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REVIEW

# A review of antioxidant strategies to improve reproduction in aging male broiler breeders

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**Abstract** As only 10% of the broiler breeder flock is roosters, their fertility is very important. The rooster sperm plasma membrane has high concentrations of polyunsaturated fatty acids that are sensitive to oxidative stress. Lipid peroxidation can change membrane structure, permeability, and fluidity, adversely affecting the acrosome reaction and fertility. Aging roosters have decreases in sexual behavior, serum androgen concentrations, sperm quantity and quality, and fertility. Low fertility in aging roosters is attributed to an imbalanced testicular oxidant-antioxidant system, with increased reactive oxygen species (ROS) damaging spermatogenic epithelium. However, antioxidant components can enhance antioxidant defenses in aging broiler breeder roosters. Protection against oxidative damage, particularly in the testes, improves reproductive hormone concentrations, testicular histology, sperm membrane function, and mitochondrial activity and thereby improves semen volume, sperm concentration, viability, motility, and sperm polyunsaturated fatty acid content, sperm-egg penetration, fertility, and reproductive performance. This review summarizes antioxidants that could improve fertility and reproductive performance and delay or prevent age-related declines in broiler breeder roosters, with benefits for poultry production.

**Keywords** Roosters · Sperm · Reproductive senescence · Oxidative stress · Fertility

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## Introduction

Broiler breeders are raised to produce fertile broiler eggs. Furthermore, as one rooster can be responsible for > 1000 fertilized eggs annually [1, 2], rooster fertility is critical [3].

Poultry has many sources of stress, including environmental, technological, nutritional, and internal/biological [4]. Oxidative stress occurs when effects of oxidants exceed antioxidant protection [5, 6]. Oxidative stress increases the production of reactive oxygen species (ROS), leading to lipid

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peroxidation (LPO), apoptosis, and DNA damage [5, 7, 8]. The ROS are extremely active oxidizing factors, with single or multiple unpaired electrons. These molecules are capable of succession reactions, with the aim of "radical begets radical." Hydrogen peroxide ($H_2O_2$), superoxide ($O_{2-}$), peroxyl ($ROO^-$), and hydroxyl ($OH^-$) radicals are very reactive and adversely affect reproduction, whereas peroxynitrite anion ($ONOO^-$) and nitrogen resultant–free radical nitric oxide (NO) have physiologic roles in fertilization and other aspects of animal reproduction [9]. There is a high proportion of polyunsaturated fatty acids (PUFAs) in the sperm plasma membrane [10], and the main PUFA in rooster semen (22:4n-6, docosatetraenoic acid) (Fig. 1) is very susceptible to peroxidation [11]. Those sperm characteristics, coupled with age-related reductions in seminal antioxidant capacity and gonadal axis activity, predispose numerous avian species to have reduced fertility as they age. Rooster fertility peaks at ~ 37 weeks of age, with an age-related decline in fertility starting at ~ 40 weeks of age (across genetic lines), negatively affecting the production of hatching eggs and causing substantial economic losses [12]. In roosters > 40 weeks old, there are decreases in testicular weight, serum testosterone concentrations [13–15], semen volume, sperm concentration, viability and progressive motility, sperm polyunsaturated fatty acids (PUFAs), and antioxidant capacity (Fig. 2) [16–19].

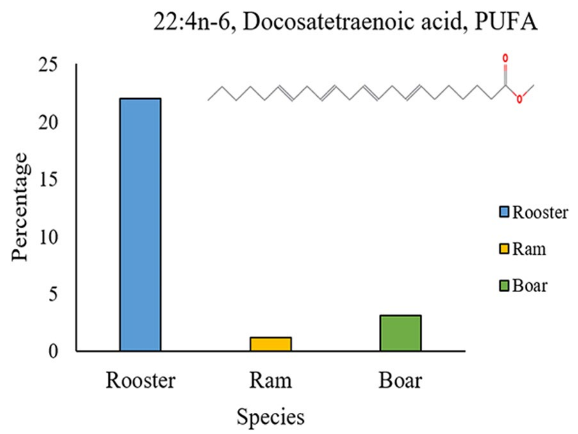


**Fig. 1** A high proportion of polyunsaturated fatty acids (PUFAs) in the sperm plasma membrane, the main PUFA in the rooster semen (22:4n-6, docosatetraenoic acid) being most susceptible to peroxidation, plus age-related reductions in seminal antioxidant capacity, make the plasma membrane of avian sperm susceptible to lipoperoxidation once exposed to reactive oxygen species

Aging generally has adverse effects on the reproductive performance of birds, causing age-related reductions in fertilized eggs produced by broiler breeder flocks. Therefore, a common practice to maintain fertility in aging flocks is to spike (add replacement roosters) breeder flocks at 40 to 50 weeks of age, although this approach is costly,

