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Review

The Role of Methionine Supplementation on Oxidative Stress and Antioxidant Status of Poultry-A Review

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Abstract: The physiological status of poultry can be disturbed by different stressors that may lead to oxidative stress conditions. Oxidative stress activates defense systems, which mitigates the adverse effects. Several lines of the poultry defense system exist, including enzyme systems such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and non-enzymatic antioxidants such as Glutathione (GSH). Methionine—a vital amino acid in poultry nutrition—plays a significant role in protein synthesis, transsulfuration, and transmethylation and is also involved in several biochemical pathway activations that can affect the antioxidant system. Therefore, this review aims to summarize the current knowledge on the role of methionine in poultry under heat stress or managing stress, on the antioxidants responsible for scavenging free radicals (GSH) and those responsible for detoxification (SOD, CAT, and GPx). Different levels of methionine supplementation above the requirement (up to 1% Met added on the basal diet) have been tested on the antioxidant status of poultry. It has been shown to improve the antioxidant status and reduce oxidative stress. The results of many experiments on poultry supplemented with diets of different methionine sources indicate that L-Met has good potential to stimulate the antioxidant status of poultry.

Keywords: poultry; antioxidant defense system; methionine sources; oxidative stress

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1. Introduction

Poultry production is affected by many factors, mainly genetics, environment, and nutrition. About 85% of a broiler's performance is due to genetics, while the environment and nutrition contribute by 15% [1]. However, the environment and nutrition should be optimal to allow the animal to express its maximum genetic potential [2,3]. Nutrition, especially amino acids, and energy supply greatly influence the performance of both genetically improved and autochthonous poultry by modulating different pathways responsible for maintaining the physiological status [4–6]. Maintaining normal physiological status, especially in intensive poultry production, is nearly impossible due to stress factors such as high temperature (in summer and tropical regions), high stocking density, and diseases [7–9]. Stress can be defined as a "nonspecific response of the body to any demand", while a stressor can be defined as "an agent that produces stress at any time" [10]. Stress activates the defense system mechanism, resulting in increased demand for energy, amino acids, vitamins, and trace minerals and, consequently, poor production performance. The effects of stress are mainly managed or avoided by dietary supplementation with antioxidants or environmental management. Oxidative stress caused by an environmental stressor alters an animal's physiological status [11]. Oxidative stress occurs when there is an overproduction of reactive oxygen species (ROS) or an insufficient antioxidant defense system [12]. Mitigation strategies involve elevated dietary supplementation of nutrients in antioxidant defense systems, such as vitamins, trace minerals, and amino acids.

Studies with amino acid supplementation in poultry have been conducted for about half a century [13,14]. Due to the importance of amino acids in poultry production, many studies have been conducted regarding the appropriate amount of different amino acids in the diet [15–17]. In most poultry diets, methionine (Met) is the first limiting amino acid which plays a vital role in the biosynthesis of other essential molecules such as cysteine, carnitine, and taurine, and is converted to S-adenosylmethionine (SAM), which serves as a methyl donor in the birds' metabolism [17]. Birds cannot synthesize a sufficient amount of Met to sustain average growth; hence it must be supplemented in their diet [18–20]. As Met is involved in several routes in antioxidant defense systems, oxidative stress can raise requirements.

The optimal Met requirements have been established for carcass quality, productive performance, and growth performance [21–25]. In poultry, there are different Met requirements for different species and breeds due to the breed's different growth rates and genetic potential [26]. For example, according to NRC [27], the Met requirement for commercial broilers at the starter, grower, and finisher phases are 0.50%, 0.38%, and 0.32%, respectively. Growing turkeys require a recommended Met of 0.55%, 0.45%, 0.40%, and 0.35% for 1–4 weeks, 5–8 weeks, 9–12 weeks, and 13–16 weeks, respectively [27]. Several studies have been conducted to evaluate the effects of varying the optimal Met recommended for growth rate, feed intake, feed conversion ratio, and meat quality on the antioxidant status of poultry [8,20,23,25]. From the above-cited studies and NRC 1994, commercial broilers do not need more than 0.5%, and 0.38% Met for optimum performance in the starter and grower period. However, higher Met levels have been used to evaluate the antioxidant status of broilers (Figure 1). Met levels in starter diets ranging from 0.2 to 1.32% and in grower diets from 0.24 to 1.3%, along with the Met to Lys ratio of 20 to 110%, have been used in experiments on antioxidant responses of commercial broilers under stress (Figure 1). The increased dietary Met levels improved the antioxidant status with no adverse effect on the growth performance, indicating that a high level of Met is needed to facilitate antioxidant function.

