Fermented Feed as a Potential Source of Natural Antioxidants for Broiler Chickens – A Mini Review

by Endang Widiastuti

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Summary

As a consequence of genetic selection for fast growth rate, broiler chickens are particularly susceptible to oxidative stress. Such stress may cause metabolic disfunctions, tissue disruption and pathologies that adversely affect broiler performance. Other than synthetic antioxidants that potentially harm human health (as consumers), fermented feed has gained increasing interest from nutritionist as a source of natural antioxidants for broiler chickens. The present review focuses on the effect of fermentation on antioxidative properties of substrates as well as the effect of feeding fermented feed or feed ingredients on the antioxidative status of broiler chickens. Besides improving the nutritional characteristics, fermentation can be cheap and simple means to improve the antioxidant capacity of feed or feed ingredients. It is also apparent from the literature that feeding fermented feed or feed ingredients is beneficial in balancing pro-oxidants and antioxidants and thereby improving the antioxidative status and preventing the oxidative damage in broilers. Likewise, fermented feed improves the oxidative stability and thus meat quality of broilers. Overall, fermented feed or feed ingredients can be the effective and safe alternatives to synthetic antioxidants for broiler chickens.

Key words

antioxidative status, broilers, fermentation, oxidative stress

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Introduction

Modern broiler production is associated with intensive rearing system. Such intensive production system, however, often leads to stress conditions in broilers. Indeed, severe stress implies the increased production of free radicals or reactive oxygen species (ROS), and it means that the excessive ROS production may trigger oxidative stress in chickens. The latter condition eventually causes metabolic disfunctions, tissue damage, pathologies and retarded growth rate (Fellenberg and Speisky, 2006; Surai, 2016). To alleviate the detrimental effects of oxidative stress, dietary supplementation using antioxidants especially with synthetic antioxidants has commonly been practiced in intensive broiler production (Salami et al., 2015). Yet, given that the excessive use of synthetic antioxidants may cause carcinogenic and/or mutagenic effects (Fellenberg and Speisky, 2006; Salami et al., 2015; Taghvaei and Jafari, 2015), the use of natural antioxidants seems therefore to be crucial. Due to its content of several bioactive compounds, fermented feed has recently gained a considerable attention from poultry nutritionists as a source of antioxidants for broiler chickens (Wu et al., 2015; Zhang et al., 2015; Niu et al., 2019). This present review elaborates the improving effect of fermentation on antioxidative properties of substrates as well as the effect of feeding fermented feed on the antioxidative status of broiler chickens. The oxidative stability of meat from broilers fed on fermented feed is also briefly highlighted. To prepare this review article, we did a literature search with focus on the effect of fermented feed or feed ingredients on the antioxidative status of broiler chickens. Peer-reviewed articles (in English) and chapters in an edited book were included in our database and referred in this present review. A number of scientific portals including Elsevier Science-Direct, Springerlink E-Journal, Proquest Research Library, Cambridge University Press E-Journal and Google Scholar were used for collection of the related references.

The Importance of Antioxidants in Broiler Production

Modern broiler strains are selected for the high growth capacity and efficient use of feed. Apart from their high production traits, these meat type chickens are very susceptible to stress induced by poor nutrition, common management practice, pathological and physiological factors and environment (Salami et al., 2015; Nelson et al., 2018). When the chickens are not capable of coping with the stress, they may undergo oxidative stress, which is a disturbance in the balance between the production of pro-oxidants (free radicals/ROS) and antioxidants. Such imbalance may implicate biological damage, pathologies and poor growth performance (Fellenberg and Speisky, 2006). The animal body is provided with multiple antioxidants (endogenous antioxidants) that can counterbalance pro-oxidants so that the harmful effects of ROS can be alleviated. Antioxidants are molecules that can impede the oxidation of other molecules and may be categorized into enzymatic and nonenzymatic antioxidants. The enzymatic antioxidants are naturally produced in the body of animals and include enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), thioredoxin, peroxiredoxin and glutathione transferase. The nonenzymatic antioxidants include vitamin A, vitamin C, vitamin E, β-carotene and glutathione (Ayaşan and Karakozak, 2010; Birben et al., 2012). The nonenzymatic antioxidants are not naturally found in the body of animals (Mamta et al., 2014), and are therefore needed to be supplemented (introduced through diets) to ensure the optimal antioxidant status and body functions of animals (Birben et al., 2012; Mamta et al., 2014). The mechanisms through which antioxidants neutralize pro-oxidants have extensively been reviewed elsewhere (Salami et al., 2015; Aguilar et al., 2016). In principle, antioxidants inhibit pro-oxidant enzyme activities or directly scavenge ROS in the body of chickens. It should be noticed that eradication of ROS from the animal body is not the concept of antioxidants (Aguilar et al., 2016), as, apart from its harmful effect, ROS may be beneficial in counteracting the invading pathogens (Paiva and Bozza, 2014; Dey et al., 2016). Hence, the production of ROS should be regulated (to be balanced with antioxidants), and not be eradicated (Aguilar et al., 2016).