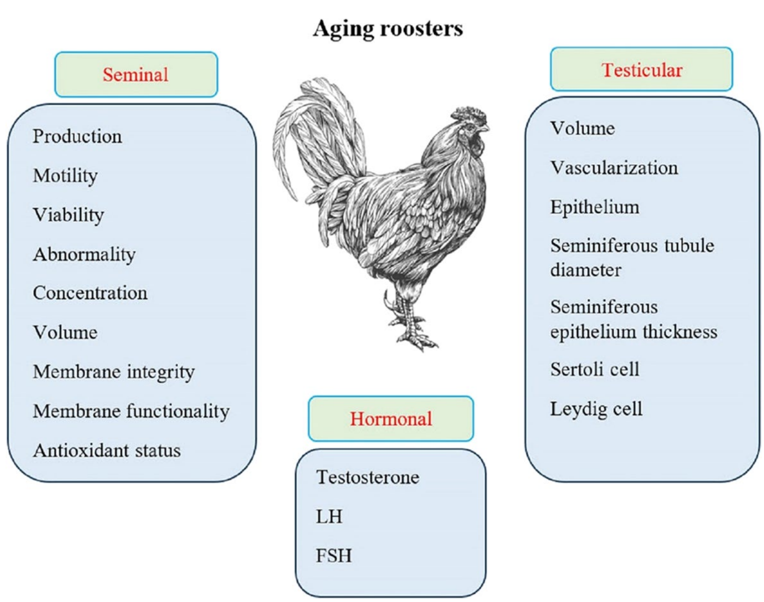


**Fig. 2** Effects of age on roosters' fertility potential. Age-related reductions in sperm quality, hormonal balance, and testicular histology. LH, luteinizing hormone; FSH, follicle-stimulating hormone

threatens biosecurity, and disrupts social flock behavior [20].

To counteract adverse effects of ROS, the antioxidant system of rooster semen includes glutathione peroxidase, glutathione, catalase, superoxide dismutase, and other natural antioxidants such as vitamin E and vitamin C [21]. However, antioxidant activity in rooster semen decreases with age [22]. Therefore, nutrition has an important role in sustaining or improving reproductive performance [23]. For example, letrozole [24], guanidinoacetic acid [25], D-aspartate [26], vitamin E [27], or zinc oxide [28] can improve reproductive performance in broiler breeders. Currently, there is a growing trend to increase antioxidant intake (especially natural antioxidants) to counteract age-induced reproductive disorders (namely, high ROS concentrations in seminal plasma, sperm, and testis) causing infertility in roosters (Fig. 3). This review summarizes antioxidants with potential to improve fertility in aging broiler breeder roosters (Table 1) and their modes of action.

Some reproductive aging processes are common among species. For example, in livestock, similar to humans, decreasing concentrations of anti-Mullerian hormone (AMH) during aging are associated with declining reproductive performance [29]. Furthermore, in male Japanese quail (*Coturnix japonica*), plasma androgen concentrations decline gradually, accompanied by a loss of reproductive behavior [30], which is overall similar to mammals. Considering effects of aging and oxidative stress on reproductive performance in males, which are common among many species, mammalian treatment models can provide insights regarding potential beneficial effects in aging birds, particularly as a means of augmenting the limited amount of literature for birds.

## Ginger

Ginger has been used for more than two millennia. Its antioxidant properties are attributed to both volatile and non-volatile compounds, with particular emphasis on its rhizome [31, 32]. Polyphenol chemicals in rhizome extracts are of importance, with 6-gingerol and its derivatives having notable antioxidant activity [33]. Terpinene-4-ol, a volatile chemical in a ginger rhizome, has antioxidant properties [33]. There are also several non-volatile phenolic compounds in ginger, including zingerone, paradol, gingerols, and shogaols [32]. In laying hens, ginger consumption enhanced reproductive performance and increased serum antioxidant capacity [34]. Similarly, in male rats, feeding ginger increased testosterone concentrations, sperm viability and motility, and sexual function [35], consistent with ginger having androgenic potential [36].

Dietary ginger significantly increased sperm concentration and reduced sperm abnormalities in roosters [37]. Supplementing a rooster diet with ginger (1 g/kg) increased blood testosterone concentrations and significantly increased testis weight, number of spermatogonia, and spermatogenic tubule diameter [38]. For male Japanese quail, 10 or 15 mg/kg ginger in the diet increased testosterone concentrations, spermatogenic tubule diameter, plus sperm volume, concentration, motility, and viability, with improved fertility; furthermore, the higher dose increased penetration of sperm into the perivitelline membrane [39]. Adding 15 or 30 g/kg of ginger rhizomes in the diet of aged (52 weeks) roosters enhanced sperm motility, viability, and membrane integrity [40], and there was a significant correlation between sperm antioxidant potential and