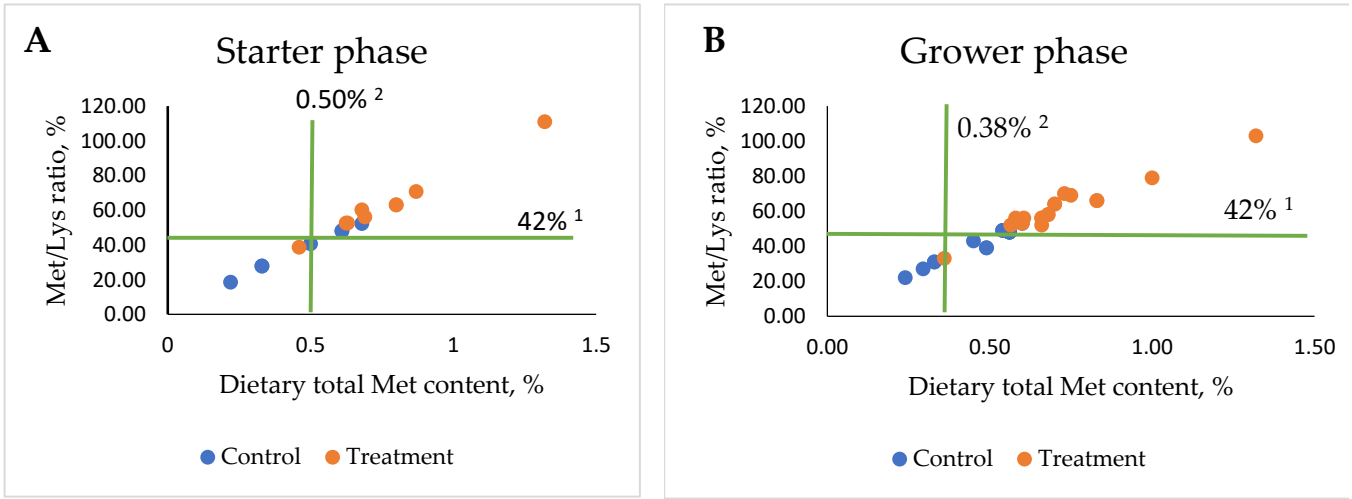

Figure 1. Dietary total Met levels and Met to Lysine ratios used in starter and grower diets from various studies conducted in broilers. (**A**) Relationship of total dietary Met content to Met/Lys ratio in the starter phase of broilers. (**B**) Relationship of total dietary Met content and Met/Lys ratio in the grower phase of broilers. ([1] NRC 1994 Methionine to Lysine ratio recommendations, [2] NRC 1994 Met recommendation in broilers diet in respective feeding phase).

Therefore, this review aims to summarize the current knowledge on the role of Met in poultry under heat stress or managing stress, on the antioxidants responsible for scavenging free radicals (GSH) and those responsible for detoxification (SOD, CAT, and GPx).

1.1. Methionine Supplementation of Poultry

Met as a feed-grade amino acid in poultry diets was first introduced in 1951. Since then, a variety of Met sources have been used to supplement bird diets, such as L-methionine (LM) [28–30], DL-methionine (DLM), a combination of the L and D enantiomers [30–34], the Met analog DL-2-hydroxy-4-(methylthio) butanoic acid (DL-HMTBA), also known as MHA, which is sold as a calcium salt (MHA-Ca) or as a free acid (MHA-FA) [35–40]. In contrast to DLM and DL-HMTBA, mostly employed as Met sources in chicken feeds, LM is a naturally occurring form of Met that may be utilized directly by birds. These sources differ in bioavailability depending on their metabolism in poultry [41,42], therefore differing in their effect on performance. While L-Met and D-Met are actively absorbed (transported against a concentration gradient), MHAs are passively absorbed through diffusion from a more concentrated medium to a less concentrated medium [35]. Physiologically, D-isomer amino acids cannot be utilized in the animal cell; instead, they must be converted to the corresponding L-isomer before being utilized in protein synthesis [43]. DLM and HMTBA must be converted to LM to be utilized in the body, mainly by the liver and kidney as the main organ [17,42]. Some experiments showed that D-Met is 90 to 100% as efficacious as L-Met, whereas HMTBA is 65 to 100% as efficacious as DL-Met [44].

