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Review

Recent Advances of Using Polyphenols to Extenuate Oxidative Stress in Animal Production: Evidence from Poultry

Nguyen Hoang QUI [1,a (*)] Nguyen Thi Anh THU [1,b]

[1] Tra Vinh University, School of Agriculture and Aquaculture, Department of Animal Science and Veterinary Medicine, 94000, VIETNAM

ORCIDs: a 0000-0002-3499-6669; b 0000-0002-6778-3631

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Abstract: Oxidative stress has become a very challenging issue to animal production due to its disturbance to animal growth. Under heat stress conditions, the ability of poultry animals to combat oxidation stress is reduced; oxidative stress minimizes the concentration of globulins in plasma, thus lowering poultry's immune status. Many researches have been conducted to solve this problem, and one of the safest solutions is the use of Polyphenols. Polyphenols are widely known as exogenous antioxidants which act as one of the cell's first lines of defence. Thus, the findings on the intracellular antioxidant activity of polyphenol as a plant-based feed supplement, has greatly helped improve poultry antioxidant activity due to the beneficial effects of polyphenols in preventing damage from oxidative stress and removing excessively produced free radicals. This review aims to synthesize information regarding the antioxidant activity of polyphenols and their influence on poultry antioxidant status.

Keywords: *Antioxidant, Monogastric, Oxidative stress, Polyphenols*

Hayvansal Üretimde Oksidatif Stresi Azaltmak İçin Polifenollerin Kullanımında Son Gelişmeler: Kanatlı Hayvan Örneği

Öz: Oksidatif stres, hayvanlarda büyümeyi engellemesi nedeniyle hayvansal üretim için çok problemli bir konu haline gelmiştir. Isı stresi koşulları altında, kanatlıların oksidasyon stresiyle mücadele etme yeteneği azalır; oksidatif stres, plazmadaki globulin konsantrasyonunu en aza indirerek kanatlı hayvanların bağışıklığını azaltır. Bu sorunun çözümü için birçok araştırma yapılmıştır ve en güvenli çözümlerden biri Polifenollerin kullanılması olmuştur. Polifenoller, hücrenin ilk savunma hatlarından biri olarak işlev gören eksojen antioksidanlar olarak bilinir. Bu nedenle, bitki bazlı bir yem takviyesi olarak polifenolün hücre içi antioksidan aktivitesine ilişkin bulgular, polifenollerin oksidatif stresten kaynaklanan hasarı önlemedeki ve aşırı üretilen serbest radikalleri gidermedeki faydalı etkilerinden dolayı kanatlıların antioksidan aktivitesinin geliştirilmesine büyük ölçüde yardımcı olmuştur. Bu derleme, polifenollerin antioksidan aktivitesi ve kanatlıların antioksidan durumu üzerindeki etkileri ile ilgili bilgileri sentezlemeyi amaçlamaktadır.

Anahtar sözcükler: *Antioksidan, Monogastrik, Oksidatif stress, Polifenoller*

Introduction

Due to public health concerns, many countries strictly regulate or even ban to decelerate antibiotic resistance in animals. Therefore, scientists are seeking for alternatives to solve this issue, and the prime alternatives are phytogenic chemicals that are found to be potent feed additives and, too, possess clinical effects [1]. Phytochemicals distribute benefits to, both, animal health and performance, especially, polyphenols that are produced by secondary metabolites [2,3].

