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EVALUATION OF PHENOLIC COMPOUNDS IN BROILERS ON PERFORMANCE PARAMETER AND OXIDATIVE STATUS

EVALUACIÓN DE COMPUESTOS FENÓLICOS EN POLLOS DE CARNE SOBRE EL REDEMIENTO PRODUCTIVO Y SU STATUS OXIDATIVO

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ABSTRACT

Lipid oxidation occurs mainly in poultry due to an excessive formation of free radicals, as a result of high intake of polyunsaturated fatty acids or a low antioxidant intake. It is generally accepted that lipid oxidation is the major cause of the deterioration of tissues that directly affects chicken meat. This process could be prevented by the supplementation of diets with antioxidants that minimize the formation of free radicals and increase lipids stability. At present, various phenolic compounds have received substantial attention, because of their potential. Phenolic compounds are secondary plant metabolite characterized by minimizing the negative consequence of lipid oxidation, which present an interest for their use in animal feed. The global aim of this study is to evaluate the effect of phenolic compounds as natural sources of antioxidants in broiler chickens. Ninety-six female broiler of Ross 308 strain were randomly allocated in 24 cages (4 animals per cage) and cages were randomly distributed in 4 treatments (6 cages per treatment). Treatments were: C-, basal diet with 3% of fish oil; C+, C- with 250 ppm of vitamin E; Diet A, C- with 1500 ppm of product A; Diet B, C- with 1500 ppm of product B. Product A and B are products with a high content of phenolic compounds. The results of this study showed that dietary supplementation with the tested products did not modify growth performance, neither lipid profile and oxidation degree in blood ($P>0.05$). Further studies are needed to evaluate the efficacy of these products at different levels of incorporation, as well as the concentration and combination of these active ingredients.

Keywords: phenolic compounds, antioxidants, broiler diet.

RESUMEN

La oxidación de los lípidos se puede producir por la presencia de una elevada cantidad de radicales libres, debido a un elevado consumo de ácidos grasos poliinsaturados o como resultado de una falta de antioxidantes. En general, se acepta que la oxidación de los lípidos es la principal causa del deterioro de los tejidos y que afecta directamente al valor comercial de la carne de pollo. Este proceso podría prevenirse mediante la suplementación de antioxidantes en las dietas, que minimicen la formación de los radicales libres y aumenten la estabilidad de los lípidos. En la actualidad, diversos compuestos fenólicos están siendo estudiados, debido a su potencial antioxidante. Los compuestos fenólicos son metabolitos secundarios de las plantas, que se caracterizan por minimizar las consecuencias negativas de la oxidación lipídica, pudiendo ser de interés para su uso en alimentación animal. El objetivo global de este estudio es evaluar el efecto de compuestos fenólicos, como fuente natural de antioxidantes, en pollos de carne. Noventa y seis hembras Ross 308 se asignaron al azar a 24 jaulas (4 animales por jaula) y las jaulas se distribuyeron al azar en 4 tratamientos (6 jaulas por tratamiento). Los tratamientos fueron: C-, dieta basal con 3% de aceite de pescado; C+, C- con 250 ppm de vitamina E; Dieta A, C- con 1500 ppm de producto A; Dieta B, C- con 1500 ppm de producto B. El producto A y B son productos con alto contenido de compuestos fenólicos. La suplementación dietética de los productos evaluados en este estudio, no modificó los parámetros productivos, el perfil lipídico ni el grado de oxidación de la sangre ($P > 0.05$). Hacen falta más estudios para valorar la efectividad de estos productos a diferentes dosis de incorporación así como la concentración y combinación de sus principios activos.

Palabras clave: compuestos fenólicos, antioxidantes, dieta broiler.

RESUME

L'oxydation des lipides se produisent principalement chez les volailles en raison d'une formation excessive de radicaux libres, d'un apport élevé en acides gras polyinsaturés ou d'un faible apport en antioxydant. Il est généralement admis que l'oxydation des lipides est la principale cause de la détérioration des tissus qui affecte directement la valeur commerciale de la viande chez le poulet. Ce processus pourrait être prévenu en supplémentant l'aliment avec des antioxydants qui minimisent la formation de radicaux libres et augmentent la stabilité des lipides. Actuellement, divers composés phénoliques ont fait l'objet d'une attention considérable en raison de leurs potentiel. Les composés phénoliques sont des métabolites secondaires des plantes, caractérisés par la minimisation des conséquences négatives de l'oxydation des lipides, ce qui présente un intérêt pour leur utilisation dans l'alimentation animale. L'objectif global de cette étude est d'évaluer l'effet des composés phénoliques, en tant que sources naturelles d'antioxydants chez le poulet de chair. Quatre vingt seize poulets de chair femelles de souche Ross 308 ont été répartis au hasard dans 24 cages (4 animaux par cage) et réparties de manière aléatoire dans 4 traitements (6 cages par traitement). Les traitements étaient : C-, aliment de base avec 3% d'huile de poisson; C +, C- avec 250 ppm de vitamine E; Aliment A, C- avec 1500 ppm de produit A; Aliment B, C- avec 1500 ppm de produit B. Le produit A et B sont des produits à forte teneur en composés phénoliques. La supplémentation alimentaire des produits évaluer dans cette étude n'a pas modifié les paramètres de production, ni le profile lipidique ni le degré d'oxydation dans le sang ($P > 0,05$). D'autres études sont nécessaires pour évaluer l'efficacité de ces produits à différentes doses d'incorporation, ainsi qu'à une concentration et une association de ces principes actifs.

Mots-clés: composés phénoliques, antioxydants, aliments du poulet de chair

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ABBREVIATION USED

ADFI: Average daily feed intake

ADG: Average daily gain

BW: Body weight

FCR: Feed convertion ratio

GP: Grape pomace

GS: Grape seed

GT: Green tea

GTP: Green tea powder

OMWW: Olive mil waste water

EVOO: Extra vergin olive oil

PUFA: Poly unsaturated fatty acids

SOD: Superoxide dismutase

GPx: gluthatione peroxidase

CAT: Catalase

TBARS: Thiobarbituric acid reactive substances

TAC: Total antioxidant capacity

MDA: Malondialdehydes

1. INTRODUCTION

In the animal, lipid oxidation process takes place continuously, and is due to an imbalance between the production of reactive oxygen species and the defense mechanism of the animal against oxidative factors. The antioxidant mechanism aimed at minimizing the effect of these reactive oxygen species which, in turn, represent a broad category of molecules that give free radicals such as superoxide, hydrogen peroxide, and hydroxyl radicals. These free radicals are responsible for damage organic substrates, such as lipids, protein and DNA of living organism (Kalam et al. 2012).

Several factors on poultry could exacerbate the biological formation of this oxidation process. Among nutritional factors, the major known factors involved in this process include the high levels in polyunsaturated fatty acids in diets, lipid structure and its environment. The degrees of the instauration in fatty acids, and the presence of molecular oxygen, are main factors affecting the oxidative stability of lipids.

Natural components found in muscle tissue such as iron, myoglobin, hydrogen peroxide (H_2O_2), and ascorbic acid can cause lipid oxidation, acting as catalysts or promoting the formation of free radicals (Wójciak & Dolatowski, 2012).

Lipid oxidation is much more severe in poultry phospholipid fatty acids, because it is conditioned by a wide range of species-specific mechanism such as lower activity of glutathione peroxidase (GPx), superoxide dismutase (SOD) and other antioxidant enzymes. Consequently, the risk of post-mortem oxidation and rancidity in meat will be increased and impact negatively on their organoleptic, nutritional properties and shelf-life.

The nutritional strategies to reduce the effect of lipid oxidation involve changes in the composition of the feeds and antioxidant supplementation. In particular, the supplementation with exogenous antioxidants is important to prevent feed oxidation and avoid the intake of free radicals by animals.

In one hand, supplementation with antioxidant with *in vitro* activity is used to prevent the oxidation of feeds and feedstuffs. In particular, oxidation results in rancidity of fats and nutrients deficiency as vitamin A and E by destruction them which result a lower nutrient values for the diet. Moreover, if this destruction took place in feed, or even in single ingredient, may result in disastrous nutrient deficiencies. The antioxidants prevent oxidative losses of vitamins A and E in stored mixed feeds. Moreover, stabilize critical oxidation-susceptible nutrients that are naturally present in feed.