1.2. The Antioxidant System of Poultry

Stress control, which may be due to management, nutrition, technology, and environmental elements, as well as internal stress, is one of the unavoidable issues in poultry production. One typical source of stress is an excessive generation of free radicals, which causes oxidative stress at the molecular level [45]. As a result, animals' antioxidant defense mechanisms developed over time to provide sufficient protection in an environment with high oxygen levels. There are multiple lines of antioxidant defense in the antioxidant defense network. The first line comprises antioxidant enzymes that are in charge of detoxifying the superoxide radical (the prominent biological radical) and its metabolic products. Superoxide dismutase, glutathione peroxidases (six different types in avian species), and catalase are all part of the first line of antioxidant defense [46,47]. Some metals, such as free iron and copper, which play a significant role in catalyzing free radical production and metal-binding protein, are included in the first line of antioxidant defense [46]. Chain-breaking antioxidants (such as vitamin E, carotenoids, ascorbic acid, glutathione, and uric acid) are responsible for chain oxidation reaction limitation and termination in the second line of antioxidant defense [46].

Specific enzymes that deal with the damage caused by free radicals and hazardous products of their metabolism make up the third level of antioxidant defense (e.g., methionine sulfoxide reductase, and DNA-repair enzymes) or removal (phospholipases, proteasomes) [44,45]. Protective protein modifications that prevent inactivation, such as glutathionylation and other modifications, comprise the fourth stage of antioxidant defense. Apoptosis, interestingly, can be included in the antioxidant defense network to cope with terminally damaged cells that cannot be restored. In general, the integrated antioxidant defense network's principal purpose is to maintain optimum redox status—the balance between the process of production and inactivation/detoxification of ROS/Reactive Nitrogen species (RNS) [46,47].

The antioxidant defense system in commercial poultry production requires external assistance, such as dietary supplementation of traditional antioxidants such as vitamin E or other nutrients with regulatory functions in the antioxidant defenses, such as taurine, carnitine, and branched-chain amino acids.

1.3. How Antioxidants Network Work under Oxidative Stress

When ROS/RNS overpowers the antioxidant defense system's ability, oxidative stress damages several biological compounds such as polyunsaturated fatty acids, proteins, and DNA [48]. In response to oxidative stress, the antioxidant defense system employs some strategies to restore the situation to normal [46] (Figure 2). Foremost, they reduce the

formation of free radicals, decreasing the activities of enzymes involved in ROS/RNS formation, such as NADPH oxidase and xanthine oxidase, and by the action of iron and copper bound to protein which prevents the formation of new free radicals. Second, the critical maintenance of mitochondrial integrity is the biological system's principal source of free radicals. Third, crucial elements of an antioxidant defense strategy include detoxification/decomposition of free radicals and non-radical harmful chemicals (SOD, GPx, catalase, etc.) and scavenging free radicals (e.g., vitamin E, vitamin C, GSH, coenzyme Q). Fourth, vitamin E's biological antioxidant effectiveness may be increased by the recycling mechanism that maintains it active (ascorbic acid, thioredoxin reductase (TrxR), vitamins B1 and B2). Fifth, redox signaling, transcription factor (Nrf2), and vitagene activation are the main components of the anti-stress strategy. Additional protective molecules with antioxidant and detoxifying properties are also produced. Sixth, enzymatic mechanisms (heat shock proteins, HSP; methionine sulfoxide reductase, Msr; DNA repair enzymes, etc.) are involved in repairing damaged molecules, after which the damaged molecules are removed to prevent accumulation. Last but not least, the antioxidant defense network consists of apoptosis, autophagy, and other procedures that eliminate fatally injured cells and stop the damage from spreading to neighboring cells and tissues [46].