Polyphenols, also known as phenolic compounds, is a class of chemical present in various plant species. They are distinguished by the presence of one or more aromatic rings and the presence of one or more than two hydroxyl groups. There are three main groups of polyphenols: Flavonoids, Non-Flavonoids and Tannin, and their biological functions mainly rely on chemical structures [4]. More than 8000 polyphenols have been identified and believed to exhibit immunomodulatory, anti-microbial, anti-inflammatory, antiallergic, antimutagenic, and detoxifying properties which benefit to animals and

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(*) Corresponding Author
Tel: +84 334 933577 Cellular Phone: +84 334 933577
E-mail: nhqui@tvu.edu.vn (N.H. Qui)

humans [2,3,5]. Additionally, polyphenols are potent in combating heat stress through its antioxidant activities since the consequence of heat stress partly causes the oxidative stress in animals. Lykkesfeldt and Svendsen [6] and Chauhan et al.[7] defined oxidative stress occurs once there is an imbalance between Reactive Oxygen Species (ROS) and antioxidant. Administration of antioxidant compounds is necessary to prevent regeneration of free radicals, and polyphenols have been accounted as prominent compounds to take the action. Reactive oxygen species must be removed by administering antioxidant compounds to prevent the further generation of other free radicals, which eventually causes oxidative stress in animals, including poultry. Moreover, some studies proved the positive results of polyphenols via *in vivo* experiment which indicates their potential as natural antioxidants [8-10].

As mentioned previously, this review tries to identify the capability of polyphenols in combating heat stress and oxidative stress of mono-gastric animals, more specifically the poultry animals. Therefore, the objective of this review is to identify the capability of polyphenol compounds to extenuate oxidative stress in poultry which includes modes of action of those compounds in preventing and combating oxidative stress.

Polyphenol's Sources and Modes of Action

Flavonoids are the most abundant polyphenol class, which accounts around 50% of polyphenol class, that have more than 4000 compounds having been identified to date. They have a popular structure consisting of two benzene rings associated with three carbon atoms, resulting in an oxygenated heterocycle. Their role is to protect against free radical damage through the following modes of action: the direct scavenging of ROS occurring in different processes of the body, the activation of antioxidant enzymes, inhibition of oxidases, enhancing antioxidant properties of low molecular antioxidants and mitigation of oxidative stress caused by reduction of α-tocopherol radicals, nitric oxide, metal chelating activity, growth in uric acid levels [4,11]. Flavonoids only occur in plants by biosynthesis via phenylpropanoid pathway, converting phenylalanine into 4-coumaroyl-CoA, which enters the flavonoid biosynthesis pathway. The first enzyme, chalcone synthase, produces chalcone scaffolds from which all flavonoids derive. Flavonoid skeleton modification highly depends on the species, enzymes, such as isomerases, reductases, hydroxylase, and several Fe^{2+}/2-oxoglutarate-dependent dioxygenases which leads to different flavonoid subclasses in plants, and transferases modify the flavonoid backbone with sugars, methyl groups and/or acyl moieties, regulating the physiological activity of the resulting flavonoid by altering their solubility, reactivity and interaction with cellular targets [12]. There are 3 main representative substances to take the action: Flavonols, Procyanidins and Anthocyanins. The functions of Flavonols are to form intramolecular hydrogen bonds by reacting with free radical, improve the activity of antioxidant enzymes such as Glutathione peroxidase (GSH-Px) and Superoxide dismutase (SOD), and increase the mitochondrial membrane potential and decreasing the oxidative damage level of mitochondria. Procyanidins provide hydrogen atoms to react with free radicals chelate with metal ions, downregulate stress-activated Mitogen-activated protein kinase (MAPK) pathway activity, promote Extracellular signal-regulated kinases (ERK) phosphorylation and upregulation the expression of anti-oxidant genes Nuclear factor erythroid 2-related factor 2 (Nrf2) and Heme oxygenase-1 (HO^{-1}), protect Adrenal phaeochromocytoma (PC12) from Hydrogen peroxide (H_2O_2), inhibit lipid peroxidation activity and slow down the enzymatic oxidation of fat. Moreover, Anthocyanins' functions are to regulate oxidase activity by activate Nrf2 and inhibit Nuclear factor kappaB (NF-kB) signaling pathway, chelate with metal ions, inhibit Peroxynitrite (ONOO-) by destroying mitochondrial apoptosis pathway and inhibit Bcl-2-associated X protein (Bax) nuclear translocation [13].

