$^{x}0.039^{ab}$	27.6^{x}	41.2^{y}	30.2^{x}	71.5^{x}	0.390^{x}
Oxidized	Yes	No	0.164	0.194	$^{x}0.048^{a}$	28.7^{x}	42.8^{x}	27.3^{x}	71.2^{x}	0.403^{x}
Oxidized	Yes	Yes	0.144	0.195	0.034^{b}	28.2	42.5	29.4	71.3	0.399
Poled SD			0.025	0.053	0.012^{ab}	0.740	1.292	1.734	0.954	0.018
PC[2]			0.163	0.201	0.044	28.4^{y}	42.0^{x}	29.5^{x}	71.5^{x}	0.398^{x}
Pooled SD[3]			0.021	0.050	0.007	1.023	1.387	2.086	1.099	0.020
			----------Probability----------							
Oil quality			0.532	0.631	0.641	0.284	0.003	0.326	0.065	0.111
Phytase			0.259	0.365	0.254	0.931	0.657	0.779	0.921	0.690
Vitamin E			0.947	0.117	0.802	0.065	0.367	0.031	0.043	0.004
Oil quality x vitamin E			0.377	0.726	0.852	0.040	0.332	0.955	0.128	0.170
Phytase x oil quality			0.887	0.723	0.850	0.450	0.850	0.648	0.729	0.781
Phytase x vitamin E			0.303	0.853	0.079	0.039	0.734	0.244	0.265	0.600
Phytase x oil quality x vitamin E			0.473	0.100	0.032	0.812	0.242	0.871	0.168	0.141
PC vs. NC			0.949	0.950	0.893	0.149	0.780	0.461	0.127	0.119

[a–b]Means with different superscripts within the same column differ significantly based on Tukey HSD ($P < 0.05$)

[1]Negative control (NC) diet

[2]PC=positive control

[3]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

Table A.3. 3 The effects of oil quality, phytase, vitamin E supplementation on the fatty acid profile of the liver (Chapter 3)

Oil quality	Phytase	Vitamin E	C14:0	C14:1	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1
Main effect										
Fresh			0.484	0.159	23.0	4.4	0.088	0.091	16.2	23.6
Oxidized			0.458	0.157	23.2	4.7	0.075	0.076	16.3	25.1
	No		0.474	0.159	23.0	4.7	0.085	0.108^{a}	16.1	24.5
	Yes		0.468	0.157	23.2	4.4	0.077	0.060^{b}	16.4	24.2
		No	0.443	0.156	23.0	4.5	0.085	0.103^{a}	16.6	23.4
		Yes	0.499	0.160	23.2	4.6	0.077	0.065^{b}	15.9	25.3
2-way interaction effect										
Oil quality x vitamin E										
Fresh	-	No	0.489	0.166	23.1	4.6	0.084^{ab}	0.105	16.5	22.4
Fresh	-	Yes	0.478	0.151	23.0	4.2	0.091^{a}	0.077	15.9	24.9
Oxidized	-	No	0.397	0.146	23.0	4.4	0.086^{ab}	0.100	16.7	24.5
Oxidized	-	Yes	0.519	0.169	23.4	4.9	0.063^{b}	0.053	15.9	25.8
Phytase x oil quality										
Fresh	No	-	0.456	0.148^{b}	22.6	4.5	0.089	0.110	15.5	24.5
Fresh	Yes	-	0.492	0.169^{a}	23.4	4.9	0.082	0.106	16.7	24.6
Oxidized	No	-	0.512	0.169^{a}	23.5	4.2	0.086	0.073	16.9	22.7
Oxidized	Yes	-	0.424	0.146^{b}	23.0	4.5	0.067	0.047	16.0	25.7
Phytase x vitamin E										
-	No	No	0.434	0.145^{b}	22.9	4.6	0.095	0.141^{a}	16.7	23.1
-	No	Yes	0.514	0.172^{a}	23.1	4.8	0.076	0.074^{b}	15.5	26.0
-	Yes	No	0.453	0.167^{ab}	23.2	4.4	0.075	0.064^{b}	16.5	23.7
-	Yes	Yes	0.484	0.147^{ab}	23.3	4.3	0.079	0.056^{b}	16.3	24.7
Dietary treatments										
Fresh	No	No[1]	0.427	0.134^{b}	22.7	4.4	0.092	0.139^{x}	16.3	22.3
Fresh	No	Yes	0.484	0.162^{ab}	22.4	4.5	0.086	0.080	14.7	26.8^{x}
Oxidized	No	No	0.440	$^{x}0.156^{ab}$	23.0	4.8	0.097	0.143^{x}	17.0	24.0
Oxidized	No	Yes	0.544	0.182^{ab}	23.8	5.0	0.066	0.068^{x}	16.3	25.1

Fresh	Yes	No	0.551	[x]0.199[a]	23.5	4.7	0.075	0.072	16.6	22.4[x]
Fresh	Yes	Yes	0.473	0.139[ab]	23.5[x]	3.8	0.097	0.074	17.1	23.0
Oxidized	Yes	No	0.355	[x]0.136[ab]	23.0	4.1	0.074[x]	0.056	16.4	25.0
Oxidized	Yes	Yes	0.494	0.155[ab]	23.0[x]	4.9	0.061	0.038[x]	15.6	26.4
Poled SD			0.120	0.035	1.452	0.946	0.026	0.046	1.993	3.459
PC[2]			0.513	0.168	22.7[x]	5.2	0.090	0.083	14.9	24.0
Pooled SD[3]			0.095	0.053	0.704	0.845	0.023	0.058	1.729	3.871
			----------Probability----------							
Oil quality			0.445	0.894	0.657	0.258	0.067	0.259	0.808	0.116
Phytase			0.872	0.910	0.511	0.216	0.218	0.001	0.515	0.713
Vitamin E			0.105	0.707	0.740	0.789	0.303	0.006	0.225	0.051
Oil quality x vitamin E			0.054	0.058	0.500	0.106	0.037	0.484	0.829	0.509
Phytase x oil quality			0.071	0.026	0.090	0.732	0.442	0.401	0.068	0.118
Phytase x vitamin E			0.464	0.020	0.849	0.701	0.102	0.029	0.390	0.333
Phytase x oil quality x vitamin E			0.210	0.046	0.437	0.161	0.747	0.928	0.305	0.294
PC vs. NC			0.136	0.254	0.987	0.141	0.823	0.110	0.161	0.423

[a–b]Means with different superscripts within the same column differ significantly based on Tukey HSD ($P < 0.05$).

[1]Negative control (NC) diet

[2]PC=positive control

[3]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

Table A.3.3 continued. The effects of oil quality, phytase, vitamin E supplementation on the fatty acid profile of the liver (Chapter 3)

Oil quality	Phytase	Vitamin E	C18:2	C18:3 n-6	C18:3 n-3	C20:0	C20:1	C20:2	C20:3	C20:4	C22:0
Main effect											
Fresh			17.0[a]	0.267	0.384[a]	0.070	0.215	0.334[a]	1.747	8.1	0.429[a]
Oxidized			15.8[b]	0.303	0.312[b]	0.076	0.227	0.290[b]	1.711	7.7	0.380[b]
	No		16.7	0.283	0.354	0.071	0.221	0.303	1.708	8.2	0.385
	Yes		16.2	0.287	0.343	0.075	0.221	0.321	1.750	7.7	0.424
		No	16.6	0.273	0.339	0.068	0.238	0.308	1.719	8.0	0.419
		Yes	16.2	0.297	0.358	0.078	0.204	0.316	1.739	7.8	0.390
2-way interaction effect											
Oil quality x vitamin E											
Fresh	-	No	17.0	0.249	0.382	0.080[ab]	0.232	0.318	1.686	8.1	0.437
Fresh	-	Yes	17.1	0.285	0.387	0.059[ab]	0.199	0.351	1.730	8.2	0.421
Oxidized	-	No	16.3	0.297	0.296	0.057[b]	0.245	0.299	1.752	8.0	0.401
Oxidized	-	Yes	15.4	0.308	0.329	0.095[a]	0.209	0.281	1.748	7.4	0.360
Phytase x oil quality											
Fresh	No	-	17.2	0.258	0.396	0.077	0.239[ab]	0.336	1.744	8.4	0.418
Fresh	Yes	-	16.1	0.308	0.312	0.066	0.203[ab]	0.270	1.672	7.9	0.352
Oxidized	No	-	16.9	0.277	0.373	0.063	0.192[b]	0.333	1.750	7.8	0.440
Oxidized	Yes	-	15.6	0.297	0.312	0.086	0.251[a]	0.309	1.750	7.5	0.408
Phytase x vitamin E											
-	No	No	16.9	0.257	0.335	0.072	0.239	0.286	1.686	8.7	0.403
-	No	Yes	16.4	0.309	0.373	0.070	0.203	0.320	1.730	7.7	0.367
-	Yes	No	16.4	0.289	0.343	0.064	0.238	0.330	1.752	7.4	0.435
-	Yes	Yes	16.1	0.285	0.342	0.085	0.205	0.312	1.748	7.9	0.414
Dietary treatments											
Fresh	No	No[1]	17.4	0.248	0.392	0.094	0.240	0.303[x]	1.701	9.1	0.432
Fresh	No	Yes	17.0	0.267	0.400	0.059	0.238	0.368	1.786	7.8	0.404
Oxidized	No	No	16.4	0.266	0.277	0.051	0.238	0.270[x]	1.671	8.3	0.375[x]
Oxidized	No	Yes	15.8	0.350	0.347	0.081	0.168	0.271	1.674	7.6	0.329