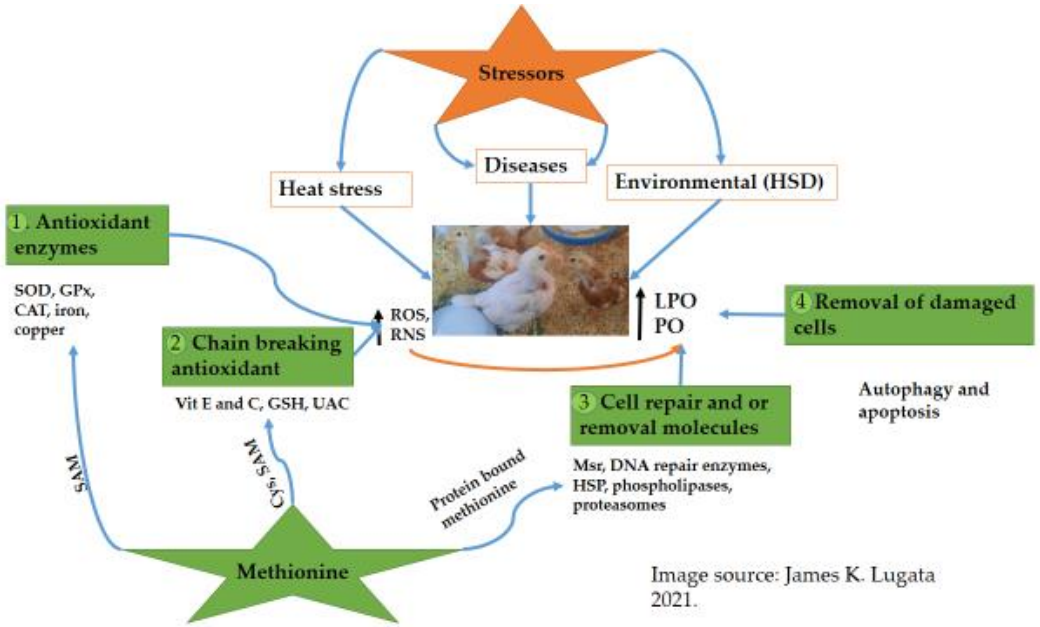

Figure 2. Overview of the effect of stressors, the antioxidant system function, and the influences of Met on poultry's antioxidant system/status. Adapted from [17,46,49]. The stressors greatly affect poultry production, including heat stress, diseases, environmental, technological, and nutrition, by altering the physiological status due to an increase in the production of Reactive Oxygen Species (ROS) and Reactive Nitrogen species (RNS). The poultry antioxidant defense system plays a great role in detoxifying and preventing ROS's effect. Four levels of antioxidant defense have been suggested. (1). The first level deals with detoxifying free radicals at the beginning of forming (superoxidase dismutase -SOD, glutathione peroxidase -GPX, catalase -CAT, and chelating metals such as iron and copper). (2). The second level of antioxidant defense deals with scavenging the free radicals (vitamin E and C, glutathione -GSH, and uric acid -UAC). (3). The third level involves cell repairs and removing the damaged molecules; this includes methionine sulfoxide reductase (Msr), heat shock proteins (HSPs), and DNA-repairing enzymes. (4). The 4-fourth level deals with removing damaged cells and preventing the spread of the damage (autophagy and apoptosis). Met, directly and indirectly, influences all the major antioxidant defense levels through the ROS scavenging role of protein Met residues and cysteine (Cys) and SAM, which then influence the activity of the antioxidant enzymes such as SOD and CAT.

A growing body of research suggests that all antioxidants in the body operate collectively as a "team" to maintain adaptive homeostasis. There are cooperative interactions when one team member helps another to work more effectively. All cell compartments, including the mitochondria, nucleus, and cytoplasm, have antioxidant defense mechanisms that are expressed tissue-specifically. These mechanisms include internal antioxidants (AO enzymes, GSH, CoQ, uric acid, carnitine, taurine, etc.) and antioxidants consumed through diet (vitamin E, carotenoids, synthetic antioxidants, carnitine, silymarin, etc.). The degree of stress affects the expression and activity of numerous antioxidant enzymes, including SOD, GPx, and other selenoproteins [46].