In the other hand, antioxidants with *in vivo* activity play a key role in the stability of lipids, sustain the endogenous antioxidant system in the organism, and then, can improve the meat shelf life and increase the animal performance.

Presently, great attention in poultry is being paid to the supplementation of feed by phytogenic compounds to improve animal productivity and the quality of food derived from those animals. These compounds include various substances with different chemical characteristics, which can be found in any plant part such as grains, fruits, kernels, seeds, leaves. However, they could be alternative to conventional antioxidants because of their benefits, due to poultry health implications. Moreover, meat products with natural antioxidants are becoming more acceptable to consumers than the use of their synthetic counterparts.

Phenolic compounds are one from the phytochemicals group, used as exogenous antioxidants because of two fold's functions: to prevent feed lipid oxidation, and at the same time to increase the amount of antioxidant agents present in the broiler animal tissue, protecting against antioxidant system disorder. However, numerous studies for humans have demonstrated the beneficial effects of phenolic compounds, including anticancer, anti-inflammatory and cardiovascular protective effects, as well as a protective role in degenerative disease (McCullough et al. 2012), so they would have beneficial effects on broilers production and meat characteristics.

1.1. Antioxidant effect and protective role of phenolic compounds in broilers

Phytochemicals are the substances found naturally in all the fruits, vegetables and medicinal plants that ingested daily or rarely, can be favorable for the preclusion of chronic and degenerative diseases. Into phytochemicals, we can find a several compounds, including phenolic compounds.

Phenolic compounds are secondary metabolites that are commonly found in herbs and fruits such as berries, apples, citrus fruits, grapes, vegetables like olive, green and black teas, soybeans and broccoli (Brit & Wang, 2001). These compounds are one of the most widely occurring groups of phytochemicals. All of these phenolic compounds are found in plants, esterified with glucose and carbohydrates (glycosides), or as free aglycones. They play an important morphological role in plants because many of them are responsible for the attractive colour of leaves, fruits and flowers (Hermann, 1993).

Phenolic compounds have been associated with beneficial effects, attributed to their antioxidant activity. Therefore, they can be a natural source of antioxidants, because of their capability towards free radicals normally produced by cells metabolism or in response to external factors that can damage biomolecules such as lipids, nucleic acids, proteins, cause membrane peroxydation (Degroot, 1994).

Structurally, all phenolic compounds possess a common phenol which is an aromatic ring, bearing one or more hydroxyl substituent, and range from simple phenolic molecules to highly polymerized compounds (Bravo, 1998). A further classification divided them into two major groups: Polyphenols and simple phenols (Table 1.1.1.) depending on the number of phenol subunits. Simple phenols include the phenolic acids with only one phenol subunits (Robbins, 2003). Among, polyphenols possessing two phenols subunits include the flavonoids and the stilbenes. Moreover, polyphenols possessing three or more phenols subunits are referred to as the tannins (King & Young, 1999).

In the following **table.1.1.1** is presented the classification of the different phenolic compounds.

Table 1.1.1. Classes of phenolic compounds adapted from (Ebrahimi & Schluesener, 2012)

	Group	Class	Compounds
Simple phenols	Phenolic acids	Hydroxybenzoic	Gallic acid, gentisic acid, protocatechuic acid, syringic acid, vanillic acid, etc.
		Hydroxycinnamic	p-coumaric acid, caffeic acid, chlorogenic acid, ferulic acid, sinapic acid, etc.
Polyphenols	Flavonoids	Flavone	Pigenin, luteolin, tangeritin, chrysin, 6-hydroxyflavone.
		Flavanone	Butin, eriodictyol, hesperetin, hesperidin, homoeriodictyol, etc.
		Flavanols	Catechin, epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate, epiafzelechin, fisetinido, gallactechin etc.
		Flavonols	Natsudaidain, pachypodol, quercetin, rhamnazin, rhamnetin, etc
		Isoflavones	Genistein, daidzein, lonchocarpane, laxiflorane, etc
		Anthocyanidins	Aurantinidin, cyanidin, delphinidin, europinidin, luteolinidin, etc.
	Stilbenes		Resveratrol analogs, etc.
	Tannins		Theaflavins, thearubigins, condensed tannins, proanthocyanidins, ester of gallic acids, etc.

Phenolic acids consist of two subgroups. The hydroxybenzoic and hydroxycinnamic acids **(Figure 1.1.1).** Hydroxybenzoic acids include gallic, p-hydroxybenzoic, protocatechuic, vanillic and syringic acids among others, which in common have the C6–C1 structure. Hydroxycinnamic acids, on the other hand, are aromatic compounds with a three-carbon side chain C6–C3, with caffeic, ferulic, p-coumaric and sinapic acids being the most common (Bravo, 1998).

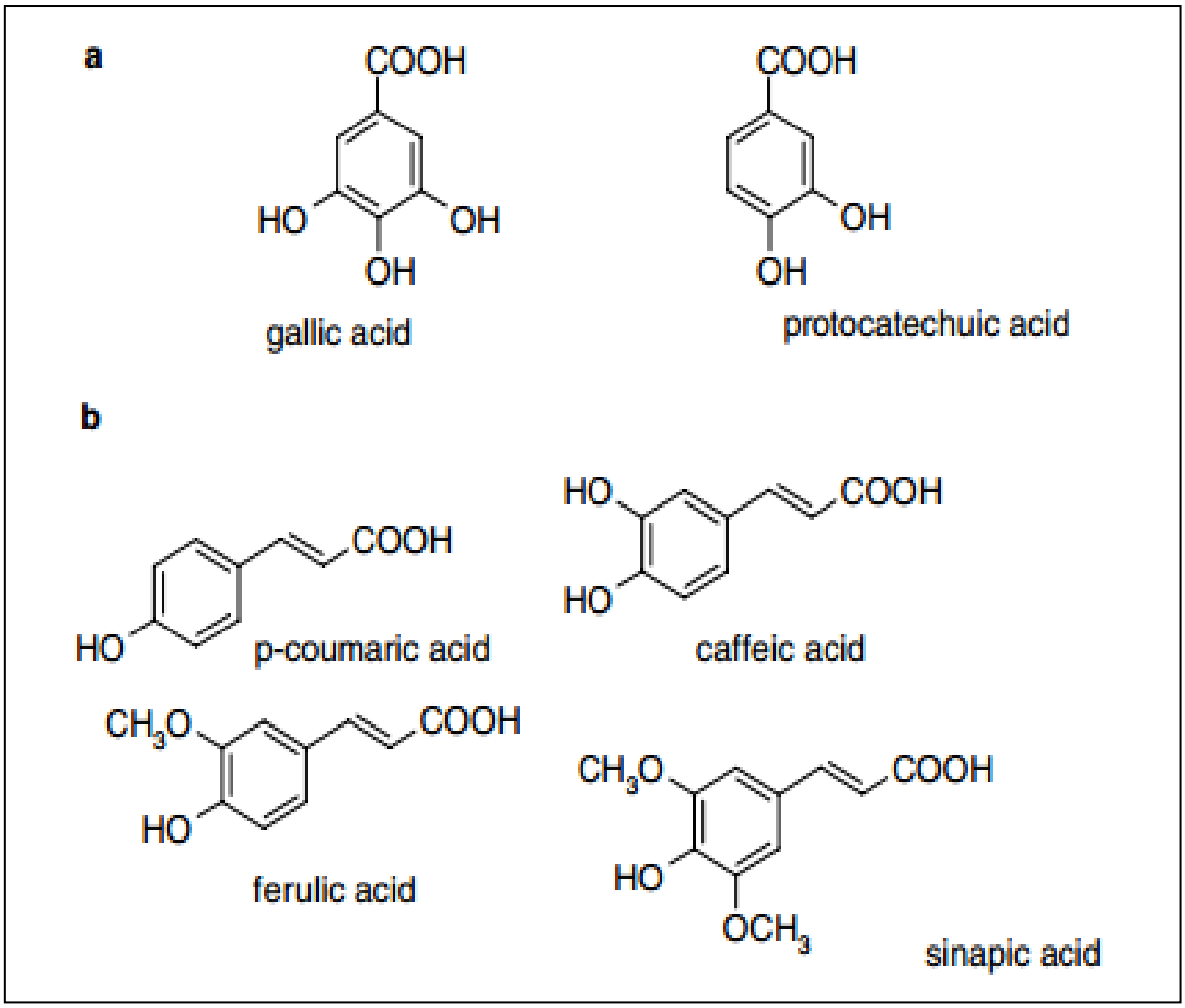

Figure 1.1.1. Examples of simple phenols structures. a. Hydroxybenzoic class and b. Hydroxycinnamic class ((Balasundram 2006)