During the normal state, the production of ROS may be in balance with the production of antioxidants. However, during stress condition the production of ROS may exceed the antioxidants production (Panda and Cherian, 2014; Salami et al., 2015). To avoid the excessive production of ROS, raising broilers in the comfort zone and away from the stress-inducing factors is crucial. This, however, seems difficult to be practiced in commercial (intensive) broiler production as multiple stressor may arise with conventional farming practice such as vaccination, high environmental temperature, high stocking density, wet litter, etc. Dietary supplementation of antioxidants (exogenous antioxidants) has conventionally been applied to neutralize free radicals in broilers (Surai, 2016). Aguilar et al. (2016) noticed that exogenous antioxidants may simultaneously work with endogenous antioxidants to decrease the level of ROS, and hence oxidative stress can be prevented. There are several examples of antioxidants that have commonly been supplemented to broiler chickens, including vitamin E, vitamin C, selenium (Baylan et al., 2010), plant extracts, herbs and other synthetic antioxidants such as ethoxyquin, propyl gallate and butylated hydroxytoluene (Salami et al., 2015).

Effect of Fermentation on Antioxidative Properties of Feed

Fermentation is the simple method involving microorganisms to break down the complex substrates into simpler components. Such technique has widely been applied in poultry feed production particularly to improve the nutritional characteristics and palatability of non-conventional feed ingredients (Sugiharto et al., 2018; Sugiharto and Ranjitkar, 2019). Apart from the improved nutrition, fermentation has been subjected to improve the functional properties of the substrates, one of which is the increased antioxidant potentials of the fermented products. The latter property is of importance in modern broiler production due to the exposure of broiler chickens to myriads of stressors. The effect of fermentation on antioxidant activity in several plant-based materials, which are the major feed ingredients for broilers, has comprehensively been reviewed by Hur et al. (2014). There are several explanations for the improvement of antioxidant potentials in the substrates following fermentation, including the breakdown of cell wall leading to the liberation and/or synthesis of a number of bioactive compounds. In the latter case, fermentation may increase the production of α-amylase enzyme responsible for the degradation of cell wall matrix resulting in the release of phenolics (Sadh et al., 2017). Fermentation may also increase the production of phenolic compounds and anthocyanins (Martins et al., 2011) as well as flavonoids in the substrates (Cheng et al., 2016). Likewise, the change in structures of phytochemicals may be responsible for the increased antioxidative activity of the fermented products. In the latter case, Hur et al. (2014) noticed conversion of glycosides into their aglycone after fermentation resulting in increased antioxidant activity in plant-based materials. Moreover, the increased antioxidant capacity in fermented products may be attributable to several active peptides derived from the protein hydrolysates in the substrates, which may neutralize free radicals (Wang et al., 2017a). The production of amino acids, lactic acid and antioxidant vitamins during fermentation may also contribute to the increased antioxidant capacity of the fermented feed (Doblado et al., 2005). In addition to the release of bioactive compounds from the substrates during fermentation process, phenolic compounds may also be produced by the microorganisms used as fermentation starters through secondary metabolic pathway (Dey et al., 2016). In line with this, our earlier data showed the capacity of filamentous fungus *Acremonium charticola* and *Rhizopus oryzae* in producing phenolic compounds (Sugiharto et al., 2015; 2016). Furthermore, starter microorganisms (especially fungi and bacteria) may also produce bioactive polysaccharides that can act as antioxidants (Sánchez et al., 2015). Indeed, the extent of enhancement of antioxidant activity with fermentation may vary depending on starter (microorganism species), nature of substrates and fermentation conditions (such as pH, temperature, solvent, water content, duration of fermentation process and aerobic conditions). With regard to effect of fermentation starters, Sadh et al. (2017) reported an increase in phenolics and antioxidant activities in *Oryza sativa* (rice) and *Lablab purpureus* (seim) following fermentation with *Aspergillus oryzae* and *Aspergillus awamori*. Similarly, Shin et al. (2014) found increased total of polyphenols, isoflavone-aglycones and thus antioxidant activities in *cheonggukjang* of brown soybeans following the fermentation with *Bacillus subtilis* CSY191. In contrast to the above studies, Yoon et al. (2016) reported that fermentation with *Bacillus subtilis* U3 decreased total phenolic and anthocyanin contents as well as antioxidant activities (measured by 1,1-diphenyl-2-picrylhydrazyl radical scavenging, β-carotene bleaching and ferric thiocyanate assay) of black rice bran.