Fresh	Yes	No	16.6	0.250	0.372	0.066	0.223	0.333[x]	1.719	7.1	0.443
Fresh	Yes	Yes	17.1	0.303	0.374	0.061	0.161	0.333[x]	1.780	8.6	0.437[x]
Oxidized	Yes	No	16.2	0.327	0.314	0.062	0.253	0.328	1.784	7.7	0.427
Oxidized	Yes	Yes	15.0	0.267	0.310	0.110	0.250	0.291[x]	1.716	7.3	0.390[x]
Poled SD			1.655	0.078	0.059	0.046	0.081	0.071	0.206	1.430	0.082
PC[2]			17.2	0.280	0.441	0.073	0.216	0.318	1.656	7.8	0.442
Pooled SD[3]			1.224	0.040	0.060	0.044	0.065	0.059	0.122	1.466	0.059
			----------Probability----------								
Oil quality			0.011	0.116	<.001	0.647	0.597	0.032	0.549	0.301	0.036
Phytase			0.341	0.855	0.500	0.792	0.982	0.373	0.477	0.196	0.089
Vitamin E			0.383	0.287	0.252	0.473	0.129	0.708	0.729	0.561	0.210
Oil quality x vitamin E			0.305	0.586	0.396	0.029	0.933	0.219	0.370	0.396	0.600
Phytase x oil quality			0.886	0.499	0.491	0.202	0.037	0.304	0.541	0.870	0.451
Phytase x vitamin E			0.852	0.219	0.234	0.369	0.937	0.204	0.688	0.060	0.728
Phytase x oil quality x vitamin E			0.386	0.050	0.299	0.828	0.162	0.743	0.844	0.116	0.878
PC vs. NC			0.766	0.196	0.175	0.440	0.523	0.660	0.518	0.136	0.767

[a–b]Means with different superscripts within the same column differ significantly based on Tukey HSD ($P < 0.05$).

[1]Negative control (NC) diet

[2]PC=positive control

[3]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

Table A.3.3 continued. The effects of oil quality, phytase, vitamin E supplementation on the fatty acid profile of the liver (Chapter 3)

Oil quality	Phytase	Vitamin E	C22:2	C22:4	C24:0	C24:1	∑SFA	∑MUFA	∑PUFA	∑UFA	SFA:UFA
Main effect											
Fresh			1.800	0.200	0.114	0.447^{a}	40.2	28.6	29.4^{a}	58.0	0.694
Oxidized			1.594	0.160	0.102	0.244^{b}	40.4	30.0	27.6^{b}	58.0	0.707
	No		1.793	0.204	0.111	0.314	40.2	29.5	29.4^{a}	59.0^{a}	0.682
	Yes		1.601	0.157	0.105	0.377	40.5	29.0	27.6^{b}	56.9^{b}	0.720
		No	1.773	0.216	0.111	0.415	40.5	28.2	28.6	57.2	0.721^{a}
		Yes	1.621	0.144	0.105	0.276	40.2	30.3	28.4	58.9	0.680^{b}
2-way interaction effect											
Oil quality x vitamin E											
Fresh	-	No	1.843	0.198	0.108	0.447	40.7	27.5	29.0	57.0	0.722
Fresh	-	Yes	1.743	0.203	0.120	0.180	39.8	29.6	29.9	59.1	0.667
Oxidized	-	No	1.703	0.234	0.114	0.383	40.4	29.0	28.3	57.3	0.721
Oxidized	-	Yes	1.499	0.086	0.090	0.372	40.5	30.9	26.9	58.7	0.694
Phytase x oil quality											
Fresh	No	-	1.867	0.170^{ab}	0.108	0.400	39.3^{b}	29.7	30.7	59.9^{a}	0.645^{b}
Fresh	Yes	-	1.719	0.238^{a}	0.113	0.227	41.1^{a}	29.4	28.1	58.1^{ab}	0.718^{a}
Oxidized	No	-	1.733	0.231^{a}	0.120	0.494	41.2^{a}	27.5	28.2	56.1^{b}	0.744^{a}
Oxidized	Yes	-	1.469	0.082^{b}	0.091	0.261	39.8^{ab}	30.5	27.1	57.9^{ab}	0.696^{ab}
Phytase x vitamin E											
-	No	No	1.843	0.315^{a}	0.128^{a}	0.447	40.6	28.0	29.9	58.8	0.698
-	No	Yes	1.743	0.092^{b}	0.093^{b}	0.180	39.9	31.1	28.9	59.3	0.666
-	Yes	No	1.703	0.117^{b}	0.094^{b}	0.383	40.5	28.5	27.4	55.5	0.745
-	Yes	Yes	1.499	0.196^{ab}	0.116a^{b}	0.372	40.5	29.5	27.9	58.5	0.695
Dietary treatments											
Fresh	No	No[1]	1.932	0.251^{x}	0.125^{x}	0.581	40.1	27.2	31.2^{x}	58.9^{x}	0.679^{x}
Fresh	No	Yes	1.802	0.088^{x}	0.092	0.219	38.5^{x}	32.2^{x}	30.2^{x}	61.1^{x}	0.611^{x}
Oxidized	No	No	1.753	0.379	0.131^{x}	0.313	41.0	28.9	28.7	58.7^{x}	0.717

Oxidized	No	Yes	1.684	0.096^{x}	0.095	0.141^{x}	41.2	30.0	27.5	57.5	0.720
Fresh	Yes	No	1.770	0.145^{x}	0.092^{x}	0.502	41.2	27.8^{x}	26.8	55.1^{x}	0.765^{x}
Fresh	Yes	Yes	1.695	0.318	0.149	0.486	41.2^{x}	27.1	29.5^{x}	57.1^{x}	0.723^{x}
Oxidized	Yes	No	1.636	0.090^{x}	0.097	0.265^{x}	39.8^{x}	29.1	27.9^{x}	55.9	0.725
Oxidized	Yes	Yes	1.303	0.075^{x}	0.084	0.258	39.8^{x}	31.9	26.4^{x}	59.9^{x}	0.667^{x}
Poled SD			0.592	0.151	0.041	0.291	4.502	4.461	4.502	3.820	0.074
PC[2]			1.771	0.095^{x}	0.095^{x}	0.180^{x}	36.4^{x}	31.0^{x}	29.1	58.2	0.626^{x}
Pooled SD[3]			0.551	0.128	0.044	0.351	2.133	4.153	2.864	3.107	0.067
			----------Probability----------								
Oil quality			0.214	0.350	0.279	0.015	0.743	0.218	0.029	0.941	0.492
Phytase			0.246	0.279	0.639	0.439	0.632	0.633	0.034	0.026	0.051
Vitamin E			0.356	0.100	0.589	0.081	0.536	0.076	0.762	0.057	0.034
Oil quality x vitamin E			0.765	0.079	0.116	0.531	0.425	0.920	0.174	0.687	0.477
Phytase x oil quality			0.726	0.015	0.128	0.676	0.009	0.148	0.347	0.044	0.002
Phytase x vitamin E			0.751	0.001	0.015	0.109	0.582	0.386	0.320	0.164	0.639
Phytase x oil quality x vitamin E			0.628	0.693	0.141	0.569	0.424	0.113	0.232	0.138	0.256
PC vs. NC			0.609	0.061	0.274	0.065	0.164	0.151	0.133	0.744	0.244

[a–b]Means with different superscripts within the same column differ significantly based on Tukey HSD ($P < 0.05$).

[1]Negative control (NC) diet

[2]PC=positive control

[3]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

Appendix 4. Analyzed fatty acids profile of adipose and liver tissues for chapter 4

Table A.4. 1 The effects of oil quality, oil type, and vitamin E supplementation on abdominal fat fatty acid profile (chapter 4)