Into polyphenols **(Fig 1.1.2)**, the flavonoids consist of the large group of low molecular weight .The basic structural feature of all flavonoids is the flavane (2-phenyl-benzo-ϒ-pyrane) nucleus , it is a system of two benzene rings (A and B) linked by oxygen containing pyrane ring (C). According to the degree of oxidation of the C ring, the hydroxylation pattern of the nucleus, and the substituent at carbon 3, we find several subclasses as flavones, flavanones, flavanols (catechins), flavonols, isoflavone, chalcones, anthocyanins and stilbenes (Fig 2). Flavanols differ from flavanones by hydroxyl group at the C3, and by a C2-C3 double bond. Anthocyanins differ from the other flavonoids by possessing a charged oxygen atom in the ring C. The ring C is open in the chalcones. Also, the stilbenes are another family of polyphenols characterized by a double bond connecting the phenolic rings.

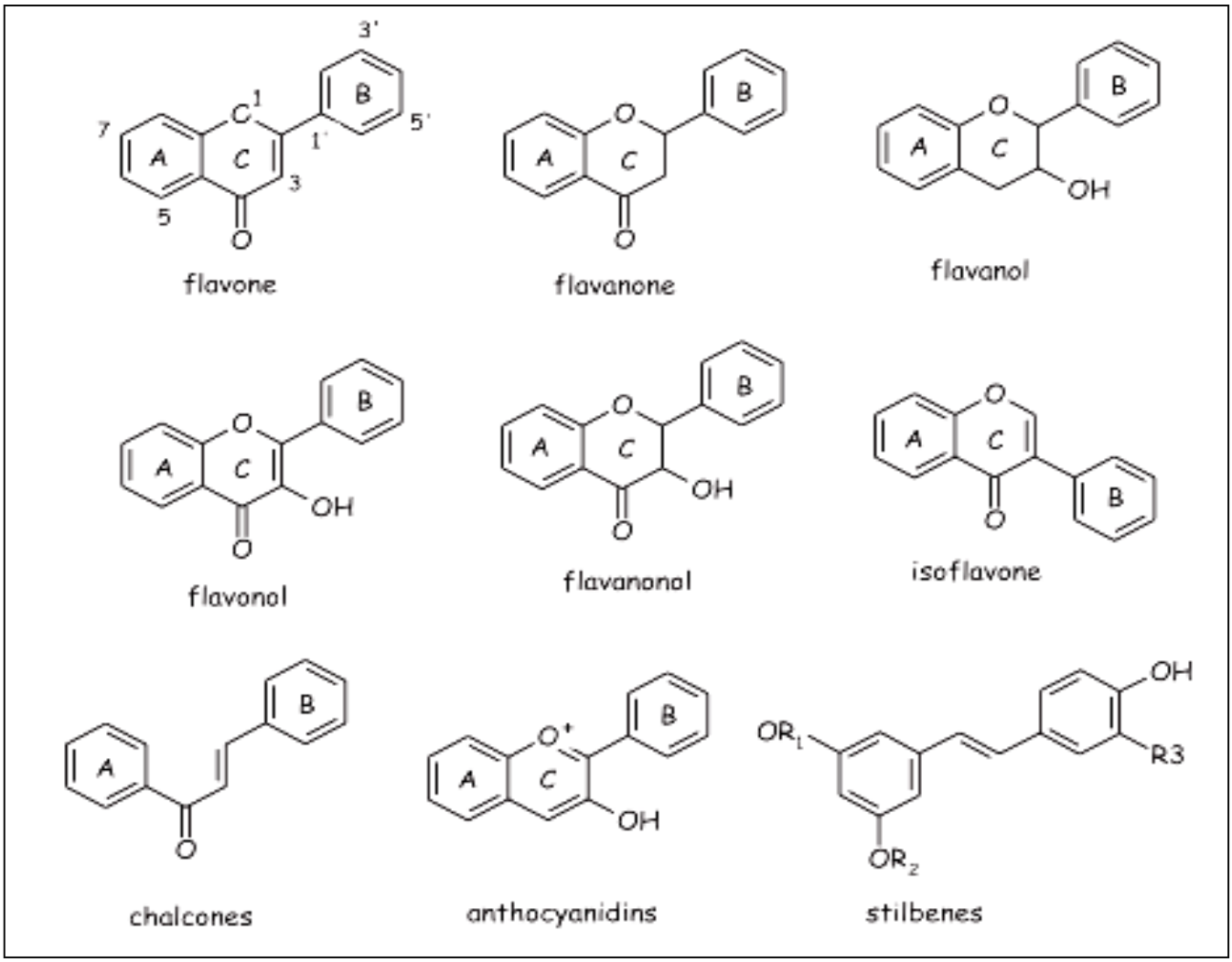

Figure 1.1.2. Different structure of polyphenols (Leopoldini et al. 2011)

Tannins have the higher polyphenolic molecular weight, which constitute the third important group of phenolics. Tannins are divided into hydrolysable tannins included the ester of gallic acid, and condensed tannins included the polymers of flavonoids. Tannins have been in relation to the perceived role as antinutrients, particularly due to their ability to reduce the digestibility of proteins and minerals, but their antioxidants activities could sometime be beneficial.

The metabolism of phenolic compounds is determinate by their structure including their conjugation, degree of glycosilation/acylation, molecular size and solubility (Bravo, 1998).

Much of the earlier studies on the bioavailability of phenolic compounds had concentrated on flavonoids to understand their absorption and metabolism. Most of

flavonoids are absorbed into the small intestinal cells by passive mechanisms (Barnes et al. 2003). They are characterized by low bioavailability and urinary excretion ranges from 0.3% for anthocyanins to 43% for isoflavones (Landete, 2013).

The absorbed compounds will be metabolized in the liver and excreted in the bile. However the enzymes secreted by colonic microflora, hydrolyse the unabsorbed compound, strip the conjugates of their attached moieties and break the larger compounds to simpler molecules such as phenolic acids.

Phenolic compounds have antiviral, antiallergic, antithrombotic and anti-inflammatory effects, as well as an antioxidative effect which is the principal property of these compounds and the antioxidant activity depends of the hydroxyl group and increase with increasing degree of hydroxylation.

The antioxidant properties divide into two main mechanisms: scavenging the free radicals and chelation of free metals. The first one, arising from the scavenge and the direct reaction with free radicals which are very reactive species resulting in cell damage and subsequently in manifestation of pathological conditions (Papadopoulou et al. 2017).

The hydroxyl group (OH), react with the free radical by transferring it to a hydrogen atom, through hemolytic rupture of the O-H bond and leads to the formation of another radical, but it is less reactive.

The second antioxidant mechanism is TMC (Transition Metals Chelation) where the phenolic compounds entrap metals and avoid them to take part in the reactions generating free radicals. Like the reaction of Fe^{2+} in its low oxidation state with hydrogen peroxide which result reactive oxygen species HO, as subsequently, adversely affect growth performance and increase lipid peroxidation in broilers (Surai, 2014). Also, redox reaction of the phenolic compounds and molecule Cu^{2+} in the ternary complex may occur the reduction of Cu^{2+} to Cu^{+}, whose reoxidation generates a variety of reactive oxygen species.

About the antioxidants activity, it is still unclear whether phenolic compound have any direct antioxidant effects *in vivo*, although they might be capable of exerting

such effects within the gastrointestinal tract. Like the majority of flavonoids display potent antioxidant *in vitro*, the bioactive forms of flavonoids *in vivo* are not those forms found in plants, due to their extensive biotransfrormation in the small intestine and hepatic metabolism on absorption (Prochazkova et al. 2011).

1.2. Effect of supplemental grape seed and grape pomace.

Into phenolic compounds, grape pomace (GP) and grape seed (GS) are the residue left after juice extraction by pressing grapes in wine industry, are known to contain polyphenols with high antioxidant capacity.