In general, fermentation may be divided into solid-state fermentation (SSF) and submerged fermentation (SmF). Compared to SmF, SSF seems to be more popular in poultry feed production as SSF produces greater yields and better characteristics of the products (Martins et al., 2011; Sugiharto and Ranjikar, 2019). According to Dey et al. (2016), both SSF and SmF may be employed to improve the antioxidative properties in plant-based materials. Yet, SSF has received more attention to enhance the production of phenolic compounds and thus antioxidant activities in the substrates (Martins et al., 2011). In line with this, Mokochinski et al. (2015) documented that antioxidant activities in apple pomace, grape pomace, pineapple pomace, pineapple peel, wheat and malt were higher after SSF than SmF with *Agaricus brasiliensis*. No exact reason for more superior SSF than SmF in term of improving antioxidative activity could be elaborated in the latter case, but it seems that SSF produced more antioxidant enzymes such as manganese superoxide dismutase, peroxidase and laccase (Dey et al., 2016). Moreover, the SSF may produce more antioxidant peptides, amino acids, lactic acid and antioxidant vitamins and thereby antioxidative capacity of the fermented products (Hu et al., 2016).

Effect of Feeding Fermented Feed on Antioxidative Status of Broilers

Due to their relatively low price, synthetic antioxidants have commonly been used in intensive broiler production to deal with stress conditions. However, the use of such antioxidants in broiler production has become of a great concern as at high doses some synthetic antioxidants may exert carcinogenic and/or mutagenic effects (Fellenberg and Speisky, 2006; Taghvaei and Jafari, 2015). In response to this, it is necessary to use cheap natural antioxidants to balance the level of pro-oxidants and antioxidants in broilers. It should be noted that not all materials can be used as a source of antioxidants for chickens, only the materials with particular characteristics can be considered as antioxidant source, such as reduction potential (hydrogen or electron donating potentials), transition metal chelating potential and unpaired electron stabilization and delocalization capacity (Dey et al., 2016). In the recent time, fermented feed has received a considerable interest from nutritionists as a source of antioxidants for broiler chickens. Table 1 provides some examples of the improvement effect of feeding fermented feed or feed ingredients on the antioxidant status of broiler chickens. There are some possible mechanisms by which fermented feed improves the antioxidative status in broilers. One possibility could be that polysaccharides and flavonoids in the fermented feed modulate the cellular production of ROS and antioxidants resulting in balanced pro-oxidants and antioxidants (Cao et al., 2012). In accordance with this, Wu et al. (2015) pointed out the role of polysaccharides, phenolics and flavonoids in balancing the production of pro-oxidants and antioxidant in the body of chickens. Moreover, active peptides derived from protein hydrolysates in fermented feed may also help to scavenge the ROS (Wang et al., 2017b). Furthermore, the live microorganisms in fermented feed may contribute to balancing the pro-oxidants and antioxidants in the body of chickens. Note that some microorganisms may have antioxidant properties, for instance lactic acid bacteria (Liu and Pan 2010), filamentous fungi (Sugiharto et al., 2015; 2016) and *Bacillus* spp. (Kadaikunnan et al., 2015). Apart from the substantial improvement effect of fermented feed on the antioxidative status in broilers, different result was reported by Elmasry et al. (2017), in which feeding wheat bran fermented with *Trichoderma longibrachiatum* (SF1) resulted in no effect on total antioxidant capacity (T-AOC) of chickens. No definite reason could be elaborated in such case, but the differences in the microorganisms used for fermentation, the nature of substrate and other *in vivo* experimental conditions may be responsible for the above discrepancy.