As demonstrated by Molina et al.[14], who performed an experiment on ethanol-induced oxidative stress in mouse liver, pre-treatment of flavonoid may protect oxidative stress by directly quenching lipid peroxides and indirectly by enhancing the production of the endogenous anti-oxidant glutathione (GSH). Moreover, flavonoids exert protective antioxidant effects on bovine mammary cells, according to Perruchot et al.[15], and the finding suggests that flavonoid could be used to prevent oxidative meta-bolic disorders, too. Evidently, flavonoids can be future alternatives of antioxidants in animal production too.

Beside flavonoids, non-flavonoid polyphenol groups are identified as any substance consisting of a benzene ring with one phenol or polyphenol hydroxyl groups with more than one benzene ring such as methyl esters, esters, and other similar compounds which include lignans, stilbenes and phenolic acids [4]. Typically, phenolic acids are divided into hydroxybenzoic and hydroxycinnamic acids. The position and number of methoxy and hydroxyl groups attached to the aromatic ring define the structure of phenolic acids, and they contribute to their antioxidant properties. Phenolic acids are synthesized from aromatic amino acids produced via the shikimate pathway. The shikimic acid is transformed into L-phenylalanine through a chorismic acid intermediate, and then the L-phenyl-alanine is turned into p-oumaric, salicylic, and p-hydroxy-benzoic acids which serve as precursors for other derivatives of phenolic acids [16]. Chlorogenic acid and gallic acid are the representative substances of phenolic acids. While chlorogenic acid supplies hydrogen atoms to free radicals, chelates with metal ions, prevent NH_2Cl-induced plasmid DNA fragmentation, reduces the expression of Forkhead box O (FOXO) family genes, activates Nrf2 transcription and upregulates the expression of cellular antioxidant enzymes, gallic acid reduces the accumulation of malonaldehyde and nitric oxide (NO^-) to enhance to activities of antioxidant enzymes and clears radicals produced by the Fenton reaction [13]. According to Zhang et al.[17], chlorogenic acid can effectively improve growth performance and enhance antioxidant capacity in chickens challenged with Clostridium perfringens type A. Additionally, Zhao et al.[18] proved that gallic acid supplementation on high weaning weight pigs enhanced plasma antioxidant capacity. Therefore, non-flavonoid polyphenol groups, too, possess high antioxidant properties to improve farm animal health.

Tannins, one of the three main groups of polyphenols, are water-soluble phenolic compounds found in various plant foods, also known as tannic acid, including tea and coffee [4]. Tannins are classified into two groups: hydrolysable and condensed tannins. Physiochemical properties and biogenesis are the indicators of this classification. Hydrolysable tannins result from binding to sugar fragments (mainly to the D-glucose moiety) of gallic, meta-digallic or hexahydroxy diphenic acid residues. Shikimic or dehydroshikimic acids are the precursors of these phenol carboxylic acids. On another hand, condensed tannins are created by oxidative condensation of flavonoids (mostly flavan-3,4-diol monomers, catechins, stilbetes and dihydrochalcones). The precursors of condensed tannins are malonyl-CoA and para-hydroxycinnamoyl-CoA. While condensed tannins are usually stored in heartwood and bark, hydrolysable tannins are stored in leaves, fruit pods and galls [19]. Tannins exert antioxidant effects such as inhibition of lipid peroxidation, scavenging of oxygen radicals, binding and inactivation of pro-oxidative medallions (Fe and Cu), and binding of proteins with suppression of their enzymatic activity (protease inhibition) [20]. Ebrahim et al.[21] suggested that tannins could be potentially applied as a biological antioxidant for poultry nutrition on hot climate condition. Moreover, hydrolysable tannins could be a replacement of Zinc Oxide to reduce diarrhea and improve antioxidant capacity of weaned piglets, too [22]. For that reason, tannins can be used as antioxidant which can be beneficial to animal and human health.