Oil quality	Oil type	Vitamin E	C12:0	C14:0	C14:1	C15:0	C16:0	C16:1	C17:0	C17:1
Main effect										
Fresh			0.022[a]	0.446[a]	0.138	0.059	21.0	5.8	0.249[a]	0.127
Oxidized			0.019[b]	0.412[b]	0.147	0.060	20.6	6.2	0.201[b]	0.122
	Corn oil		0.020[b]	0.419[b]	0.138	0.061	20.6	5.9	0.234[a]	0.123
	Soy oil		0.022[a]	0.439[a]	0.146	0.058	21.1	6.0	0.216[b]	0.126
		No	0.021	0.421[b]	0.142	0.060	20.7	6.1	0.225	0.120
		Yes	0.021	0.438[a]	0.142	0.059	20.9	5.9	0.225	0.129
2-way interaction effect										
Oil quality x vitamin E										
Fresh	-	No	0.022	0.440	0.131[b]	0.062	20.6	5.6	0.251	0.127
Fresh	-	Yes	0.022	0.452	0.144[ab]	0.056	21.3	5.9	0.248	0.127
Oxidized	-	No	0.019	0.401	0.154[a]	0.058	20.8	6.5	0.200	0.114
Oxidized	-	Yes	0.020	0.423	0.140[ab]	0.061	20.5	5.9	0.201	0.131
Oil type x oil quality										
Fresh	Corn oil	-	0.021	0.440	0.129	0.062	20.5	5.5	0.259	0.129
Oxidized	Corn oil	-	0.019	0.399	0.148	0.060	20.6	6.3	0.208	0.117
Fresh	Soy oil	-	0.023	0.453	0.147	0.057	21.4	6.0	0.239	0.125
Oxidized	Soy oil	-	0.020	0.426	0.146	0.059	20.7	6.1	0.193	0.128
Oil type x vitamin E										
-	Corn oil	No	0.019	0.406	0.133	0.062	20.1[b]	5.9	0.237	0.120
-	Corn oil	Yes	0.020	0.432	0.144	0.059	20.9[ab]	5.9	0.230	0.126
-	Soy oil	No	0.022	0.435	0.152	0.058	21.3[a]	6.2	0.213	0.121
-	Soy oil	Yes	0.022	0.444	0.141	0.058	20.8[ab]	5.9	0.219	0.132
Dietary treatments										
Fresh	Corn oil	No	0.021	0.430[x]	0.118[x]	0.064	19.8[x]	5.3	0.261	0.130
Fresh	Corn oil	Yes	0.021[x]	0.450[x]	0.139	0.060	21.1[x]	5.7	0.257	0.128

Oxidized	Corn oil	No	0.018[x]	0.383[x]	0.147	0.061	20.4[x]	6.5	0.213[x]	0.109
Oxidized	Corn oil	Yes	0.020	0.414[x]	0.149	0.058	20.7[x]	6.1[x]	0.202[x]	0.124
Fresh	Soy oil	No	0.024	0.451	0.144[x]	0.061	21.4	5.9[x]	0.240[x]	0.123
Fresh	Soy oil	Yes	0.023[x]	0.455	0.150[x]	0.053	21.4	6.1	0.239	0.126
Oxidized	Soy oil	No	0.020[x]	0.419[x]	0.160	0.055	21.2	6.5[x]	0.186[x]	0.119
Oxidized	Soy oil	Yes	0.020	0.433[x]	0.132[x]	0.064	20.1	5.6	0.200[x]	0.137
	Pooled SD[1]		0.002	0.032	0.024	0.010	1.162	0.918	0.022	0.019
			----------Probability----------							
Oil quality			<.001	0.000	0.131	0.894	0.287	0.086	<.001	0.375
Oil type			0.008	0.020	0.210	0.318	0.096	0.619	0.004	0.459
Vitamin E			0.110	0.396	0.097	0.375	0.165	0.132	0.656	0.109
Oil quality x vitamin E			0.382	0.548	0.034	0.077	0.092	0.051	0.708	0.088
Oil type x oil quality			0.454	0.045	0.984	0.516	0.644	0.470	0.888	0.097
Oil type x vitamin E			0.370	0.311	0.072	0.505	0.025	0.551	0.234	0.665
Oil type x oil quality x vitamin E			0.861	0.987	0.532	0.143	0.838	0.828	0.323	0.934

[a–b]Means with different superscripts within the same column differ significantly based on Tukey-Kramer comparison ($P < 0.05$).

[x-y]Values represent the mean of 8 replicates cages (pooled excreta of 6 birds per replicate) except for mean values with x and y where the number of replicates (n) was 7 and 6, respectively.

[1]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

Table A.4.1 contd. The effects of oil quality, oil type, and vitamin E supplementation on abdominal fat fatty acid profile (chapter 4)

Oil quality	Oil type	Vitamin E	C18:0	C18:1	C18:2	C18:3 n-6	C18:3 n-3	C20:0	C20:1	C20:2
Main effect										
Fresh			5.2^{a}	35.9^{b}	28.4	0.284^{b}	1.756	0.093	0.379	0.134
Oxidized			4.9^{b}	36.8^{a}	27.4	0.320^{a}	1.652	0.111	0.381	0.128
	Corn oil		4.9^{b}	37.0^{a}	28.3	0.323^{a}	1.048^{b}	0.099^{b}	0.385	0.132
	Soy oil		5.3^{a}	35.7^{b}	27.5	0.281^{b}	2.360^{a}	0.105^{a}	0.375	0.130
		No	5.0^{b}	36.4	28.0	0.296	1.694	0.098^{b}	0.377	0.130
		Yes	5.2^{a}	36.3	27.8	0.308	1.714	0.106^{a}	0.383	0.133
2-way interaction effect										
Oil quality x vitamin E										
Fresh	-	No	5.1	35.5	29.1	0.280	1.803	0.086	0.366	0.139
Fresh	-	Yes	5.3	36.3	27.8	0.289	1.708	0.100	0.392	0.129
Oxidized	-	No	4.8	37.2	27.0	0.311	1.584	0.109	0.388	0.121
Oxidized	-	Yes	5.1	36.3	27.9	0.328	1.720	0.112	0.374	0.136
Oil type x oil quality										
Fresh	Corn oil	-	5.0	36.0^{b}	29.6^{a}	0.302	1.074	0.089	0.384	0.142^{a}
Oxidized	Corn oil	-	4.7	37.9^{a}	27.0^{b}	0.343	1.021	0.108	0.385	0.122^{b}
Fresh	Soy oil	-	5.5	35.7^{b}	27.2^{b}	0.266	2.438	0.097	0.374	0.126^{ab}
Oxidized	Soy oil	-	5.2	35.6^{b}	27.8^{ab}	0.297	2.283	0.113	0.376	0.135^{ab}
Oil type x vitamin E										
-	Corn oil	No	4.6^{b}	36.9	28.9	0.315	1.076	0.097	0.390	0.133
-	Corn oil	Yes	5.1^{a}	37.1	27.7	0.330	1.020	0.100	0.379	0.131
-	Soy oil	No	5.3^{a}	35.9	27.1	0.276	2.312	0.098	0.363	0.127
-	Soy oil	Yes	5.3^{a}	35.6	27.9	0.287	2.409	0.112	0.386	0.134
Dietary treatments										
Fresh	Corn oil	No	4.7^{x}	35.4	30.5	0.292^{x}	1.117	0.085^{x}	0.379	0.147^{x}
Fresh	Corn oil	Yes	5.3^{x}	36.6^{x}	28.7	0.312	1.030^{x}	0.093^{x}	0.389	0.137
Oxidized	Corn oil	No	4.5	38.3	27.3^{x}	0.337	1.034^{x}	0.109	0.402	0.118
Oxidized	Corn oil	Yes	4.9	37.5	26.7	0.349	1.009	0.107	0.369	0.126^{x}

Fresh	Soy oil	No	5.6	35.6	27.6	0.267	2.489	0.087^{x}	0.353	0.131
Fresh	Soy oil	Yes	5.4^{x}	35.9^{x}	26.8^{x}	0.266	2.387^{x}	0.107^{x}	0.394	0.121^{x}
Oxidized	Soy oil	No	5.0	36.1^{x}	26.7^{x}	0.286^{x}	2.134^{x}	0.109	0.374	0.123^{x}
Oxidized	Soy oil	Yes	5.3^{x}	35.1	29.0	0.307^{x}	2.432	0.117^{x}	0.378	0.146
	Pooled SD[1]		0.453	1.667	2.723	0.051	0.225	0.016	0.047	0.026
			----------Probability----------							
Oil quality			0.014	0.043	0.172	0.009	0.082	<.001	0.878	0.397
Oil type			0.000	0.004	0.259	0.003	<.001	0.134	0.400	0.803
Vitamin E			0.997	0.025	0.026	0.679	0.383	0.766	0.977	0.040
Oil quality x vitamin E			0.816	0.064	0.125	0.773	0.053	0.164	0.094	0.060
Oil type x oil quality			0.036	0.879	0.771	0.329	0.723	0.045	0.618	0.704
Oil type x vitamin E			0.046	0.519	0.152	0.833	0.193	0.206	0.159	0.566
Oil type x oil quality x vitamin E			0.101	0.715	0.510	0.563	0.155	0.943	0.885	0.551

[a–b]Means with different superscripts within the same column differ significantly based on Tukey-Kramer comparison ($P < 0.05$).

[x-y]Values represent the mean of 8 replicates cages (pooled excreta of 6 birds per replicate) except for mean values with x and y where the number of replicates (n) was 7 and 6, respectively.