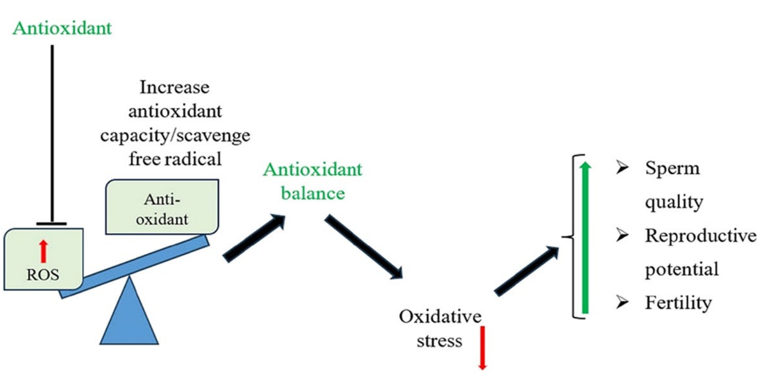


**Fig. 3** Antioxidants improve the reproduction potential of aging roosters; they are ROS "scavengers " that promote oxidant/antioxidant balance, thereby improving sperm quality, reproductive performance, and fertility. ROS, reactive oxygen species

**Table 1** Effects of antioxidant intake in aging broiler breeder roosters

| Antioxidant | Amount (dose) | Age (weeks) | Relevant findings | Reference |
|---|---|---|---|---|
| Ginger | 15 and/or 30 g/kg, for 98 days | 52 | ↑Sperm quality (↑motility, ↑viability, ↑membrane integrity, ↓abnormalities), ↑sperm penetration, ↓seminal MDA and TAC, ↑fertility | [40] |
| L-Carnitine | 50 and/or 150 mg/kg BW/day, for 84 days | 55 | ↑Testosterone, ↑testicular antioxidant activity (CAT and GSH-$P_X$), ↓seminal MDA, ↑fertility | [60] |
| Chrysin | 25, 50, and/or 75 mg/bird/day, for 84 days | 40 | ↑Sperm quality (↑motility, ↑concentration, ↑membrane integrity, and functionality), ↓seminal MDA, ↑fertility, ↑hatchability | [66] |
| Alpha-lipoic acid | 15, 40, 70, 95, 120, and/or 145 mg/bird/day, for 56 days | 45 | ↑Sperm quality (↑motility, ↑viability, ↑concentration, ↑membrane integrity), ↑testosterone, ↓seminal MDA, ↑fertility, ↑hatchability | [75] |
| Coenzyme Q10 | 300 and/or 600 mg/kg, for 49 days | 47 | ↑Sperm quality (↑volume, ↑concentration, ↑membrane integrity, and functionality), ↑sperm penetration ↑fertility, ↑seminiferous tubule diameter, ↑seminiferous epithelium thickness, ↑testosterone | [81] |
| Organic selenium | 0.30 and/or 0.45 mg/kg, for 70 days | 64 | ↑Sperm quality (↑motility, ↑concentration, ↑membrane integrity), ↑seminal TAC and MDA, ↑fertility | [87] |
| Curcumin | 10, 20, and/or 30 mg/bird/day, for 91 days | 48 | ↑Sperm quality (↑motility, ↑concentration, ↑membrane integrity, and abnormality), ↑sperm penetration, ↓seminal MDA, TAC, SOD and GSH-$P_X$, ↑fertility | [95] |
| Astaxanthin | 25, 50, and/or 100 mg/kg, 42 days | 53 | ↑Sperm quality (↑volume, ↑concentration), ↑testosterone, ↑SOD, ↑GSH-$P_X$, ↑CAT | [101] |
| *Lasia spinosa* Thw | 2 and/or 4%, for 44 days | 71 | ↑Sperm quality (↑motility, ↑volume, ↑concentration), ↑fertility, ↑seminiferous tubule diameter, ↑seminiferous epithelium thickness, ↓seminal MDA, ↑testis weight | [110] |
| Rosemary | 2.5, 5, and/or 7.5 g/kg, for 56 days | 70 | ↑Sperm quality (↑motility, ↑viability, ↑volume, ↑concentration), ↑sperm penetration, ↓seminal MDA, ↑fertility, ↑hatchability | [115] |
| Rooibos | 0, 1.5, and/or 3%, for 91 days | 47 | ↑Sperm quality (↑motility, ↑viability, ↑concentration, ↑volume), ↑testosterone, ↑testes weight, ↑fertility | [121] |
| Tomato pomace | 5, 10, and/or 15%, for 84 days | 58 | ↑Sperm quality (↑concentration, ↑volume, ↑viability, ↑motility), ↑sperm penetration, ↑fertility | [126] |
| Apple pomace | 10, 25, and/or 25%, for 98 days | 54 | ↑Sperm quality (↑motility, ↑viability, ↑concentration, ↑membrane integrity), ↑sperm penetration, ↓seminal MDA, ↑fertility, ↑hatchability | [132] |
| Black seed | 0.5 and/or 1.0%, for 63 days | 45 | ↑Sperm quality (↑motility, ↑volume, ↑concentration, ↓abnormality), ↑fertility, ↑hatchability | [141] |

**Table 1** (continued)

| Antioxidant | Amount (dose) | Age (weeks) | Relevant findings | Reference |
|---|---|---|---|---|
| Olive oil | 0.2 and/or 0.4 ml/bird/day, for 56 days | 54 | ↑Sperm quality (↑motility, ↑viability, ↑concentration) | [151] |
| Lemongrass | 200, 300, and/or 400 ml/l, for 42 days | 50 | ↑Testosterone and ↑LH | [154] |

*CAT* catalase, *GSH-P$_X$* glutathione peroxidase, *LH* luteinizing hormone, *MDA* malondialdehyde, *SOD* superoxide dismutase, *TAC* total antioxidant capacity

reductions in defective sperm, with fertility highest in roosters fed 30 g/kg ginger.