In general, polyphenols protect cells from the free-radical production. Exogenous antioxidants, specifically polyphenols, act as the defenders in the first line of cells combating excessive free radical production, shielding their constituents from oxidation damage [3]. However, polyphenol compounds are not able to be absorbed in its natural forms such as glycosides and polymers, and they have to be hydrolyzed in the host intestine [23]. Some flavonoids can be absorbed at gastric level, as well. The further study and understanding of the role of polyphenol's class in animal diets will be worthwhile.

To sum up, the antioxidant polyphenols have the ability to act on radical scavengers in various ways. Sandoval-Acua et al.[24] defined two ways to scavenge ROS: indirect activity and direct activity of scavenging ROS by inducting the synthesis of reactive oxygen species-removing enzymes. Polyphenols exhibit the indirect activity of antioxidants by activating Nrf2 to reduce oxidative stress [25]. Overall, Polyphenols protect cells against the free radical generation through the following modes of action [2]:

(1) Inhibiting the activities of pro-oxidant enzymes

(2) Activating the enzymes of antioxidant

(3) Scavenging ROS directly through an electron donor participation

(4) Chelation of transition metals which regulates formation of reactive hydroxyl radicals

(5) α-tocopherol radicals' reduction

(6) Alleviation of nitric oxide oxidative stress

(7) Prevents oxidation of low molecular antioxidants such tocopherols and ascorbate which boosts its antioxidant activities.

However, the effects of polyphenol's mechanism on oxidative stress can be different due to the difference in gastrointestinal absorption of various polyphenols, as well as their related interactions with the glucuronide, sulphate, or methyl groups of amino acids, and the type of the circulating metabolites in the blood, which are categorized following polyphenols' chemical forms [3]. Therefore, further studies are required to have proper usage on polyphenol compounds.

Polyphenols in Combating Oxidative Stress

Oxidative Stress

As a result of a disruption in the equilibrium between the accumulation of ROS and the body's antioxidants, cells and tissues are damaged. This results in oxidative stress and a precursor for the entry of various diseases. The reactive species are primarily responsible for generating oxidative stress that could be separated into three categories: free radicals, nonradicals, and redox-active transition metal ions [26]. Free radicals are considered unstable because they contain one or more than two unpaired electrons. In essence, its primary objective is to achieve stability. Therefore, these molecules either transfer the unpaired electrons to others or simply accept an electron in order to achieve a stable formation [27]. Furthermore, when reactive species are created in excess, stress from oxidation emerges and the systems of antioxidant defense in animals might be overwhelmed while antioxidant enzymes including SOD, catalase, GSH-Px diminishes its activity [28]. Lipiński et al.[2] found that under an oxidative stress environment, the body of animals is unable to effectively counteract the excessively generated free radicals, consisting of reactive nitrogen species (RNS) and/or ROS, which further results to detrimental effects to chromosomes. Additionally, it also modifies the encoded amino acids and consequently many other biological processes [2].

In order to prevent the generation of free radicals, which are known to cause some redox-related diseases and oxidative stress, antioxidant compounds should be administered to remove those [4]. According to Procházková et al.[11], dietary polyphenol's extensive biotransformation such as dietary flavonoids, may modify its bioactive forms in the small intestine and liver, thus affecting their antioxidant activity. In another trial, the diets with higher amounts of polyphenols boosted SOD activity and GSH-Px activity in the blood of poultry species or enhanced the quantities of vitamin E in the plasma of poultry species [29]. The addition of polyphenols to the diet can dramatically boost the activities of GSH-Px, catalase, and SOD in the serum of broiler. Under heat stressed conditions, adding polyphenols to broiler diets boosted antioxidant enzyme activity in broiler serum, such as GSH and SOD, and improved the health status of broilers. Polyphenols supplemented *in vivo* and *in vitro*, at a dose of 0.2 g/litter in water could ameliorate pathological damage and downregulate creatine kinase, lactate dehydrogenase, and creatine kinase-MB isoenzyme levels in cardiomyocytes caused by heat stress [8].