[1]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

Table A.4.1 contd. The effects of oil quality, oil type, and vitamin E supplementation on abdominal fat fatty acid profile (chapter 4)

Oil quality	Oil type	Vitamin E	C20:3	C20:4	C22:0	∑SFA	∑MUFA	∑PUFA	∑UFA	∑SFA:UFA
Main effect										
Fresh			0.179[b]	0.268	0.031[b]	26.9	42.3	31.0	72.8	0.370
Oxidized			0.200[a]	0.300	0.044[a]	26.4	43.4	30.0	73.5	0.360
	Corn oil		0.196[a]	0.315[a]	0.029[b]	25.8[b]	43.3	30.3	73.5	0.353[b]
	Soy oil		0.183[b]	0.253[b]	0.046[a]	27.4[a]	42.4	30.7	72.9	0.374[a]
		No	0.189	0.277	0.038	26.5	43.1	30.6	73.3	0.360
		Yes	0.191	0.291	0.037	26.8	42.6	30.4	73.1	0.370
2-way interaction effect										
Oil quality x vitamin E										
Fresh	-	No	0.177	0.270	0.031	26.5	41.9[b]	31.7	73.0	0.365
Fresh	-	Yes	0.181	0.266	0.031	27.2	42.7[ab]	30.3	72.6	0.367
Oxidized	-	No	0.201	0.284	0.045	26.4	44.3[a]	29.5	73.5	0.360
Oxidized	-	Yes	0.200	0.316	0.043	26.4	42.4[b]	30.6	73.5	0.363
Oil type x oil quality										
Fresh	Corn oil	-	0.189	0.305	0.022	25.6[b]	42.1[b]	31.5	73.2	0.349
Oxidized	Corn oil	-	0.204	0.324	0.036	26.1[b]	44.5[a]	29.0	73.7	0.357
Fresh	Soy oil	-	0.169	0.230	0.040	28.1[a]	42.5[b]	30.4	72.4	0.383
Oxidized	Soy oil	-	0.196	0.276	0.052	26.7[b]	42.3[b]	31.0	73.3	0.366
Oil type x vitamin E										
-	Corn oil	No	0.200	0.308	0.031	25.2[c]	43.4	30.9	74.0[a]	0.339[b]
-	Corn oil	Yes	0.193	0.322	0.027	26.5[b]	43.1	29.6	72.9[ab]	0.366[a]
-	Soy oil	No	0.178	0.246	0.045	27.8[a]	42.8	30.3	72.6[b]	0.384[a]
-	Soy oil	Yes	0.188	0.260	0.047	27.1[ab]	42.0	31.2	73.2[ab]	0.364[a]
Dietary Treatments										
Fresh	Corn oil	No	0.196	0.312[x]	0.026[x]	22.3[x]	41.4	32.5	73.9	0.332[x]
Fresh	Corn oil	Yes	0.182	0.299	0.019	24.0[x]	38.4[x]	30.5	68.9[x]	0.366[x]
Oxidized	Corn oil	No	0.204[x]	0.304[x]	0.037	23.1[x]	45.4	25.7[x]	71.2[x]	0.348[x]

Oxidized	Corn oil	Yes	0.205	0.345	0.036	23.8 [x]	43.5	28.8	72.2 [x]	0.366 [x]
Fresh	Soy oil	No	0.158	0.227	0.037	27.9 [x]	41.3 [x]	30.8	72.2	0.397 [x]
Fresh	Soy oil	Yes	0.180	0.232	0.043	27.0 [x]	38.2 [x]	26.4 [x]	64.5 [x]	0.369 [x]
Oxidized	Soy oil	No	0.198 [x]	0.265	0.053	27.1	37.9 [x]	26.0 [x]	63.9 [x]	0.371 [x]
Oxidized	Soy oil	Yes	0.195	0.287	0.051	25.5 [x]	41.4	32.3	73.8	0.360 [x]
	Pooled SD[1]		0.027	0.086	0.012	1.677	2.294	2.964	1.513	0.030
			----------Probability----------							
Oil quality			0.003	0.141	<.001	0.315	0.064	0.219	0.095	0.554
Oil type			0.050	0.006	<.001	0.001	0.145	0.549	0.143	0.010
Vitamin E			0.381	0.538	0.774	0.486	0.362	0.821	0.595	0.705
Oil quality x vitamin E			0.705	0.413	0.879	0.489	0.027	0.112	0.615	0.975
Oil type x oil quality			0.801	0.525	0.803	0.039	0.038	0.051	0.498	0.119
Oil type x vitamin E			0.235	0.993	0.245	0.031	0.652	0.154	0.040	0.006
Oil type x oil quality x vitamin E			0.149	0.670	0.249	0.457	0.578	0.501	0.970	0.293

[a–b]Means with different superscripts within the same column differ significantly based on Tukey-Kramer comparison (P < 0.05).

[x-y]Values represent the mean of 8 replicates cages (pooled excreta of 6 birds per replicate) except for mean values with x and y where the number of replicates (n) was 7 and6, respectively.

[1]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

Table A.4. 2 The effects of oil quality, oil type, and vitamin E supplementation on subcutaneous fat fatty acid profile (chapter 4)

Oil quality	Oil type	Vitamin E	C12:0	C14:0	C14:1	C15:0	C16:0	C16:1	C17:0	C17:1
Main effect										
Fresh			0.021[a]	0.457[a]	0.128	0.058	22.1	5.4	0.121	0.074[b]
Oxidized			0.018[b]	0.431[b]	0.134	0.059	21.6	5.8	0.124	0.077[a]
	Corn oil		0.019	0.440	0.127	0.058	21.7	5.6	0.119[b]	0.073[b]
	Soy oil		0.020	0.448	0.135	0.058	22.0	5.7	0.126[a]	0.079[a]
		No	0.019	0.436	0.132	0.059	21.7	5.6	0.119[b]	0.076
		Yes	0.020	0.452	0.131	0.058	22.0	5.6	0.126[a]	0.076
2-way interaction effect										
Oil quality x vitamin E										
Fresh	-	No	0.021	0.445	0.126	0.060[a]	21.8	5.3	0.124[ab]	0.076[ab]
Fresh	-	Yes	0.021	0.470	0.130	0.055[c]	22.4	5.6	0.118[b]	0.073[b]
Oxidized	-	No	0.018	0.426	0.137	0.057[abc]	21.5	6.0	0.114[b]	0.076[ab]
Oxidized	-	Yes	0.019	0.435	0.132	0.061[ab]	21.7	5.6	0.134[a]	0.079[a]
Oil type x oil quality										
Fresh	Corn oil	-	0.020	0.454	0.123	0.057	21.8	5.2[b]	0.123[ab]	0.073
Oxidized	Corn oil	-	0.018	0.425	0.132	0.059	21.6	6.0[a]	0.114[b]	0.073
Fresh	Soy oil	-	0.021	0.460	0.133	0.058	22.4	5.7[ab]	0.119[b]	0.076
Oxidized	Soy oil	-	0.019	0.436	0.136	0.059	21.6	5.6[ab]	0.133[a]	0.081
Oil type x vitamin E										
-	Corn oil	No	0.019	0.430	0.125	0.059	21.3	5.5	0.117	0.074
-	Corn oil	Yes	0.019	0.449	0.130	0.057	22.1	5.7	0.120	0.072
-	Soy oil	No	0.019	0.441	0.138	0.058	22.0	5.8	0.121	0.077
-	Soy oil	Yes	0.021	0.455	0.131	0.059	21.9	5.5	0.132	0.080
Dietary treatments										
Fresh	Corn oil	No	0.021[x]	0.441	0.123	0.060	21.4	4.9	0.127[x]	0.074[x]
Fresh	Corn oil	Yes	0.020	0.468	0.123	0.055[x]	22.2	5.4	0.119[x]	0.071
Oxidized	Corn oil	No	0.017[x]	0.420[x]	0.126	0.059	21.2[x]	6.0	0.108[x]	0.073
Oxidized	Corn oil	Yes	0.019[x]	0.431[x]	0.138	0.059[x]	22.0	5.9	0.120[x]	0.073[x]

Fresh	Soy oil	No	0.020	0.449	0.130	0.060 [x]	22.1	5.6	0.122 [x]	0.077 [x]
Fresh	Soy oil	Yes	0.023 [x]	0.471	0.137	0.056 [x]	22.6	5.8	0.117 [x]	0.074
Oxidized	Soy oil	No	0.018	0.433 [x]	0.147	0.055	21.9 [x]	6.0 [x]	0.120 [x]	0.078 [x]
Oxidized	Soy oil	Yes	0.020 [x]	0.439	0.125	0.062	21.3	5.3	0.147 [x]	0.085
	Pooled SD[1]		0.003	0.036	0.022	0.008	1.137	0.878	0.012	0.006
	Probability									
Oil quality			0.003	0.005	0.288	0.556	0.111	0.100	0.443	0.049
Oil type			0.155	0.379	0.197	0.923	0.384	0.672	0.020	0.001
Vitamin E			0.123	0.072	0.896	0.819	0.223	0.901	0.033	0.956
Oil quality x vitamin E			0.486	0.385	0.471	0.039	0.412	0.114	0.000	0.041
Oil type x oil quality			0.836	0.796	0.595	0.788	0.264	0.048	0.001	0.121
Oil type x vitamin E			0.341	0.793	0.260	0.367	0.150	0.315	0.179	0.194
Oil type x oil quality x vitamin E			0.373	0.990	0.070	0.490	0.345	0.684	0.375	0.375

[a–b]Means with different superscripts within the same column differ significantly based on Tukey-Kramer comparison ($P < 0.05$).

[x-y]Values represent the mean of 8 replicates cages (pooled excreta of 6 birds per replicate) except for mean values with x and y where the number of replicates (n) was 7 and 6, respectively.