The polyphenolic constituent found in GS are mainly flavanols (epicatechins, gallactechins, epigallocatechins and epicatechins 3-O-gallate) (Farahat et al., 2016). The use of those antioxidants in broilers feed has been investigated by various authors and the resume of these results is shown in the **table 1.2.1.**

In the experiment of Ibrahim (2017), the supplementation with 20 g/kg of GS powder to the basal diet increased final body weight (BW) and body weight gain (BWG), improved the feed conversion (FCR), increased the percentage of carcass yield but did not affect feed intake (FI) on Cobb-500 chicks for 42 days. However the supplementation with 40 g/kg of GS significantly reduced the percentage of abdominal fat in the birds. Moreover this doses (40 g/kg of GS) significantly ($p < 0.05$) enhanced the activity of glutathione (GSH), catalase (CAT) , superoxide dismutase (SOD), glutathione peroxidase (GPx), and correlated with significantly decreased thiobarbituric acid-reactive substances (TBARS) levels compared with the control group. The GS supplementation no showed any significant change on protein, albumin, globulin, aspartate aminotransferase, and alanine aminotransferase concentration compared with the basal diet, as well as on the value of ileal pH. However, broilers fed diets supplemented with GS had lower ileal Streptococcus spp. and Escherichia coli populations but higher Lactobacillus spp. populations ($p < 0.05$). No adverse effects on birds' health were detected due to the use of GS (Ibrahim 2017)

Elnaggar et al.(2018) showed that natural sources of polyphenols either GS or medicago sativa seeds (alfalfa MSS), could be used safely to improve productive performance, economical efficiency and immune response of broilers chickens. Broilers fed basal diet supplemented with these natural sources of polyphenols at 0.5 and 1 g/kg had greater BW, BWG and FI, and better FCR, economical efficiency, production index compared with Vitamin-E and control groups. All supplementations decreased serum urea, creatinine, total lipids, triglycerides, cholesterol, LDL and increased glucose, HDL, alkaline phosphatase (ALP), total antioxidant capacity (TAC), GPx, GSH, and SOD. Moreover, all supplementations decreased total bacterial count, Salmonella, E.Coli compared to control. Similar results were reported by Ibrahim (2017).

In another study using 5, 7.5 and 10 g/kg of GP supplemented in broiler diets, Aditya et al. (2018) showed positive effects of 7.5 and 10 g/kg of GP on BW gain during early growth stages (0 to 14 days), however FI and FCR remained unaffected. Moreover, GP supplementation was effective in reducing serum cholesterol level ($P<0.05$).

The GS and GP could be recommended as phenolic compounds supplement in the diet of broilers chickens, to improve performance, reduce blood lipids, enhance antioxidant capacity and decrease detrimental bacteria in intestine tract according to the summarized studies.

The following **table.1.2.1**. summarized the different result of the the GS and GP inclusion in the diets of broilers

Table 1.2.1. Effects of supplementary grape seed (GS) and grape pomace (GP) as phenolic compounds on performance, oxidative status and microbiota of broilers chickens.

Polyphenols	Animal and diets	Results			
Sources	Treatment	**Performance**	**Carcass**	**Blood parameter**	**Microbiota**
Grape seed **GS** Ibrahim. 2017	1.Control, 2. GS 10g/kg, 3. GS 20g/kg, 4. GS 40g/kg 1 → 42d	BWG ↑ 20g FBW ↑ 20g FCR GS 20 g < FCR 10 and 40 g	Yield % ↑ 20g/kg Dressing % ↑ 20g/kg Gizzard % ↑ 20g/kg Abdominal fat % ↓ 40g/kg	SOD, CAT, GPx, GSH ↑ 10 ; 20; 40 g/kg TBARS ↓10; 20 ;40 g/kg Total lipid , triglyceride and total cholesterol mg/dll ↓ 10; 20; 40 g /kg	Lactobacilli spp ↓ Escherichia coli ↓ Streptococcus spp ↓
Grape seed **GS** and Medicago Sativa seed **MSS** Elnaggar et al. 2018	1. Control; 2. Vit E 200 IU; 3. GS 0.5g/kg; 4. GS 1 g/kg 5. MSS 0.5g/kg; 6. MSS 1 g/kg 1 →36 d; 7→36 d	BWG ↑ GS 0.5; 1 g/kg FBW ↑ GS 0;1 g/kg	No significance	serum urea, creatinine, total lipids, triglycerides, LDL-cholesterol ↓ 200 IU; 0.5;1 g/kg glucose, HDL-cholesterol, ALP , TAOC, GPx, GSH, SOD ↑ 200 IU; 0.5;1 g/kg	Salmonella ↓ Escherichia coli ↓
Grape seed **GP** Aditya et al. 2018	1.Control; 2. GP 5 g/kg; 3. GP 7.5 g/kg; 4. GP 10g/kg 3 → 28 d	BWG ↑7.5; 10 g/kg	No significance	Total cholesterol ↓	No significance

GS, grape seed; GP grape pomace ; FBW, final body weight; BWG, body weight gain; LDL-cholesterol, low-density lipoprotein-cholesterol; FCR, feed convertion ratio; SOD, superoxide dismutase; FI, feed intake; CAT, catalase; GPx, glutathione peroxidase; GSH, glutathione ; TBARS, thiobarbituric acid reactive substances ; AST, aspartate aminotransferase ; ALT, alanine aminotransferase ; MSS, medicago sativa seed(plant secondary metabolite); TAOC, total antioxidant capacity ; ALP, alkalin phosphat. HDL-cholesterol, high-density lipoprotein-cholesterol.

1.3. Effect of supplemental green tea powder

Green tea (GT) contains phenolic compounds up to 30% of dry weight, including flavonoids and phenolic acids The most important flavonoids are flavanols (catechins) that are present at about 10% of the dry weight basis. Particularly, epigallocatechin- 3-gallate is the most biologically active ingredient in GT. Different results of studies with GP are summarized in table 1.3.1.

Liu et al.(2017) evaluate the possible effect of waste tea powder of GT Camellia sinenis (GTP) in feed supplementation on blood parameters of broiler chickens up to 42 days. Diet has been supplemented with 0, 0.25, 0.50, 0.75, and 1.00% (w/w) of GTP. Differences among treatments were observed in all the parameters. Especially, the supplementation of high feeding GTP at 0.50, 0.75, and 1 % decreased the abdominal fat content and some lipid metabolites, including VLDL cholesterol, LDL cholesterol, triglycerides, and aspartate aminotransferase of broiler chicks.

Huang et al. (2013) observed no influence of GTP consumption on the BWG and hepatic function of broilers aged 35 days old. However the supplementation by 0.5 mg/kg as lower level and 100 mg/kg as higher level of GTP for 20 days, decreased abdominal fat by 45.4 % ($p < 0.001$) and 52.5 % ($p < 0.001$) respectively. Besides, the serum triglyceride, total cholesterol, and low-density lipoprotein cholesterol (LDL-C) levels were significantly decreased by 37.9% ($p < 0.001$), 6.32% ($p < 0.05$), and 20.9% ($p < 0.001$), respectively, from 100 mg/kg GTP-treated broilers compared to control. However, these three serum parameters were not altered by the 0.5 mg/kg green tea powder.

It seems that, GTP supplementation could possess antioxidant properties in broilers diets.

The following **table.1.3.1**. summarized the different result of the the GTP inclusion in the diets of broiler.

Table 1.3.1. Effects of supplementary green tea powder (GTP) as phenolic compounds on carcass parameter and oxidative status of broilers chickens.