To determine the antioxidative status in broiler chickens, several parameters have usually been evaluated including T-AOC, total SOD, GSH-Px, CAT and malondialdehyde (MDA) (Wu et al., 2015; Zhang et al., 2015; Yin and Huang, 2016). With regard particularly to fermented feed, feeding such feed has been reported to increase the levels of T-AOC, T-SOD, GSH-Px and CAT in serum and liver, indicating that fermented feed was able to promote the antioxidative defence mechanisms (against ROS) and thus protect broiler chickens from the detrimental effect of oxidative stress (Niu et al., 2019).

Table 1. The examples of effects of feeding fermented feed on antioxidative status of broilers

References	Findings
Cao et al. (2012)	*Ginkgo biloba* leaves fermented with *Aspergillus niger* increased the serum α-tocopherol concentration, total SOD activities and T-AOC
Wu et al. (2015)	*A. niger*-fermented pine needles increased total SOD activities and T-AOC, while decreased MDA levels and total NOS activity in serum and liver
Zhang et al. (2015)	- *Ginkgo*-leaves (GL) fermented with combination of *Candida utilis* and *A. niger* increased serum concentrations of α-tocopherol - GL fermented with either *C. utilis*, *A. niger* or combination of both increased hepatic α-tocopherol - GL fermented with either *A. niger* or combination of *C. utilis* and *A. niger* decreased hepatic ROS, protein carbonyls and MDA, as well as jejunal and ileal protein carbonyls - GL fermented with combination of *C. utilis* and *A. niger* increased total SOD activities and GSH-Px of both jejunum and ileum
Hu et al. (2016)	Rapeseed meal fermented with *Bacillus subtilis*, *C. utilis* and *Enterococcus faecalis* increased the levels of serum T-AOC and total SOD
Wang et al. (2016)	Wheat bran fermented with white rot fungi increased the scavenging capacity of 1, 1-diphenyl-2- picrylhydrazyl, trolox equivalent antioxidant capacity, and chelating capacity of ferrous iron as well as regulated the expression of antioxidant molecular targets in chicken peripheral blood mononuclear cells
Yin and Huang (2016)	*B. subtilis*-fermented alfalfa meal increased the activities of GSH-Px, SOD and CAT, but decreased MDA in serum
Wang et al. (2017b)	Wheat bran fermented with white rot fungi increased the gene expression of haem oxygenase-1 and glutathione-S-transferase of chicken peripheral blood mononuclear cells, while decreased the gene expression of nicotinamide adenine dinucleotide phosphate oxygenase 1 and reactive oxygen species modulator protein 1
Wang et al. (2017a)	Cottonseed meal fermented with *B. subtilis* ST-141 and *Saccharomycetes* N5 increased the levels of T-AOC, GSH-Px and T-SOD, while decreased MDA in serum and liver
Niu et al. (2019)	*Ginkgo* biloba leaves fermented with *A. niger* and C. utilis increased the activity of GSH-Px in serum, T-AOC and total SOD activities in liver and the mRNA expression of Nrf2 and GSH-Px, while decreased MDA concentration in serum and liver
Qiao et al. (2018)	Fermented *Astragalus* with *L. plantarum* increased glutathione superoxide dismutase, T-AOC and total SOD, while decreased MDA in serum
Cao et al. (2012)	*Ginkgo biloba* leaves fermented with *A. niger* decreased muscle MDA concentration
Ahmed et al. (2014a)	*Citrus junos* by-products fermented with mixed of *S. cerevisiae, Enterococcus faecium, Lactobacillus acidophilus* and *B. subtilis* reduced the values of thiobarbituric acid reactive substances (TBARS) of breast and thigh meat
Ahmed et al. (2014b)	Fermented kelp with *B. subtilis* and *Aspergillus oryzae* decreased MDA values in meat
Hossain et al. (2012)	Fermented *Alisma canaliculatum* using *L. acidophilus* KCTC 3111, *E. faecium* KCTC 2022, *B. subtilis* KCTC 3239 and *S. cerevisiae* KCTC 7928 reduced MDA level in breast and thigh meat
Hossain and Yang (2014)	Fermented water plantain (using *L. acidophilus* KCTC 3111, *E. faecium* KCTC 2022, *B. subtilis* KCTC 3239 and *S. cerevisiae* KCTC 7928) decreased MDA levels in breast and thigh muscle
Kim and Kang (2016)	Barley or wheat fermented with *L. plantarum* KCTC 1048 or *B. subtilis* ATCC 21322 improved 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and (ferric reducing antioxidant power) FRAP in breast meat
Liu et al. (2018)	Fermented broccoli residue with lactic acid bacteria, *S. cerevisiae* and *B. subtilis* increased the activities of SOD, GSH-Px and T-AOC in the breast and leg muscle