[1]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

Table A.4.2 continued. The effects of oil quality, oil type, and vitamin E supplementation on subcutaneous fat fatty acid profile (chapter 4)

Oil quality	Oil type	Vitamin E	C18:0	C18:1	C18:2	C18:3 n-6	C18:3 n-3	C20:0	C20:1	C20:2
Main effect										
Fresh			5.6[a]	35.8	27.4	0.284[b]	1.574	0.092[b]	0.374	0.129
Oxidized			5.3[b]	36.5	27.0	0.322[a]	1.572	0.106[a]	0.375	0.136
	Corn oil		5.3[b]	36.7[a]	27.7	0.322[a]	0.969[b]	0.098	0.394[a]	0.133
	Soy oil		5.6[a]	35.6[b]	26.8	0.284[b]	2.176[a]	0.099	0.355[b]	0.132
		No	5.4	36.1	27.6	0.297	1.589	0.099	0.367	0.131
		Yes	5.5	36.2	26.9	0.309	1.557	0.099	0.382	0.134
2-way interaction effect										
Oil quality x vitamin E										
Fresh	-	No	5.6	35.3[b]	28.3	0.287	1.647	0.092	0.365	0.130
Fresh	-	Yes	5.6	36.3[ab]	26.5	0.280	1.500	0.091	0.384	0.127
Oxidized	-	No	5.2	36.9[a]	26.9	0.306	1.531	0.105	0.369	0.131
Oxidized	-	Yes	5.4	36.1[ab]	27.2	0.338	1.614	0.106	0.381	0.140
Oil type x oil quality										
Fresh	Corn oil	-	5.4	35.8[b]	28.7[a]	0.300	0.972	0.092	0.397	0.140[ab]
Oxidized	Corn oil	-	5.1	37.6[a]	26.7[ab]	0.344	0.967	0.103	0.392	0.125[ab]
Fresh	Soy oil	-	5.7	35.8[b]	26.2[b]	0.267	2.175	0.091	0.352	0.117[b]
Oxidized	Soy oil	-	5.5	35.4[b]	27.4[ab]	0.300	2.178	0.108	0.358	0.146[a]
Oil type x vitamin E										
-	Corn oil	No	5.1	36.6	28.5	0.320	1.001	0.099	0.394	0.138
-	Corn oil	Yes	5.4	36.9	26.8	0.325	0.938	0.097	0.394	0.128
-	Soy oil	No	5.6	35.6	26.6	0.274	2.177	0.098	0.340	0.123
-	Soy oil	Yes	5.7	35.5	26.9	0.293	2.176	0.100	0.370	0.140
Dietary treatments										
Fresh	Corn oil	No	5.3	35.3	29.8	0.305[x]	1.009[x]	0.094	0.399	0.147
Fresh	Corn oil	Yes	5.6[x]	36.4	27.5	0.296	0.936[x]	0.091	0.394	0.134
Oxidized	Corn oil	No	4.9	37.9	27.2[x]	0.335	0.994[x]	0.104	0.389[x]	0.129[x]

Oxidized	Corn oil	Yes	5.3	37.4	26.1	0.353	0.939	0.103	0.395	0.121 [x]
Fresh	Soy oil	No	5.8	35.3	26.8	0.270	2.286	0.090	0.331 [x]	0.113 [x]
Fresh	Soy oil	Yes	5.7	36.2	25.6 [x]	0.264	2.064	0.091	0.373	0.121
Oxidized	Soy oil	No	5.4	35.9 [x]	26.5 [x]	0.278 [x]	2.067 [x]	0.107	0.349 [x]	0.134 [x]
Oxidized	Soy oil	Yes	5.6 [x]	34.8	28.3	0.323 [x]	2.288	0.109	0.367	0.158
	Pooled SD[1]		0.409	1.579	2.495	0.049	0.233	0.019	0.058	0.024
			----------Probability----------							
Oil quality			0.009	0.079	0.521	0.004	0.979	0.005	0.978	0.286
Oil type			0.001	0.005	0.189	0.004	<.001	0.734	0.011	0.829
Vitamin E			0.098	0.810	0.271	0.344	0.595	0.999	0.313	0.666
Oil quality x vitamin E			0.326	0.029	0.113	0.133	0.061	0.873	0.815	0.380
Oil type x oil quality			0.513	0.007	0.018	0.673	0.945	0.512	0.733	0.001
Oil type x vitamin E			0.259	0.643	0.127	0.553	0.603	0.676	0.331	0.037
Oil type x oil quality x vitamin E			0.710	0.866	0.461	0.630	0.083	0.968	0.569	0.670

[a–b]Means with different superscripts within the same column differ significantly based on Tukey-Kramer comparison ($P < 0.05$).

[x-y]Values represent the mean of 8 replicates cages (pooled excreta of 6 birds per replicate) except for mean values with x and y where the number of replicates (n) was 7 and 6, respectively.

[1]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

Table A.4.2 continued. The effects of oil quality, oil type, and vitamin E supplementation on subcutaneous fat fatty acid profile (chapter 4)

Oil quality	Oil type	Vitamin E	C20:3	C20:4	C22:0	∑SFA	∑MUFA	∑PUFA	∑UFA	SFA:UFA
Main effect										
Fresh			0.161[b]	0.202[b]	0.019[b]	28.3	41.8	28.1	70.7	0.409
Oxidized			0.197[a]	0.275[a]	0.037[a]	27.8	42.8	29.5	72.1	0.535
	Corn oil		0.187[a]	0.266[a]	0.019[b]	27.7	42.9	29.4	72.0	0.385
	Soy oil		0.171[b]	0.210[b]	0.037[a]	28.3	41.8	28.1	70.8	0.559
		No	0.178	0.234	0.027	27.6[b]	42.2	29.9	72.1	0.531
		Yes	0.180	0.242	0.028	28.5[a]	42.4	27.6	70.7	0.413
2-way interaction effect										
Oil quality x vitamin E										
Fresh	-	No	0.162	0.212	0.019	27.7	41.1	30.6[a]	72.0	0.384
Fresh	-	Yes	0.160	0.192	0.020	28.9	42.4	25.5[b]	69.5	0.435
Oxidized	-	No	0.194	0.256	0.036	27.5	43.4	29.2[a]	72.3	0.678
Oxidized	-	Yes	0.200	0.293	0.037	28.1	42.3	29.7[a]	71.8	0.391
Oil type x oil quality										
Fresh	Corn oil	-	0.178[a]	0.233	0.012	27.9	41.5[b]	30.3[a]	71.8	0.389
Oxidized	Corn oil	-	0.195[a]	0.299	0.026	27.6	44.2[a]	28.5[ab]	72.2	0.382
Fresh	Soy oil	-	0.143[b]	0.171	0.026	28.6	42.0[b]	25.9[b]	69.7	0.430
Oxidized	Soy oil	-	0.198[a]	0.250	0.047	28.0	41.5[b]	30.4[a]	71.9	0.688
Oil type x vitamin E										
-	Corn oil	No	0.186	0.270	0.017	26.9[b]	42.6	30.3	72.6	0.371
-	Corn oil	Yes	0.188	0.262	0.021	28.5[a]	43.1	28.5	71.5	0.399
-	Soy oil	No	0.170	0.198	0.038	28.1[a]	41.9	29.6	71.7	0.691
-	Soy oil	Yes	0.172	0.223	0.036	28.4[a]	41.6	26.7	69.9	0.427
Dietary treatments										

Fresh	Corn oil	No	0.181	0.248 [x]	0.009 [x]	27.5 [x]	40.8	31.4	72.2	0.381 [x]
Fresh	Corn oil	Yes	0.175 [x]	0.218 [x]	0.015	27.7 [x]	42.3	29.1	71.4	0.389 [x]
Oxidized	Corn oil	No	0.190 [x]	0.293 [x]	0.025	24.1 [x]	44.4	25.6 [x]	70.0 [x]	0.335 [x]
Oxidized	Corn oil	Yes	0.200	0.306	0.027	27.9 [x]	43.9	28.0	71.9 [x]	0.390 [x]
Fresh	Soy oil	No	0.142	0.176	0.028	28.7 [x]	41.4	29.8	71.2 [x]	0.403 [x]
Fresh	Soy oil	Yes	0.144 [x]	0.165	0.025	29.0	42.6	21.9	64.5 [x]	0.466
Oxidized	Soy oil	No	0.197 [x]	0.220 [x]	0.048 [x]	25.3 [x]	37.1 [x]	25.7 [x]	62.8 [x]	0.989
Oxidized	Soy oil	Yes	0.199	0.280	0.047	27.0 [x]	40.7	31.5	72.2	0.375 [x]
Pooled SD[1]			0.026	0.079	0.013	1.064	2.238	5.023	3.106	0.632
			----------Probability----------							
Oil quality			<.001	0.001	<.001	0.100	0.061	0.278	0.106	0.453
Oil type			0.023	0.009	<.001	0.057	0.056	0.322	0.133	0.303
Vitamin E			0.744	0.694	0.753	0.002	0.822	0.079	0.080	0.481
Oil quality x vitamin E			0.551	0.171	0.950	0.287	0.032	0.032	0.222	0.315
Oil type x oil quality			0.006	0.746	0.249	0.600	0.008	0.018	0.257	0.430
Oil type x vitamin E			0.998	0.433	0.346	0.036	0.496	0.666	0.651	0.384
Oil type x oil quality x vitamin E			0.536	0.740	0.644	0.363	0.746	0.089	0.112	0.316

[a–b]Means with different superscripts within the same column differ significantly based on Tukey-Kramer comparison (P < 0.05).

[x-y]Values represent the mean of 8 replicates cages (pooled excreta of 6 birds per replicate) except for mean values with x and y where the number of replicates (n) was 7 and 6, respectively.