Polyphenols	Animal and diets	Results	
Sources	Treatment	**Carcass**	**Blood parameter**
Green Tea Powder **GTP** Liu et al. 2017	1. Control , 2. GTP 0.25% 3. GTP 0.50% 4. GTP 0.75% 5. GTP 1.00 % 1 → 21d; 21→42d	Abdominal fat % ↓ 0.50; 0.75; 1.00 %	LDL-cholesterol ↓ 0.50; 0.75; 1.00 % HDL -cholesterol ↓ 0.50; 0.75; 1.00 % Triglyceride ↓ 0.50; 0.75; 1.00 % AST ↓ 0.50; 0.75; 1.00 %
Green Tea Powder **GTP** Huang et al. 2013	1. Control , 2. GTP 0.50 mg /kg , 3. GTP 100 mg/kg 35→55 d	Abdominal fat % ↓0.50; 100 mg/kg	LDL -cholesterol ↓ 100 mg/kg Triglyceride ↓ 100 mg/kg

GTP, green tea powder; AST, aspartate aminotransferase; HDL-cholesterol , high-density lipoprotein-cholesterol; LDL-cholesterol, low-density lipoprotein-cholesterol;

1.4. Effect of supplementation with olive mill wastewater

Olive mill wastewater (OMWW) is a liquid effluent derived mainly from the water used for the various stages of olive oil production. It contains amounts to 0.5–3.25 m3 per 1000 kg of olives (Kapellakis, 2012). Moreover, OMWW has a dark brown color, high organic content, strong specific olive oil smell and acidic pH (3–6) (Paraskeva & Diamadopoulos, 2006). The phenolic compounds found in OMWW can be simple phenols or polyphenols resulting from polymerization of simple phenols and their concentration varies from 0.5 to 24 g/L (Frankel & Fernàndez-Gutérrez., 2013). The polyphenols identified in OMWW include phenolic acids such as p-coumaric acid, caffeic acid, protocatechuic acid, 4- vanillic acid and ferulic acid. The OMWW is waste of production which has a toxic effect for the aqua world and environment, but because of its constituents of phenolic compounds could represent an antioxidant source in feed, pharmaceutical and cosmetic industry, while reducing the environmental toxicity of OMWW.

Several authors have evaluated the effect of OMWW as an antioxidant in poultry feed and the result are summarized in the table 1.4.1.

Papadopoulou et al. (2017) examined the effect of polyphenolic powder from OMWW administrated through drinking water, and 3 groups of broilers were given drinking water containing 0.02 g/L and 0.05 g/L of polyphenols, for 45 days. The results showed that CARB and TBARS were significantly decreased by levels 44.7 and 33.8 % at days of 25 and 45, respectively ; TBARS 34.1 and 19.4 % at 25 and 45 days respectively, catalase CAT was increased significantly by 13.9 and 19.5% at 25 and 45 days, respectively.

Gerasopoulos et al. (2015) observed similar results with OMWW supplementation at 0.5 %, had significantly lower protein oxidation and lipid peroxidation levels in blood. The OMWW showed the highest decrease in protein oxidation levels by 52.6, 46.3 and 42.7% at 17, 2 and 37 days. TBARS was also decreased by 69.8, 69.0 and 54.0 % at 17, 3 and 37 days respectively. The author

showed also the antioxidant status in blood. CAT increased by levels of 16.8, 16.6 and 17.6 % at the three period of control in birds compared to the control group.

Tufarelli et al. (2015) compared the effect of supplementation with 2.5 % of extra-virgin olive oil (EVOO) in contrast to sunflower oil and lard for 49 days. Extra-virgin olive oil improved significantly ($p< 0.05$) the final body weight (FBW) compared to those supplemented with sunflower oil and lard. For the oxidative status, the supplementation with EVOO significantly ($p<0.05$) reduced the lipid oxidation by increasing the levels of SOD, CAT and GPx compared with sunflower oil and Lard.

OMWW improved the antioxidants capacity of broilers chicks and then, it would be used for developing high-added value products for animal feed supplementation, thus in turn, it is positive in order to reduce environmental (Papadopoulou et al. 2017).

The following **table 1.4.1**. summarized the different result of the the phenolic compounds inclusion in the diets of broilers.

Table 1.4.1. Effects of supplementary olive mil waste water (OMWW) as phenolic compound on oxidative status and performance of broilers

Polyphenols	Animal and diets	Results	
Sources	Treatment	**Performance**	**Blood parameter**
Olive Mill Waste Water **OMWW** Papadopoulou et al. 2017	1. Control ; 2. OMWW 0.02 g/L in water; 3. OMWW 0.05 g/L in water 1→21d ; 21→45d	No significance	CARB ↓ 0.02; 0.05 g/L TBARS ↓0.02; 0.05 g/L CAT ↑0.02; 0.05 g/L
Olive Mill Waste Water **OMWW** Gerasopoulos et al. 2015	1. Control; 2. OMWW/ 0.5 % 13→17d; 17→27d; 27→37d	No significance	CARB ↓ 0.5 % TBARS ↓ 0.5 % CAT ↑ 0.5 %
Extra-virgin olive oil **EVOO** Tufarelli et al. 2015	1. 2.5% SFO; 2. 2.5% LRD; 3. 2.5% EVOO 1→49d	FBW ↑ by EVOO vs SFO and LRD	SOD, CAT ↑ EVOO vs LRD GPx ↑ EVOO vs SFO

FBW, final body weight; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; TBARS, thiobarbituric acid reactive substances ; OMWW, olive mill wastewater ; CARB, protein carbonyl ; EVOO, extra vergin olive oil ; SFO, sunflower oil ; LRD, lard oil

So, because of all of these reasons, the many benefits that phenolic compounds could offer us, need to be taken into account in order to do more research intended for animal nutrition. In fact, the present study is behind this purpose.

2. HYPOTHESES AND OBJECTIVES

As we have seen in the state of the art, many authors described many benefits of the phenolic compounds as new natural source of antioxidants intended for animal nutrition. First, Ibrahim (2017), Elnaggar et al.(2018) and Papadopoulou et al.(2017), among others pointed out that phenolic compounds are good natural sources of antioxidants because of their efficiency on performance and oxidative status. Second, Liu (2017) and Huang et al.(2013) reported that phenolic compounds posses antioxidant characteristics, resulting in changes of lipid profile in blood.

Taking these point into account, we hypothesized that phenolic compounds can be introduced in broiler feeding achieving at least similar productive performance than obtained with conventional diets, and improving the oxidation status on broilers.

The global aim of this study is to evaluate the effect of the phenolic compounds in broiler chicken, as natural source of antioxidant.

The specific objectives are:

- To test the effect of the dietary supplementation of two different phenolic compounds (Product A and Product B) on broiler productive performance.
- To test the effect of dietary supplementation of two different phenolic compounds (Product A and Product B) on blood oxidative status and serum lipid profile in broilers.

3. MATERIAL AND METHODS

3.1. Animal and facilities

The study was carried out at the animal experimental facilities of the *Servei de Granges i Camps Experimentals (SGiCE), Universitat Autònoma de Barcelona (UAB; Barcelona, Spain)* (see **Figure 3.1.1).**

Figure 3.1.1. Seventeen days old female chicks.

All the animal experimentation procedures had been approved by the animal Ethics Committee of the UAB (CEEAH 4006) and were in compliance with the European Union guidelines for the care and use of animals in research (European Parliament, 2010).

Ninety-six female broiler chickens of Ross 308 strain (Pondex SAU; Lleida, Spain) were housed in the growing poultry unit, in particular, in 24 identical sized battery cages arranged in three floors, under identical controlled conditions **(Figure 3.1.2**). The cage was the experimental unit.

Figure 3.1.2. General overview of the facilities, where the study took place**.**

Figure 3.1.3. Fourteen days old broiler (Ross 308 stain) allocated in cage.

he birds were randomly allocated to 24 cages (4 animals per cage) and cages were randomly distributed in 4 treatments (6 cages per treatment). The birds were individually identified (by steel ring number on the wings).

The study was performed from 0 (hatch) until 38 days old. Throughout the study, feed and water were supplied *ad libitum*, and birds were raised under controlled conditions of light and temperature consistent with the specifications in the Ross 308 lineage management handbook (2018).

The temperature was maintained at 30 °C for the first 3 days and then reduced gradually to 22 °C. Air ventilation was arranged by automatic ventilator to manage the humidity. The lighting program provides 23 hours of light and 1 hour of dark for the first 2 days after placement and then reduced gradually to 14 hours of light since 10 days until the end. Light intensity was between 80-100 lux in the first week and decreased to 30-60 lux until the end.

3.2. Diets and experimental design

The feeding program was in two phases: Starter diet, from 0 to 21 days and grower-finisher diet (Grower-Finisher) from 22-38 days of age.