Certain phytochemical components of ginger enhanced sperm viability and motility [41]. Potential beneficial impacts of ginger on sperm quality could be attributed to its phenolic components, which can enhance sperm membranes and protect against DNA damage. Impacts of ginger on fertility are attributed to several mechanisms. Firstly, ginger elevates testicular cholesterol concentrations, which may enhance reproductive function [42]. In addition, ginger also promoted testicular blood flow, stimulated the release of luteinizing hormone (LH), and enhanced the testicular antioxidant defense system, all likely to increase reproductive function [42]. Finally, ginger increased serum testosterone concentrations plus enhanced total antioxidant capacity (TAC) and reduced malondialdehyde (MDA), consequently decreasing DNA damage and oxidative stress in sperm [35].

## L-Carnitine

Carnitine ($C_7H_{15}NO_3$; MW = 162.2 g/mol) is a water-soluble compound. L-Carnitine (LC), the active isomer, is a non-protein amino acid known as β-hydroxy-γ-trimethylaminobutyric acid, is generated from lysine and methionine, and has various roles in reproduction [43]. The primary function of LC is the facilitation of fat metabolism and transportation of long-chain fatty acids into cellular structures, including the movement of active fatty acids (acyl coenzyme A) into the mitochondrial matrix [44]. During beta-oxidation, free fatty acids are converted into acyl-CoA that enters the Krebs cycle and produces ATP and water, decreasing oxygen concentrations and reducing ROS formation [45]. Furthermore, LC is an antioxidant [46, 47]. Natural antioxidant molecules have crucial roles in protecting sperm from oxidative stress [48]. The epididymal lumen has ample LC, with a vital role in preserving the energy equilibrium of sperm and facilitating their maturation [49, 50], plus enhancing sperm motility [51].

Dietary LC enhanced sperm characteristics and reproductive parameters in roosters, Japanese quail, and African black neck ostrich [52–54]. Furthermore, LC enhanced the viability and integrity of sperm chromatin, plus promoted glucose uptake in sperm [55]; these beneficial effects, plus its antiapoptotic and antioxidant properties, improved sperm indices. Moreover, with aging, LC deficiencies disrupt metabolic processes, particularly mitochondrial function [56]. However, exogenous LC enhanced metabolic functions in rats, particularly old rats [57]. In aging males, there is a depletion of cytoplasmic material in sperm, increasing susceptibility to oxidative stress, particularly in birds, with the potential to cause reproductive malfunctions [58, 59]. However, adding 150 mg/kg LC to the diet of aged (55 weeks) roosters enhanced testosterone concentrations, activity of antioxidant enzymes (CAT and GPX), and sperm quality and fertility [60].

## Chrysin

Flavonoids are polyphenolic chemicals with a 15-carbon skeleton, including two phenyl rings (A and B) and a heterocyclic ring (C). These compounds are widely distributed in many fruits, vegetables, and drinks. Chrysin, also known as 5,7-dihydroxyflavone, is a naturally occurring polyphenolic substance in *Passiflora caerulea*, honey, and other botanical sources. Chrysin has protective properties against oxidative and inflammatory damage [61]. Numerous studies, both in vitro and in vivo, supported the antioxidant action of chrysin; it reduces seminal MDA, which enhances sperm antioxidant status and membrane integrity. Likewise, chrysin has been extensively validated for its effects on improving testicular antioxidant enzymes, specifically to enhance GSH, CAT, SOD, and GSH-Px [62, 63]. Furthermore, it increased testosterone concentrations and male fertility [63, 64] and in male rats, sperm count, and sperm motility [62].

In male broiler breeders, 50 mg of chrysin improved semen traits and fertility of both fresh and frozen-thawed semen [65]. In another study [66], chrysin (50 or 75 mg per bird) in the diet of aging roosters (40 weeks) significantly improved sperm count, motility, and progressive motility, plus membrane integrity and seminal MDA, with better fertility and hatchability. Furthermore, the higher dose (75 mg) increased blood testosterone concentrations.