Oxidative Stress by Heat Stress

One of the most common sources of stress affecting animal performance is heat stress, which occurs when temperatures rise above a certain threshold [30]. Heat stress is partly affected by the temperature of the environment, humidity, ventilation, and the quantity of heat exchange due to the density of animals at one place [31]. Recent researches have repeatedly demonstrated the importance of dietary antioxidants in increasing the performance of broilers raised in high temperatures [32]. Under high environmental temperature settings, the activity of diverse plant extracts containing polyphenols showed great results in stabilizing poultry health. Furthermore, stress caused by heat is a significant barrier in poultry industry and a growing concern for many experts studying food safety and global warming [30].

An increase in cellular energy requirement appears to be the initial step in the pathophysiology of heat stress [33]. Yang et al.[34] found that the energy expenditure of cells increases by 2-fold after being exposed to acute heat stress. After 6 h of acute heat stress, mitochondrial transportation as well as β-oxidation of fatty acids were increased, and this was established in the study of Mujahid et al.[35]. To meet the increased energy requirement of the cells for its mitochondrial biogenesis, the production of the enzymatic activity and reducing equivalents of subunits of respiratory chain complexes are enhanced. Furthermore, electron transport is intimately linked to electron transport-linked phosphorylation (oxidative phosphorylation) under normal physiological conditions. Electron transport chain and mitochondrial substrate oxidation activity increases during the early stages of heat stress, thereby resulting in excess superoxide. Down-regulation of animal uncoupling protein increases the stress from oxidation, leading to tissue damage and mitochondrial dysfunction during the later periods of heat stress. The activities of antioxidant enzymes are usually increased. Additionally, acute heat stress causes oxidative capability of mitochondrial metabolism to be reduced, uncoupling protein to be upregulated, the activity pattern of antioxidant enzymes to be altered and stores of antioxidants to be depleted [36]. Furthermore, poultry are unable to sweat, and their body feathers restrict their capacity to expel heat into the intermediate environment greatly [36]. Heat stress is known to inflict severe damage to organisms, as mentioned in all the previous studies above. Physiological and biological changes, such as the development of stress hormones, increased free radical generation, and poorer antioxidant status, as well as diminished resistance and disturbance of homeostasis, are all possible [36].

The Role of Polyphenols in Oxidative Stress Reaction

It has been demonstrated that the application of polyphenol-enriched diets increases the meat's oxidative stability by decreasing the oxidative processes that contribute to meat lipid peroxidation. This is achieved through decreasing the malondialdehyde (MDA) concentration in meat [37]. Therefore, there was a decrease in the level of GSH inside the liver tissue which is an essential antioxidant found inside the living cells and this is a reliable index of the antioxidant activity of the tissue [37]. According to the findings of Rahman et al.[38], broilers fed with diets containing 0.5% - 2% of polyphenols (tea polyphenols) have improved muscle antioxidant activity, growth performance and as well as the meat quality. Furthermore, many studies have shown that polyphenols-based feed could be applied as an alternative for vitamin E in the diet to prevent the oxidative stress for breast meat and thigh meat in poultry without causing any depletion of vitamin E stores in the poultry's tissue reserves. These polyphenols can be used partially as an alternative to vitamin E or exert antioxidant benefits on their own and it is identified by the amount of α-tocopherol in the diet [9].