Basal diet was based in corn, wheat and soybean meal (meal form), and it was formulated to meet the requirements for maintenance and growth according to FEDNA 2018 and was manufactured in Pinsos Molinet factory (Gaià, Spain).

Animal were fed of the four experimental diets (6 replicate per treatment):

1. Negative control (C-): Basal diet with 3% of fish oil (high level in polyunsaturated fatty acids PUFA)
2. Positive control (C+): C- supplemented with 250 ppm of vitamin E (α-tocopherol acetate)
3. Diet A: C- supplemented with 1500 ppm of product A (high content of phenolic compounds).
4. Diet B: C- supplemented with 1500 ppm of product B (high content of phenolic compounds).

In the **Table 3.2.1**, it is shown the ingredients and nutrient composition of the experimental diets.

Table 3.2.1. Ingredients, EMA and nutrient composition of experimental diets.

Item	Starter diet (d 0 to 21)	Grower-finisher diet (d 22 to 38)
Ingredientes (%)		
Corn	40,01	39.98
Wheat	24.46	30.76
Soy-Bean 47% (PB)	28.97	22.90
Yellow pigment	0.10	0.10
Red pigment	0.04	0.04
L-Lysine CLH 79	0.34	0.28
DL-Metionine 99	0.31	0.23
Solid threonine	0.15	0.10
Solid Hill 60	0.03	0.04
Calcium carbonate	0.78	1.06
Bicalcium phosphate	1.14	0.69
Salt	0.35	0.29
Sodium bicarbonate	0.05	0.04
L-Valine	0.03	0.20
Fish oil	2.96	3.00
Mineral and vitamin premix[1]	0.3	0.3
Theoretical analysis		
ME (Kcal/kg)	2.950	3.120
Crude protein (%)	20.00	18.00
Lys (%)	1.25	1.05
Met (%)	0.64	0.54
Crude fat (%)	5.09	5.10
Crude fiber (%)	2.72	2.66
Ash(%)	4.81	4.36
Ca (%)	0.90	0.88
P (%)	0.56	0.46
Na (%)	0.20	0.10

[1] Provides per Kg of feed (as mg of diet): Vitamin A (1000) 9.6 mg; Vitamin D-3 (500) 3.6 mg; Vitamin E (alfa-tocpherol) 30 mg; Vitamin K-3 (MNB 43%) 2.4 mg; Vitamin B-2 4.8 mg; Vitamin B-12 (0.1%) 9.6 mg; Vitamin B6 1.2 mg; nicotinic acid 25.2 mg; folic acid 1.2 mg; Cu 32.4 mg; mg; Fe 82.2 mg; Mn 249.6 mg; Zn 51.6 mg; Chloride 583.2 mg; I 8.4 mg; Se 7.2 mg.

3.3. Controls and sampling

Individual broiler body weight (BW) and feed intake per cage were recorded at day of hatch, 21, and 38 days old of age. Average daily gain (ADG), average daily feed intake (ADFI) and the feed conversion ratio (FCR) were calculated along the whole experiment. Mortality was recorded to adjust ADFI and ADG.

At the end of the experiment, one bird per replicate was euthanized and 3 ml of whole blood were collected by the use of syringe from wing vein.

One ml of whole blood was collected into no-heparinized tubes (Lithium Heparin, REF 368494) to analyze oxidative status and, 2 ml were collected into heparinized tubes (AQUISEL® Z/SERUM) and immediately centrifuged (before one hour of extraction), and stored at -80ºC to analyze lipid peroxidation biomarkers and serum lipid profile.

In addition, 2 birds per cage (6 replicate/ treatment) were stunned, bled, plucked in a commercial slaughterhouse (GIMAVE SA; Ripollet, Spain) and carcasses were recovered for another study. Carcasses (total BW excluding blood and feathers) were weighed, and abdominal fat pad (AFP, from the proventriculus, surrounding the gizzard down to the cloaca) of each bird were removed and weighed in order to calculate carcass yield and AFP percentage.

3.4. Analytical determinations

Analytical determination of oxidative status and serum lipid profile in blood, were carried out by the *Servei Bioqumica Clínicq Veterinària (SBCV) from UAB (Barcelona, Spain).*

Oxidative status was measured by the changes in antioxidants enzyme activity in erythrocytes.

Superoxide dismutase (SOD) was determined according to the kit (Rx Monza) instructions from Randox Laboratories Ltd (Co. Antrim, United Kingdom). This

technique consist of xanthine oxidase (XOD) to generate superoxide radicals witch react with chloride (I.N.T) to form a red formazen dye. The SOD activity is then measured by the degree of inhibition of this reaction in blood. Samples are washed with 0.9% with NaCl solution, centrifuged at 3000 rpm for 10 minutes then diluted.

Glutathione peroxidase (GPx) was determined according to the method of Randhir and Shetty (2004). This technique consists of measuring the GPx by the reduced levels of catalysing the oxidation of glutathione (GSH) in whole blood.

Malondialdehydes (MDA) as lipid peroxidation biomarker in blood was determined according to the kit instructions from Cayman Chemical Company (Ann Arbor, MI, USA). From 2 ml placed into heparinized tube, 1 ml of plasma was taken and samples were measured calorimetrically and centrifuged at 1000 rpm for 10 minutes at 4ºC.

Total antioxidants status (TAS) was also measured in plasma (placed into heparinized tubes) by using kit manufactured by Randox Laboratories Ltd (Co. Antrim, United Kingdom).

Serum lipid profile was analysed by taken 1 ml of plasma from 2 ml placed into heparinized tube to analyse triglycerol, high-density lipoprotein-cholesterol (HDL-Cholesterol), low-density lipoprotein-cholesterol (LDL-Cholesterol), and total cholesterol.

3.5. Statistical analysis

Statistical analyses were conducted using R software (version 3.4.2) for Animal Sciences. With this software, all values were analyzed by using the one-way analysis of variance (ANOVA) by the General Lineal Model (GLM) procedure. Tukey's multiple range test was performed to determine whether means were significantly different

Normality and equal variances were checked in all continuous variables classified by dietary treatment respectively.

Initial and final body weight; ADFI and ADG variables had a normal distribution and presented equal variances between groups.

The general model for the one way-analysis of variance for the fixed effects is:

$$y_{ij} = \mu + T_i + \varepsilon_{ij}$$

Where y_{ij} is the observed dependent variable; μ is the overall mean; T_i is the fixed effect of group or treatment (type of diet in our case) and finally ε_{ij} is the random error.

Performance results, oxidative status and serum lipid profile in blood were analyzed using every cage as replicate (6 replicates per treatment, except BW). On the other hand, abdominal fat pad was analyzed using two birds from each replicate (the experimental unit was the bird, 12 chicks per treatment).

4. RESULTS AND DISCUSSION

4.1. Performance parameters

The effect of different dietary phenolic compounds on growth performance in the starter (from 0 to 21), the grower-finisher (from 22 to 38) and the global (from 0 to 38) periods, and abdominal fat deposition are presented in **Table 4.1.1.**

Table 4.1.1. Growth performance and carcass fat depot of broilers chickens according to different treatment diet [1]

	Dietary Treatment [2]				Statistics	
Item	**C -**	**C +**	**A**	**B**	**RSE**	**P-value**
From 0 to 21 d						
BW at 0 d (g)	38.5	38.5	38.4	38.5	0.17	0.985
BW at 21 d (g)	765	816	798	721	95.2	0.350
ADFI (g/d/bird)	51.3	54.0	51.0	48.3	3.54	0.134
ADG (g/d/bird)	34.6	37.0	36.3	33.9	4.37	0.609
FCR (g/g)	1.40	1.38	1.35	1.42	0.16	0.903
From 22 to 38 d						
BW at 38 d (g)	2043	2185	2140	2019	127.7	0.216
ADFI (g/d/bird)	128	133	132	130	6.05	0.470
ADG (g/d/bird)	73.7	76.5	76.6	75.3	2.71	0.286
FCR (g/g)	1.74	1.74	1.75	1.73	0.05	0.921
From 0 to 38 d						
ADFI (g/d/bird)	85.6	87.8	87.6	83.0	4.70	0.360
ADG (g/d/bird)	52.6	56.4	54.5	52.0	3.29	0.151
FCR (g/g)	1.60	1.59	1.57	1.56	0.04	0.432
Carcass weight (g)	1871	1881	1932	1843	153.6	0.599
Abdominal fat depot						
g	38.6	36.0	40.7	43.3	9.69	0.367
(%)[3]	1.89	1.78	1.90	2.14	0.43	0.263

[1] C - = fish oil at 3%; C + = fish oil at 3% and α-tocopherol at 250 ppm; Diet A= fish oil at 3% and product A at 1500 ppm; Diet B= fish oil at 3% and product B at 1500 ppm.[2] Value are means of 6 replicate with 4 chickens/replicate from 0 to 21 d and 3 chickens/replicate from 22 to 28 d. [3] As percentage of live weight of animal before slaughter. In the case of carcass weight and abdominal fat depot, values are means of 12 chickens for each treatment. BW = body weight; ADFI = average daily feed intake; ADG = average daily gain; FCR = feed conversion ratio; RSE = residual standard error. Statistical signification was declared at a probability of $p \leq 0.05$ and tendency was defined as $p \leq 0.15$

The incorporation of Product A and Product B, did not affect any performance parameter in any phase, neither the global period ($P > 0.05$), compared with two controls (negative and positive) diets.