## Alpha-lipoic acid

Alpha-lipoic acid (ALA) is commonly used to mitigate or delay effects of oxidative stress [67]. ALA, also known as lipoic acid or thioctic acid, has the chemical formula $C_2H_{14}O_2S_2$, a relatively low POP ID value of 6112, and a molar mass of 206.33 [68]. In contrast to other antioxidants, ALA has both hydrophilic and lipophilic characteristics, functioning in both aqueous and lipid phases [69], enabling its active involvement in several cellular compartments including cytoplasm, lipoproteins, serum, and plasma membrane [69]. Within mitochondria, ALA functions as a coenzyme for a-ketoglutarate dehydrogenase and pyruvate dehydrogenase. Furthermore, exogenous ALA is an antioxidant, suppressing oxidative stress in vitro and in vivo, with synergistic effects when combined with other antioxidants. It can inhibit oxygen-free radical species in both aqueous and lipid phases, impeding lipid peroxidation in the membrane [70, 71] and counteracting ROS, including superoxide radicals, hydroxyl radicals, hypochlorous acid, peroxyl radicals, and singlet oxygen [72]. It has a crucial protective function by inhibiting apoptosis and mitigating degenerative processes in testes and chromosomes [73, 74], plus promoting the creation of adenosine triphosphate (ATP) in the Krebs cycle. When birds (45 weeks old) were given 5 to 145 mg of ALA once daily, those fed 95 mg had reduced sperm MDA concentrations, although testicular histology was not determined [75].

## Coenzyme Q10

Coenzyme Q10 (CoQ10), chemically represented as $C_{59}H_{90}O_4$, is a lipophilic androgen benzoquinone molecule regarded as essential for overall well-being. It is produced endogenously, although production decreases with age [76]. In addition, there are various dietary sources. CoQ10 is involved in cellular ATP production and is primarily located within the inner membrane of mitochondria, acting as an electron acceptor in the mitochondrial respiratory chain. Additionally, it aids in membrane synthesis and functions as an antioxidant, potentially facilitating the removal of free radicals. Its antioxidant capacity is ~ 50-fold that of vitamin E. Consequently, it effectively mitigates oxidative damage, peroxidation of membrane lipids, and genomic instability [77]. Supplementing rooster semen extender with CoQ10 may improve semen quality and fertility [78].

CoQ10, an essential component in energy metabolism, has a high concentration within mitochondria, located in the sperm midpiece [79]. Furthermore, CoQ10 functions as an antioxidant and contributes to the replenishment of endogenous antioxidants such as superoxide dismutase, thereby impeding lipid peroxidation [76]. Antioxidants can improve fertility by increasing antioxidant capacity (both enzymatic and non-enzymatic; Fig. 4) by preventing oxidation of abundant fatty acids in sperm and reducing MDA concentrations. In addition, dietary supplementation with CoQ10 had beneficial effects on productive and reproductive functions in aged hens [80].

Aging roosters (47 weeks) were given a 300 or 600 mg CoQ10/kg diet to determine protection against oxidative stress and enhance various aspects of sperm quality, with many benefits, including increases in ejaculate volume, sperm concentration, and sperm membrane integrity [81]. CoQ10 is one of many poultry diet additives that can activate the recycling and regeneration of some antioxidants (e.g., Vit E), providing an impetus to combine it with another antioxidant and determine effects on reproduction in aging roosters.

## Organic selenium

Selenium has a vital role in several metabolic processes in animals, including activating enzymes and