On the other hand, fermented feed was capable of decreasing MDA concentrations (Cao et al., 2012). Considering that MDA is the final product of lipid peroxidation, the decreased MDA level may indicate that fermented feed was capable of alleviating the tissue lipid peroxidation in broilers (Hu et al., 2015; Wang et al., 2017b). According to Kobayashi and Yamamoto (2005) and Zhang et al. (2010), some antioxidant proteins such as GSH-Px, SOD and CAT are regulated by nuclear factor erythroid 2-related factor 2 (Nrf2). Hence, the increased activities of antioxidant enzymes may be attributed to the activation of Nrf2 gene expression. In the study of Niu et al. (2019), it was apparent that feeding fermented *Ginkgo* biloba leaves increased the mRNA expression of Nrf2 and eventually increased the activity of GSH-Px in serum, T-AOC and total SOD activities in liver. In addition to the above mentioned antioxidant parameters, total nitric oxide synthase (NOS) activity may be used to assess the antioxidative status in broiler chickens. It is generally accepted that low level of total NOS activity is beneficial for protecting the cells from oxidative stress, while in high level total NOS may act as pro-oxidant. In the study of Wu et al. (2015), feeding of fermented pine needles decreased the activity of total NOS in the serum and liver, thus alleviating the negative effect of ROS in broiler chickens. Other parameter that may be used to assess the antioxidative status in broiler chickens is the level of protein carbonyls, in which the lower level of protein carbonyls would be beneficial in protecting the birds from oxidative damage. Indeed, Zhang et al. (2015) showed the potential of fermented *Ginkgo*-leaves in decreasing the level of jejunal and ileal protein carbonyls in broiler chickens and thereby protecting the jejunum and ileum from tissue damage.

Feeding fermented feed has been reported to improve the oxidative stability of broiler meat (Table 1). It seems that fermented feed promote the activities of antioxidant enzymes resulting in improved meat oxidative stability (Liu et al., 2018). Likewise, the antioxidative substances in fermented feed may intercept and scavenge free radicals and thus prevent the oxidation process in meat (Hossain et al., 2012). In contrast to the above mentioned studies (Table 1), Kovalík et al. (2018) reported that feeding spelt brans fermented with *Cunninghamella elegans* CCF 2591 increased the concentration of MDA in broiler meat during 7-day storage in refrigerator, suggesting that feeding fermented feed resulted in lower oxidative stability in broiler meat. Such discrepancy may be explained by the increased polyunsaturated fatty acids (PUFA) content in meat as a result from feeding fermented feed. In this regard, the improvement effect of fermented feed on the meat oxidative stability may be hampered by the high PUFA content in meat.

Conclusion

Fermentation increases the antioxidant capacity of feed or feed ingredients. Feeding fermented feed or feed ingredients increases the antioxidant activity and thereby alleviate the negative effect of free radicals in broiler chickens.

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