Many references exist in literature in relation to the use of phenolic compounds in chicken feed. Our findings are in agreement with Chamorro et al. (2013). They reported that dietary supplementation levels of phenolic compounds up to 2500 ppm did not affect chick performance. The supplementation level of phenolic compounds in the current study at 1500 ppm might be considered low to affect positively in growth performance, and the variation in response to inclusion of phenolic compounds could be attributed to the difference in supplementation levels. Nevertheless, Wang et al. (2010) observed that, supplementation level at 80 ppm of phenolic compounds had positive results in the growth performance.

Previous data regarding supplementation at 1500 ppm on performance parameters are scarce. Results (**Table 4.1.1)** show that the supplementation of Product A and Product B at 1500 ppm does not negatively affect broiler chicken performance ($P > 0.05$). Nevertheless, different studies showed controversial results concerning the inclusion of phenolic compounds. Viveros et al. (2011), and Chamorro et al. (2013) reported a negative influence on growth performance efficiency due to high dosage of phenolic compounds at 7200 ppm and 30000 ppm, respectively.

However, high level of phenolic compounds could interact with protein and form tannin-protein complexes that decrease efficiency of nutrient utilization and depress growth performance (Brenes et al. 2008). Consequently, the supplementation at 1500 ppm of phenolic compounds in our study might be low to produce such growth depression.

Our results are in the line with the study performed by Chamorro et al.(2013), using the same level of dietary supplementation of grape seed extract at 1500 ppm with low proportion of gallic acid (phenolic acid) at 29.6 g/100 g of dry matter. Also, we agree with Brenes et al. (2010) who observed that the supplementation of grape seed extract at the previous level with the proportion of gallic acid at 45.5 g/100 g of dry matter did not affect the growth performance. Nevertheless, Ibrahim (2017)

reported that the supplementation of grape seed with proportion of gallic acid at 55.7 g/100 g of dry matter affect positively the growth performance. These differences in response due to the different proportions in dry matter may explain the importance to characterize the phenolic compounds profile and proportion of different tested products in our experiment.

For abdominal fat deposit, our results are similar than those obtained by Brenes et al. (2010) using GSE at 1800 ppm as supplement for the chickens, but differ from Ibrahim (2017), in which the abdominal fat deposit was significantly lower in birds fed on phenolic compounds at 40000 ppm.

4.2. Plasma lipid profile and enzymatic antioxidant activity

The effect of different dietary phenolic compounds on serum lipid profile and antioxidant status are presented in the **Table 4.2.1**

Table 4.2.1. Plasma parameters and enzymatic antioxidant activity of broilers chickens according to different treatments.

	Dietary Treatment[1]				Statistical	
Item	**C -**	**C +**	**A**	**B**	**RSE**	**P-value**
Triglyceride (mg/dl)	72.5	61.2	73.0	65.6	26.4	0.841
Cholesterol (mg/dl)	103	100	96	106	17.55	0.863
HDL-cholesterol (mmol/L)	1.85	1.90	1.88	1.97	0.268	0.869
LDL-cholesterol(mmol/L)	0.94	0.90	0.90	0.90	0.230	0.992
Enzymatic antioxidant activity						
GPx (U/L)	41436.2	42860.8	43240.2	39740.3	72.58	0.834
SOD (U/mL)	117	140	129	133	19.74	0.259
TAS (mmol/L)	1.33	1.34	1.31	1.33	0.186	0.995
MDA (μM)	18.2	17.4	18.6	18.4	2.018	0.751

[1] C - = fish oil at 3%; C + = fish oil at 3% and α-tocopherol at 250 ppm; Diet A= fish oil at 3% and product A at 1500 ppm; Diet B= fish oil at 3% and product B at 1500 ppm. GPx = glutathione peroxidase; SOD = superoxide dismutase; TAS = total antioxidant activity; MDA = malondialdehydes; HDL-cholesterol = high-density lipoprotein-cholesterol; LDL-cholesterol = low-density lipoprotein-cholesterol; RSE = residual standard error. Statistical significance was declared at a probability of $p \leq 0.05$ and tendency was defined as $p \leq 0.15$

The incorporation of Product A and Product B, did not affect any serum lipid parameter, neither the enzymatic antioxidant activity ($P > 0.05$), compared with two control (negative and positive) diets.

Our results for the serum lipid profile are similar of those obtained by Chamorro et al.,(2013), using grape seed extract (29.6 % extractable phenolic compound) at levels ranged from 25 to 5000 ppm during 32 days old broilers. In

addition, Farahat et al.(2016) observed that supplementation of phenolic compound during the 21 first days old broilers does not affect serum lipid profile, but it had significant difference in triglyceride and total cholesterol from 21 to 42 days old broilers. In our study, the 38 days feeding period may not be long enough for tested products to exert effects on the measured serum lipid profile. Nevertheless, Papadopoulou et al.(2017) observed that supplementation during the first 21 days old broilers significantly decreased the plasma cholesterol and triglyceride. These differences in the later studies may explain the importance of period applied in studies to exert the positive effect on serum lipid profile.

Regarding the effect of phenolic compounds on oxidative status, it is widely observed that the dietary phenolic compounds modify the enzymatic antioxidant activity and blood oxidation biomarker. Several authors demonstrate the increasing of SOD, GPx and TAS at the same time the decreasing of MDA, as positive effect on oxidative status in meat compositions, as compared to antioxidant effect on plasma.

Our findings are in the line with the study performed by Vossen et al. (2011), which observed that the oxidative status and lipid oxidation of plasma in broilers was not affected by fedding phenolic compound extracts at doses of 100 and 200 ppm. Previous data obtained from Brenes et al. (2010), Starčević et al. (2014) revealed that phenolic compounds present in grape seed improve sensory attributes of meat and signaficantly decreased MDA concentration in the breast muscles with no significance in plasma of broiler chickens. This situation may explain the possibility of tested products used in the current study, to affect positively in oxidative status on chickens meat, while antioxidative biomarkers in plasma were not affected.

Finally, we agree with Voljc, et al. (2013) wich observed that the supplementation of diet rich in polyunsaturated fatty acids with vitamine E at 200 IU/kg, or only the addition of 3 g/kg of a product contaning wood extract (phenolic compounds), were not sufficient to improve the antioxidant status. In cotrast, Kamboh and Zhu (2013) revealed that the combination of hesperdin and genistein (phenolic compounds) exert synergistic effects and improve the anioxidant status in plasma in comparation with those supplemented by vitamin E only. This differences may explain the importance of combination between phenolic compounds contained in the

tested products to deliver a synergitic effects which depend on phenolic compound used.

5. CONCLUSIONS

According to the result presented in this disseratation, the following conclusions can be drawn:

1. In our working conditions, the use of Product A and Product B at 1500 ppm did not modify growth performance,neither lipid profile and oxidation degree in blood.

2. Further studies are needed to evaluate the efficacy of these products at different levels of incorporation, as well as the concentration and combination of these active ingredients.

6. REFERENCES

Aditya, S., Sang, J., Musabbir, A., and Jayant L. (2018). Supplementation of grape pomace (Vitis Vinifera) in broiler diets and its effect on growth performance, apparent total tract digestibility of nutrients, blood profile, and meat quality. Animal Nutrition. 4(2), 210–14.