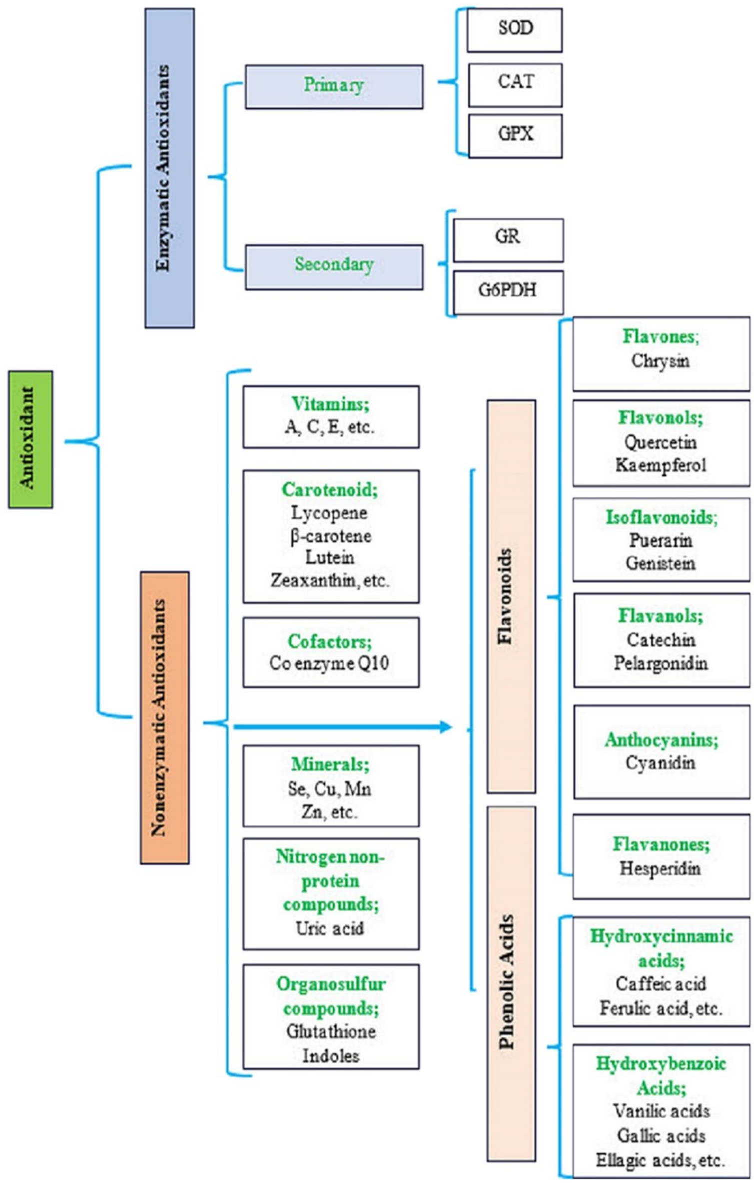


**Fig. 4** Classification of enzymatic and non-enzymatic antioxidants [155, 156]. SOD, superoxide dismutase; CAT, catalase; GPX, glutathione peroxidase; GR, glutathione reductase; G6DPH, glucose-6-phosphate dehydrogenase

ensuring the appropriate functioning of biochemical and physiological mechanisms in birds [82]. It has both inorganic and organic sources. Inorganic selenium has some restrictions, including potential toxicity, interactions with other elements, and limited capacity for storage, leading to substantial excretion [83]. Regarding selenoprotein production, selenomethionine is the sole variant capable of active engagement. Consequently, this organic selenium variant has the potential to enhance numerous metabolic processes by promoting the synthesis of selenoproteins, which are crucial in the production of various metabolic enzymes. Selenium also protects cell membranes from oxidative damage [84]. Selenium is a crucial constituent of the enzyme glutathione peroxidase, vital in detoxifying hydrogen peroxide and lipid hydroperoxides. Additionally, selenium enhances the antioxidant system [85].

Dietary supplementation of organic selenium improved reproductive potential in aged broiler breeder hens [86]. Adding 0.3 or 0.45 mg/kg organic selenium to the diet of aged (64 weeks) roosters mitigated dexamethasone-induced stress and improved fertility, sperm concentration, motility, and sperm membrane integrity [87]. Furthermore, roosters fed 0.45 mg/kg organic selenium had reduced MDA and increased total antioxidant capacity (TAC). Incorporating both organic and inorganic selenium into the diet of aging roosters (50 weeks) enhanced reproductive performance [88], with 0.45 mg/kg of organic selenium yielding the most notable improvement in reproductive performance. Mineralization of the sperm membrane was greater in comparison to control and inorganic selenium treatment, with increased sperm volume and integrity. Furthermore, organic selenium therapy enhanced fertility, hatchability, and testicular histology (seminiferous epithelium thickness and seminiferous tubule diameter).

## Curcumin

Curcumin, the bioactive compound in turmeric spice, has antioxidant properties and enhances the activity and expression of enzymes involved in neutralizing free radicals, including superoxide dismutase, glutathione peroxidase, and catalase [89, 90]. In addition, it has anti-inflammatory and anti-diabetic actions, plus it reduces fat and concentrations of glucose and cholesterol.

Adding curcumin to the diet of laboratory and field mice exposed to heavy metals, including lead and cadmium, protected testicular structures, including spermatogonia, by mitigating oxidative stress and eliminating free radicals [91]. Additionally, curcumin protected cryopreserved human sperm, including diminished intracellular ROS and DNA fragmentation [92]. However, its medicinal value is limited by poor solubility, low absorption, and rapid metabolism, resulting in low bioavailability [93], although perhaps these limitations could be overcome with encapsulation.

Feeding roosters 8 mg/kg turmeric improved sperm motility [94]. Similarly, curcumin in the diet of aging roosters after peak sperm production decreased sperm MDA concentrations and improved sperm quality and fertility [95]. Adding curcumin (10, 20, or 30 mg/bird per day) in the diet of aging (48 weeks) broiler roosters reduced MDA concentrations and enhanced reproductive efficiency, including increased sperm concentration and total and progressive motility [95]. It appeared that curcumin had its effects on spermatogenesis and sperm.

## Astaxanthin

Astaxanthin (ASTA), a xanthophyll carotenoid, can be extracted from various microorganisms, phytoplankton, and marine species [96]. ASTA is a naturally occurring and efficacious antioxidant, with superior capacity for neutralizing free radicals (~550 times greater than vitamin E) [97, 98]. Additionally, ASTA serves as a precursor to vitamin A, which also has antioxidant attributes [99].

Inclusion of 25, 50, or 100 mg/kg ASTA in the diet of aging (53 weeks) roosters enhanced sperm motility by activating the MAPK/Nrf2 pathway, thereby reinforcing the antioxidant defense system [100]; furthermore, it enhanced secretion of reproductive hormones and improved sperm quality [101]. ASTA mitigated the impacts of ROS and enhanced testicular functionality (both seminiferous tubule diameter and epithelium thickness were markedly increased). In addition, it promoted P450 function and StAR activity and increased reproductive hormone synthesis, although effects on fertility were not evaluated.