[1]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

Table A.4. 3 The effects of oil quality, oil type, and vitamin E supplementation on liver fat fatty acid profile (chapter 4)

Oil quality	Oil type	Vitamin E	C14:0	C14:1	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1
Main effect										
Fresh			0.239	0.046	18.5	2.2	0.201	0.214	17.5^{b}	17.2
Oxidized			0.256	0.050	18.8	2.2	0.179	0.216	18.4^{a}	16.7
	Corn oil		0.256	0.046	18.7	2.1	0.197	0.211	18.4	17.1
	Soy oil		0.239	0.050	18.7	2.3	0.182	0.219	17.5	16.8
		No	0.255	0.044	18.4	2.2	0.198	0.215	17.9	16.9
		Yes	0.241	0.051	19.0	2.2	0.182	0.215	18.0	16.9
2-way interaction effect										
Oil quality x vitamin E										
Fresh	-	No	0.249	0.041	17.9	1.8^{b}	0.213	0.216	18.4^{ab}	16.4^{bc}
Fresh	-	Yes	0.230	0.050	19.2	2.5^{a}	0.188	0.211	16.6^{b}	17.7^{a}
Oxidized	-	No	0.262	0.048	18.9	2.6^{a}	0.183	0.215	17.3^{bc}	17.5^{ab}
Oxidized	-	Yes	0.251	0.053	18.8	1.8^{b}	0.175	0.218	19.4^{a}	16.2^{c}
Oil type x oil quality										
Fresh	Corn oil	-	0.246	0.043	18.2	1.9^{b}	0.224	0.214	18.5^{a}	15.9^{bc}
Oxidized	Corn oil	-	0.266	0.048	19.1	2.3^{ab}	0.171	0.208	18.2^{a}	18.3^{ab}
Fresh	Soy oil	-	0.232	0.048	18.8	2.4^{a}	0.177	0.214	16.5^{b}	18.5^{a}
Oxidized	Soy oil	-	0.246	0.052	18.6	2.1^{ab}	0.187	0.225	18.5^{a}	15.2^{c}
Oil type x vitamin E										
-	Corn oil	No	0.250	0.043	18.1	2.1	0.221	0.215	18.6	16.4
-	Corn oil	Yes	0.263	0.049	19.2	2.2	0.174	0.207	18.1	17.7
-	Soy oil	No	0.261	0.046	18.6	2.4	0.175	0.216	17.2	17.5
-	Soy oil	Yes	0.218	0.054	18.7	2.2	0.189	0.223	17.8	16.2
Dietary treatments										
Fresh	Corn oil	No	0.252	0.044	17.6^{x}	1.5	0.247	0.220	19.3^{x}	14.1^{x}
Fresh	Corn oil	Yes	0.241	0.042	18.8	2.2	0.201	0.209	17.7^{x}	17.7
Oxidized	Corn oil	No	0.247	0.042	18.6	2.6	0.194	0.210	17.8^{x}	18.8
Oxidized	Corn oil	Yes	0.285	0.055	19.6	2.1	0.147	0.206	18.6	17.7

Fresh	Soy oil	No	0.246	0.039	18.1 [x]	2.1 [x]	0.179	0.213	17.5 [x]	16.8 [x]
Fresh	Soy oil	Yes	0.219	0.058	19.5	2.8	0.176	0.214	15.5	20.2 [x]
Oxidized	Soy oil	No	0.276	0.053	19.1	2.7	0.171	0.219	16.8 [x]	18.1
Oxidized	Soy oil	Yes	0.217	0.051	18.0	1.5	0.203	0.231	20.2 [x]	12.2 [x]
	Pooled SD[1]		0.118	0.026	2.088	0.743	0.067	0.039	1.625	3.448
			----------Probability----------							
Oil quality			0.573	0.508	0.533	0.729	0.216	0.800	0.047	0.594
Oil type			0.574	0.485	0.988	0.384	0.392	0.426	0.053	0.786
Vitamin E			0.627	0.308	0.259	0.739	0.355	0.960	0.781	0.995
Oil quality x vitamin E			0.884	0.821	0.200	0.000	0.629	0.661	<.001	0.000
Oil type x oil quality			0.922	0.913	0.330	0.040	0.076	0.398	0.010	0.002
Oil type x vitamin E			0.359	0.894	0.389	0.438	0.087	0.468	0.209	0.162
Oil type x oil quality x vitamin E			0.502	0.205	0.280	0.421	0.600	0.904	0.086	0.181

[a–b]Means with different superscripts within the same column differ significantly based on Tukey-Kramer comparison (P < 0.05).

[x-y]Values represent the mean of 8 replicates cages (pooled excreta of 6 birds per replicate) except for mean values with x and y where the number of replicates (n) was 7 and 6, respectively.

[1]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

Table A.4.3 continued. The effects of oil quality, oil type, and vitamin E supplementation on liver fat fatty acid profile (chapter 4)

Oil quality	Oil type	Vitamin E	C18:2	C18:3 n-6	C18:3 n-3	C20:0	C20:1	C20:2	C20:3	C20:4
Main effect										
Fresh			22.3[a]	0.922	0.633[a]	0.056[a]	0.200	0.458	1.4	12.4
Oxidized			20.8[b]	0.704	0.507[b]	0.044[b]	0.197	0.462	1.6	13.5
	Corn oil		20.8[b]	0.850	0.424[b]	0.043[b]	0.211	0.478	1.5	13.5
	Soy oil		22.4[a]	0.777	0.716[a]	0.056[a]	0.186	0.442	1.6	12.4
		No	21.5	0.771	0.595	0.048	0.195	0.476	1.6	13.0
		Yes	21.7	0.856	0.544	0.052	0.202	0.444	1.4	12.9
2-way interaction effect										
Oil quality x vitamin E										
Fresh	-	No	22.4	0.758	0.615	0.050	0.172[b]	0.465	1.5	13.2[ab]
Fresh	-	Yes	22.3	1.087	0.650	0.061	0.229[a]	0.451	1.4	11.6[b]
Oxidized	-	No	20.5	0.783	0.576	0.045	0.218[ab]	0.486	1.7	12.8[ab]
Oxidized	-	Yes	21.2	0.625	0.438	0.042	0.176[ab]	0.438	1.5	14.2[a]
Oil type x oil quality										
Fresh	Corn oil	-	21.8	0.902	0.454	0.045	0.200	0.501	1.3	13.5
Oxidized	Corn oil	-	19.8	0.799	0.394	0.041	0.222	0.455	1.6	13.5
Fresh	Soy oil	-	22.9	0.943	0.811	0.066	0.200	0.415	1.5	11.3
Oxidized	Soy oil	-	21.9	0.610	0.620	0.046	0.172	0.469	1.6	13.5
Oil type x vitamin E										
-	Corn oil	No	20.8	0.794	0.482	0.043	0.193	0.504	1.5	13.7
-	Corn oil	Yes	20.8	0.906	0.367	0.043	0.229	0.452	1.4	13.2
-	Soy oil	No	22.1	0.747	0.709	0.052	0.197	0.448	1.7	12.3
-	Soy oil	Yes	22.7	0.806	0.722	0.061	0.176	0.436	1.4	12.5
Dietary treatments										
Fresh	Corn oil	No	21.4[x]	0.714	0.533[b]	0.045	0.166[x]	0.488	1.5	14.1
Fresh	Corn oil	Yes	22.2	1.090	0.376[b]	0.045[x]	0.235	0.514	1.2	12.8
Oxidized	Corn oil	No	20.1	0.875	0.431[b]	0.042[x]	0.221	0.520	1.6	13.4
Oxidized	Corn oil	Yes	19.4	0.722	0.357[b]	0.040[x]	0.224	0.390	1.6	13.6

Fresh	Soy oil	No	23.3 [x]	0.803	0.698[ab]	0.055	0.177 [x]	0.443	1.5	12.3 [x]
Fresh	Soy oil	Yes	22.5	1.083	0.924[a]	0.077	0.223	0.387	1.5	10.4
Oxidized	Soy oil	No	21.0	0.691	[x] 0.721[ab]	0.049	0.216	0.453	1.9	12.3
Oxidized	Soy oil	Yes	22.9	0.529	[x] 0.520[b]	0.044	0.128 [x]	0.485	1.4	14.7
	Pooled SD[1]		2.653	0.495	0.225	0.022	0.077	0.132	0.476	2.636
			----------Probability----------							
Oil quality			0.031	0.089	0.033	0.046	0.886	0.910	0.140	0.104
Oil type			0.019	0.561	<.001	0.025	0.206	0.298	0.448	0.116
Vitamin E			0.674	0.501	0.376	0.491	0.711	0.356	0.178	0.837
Oil quality x vitamin E			0.627	0.059	0.138	0.219	0.015	0.618	0.524	0.032
Oil type x oil quality			0.420	0.365	0.263	0.166	0.211	0.152	0.497	0.118
Oil type x vitamin E			0.682	0.834	0.269	0.418	0.152	0.562	0.655	0.569
Oil type x oil quality x vitamin E			0.134	0.863	0.030	0.302	0.393	0.080	0.075	0.291

[a–b]Means with different superscripts within the same column differ significantly based on Tukey-Kramer comparison ($P < 0.05$).