Barnes, S., D'Alesandro, T., Kirk, M., Patel, R., Boersma, B., and Darley-Usma, V. (2003). The importance of in vivo metabolism of polyphenols and their biological actions. Phytochemicals mechanism of action. Boca raton CRC Press. 51–59.

Balasundram, N. (2006). Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. Food chemistry. 99, 191–203.

Bravo, L. (1998). Polyphenols, chemistry, dieatry sources metabolism and nutritional significance. Nutrition Reviews. 56, 317–333.

Brenes, A., Viveros, A., Goñi, I., Centeno, C., Sayago-Ayerdy, S.G., and Arija, I. (2008). Effect of grape pomace concentrate and vitamin E on digestibility of polyphenols and antioxidant activity in chickens. Poultry Science. 87(2), 307–16.

Brenes, A., Viveros, A., Goñi, I., Centeno, C., and Arija, I. (2010). Effect of grape seed extract on growth performance, protein and polyphenol digestibilities, and antioxidant activity in chickens. Spanish Journal of Agricultural Research. 8(2), 326-333.

Brit, D., and Wang, W. (2001). Dietary agents in cancer prevention: Flavonoids and isoflavonoids. Pharmacology and Therapeutics. 90(2-3),157-177.

Chamorro, S,. Viveros, A., Centeno, C., Romero, C., and Brenes, A. (2013). Effects of dietary grape seed extract on growth performance, amino acid digestibility and plasma lipids and mineral content in broiler chicks. The Animal Consortium. 74, 555–561.

Degroot, H. (1994). Reactive oxygen species in tissue injury. Hepato-gastroenterology. 41(4), 328-332.

Ebrahimi, A., and Schluesener, H. (2012). Natural polyphenols against neurodegenera-tive disorders: potentials and pitfalls. Ageing Research Reviews. 11, 329–345.

Elnaggar, A., and Abdelkhalek, E., and El-Kelawy, M.I. (2018). Productive performance, blood parameters, and immune response of broiler chickens supplemented with grape seed and medicago sativa as natural sources of polyphenols. Journal of Egyptian Poultry Science. vol 38, 269-288.

Farahat, M., Abdallah, M., Ali, H.A., and Hernandez-Santana, A. (2016). Effect of supplementing broiler chicken diets with green tea extract on the growth performance , lipid profile , antioxidant status and immune response. The animal consortium. 87, 1-7.

Frankel, E., and Fernández-Gutiérrez, A. (2013). Literature review on production process to obtain extra virgin olive oil enriched in bioactive compounds. Potential use of by-products as alternative sources of polyphenols. J. Agric. Food Chem. 61 (22), 5179–5188.

Gerasopoulos, K., Stagos, D., Kokkas, S. (2015). Feed supplemented with by-products from olive oil mill waste water processing increases antioxidant capacity in broiler chickens. Food and Chemical Toxicology. 62, 4–11.

Hermann, K. (1993). Flavonoid antioxidants in food of plant origin. Gordian. 93(7–8),108-111.

Huang, J., Yong, Z., Yibin, Z., Zhengzhu, Z., and Jinsong, W. (2013). Green tea polyphenols alleviate obesity in broiler chickens through the regulation of lipid-metabolism-related genes and transcription factor expression. Journal of Agricultural and Food Chemistry. 61, 8565−8572.

Ibrahim, S A. (2017). Effect of dietary polyphenol rich grape seed on growth performance, antioxidant capacity and ileal microflora in broiler chicks. J. Anim. Physiol. Anim. Nut. 42, 1–8.

Kalam, S., Singh, R., Mani, A., Patel, J., Khan, F.N., and Pandey, A. (2012). Antioxidants: elixir of life. Int. Multidiscip. Res. J. 2, 18-34.

Kamboh, A., and Zhu, W. (2013). Effect of increasing levels of bioflavonoids in broiler feed on plasma anti-oxidative potential, lipid metabolites, and fatty acid composition of meat. Poultry. Science. 92(2), 454–61.

Kapellakis, I. P. (2012). Treatment of olive mill wastewater with constructed wetlands. Water. South African Journal of Animal Science. 4, 260–271.

King, A., and Young, G. (1999). Characteristics and occurence of phenolics phytochemicals. Journal of American Dietetic Association 99(2) 213-218.

Landete, J. (2013). Dietary intake of natural antioxidants : vitamins and polyphenols. Crit. Rev. Food. Sci. Nutr. 53, 706-721.

Leopoldini, M., Marino, T., and Toscano, M. (2011). Antioxidant properties of phenolic compounds : H-atom versus electron transfer mechanism. The Journal of the Physical Chemistry. 108(22), 4916–4922.

Liu, W., Fariba, R., Alireza, S. (2017). Effect of amount and duration of waste green tea powder on the growth performance, carcass characteristics, blood parameters, and lipid metabolites of growing broilers. Environ Sci Pollut Res. 32, 1-13.

McCullough, M., Peterson, J., Patel, R., Jacques, P., Shah, R., and Dwyer,J. (2012). Flavonoid and cardiovascular disease mortality in prospective cohort of US adults. Americain journal of clinical nutrition. 95, 454-464.

Papadopoulou, A., Konstantinos, P., Dimitrios, S., Antonio, M., and Sotiria, M. (2017). Enhancement of antioxidant mechanisms and reduction of oxidative stress in chickens after the administration of drinking water enriched with polyphenolic powder from olive mill waste waters. Oxidative Medcine and Cellular Longevity. 57, 1-10.

Prochàzkovà, D., Bousovà, I., Wilhelmovà, N. (2011). Antioxidant and prooxidant properties of flavonoids. Fitoterapia. 82, 513-523.

Paraskeva, P., and Diamadopoulos, E. (2006). Technologies for olive mill wastewater (OMW) tratment. a review. J. Chem. Technol. Biotechnol. 81, 475-1485.

Randhir, R., and Shetty, K. (2004). Phenolics, their antioxidant and antimicrobial activity in dark germinated fenugreek sprouts in response to peptide and phytochemical elicitorrs. Asia Pacific Journal of Clinical Nutrition. 13, 295–307.

Robbins, R. J. (2003). Phenolic acids in foods: An overview of analytical methodology. Journal of Agricultural and Food Chemistry. 51(10), 2866–2887.

Surai, P.F. (2014). Polyphenol compounds in the chickens animal diet: from the past to the future. J. Anim. Physio. An. N. 98, 19-31.

Starčević, Ke., Krstulovic, L., Sttojevic, Z., and Mikulec, Z. (2014). Production performance, meat composition and oxidative susceptibility in broiler chicken fed with different phenolic compounds. Journal of the Science of Food and Agriculture. 95(6), 1172–1178.

Tufarelli, V., Vito, L., and Elisabetta C. (2015). An extra-virgin olive oil rich in polyphenolic compounds has antioxidant effects in meat-type broiler chickens. Environ Sci Pollut Res. 29, 52-58.

Viveros, A., Chammoro, S., Pizarro, M., Arija, I., Centeno, C., and Brenes, A. (2011). Effects of dietary polyphenol-Rich grape products on intestinal microflora and gut morphology in broiler chicks. Poultry Science. 90, 566–578.

Vossen, E., Ntawubiz,i M., Raes, K., Smet, K., Huyghebaert, G., Arnouts, S., and De Smet S. (2011). Effect of dietary antioxidant supplementation on the oxidative status of plasma in broilers. Journal of Animal Physiology and Animal Nutrition. 95, 198–205.

Volic, M., Levart, A., Zugur, S., and Salobir, J. (2013). The effect of alpha-tocopherol, sweet chestnut wood extract and their combination on oxidative stress in vivo and oxidative stability of meat in broilers .Brit. Poultry. Sci. 54, 144–156.

Wang, Y., Yang, X., Wang, L., Cui, M., Cai, Y., Li, X., and Wu, Y. (2010). Effects of proanthocyanidins from grape seed on treatment of recurrent ulcerative colitis in rats. Canadian Journal of Physiology and Pharmacology. 88, 888–898.

Wójciak, K., & Dolatowski, Z. (2012). Oxidative stability of fermented meat products. Acta Scientiarum Polonorum. Technologia Alimentaria. 11(2), 99-109.