## Lasia spinosa Thw

*Lasia spinosa* Thw (LST) is a perennial plant in the Araceae family, growing in tropical and humid geographical settings. It has antioxidant effects [102–104], including reduction of ROS [105]. Antioxidant attributes of LST are attributed to its flavonoid, technical and carotenoid constituents, and phenolic compounds, including gentistic, apigenin, syringic acid, murine, cinnamic acid, and 4-hydroxybenzoic acid [106]. Furthermore, LST may be a reservoir for phytoestrogens and phytoandrogens [107], due to testosterone (T) in the LST rhizome [108, 109].

Oral gavage of LST extract significantly augmented sperm count and testis weight in male rats [109]. Adding 2 or 4% LST in the diet of aged roosters (70 weeks) improved testis weight, width, and thickness of the seminiferous epithelium; decreased MDA concentrations in seminal plasma; improved semen characteristics (volume, sperm concentration, and motility); and increased fertility [110]. It would be worthwhile to repeat this work with younger roosters.

## Rosemary

The botanical classification of the rosemary plant is *Rosmarinus officinalis*, a member of the Lamiaceae or Labiatae family, also known as the mint family. Its antioxidant potential is attributed to many components, including carnosol, rosmanol, isorosmanol, epirosmanol, carnosic acid, and rosmarinic acid [111]. The two primary phenolic chemicals identified in rosemary leaves are carnosic acid and rosmarinic acid, with > 90% of the antioxidant effect [112, 113]. The antioxidant capacity of pure carnosic acid substantially surpasses that of butylated hydroxytoluene and butylated hydroxyanisole [114].

In aged (70 weeks) roosters given rosemary powder (5 g/kg of diet), there were reduced MDA concentrations in semen and increased sperm volume, concentration, and overall quality, plus improved fertility [115]. Likewise, giving aging (45 weeks) roosters rosemary powder (5 g/kg encapsulated) in conjunction with omega 3 and 6 fatty acids (2% of diet) increased testosterone and LH, lowered MDA, and improved sperm quality, including concentration, viability, and membrane integrity [116].

## Rooibos

Rooibos is cultivated in the Cederberg region of the Western Cape Province in South Africa and used as tea or tisane [117]. Green shoots are collected, cut, bruised, and soaked, then allowed to ferment before being sun-dried and steam-pasteurized. The absence of caffeine and relatively low tannin content in fermented rooibos distinguishes it from camellia sinensis tea, rendering it a favored herbal beverage with perceived health benefits [118]. Phenolic compounds in rooibos include aspalathin (the predominant polyphenol specific to rooibos), notophagine, quercetin, rutin, isoquercitrin, orientin, luteolin, vitexin, and chrysoeriol. Rooibos herbal tea has potent antimutagenic capabilities. Rooibos bioactivities include anticancer, anti-inflammatory, hepatoprotective, phytoestrogenic, antispasmodic, antihemolytic, antiaging, antimicrobial, antiviral, vasodilation, and improved lipid profiles [119, 120].

In aging (47 weeks) roosters, 1.5 or 3% rooibos powder improved sperm quality and reproductive performance, with the higher dose yielding the best improvements in testosterone concentrations, semen volume, sperm motility, testis weight, and fertility [121]. Future studies could include essential oil and encapsulation (micro- or nanoparticles).

## Tomato pomace

Tomato (*Solanum lycopersicum* L.) belongs to the Solanaceae family and is the second most widely cultivated vegetable crop globally, following the potato [122]. Lycopene, the primary constituent in tomato pomace, is responsible for the red hue of tomatoes; this color arises from an absence of double bonds and beta rings [123]. The free radical neutralization property of lycopene ($C_{40}H_{56}$) is attributed to its molecular structure: 8 isoprene units, 11 normal double bonds, and 2 irregular double bonds [124].

Roosters (24 weeks) fed 15 or 30% tomato pomace had improvements in semen volume, sperm concentration, membrane integrity, and morphology [125]. In aging (58 weeks) roosters, tomato pomace (5–10% or 15%) in combination with the amino acid arginine enhanced sperm volume, concentration, viability, sperm total, and progressive motility; improved seminal antioxidants (TAC, total antioxidant capacity; SOD, superoxide dismutase; and GPX, glutathione peroxidase); and finally, enhanced fertility and hatchability [126].

The nutritional composition of dried tomato pomace varies based on strain, soil conditions, and treatment, plus processing and drying. Chemical composition varies depending on the presence of seeds and skin, as the latter has substantial phenolics, dietary fiber, and lycopene, whereas tomato seeds have much oil and protein [127]. Future studies with lycopene should quantify antioxidant compounds and include testicular histology and blood hormone concentrations.

## Apple pomace

Apples are widely grown and often used for juice, generating large amounts of apple pomace (AP), much of which is fed to domestic livestock. Apples have antioxidant properties, largely attributed to polyphenols [128]. AP has many polyphenols, including phenolic acids (particularly chlorogenic acid) and flavonoids (e.g., catechin and epicatechin), plus dihydrochalcone (fluoridzin) [129] and dihydrochalcone [130]. Most apple polyphenols are in the seed and skin, and they persist in extracted pulp after drying [131]. Adding AP to the diet (10, 25, or 25% AP for 98 days) of aging roosters (54 weeks) enhanced sperm viability and motility (7% increase in hatching and fertility) [132]. Semen quality, testicular weight, and histology were not assessed.