The effect of polyphenols is recorded in several studies. Different levels of grape pomace fed for broiler chickens at 28 days of age, which contains an abundant amount of polyphenols, have been demonstrated to reduce and regulate the level of thiobarbituric acid reactive compounds through the scavenging of free radicals [39]. The study of Pirgozliev et al.[40] indicated that broiler poultry animal-fed essential oils (cinnamaldehyde, capsicum oleoresin and carvacrol) had higher hepatic antioxidants, such as coenzyme, carotene, and total vitamin E, than those fed with non-essential oils. Furthermore, using 20 mL/100 kg of rosemary essential oil could increase antioxidant activities in heat-stressed laying hens by raising the activity of glutathione peroxidase, which is a powerful antioxidant enzyme [41]. In addition, 150 mg/kg of oregano powder was utilized in broiler meals as a source of phenolic compounds, and it was discovered that experimental broilers had greater total antioxidant capacity values and lower malondialdehyde values in their serum [42]. Resveratrol have showed a strong antioxidnat capacity in poultry. The supplement of resveratrol as a natural polyphenic compound has an effect on antioxidant activity in poultry species, particularly, Japanese quails [43]. And the results showed that MDA content reduces from 0.75 (mg/dL) in control treatment to 0.23 (mg/dL) in treatment of 400 mg/kg resveratrol. Moreover, the phenolic compound from rosemary volatile oil was recorded a positive effect on GPx from plasma antioxidant parameters in quails [44]. However, plasma SOD activity is not affected by this substance. Sahin et al.[45] also showed that quails given an additional supplement of resveratrol at a dose of 400 mg/kg had a decreased concentration of MDA in their serum. According to the findings of Mazur-Kunirek et al.[46], broilers that were given diets that contained oxidized rapeseed oil and were either supplemented with vitamin E and polyphenols or just polyphenols were found to have higher levels of GSH-Px activity in their blood as well as higher levels of tocopherol and vitamin E in their livers. An increase in the quantities of polyphenols consumed through feed led to an improvement in the antioxidant status of the blood as well as an increase in the amount of non-enzymatic antioxidants found in the liver and breast muscles of broilers. Additionally, Zhang et al.[47] found that resveratrol might protect against the heat stress-induced deterioration of the meat quality of broilers by elevating the total antioxidant capacity and activity of antioxidant enzymes in the muscle tissue (CAT, GSH-PX). According to the findings of Liu et al.[48], providing black-boned broiler chickens with a supplement of either 200, 400, or 600 mg/kg of resveratrol effectively alleviated the effects of heat stress, with the dose of 400 mg/kg demonstrating the most potent antioxidant effect. Curcumin is a yellow polyphenol, and its supplementation improved the resilience of broilers to heat stress, boosting the GSH content and GSH-related enzyme activities and stimulating the expression of Nrf2 and Nrf2-mediated phase II detoxifying enzyme genes [49]. Moreover, it was discovered that green tea has a significant quantity of polyphenol, and it was shown that drinking green tea boosted the total antioxidant activity as well as increased the activity of liver antioxidant enzymes such as GSH-Px and reduced glutathione (GSH). It was shown that increasing the dose of tea polyphenols led to an increase in the concentration of MDA in the livers of Roman laying hens. Additionally, increasing the dose of tea polyphenols led to an increase in GSH-Px activities and GSH-ST levels [50]. Tea polyphenols have the potential to raise the levels of antioxidant enzyme activity while simultaneously lowering the MDA content of Ya'an laying hens [51]. For the influence of polyphenols' compounds on oxidative stress in poultry production under heat stress, Yin et al.[8] also recorded that heat stress increases the total antioxidant capacity, heme oxygenase-1 mRNA, and decreases the superoxide dismutase, while polyphenols help improve these indicators as a powerful antioxidant compound.

Conclusion and Recomendations

Oxidative stress poses a detrimental effect on poultry performance and health which leads to an economic loss to animal production. Evidently, polyphenols from plants can be future potential feed additives. It can be used to improve poultry antioxidant capacity and mitigate oxidative stress by heat stress in intensive production climates. The application of polyphenols could reduce oxidative stress through its mechanism of action and its chemical structures. In order to achieve the advantages from the function of reducing oxidative stress of polyphenols, the dose of polyphenols in the diet and the targeted poultry species should be taken into account.

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Competing Interests

The authors declare that there are no conflicts of interest

Authors' Contributions

NHQ: Made a framework, wrote and format the manuscript, NTAT: correct, complete and revise the manuscript.

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