[x-y]Values represent the mean of 8 replicates cages (pooled excreta of 6 birds per replicate) except for mean values with x and y where the number of replicates (n) was 7 and 6, respectively.

[1]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

Table A.4.3 continued. The effects of oil quality, oil type, and vitamin E supplementation on liver fat fatty acid profile (chapter 4)

Oil quality	Oil type	Vitamin E	C22:0	C22:2	C22:4	C24:0	∑SFA	∑MUFA	∑PUFA	∑UFA	SFA:UFA
Main effect											
Fresh			0.331	1.165	1.192	1.064	36.1^{b}	20.9	40.6	60.2	0.596^{b}
Oxidized			0.361	1.101	1.251	1.142	38.5^{a}	20.5	39.7	60.5	0.643^{a}
	Corn oil		0.194^{b}	1.082	1.582^{a}	0.759^{b}	36.9	20.4	40.2	59.4	0.622
	Soy oil		0.498^{a}	1.184	0.861^{b}	1.447^{a}	37.7	21.0	40.1	61.3	0.616
		No	0.351	1.132	1.206	1.060	36.4	20.7	40.3	59.8	0.606
		Yes	0.340	1.134	1.237	1.146	38.2	20.7	40.0	60.9	0.632
2-way interaction effect											
Oil quality x vitamin E											
Fresh	-	No	0.341	1.087	1.238	1.217^{ab}	35.2	18.9	41.6	57.8^{b}	0.596
Fresh	-	Yes	0.322	1.243	1.146	0.912^{b}	37.1	22.9	39.6	62.6^{a}	0.595
Oxidized	-	No	0.362	1.177	1.174	0.903^{b}	37.5	22.5	39.0	61.8^{a}	0.616
Oxidized	-	Yes	0.359	1.024	1.329	1.381^{a}	39.4	18.5	40.4	59.1^{ab}	0.670
Oil type x oil quality											
Fresh	Corn oil	-	0.177	1.030^{b}	1.666	0.793	35.5	18.9^{c}	41.4	57.6^{b}	0.608
Oxidized	Corn oil	-	0.212	1.140^{ab}	1.498	0.724	38.2	21.8^{ab}	38.9	61.1^{ab}	0.637
Fresh	Soy oil	-	0.486	1.300^{a}	0.718	1.336	36.8	22.8^{a}	39.8	62.7^{a}	0.583
Oxidized	Soy oil	-	0.509	1.060^{b}	1.004	1.559	38.7	19.2^{bc}	40.4	59.8^{ab}	0.649
Oil type x vitamin E											
-	Corn oil	No	0.193	1.080	1.638	0.774	35.3	19.6	40.8	58.1	0.602
-	Corn oil	Yes	0.196	1.084	1.525	0.743	38.4	21.1	39.6	60.7	0.643
-	Soy oil	No	0.510	1.185	0.773	1.345	37.4	21.7	39.8	61.5	0.610
-	Soy oil	Yes	0.485	1.184	0.949	1.549	38.1	20.3	40.5	61.1	0.622
Dietary treatments											
Fresh	Corn oil	No	0.165	0.994^{x}	1.773	0.859^{bc}	33.3	16.8^{x}	42.2^{x}	53.5	0.590^{x}
Fresh	Corn oil	Yes	0.188	1.058^{x}	1.559	0.727^{c}	37.7^{x}	21.1	40.6	61.7	0.625^{x}
Oxidized	Corn oil	No	0.220	1.165^{x}	1.504	0.689^{c}	37.4^{x}	22.5	39.3	62.6^{x}	0.613^{x}
Oxidized	Corn oil	Yes	0.204	1.109^{x}	1.492	0.760^{c}	39.1	21.1	38.5	59.6	0.661

Fresh	Soy oil	No	0.517	1.180^{x}	0.703	1.575^{ab}	37.2^{x}	21.0^{x}	41.0^{x}	62.0^{x}	0.602^{x}
Fresh	Soy oil	Yes	0.455	1.427^{x}	0.733^{x}	1.096^{bc}	36.4	24.7^{x}	38.6	63.5^{x}	0.565^{x}
Oxidized	Soy oil	No	0.504	1.189^{x}	0.843	1.116^{bc}	37.7^{x}	22.4	38.6	61.0	0.619^{x}
Oxidized	Soy oil	Yes	0.515^{x}	0.940^{x}	1.166	2.002^{a}	39.7^{x}	16.0^{x}	42.3	58.7^{x}	0.678^{x}
	Pooled SD[1]		0.089	0.289	0.497	0.483	4.361	3.679	4.381	5.313	0.074
			----------Probability----------								
Oil quality			0.197	0.409	0.637	0.524	0.047	0.675	0.394	0.840	0.020
Oil type			<.001	0.190	<.001	<.001	0.445	0.490	0.962	0.170	0.744
Vitamin E			0.626	0.986	0.801	0.476	0.107	0.965	0.809	0.437	0.183
Oil quality x vitamin E			0.715	0.052	0.329	0.002	0.963	0.000	0.129	0.009	0.170
Oil type x oil quality			0.780	0.028	0.076	0.231	0.713	0.001	0.169	0.025	0.365
Oil type x vitamin E			0.519	0.972	0.254	0.336	0.278	0.151	0.404	0.282	0.445
Oil type x oil quality x vitamin E			0.215	0.229	0.857	0.020	0.224	0.261	0.236	0.175	0.307

[a–b]Means with different superscripts within the same column differ significantly based on Tukey-Kramer comparison (P < 0.05).

[x-y]Values represent the mean of 8 replicates cages (pooled excreta of 6 birds per replicate) except for mean values with x and y where the number of replicates (n) was 7 and 6, respectively.

[1]SEM can be calculated from pooled SD: SEM $= \frac{SD}{\sqrt{n}}$

Appendix 5. Titanium determination protocol

This titanium determination method is modified from the method of Short et al. (1996). For general safety, please wear all protective equipment including gloves, safety goggles, and lab coats. Also, open a bag of feed-grade Sodium Bicarbonate for neutralizing acid in crucibles and in case of spills. Finally, check to ensure the fume hood and hot plates are in good working condition.

Creating Acid for use with samples

- Samples need to be heated in 7.4 M Sulfuric Acid (H_2SO_4)
- Label a screw-top 1000 ml glass beaker "7.4 M Sulfuric Acid (H_2SO_4)"
- Set up an ice bath in the sink
    - Place 1000 ml beaker in the cooler
    - Surround with ice
- Add 600 ml of dH_2O to the beaker
- Add 400 ml of concentrated (18.4 M) Sulfuric Acid to the beaker
    - Do so in series of smaller amounts to allow the beaker to cool in between additions
        - Additional ice may be needed to be added to the cooler during this process
    - Stir with glass stir rod
- Once finished and cooled the solution may be removed from the cooler

Preparation of Titanium Stock Solutions

- Weigh out 250mg of titanium dioxide

- Add the TiO_2 to 100 ml of 7.4 M Sulfuric Acid (H_2SO_4) in a 250 ml beaker
- Heat the solution at 250 ^oC until the TiO_2 is completely dissolved and the solution becomes clear. Stir occasionally with a glass rod until this happens. This may take 1-2 hours.
- Remove from heat and transfer solution into a 500 ml beaker. Using a pipettor, rinse the solution with 10 ml ddH_2O
- Add 100 ml of 7.4M Sulfuric Acid (H_2SO_4) to the 500 ml beaker
- Bring up to 500 ml with dH_2O
- Once cool, pour contents of 500 ml beaker into a 500 ml or 1000 ml screw-top glass beaker, shake a few times and label it "Titanium Stock Solution"

Standard Solutions Preparation

- Label 6 100 ml volumetric flasks "0, 10, 20, 30, 40, 50"
- Fill flasks according to Table A.5.1 below:

Table A.5. 1 Titanium standard preparation guide

Flask	Titanium Stock Solution	7.4 M Sulfuric Acid	TiO_2 Concentration (mg/ml)
0	0 ml	10 ml	0.000
10	2 ml	8 ml	0.006
20	4 ml	6 ml	0.012
30	6 ml	4 ml	0.018
40	8 ml	2 ml	0.024
50	10 ml	0 ml	0.030

- Add 5 ml of 30% Peroxide to each flask using a 5-ml pipette
- Fill up to meniscus (100 ml) with dH_2O using a squeeze bottle
- Invert flasks 2-3 times
- Cover with parafilm
- Let the flasks sit overnight and look for a color change
    - The yellow color should be darkest in the "50" flask and stay clear in the "0" flask
    - Solutions are color stable for 2 years

Sample Preparation

- Weigh out 0.2 grams of sample into each crucible and tare the crucibles on scale first
- Weigh samples in duplicates.
- Place crucibles into the ashing oven at 580°C for 8-10 hours.
    - Use tongs to remove samples from the hot oven
    - Note that the diet sample ash may look lighter than the digesta sample ash
- Rinse out crucible into 50 ml beaker with 10-20 ml of 7.4 M H_2SO_4
    - Use pipettor and don't worry about changing tips due to acid
    - Place empty crucibles into a small tub with bicarbonate and soapy water
- Place all beakers on hot plates
- Heat at 250°C until solution dissolves approximately 1 hour after. Endeavor not to increase the temperature excessively beyond this point.
- Stir occasionally by picking up with pliers or tongs and gently swirling.