## Black seed

*Nigella sativa* L (black seed) belongs to the Alales family and is a dicotyledonous plant widely used in traditional practices [133]. Black seed is volatile and includes 36–38% essential rufen, alkaloid, and saponin [134, 135]. The essential oil derived from black seed contains thymoquinone, p-cymene, and carvacrol [136] and is commonly used for its anti-inflammatory, antioxidant, and anti-cancer effects [137]. Multiple investigations demonstrated antioxidant properties of black seed and its primary constituent, thymoquinone [135]. Seeds and oil from this plant have been used for erectile dysfunction and impotence [138]. Furthermore, antioxidant capabilities of black seed decreased sperm abnormalities [139], augmented the size of sexual organs and sperm production, and enhanced fertility indicators in male rats [139, 140].

Adding 0.5 or 1.0% black seed (seed or oil) in the diet of aging roosters (45 weeks) enhanced semen volume and sperm production, concentration, morphology, progressive motility, and viability [141]. However, reproductive hormones, seminal antioxidant function, and testicular histology were not evaluated.

## Olive oil

In Mediterranean countries, olive oil (OO) is important in the diet and is consumed to prevent cardiovascular diseases [142], alleviate gastrointestinal and fatty liver diseases [143], and prevent cancer [144]. It contains large amounts of monounsaturated and polyunsaturated fatty acids, high concentrations of phenolic compounds with antioxidant activities [145], and other minor components (esters of fatty acids, carotenoids, squalene, phytosterols, tocopherols, etc.). The main antioxidants of OO are lipophilic and hydrophilic phenols [146].

Antioxidants from OO protected cryopreserved buck sperm, mitigated ROS concentrations, and improved post-thawing sperm quality [147]. Boars fed squalene (20 or 40 mg/kg/day for 60 days) had improvements in semen volume, sperm motility, and reproductive performance [148]. Supplementing the extender of aged roosters' semen with OO improved semen quality when semen samples were stored at 5 °C for up to 72 h [149]. In aging male rats, OO improved antioxidant activity (decreased MDA and improved GPX) and increased sperm concentration and motility [150]. In aged (54 weeks) roosters, feeding 0.2 or 0.4 ml of OO improved sperm viability and motility [151]. However, further studies with more comprehensive assessments are needed.

## Lemon grass

*Cymbopogon citratus* L. (lemon grass) is a perennial plant with antioxidant properties. It has a variety of chemical constituents, including steroids, alkaloids, saponins, tannins, anthraquinones, phenols, and flavonoids, conferring antioxidant, antifungal, and antibacterial activity [152]. Lemon grass water extract has been used to treat numerous ailments and infections [153]. In aging (50 weeks) roosters, lemon grass leaves extract (200 to 400 ml/l of water) enhanced concentrations of testosterone and luteinizing hormone [154], with a need for more comprehensive assessments.

## Conclusions and perspectives

In aged roosters, excessive production of ROS and reduced antioxidant defense cause oxidative stress, with deleterious effects on reproductive hormone concentrations, testicular function (including spermatogenesis and Sertoli and Leydig cell functions), sperm (including number, viability, membrane integrity, and motility), and fertility. However, various antioxidants in the diet of aging roosters improved reproductive performance, testicular histology and function, sperm quality, and fertility. The breeding period in breeder flocks is normally 64 weeks. As fertility starts to decrease at 40–45 weeks of age, we suggest dietary supplementation of one or more antioxidants from 43 to 60 weeks of age (spermatogenesis requires ~ 14 days in birds). Antioxidants combat the production of excessive ROS and therefore can substantially reduce effects of aging on reproductive physiology in roosters. As most studies have used only one antioxidant compound, future studies should evaluate effects of a combination of at least two antioxidant compounds, assessing antioxidant interactions and redox regulation of antioxidant-related genes/vitagenes. Furthermore, the antioxidant capacity of each compound needs to be well characterized (e.g., HPLC, GC–MS, Folin–Ciocalteu, DPPH test). In addition, there should be very comprehensive assessments of effects on birds, including hormone assays, assessment of antioxidant status, detailed testicular histology, and collection and assessment of sperm, including fertility. When selecting potential compounds, consideration should be given to product availability and costs, no restrictions for feeding it to animals intended for human consumption, physical form and ease of administration, appropriate absorption, and pharmacokinetics. Based on the current review, there is good evidence that antioxidants used to supplement diets of aging roosters can improve reproduction and have beneficial impacts on poultry production and delay or eliminate the need to add additional roosters (spiking) late in the production cycle, thereby improving biosecurity and animal welfare and increasing profitability and sustainability.

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**Data Availability** The data presented in the present study are available from Dr Sarallah Yarmohammadi Barbarestani on reasonable request.

**Declarations**

**Conflict of interest** The authors declare no competing interests.

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