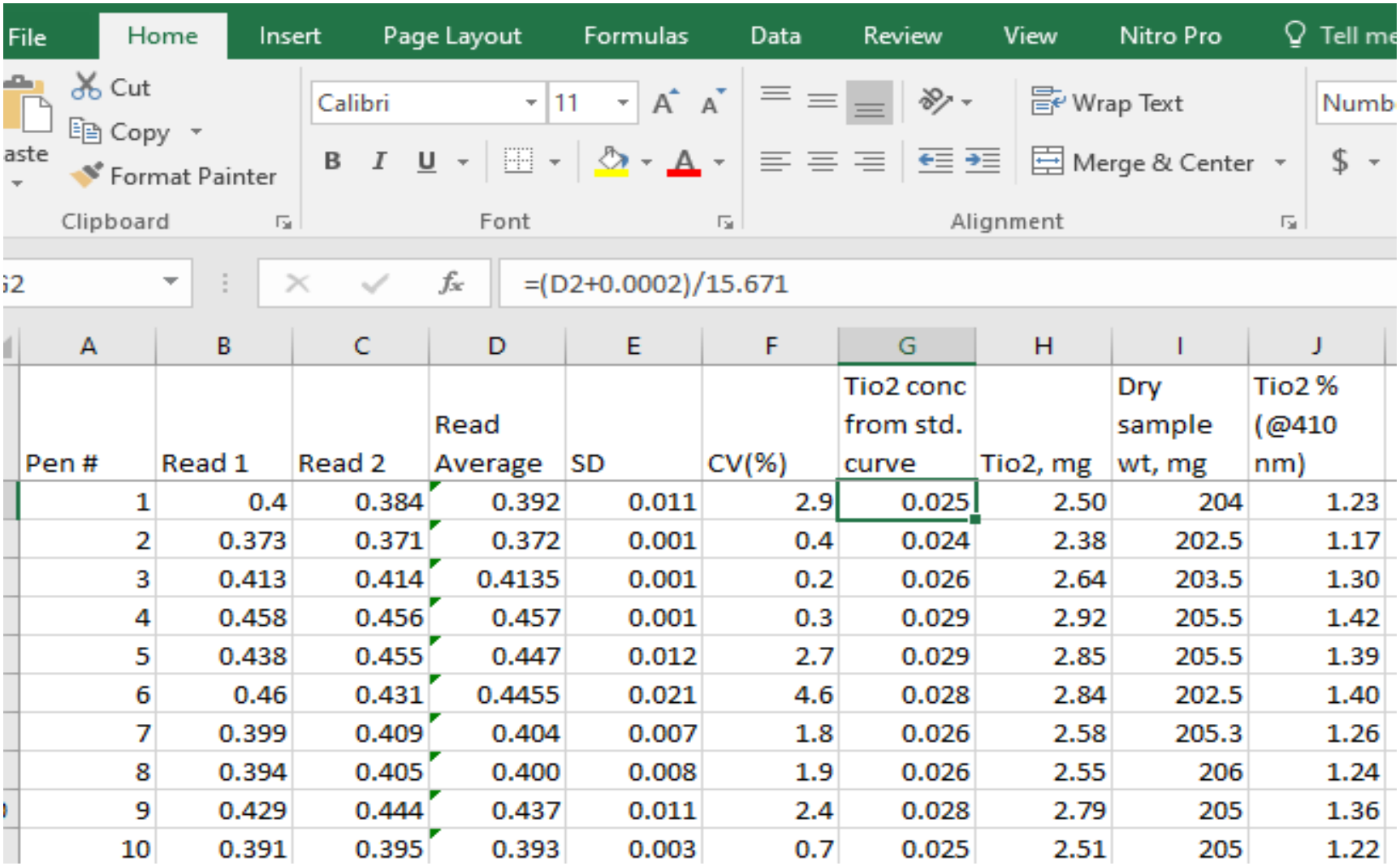
- Add a bit more acid if the solution boils off and goes below the 10 ml level.
- Once the solution dissolves and turns clear, remove it from heat
- Add 25 ml dH_2O into a 100 ml volumetric flask
- Pour contents of 50ml beaker into the volumetric flask through a glass funnel and rinse content into the flask using a squeeze bottle
- Add 5 ml of 30% peroxide to each flask using a 5-ml pipette.
- Bring contents up to volume (100ml) using ddH_2O.
- Cap with parafilm
- Invert 2-3 times
- Allow to sit overnight

Note: Diet samples will end up lighter in color than most digesta and excreta samples

<u>Determining Titanium Concentration in Samples</u>

- Read each sample in duplicate on a spectrophotometer set at 410nm wavelength
- Use standard samples to create a standard curve
    - Use "0" as blank
    - Fit linear regression
- Use sample absorbance values to calculate the concentration of the samples from the standard curve
    - Plug absorbance values into the reordered standard curve equation: *Concentration = (Absorbance – Intercept Value)/ Slope*
- Type in sample absorbance value as determined from the spectrophotometer

- Input the sample weight as milligram with one decimal place into column I (see Figure A.3.1)
- To calculate the titanium concentration from the standard curve input the unique intercept and slope value into the formula under column G (see Figure A.3.1)
- Each time you read new sets of samples, adjust the formula in the "TiO_2 from standard curve" column for the affected cells



	A	B	C	D	E	F	G	H	I	J
	Pen #	Read 1	Read 2	Read Average	SD	CV(%)	Tio2 conc from std. curve	Tio2, mg	Dry sample wt, mg	Tio2 % (@410 nm)
	1	0.4	0.384	0.392	0.011	2.9	0.025	2.50	204	1.23
	2	0.373	0.371	0.372	0.001	0.4	0.024	2.38	202.5	1.17
	3	0.413	0.414	0.4135	0.001	0.2	0.026	2.64	203.5	1.30
	4	0.458	0.456	0.457	0.001	0.3	0.029	2.92	205.5	1.42
	5	0.438	0.455	0.447	0.012	2.7	0.029	2.85	205.5	1.39
	6	0.46	0.431	0.4455	0.021	4.6	0.028	2.84	202.5	1.40
	7	0.399	0.409	0.404	0.007	1.8	0.026	2.58	205.3	1.26
	8	0.394	0.405	0.400	0.008	1.9	0.026	2.55	206	1.24
	9	0.429	0.444	0.437	0.011	2.4	0.028	2.79	205	1.36
	10	0.391	0.395	0.393	0.003	0.7	0.025	2.51	205	1.22

Figure A.5. 1 Titanium calculation sheet

Appendix 6 Fatty acid determination method

The fatty acid composition for both studies reported in this thesis was determined by gas chromatography, using a Shimadzu gas chromatograph (Model 14 A, Columbia, MD) with a flame ionization detector. The procedure was modified from Park and Goins (1994). Briefly, approximately 1-2 g of each diet sample, 100 mg of adipose tissue, 100 mg of oil, and 2.0 g of liver were used for the analysis. Tridecanoic acid (2 mg/ml in Methanol) was used as the internal standard. The samples were processed through a 2-step methylation procedure. The first was heating in 0.5 *N* sodium methoxide in methanol for 30 minutes at 90 ^{o}C, followed by the addition of boron trifluoride in methanol and heating for another 20 minutes. Methyl esters were isolated in hexane and anhydrous sodium sulfate was added to remove any residual water. Samples were stored at 4^{o}C until analyzed. Fatty acid methyl esters were separated on a Phenomenex, ZBWax Plus wide-bore capillary column (Phenomenex, Torrance, CA) with nitrogen as the carrier gas. The initial column temperature was 160 °C which was held for 10 minutes and increased at a rate of 5°C /minute to 220°C. The injector temperature was 250°C and the detector temperature was 260°C. Peaks were identified by comparison of retention times of known standards. Quantification was corrected for recovery of the internal standard and is based on the reference standard.

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VITA

Place of Birth:

Ilorin, Nigeria

Education:

B. S. – Animal Science, Obafemi Awolowo University, January 2011 - April 2016

Thesis: Effect of access to pasture of finished broilers on their abdominal fat and carcass contents

(GPA: 4.48/5)

Working Experience:

University of Kentucky
Graduate research assistant, Department of Animal and Food Sciences July 2019 – July 2021

Awards /Honors/Scholarships:

- Richards Graduate Student Research Activity Award - University of Kentucky (October 2020)
- University of Kentucky Graduate Research Assistantship Award worth over $50,000 (July 2019 – July 2021)

Publications:

- R.A. Adefioye; M.D. Lindemann; M.J. Ford; A.J. Pescatore; S.A. Adedokun (2021). "The effect of oil type, oil quality, and vitamin E supplementation on the performance, relative fat and liver percentages, and the apparent nitrogen and crude fat utilization in 21-day-old broiler chickens," in the proceedings of the 2021 Virtual International Poultry Scientific Forum. P. 20-21.

- Adefioye, R. A. and E. B. Sonaiya. 2017. Preliminary study on effect of access to pasture of finished broilers on their abdominal fat and carcass contents. Nigerian Journal of Animal Production 44(5): 1VWR16 – 121.

- Title: "Effects of oil quality, phytase, and vitamin E supplementation on the growth performance, nutrient utilization, and relative fat and liver weight in 21-day-old broiler chickens." (Presented at Poultry Science Annual Meeting, July 2021)