2. Role of Methionine on Oxidative Stress/Status of Poultry under Heat Stress

The effects of Met supplementation through different routes (in ovo, water, and diet) on poultry's oxidative stress and antioxidant status under heat and cold stress conditions are summarized in Table 1. Heat stress (HS) is the primary stress inducer that leads to significant problems in poultry production [50]. Heat stress causes several harmful effects in poultry, including disruption of mitochondrial function, increased ROS production and lipid peroxidation, reduced vitamin concentration, and altered antioxidant enzyme activity (reviewed by [47]). Both acute and chronic heat stress has been shown to alter the antioxidant system of poultry. ROS is generally produced in animals via various physiological and non-physiological processes such as the Fenton reaction, cellular respiration, mitochondrial dysfunctions, pathologies (infections), phagocytes, neutrophils, and stress [45].

Table 1. The effects of dietary Met supplement (in ovo, diet, water) on the oxidative stress on poultry under heat stress and cold stress conditions.

Met Sources/ Poultry Species	Met Levels	Results	References	Remarks
DLM and HMTBA Cornish Cross cockerels 31 °C from 14 to 42 days	BD = 0.276% MS (DLM = 0.309 and 0.404% in grower; 0.277 and 0.360% in finisher correspond to 100% or 130% required Met, respectively) MS (HMTBA = 0.719 and 0.934% in grower; 0.644 and 0.837%) (88% DLM and 58% HMTBA used as sources of Met)	130% ↑ hepatic GSH, GSSG in grower, and plasma FRAP finisher. 130% ↓ GPx, GST, and SOD in grower adipose tissues and ↓ plasma GSH, hepatic GSSG but ↑breast GSSG in the finisher broiler. In grower; DLM ↑ breast GSH and thigh GSH and GSSG than HMTBA 130% ↓ hepatic GPx and adipose tissue GPx and GST activities of the finisher broiler. In grower broiler: DLM; ↓ activities of breast GPx, thigh GR, adipose GST and all assayed antioxidant enzymes in the liver as compared with HMTBA	[7]	Chronic heat stress 30% extra Met improved the antioxidant status of broilers under HSNo effect of MS (either source or levels) on BW, average daily gain and feed conversion of birds throughout the study. DLM increased the FI in finisher birds as compared with HMTBA.
DLM DL-HMTBA HS 32 °C until 6 weeks of age Male Ross broiler	BD = 0.28% Met MS = 1.0 or 1.2 g/kg for each source (0.10% or 0.12% of either DLM or DLHMTBA added to basal diet)	In week 4: ⇀ FRAP on HS and MS ↓ MAD, but ↑UA in HS chicks In week 6: ⇀ plasma FRAP, SOD, UA by either HS or MS HS ↑ MDA in DLM but not DL-HMTBA ↑ hepatic ratios of reduced GSH to total GSH and reduced GSH to GSSG in HMTBA at HS	[50]	Chronic HS (4 weeks exposure): 0.1% and 0.12% of either DLM or HMTBA did not affect the antioxidants of broilers. HMTBA improved growth performance (FI and BW) at 5 and 6 weeks of age
DL-Met Male cobb broilers for 42 days	Met deficient (MD)-without Met supplementation (MS) Control (MD + 0.24% Met) in starter diet, (MD + 0.12% Met) in grower diet)	Met deficiency leads to oxidative stress in both the liver and kidney of growing chicks ↑ MDA ↓ SOD, CAT, GSH-PX, and GSH	[51]	Met-deficient (MD) causes injury to the kidney and liver. MD depressed body weight (BW) from 14 to 42 days

Table 1. *Cont.*

Met Sources/ Poultry Species	Met Levels	Results	References	Remarks
MHA Broilers	Basal diet + 0.46, 0.36 and 0.32% in starter, grower, and finisher, respectively	The antioxidants activity was improved by MHA supplementation	[52]	0.2% of vitamin C supplemented together with MHA, no effect on BW
Free Met DL-Met, and Met dipeptide (DL-MM Male cobb broilers, from 21 to 42 days	MD-without MS DL-M (MD + 0.27%) DL-MM (MD + 0.28%) (MD = 0.543, DL-M = 0.810, DL-MM = 0.809 digestible Met + Cys)	Heat stress increased the expression of GSS and GPX. Met supplementation (MS) reduced the expression of GPX	[53]	Thermoneutral group 27–18°CC; Heat stress continuous exposure 30°CC, 60% RH from 21 to 42 days MS increased BW
DL-Met Male cobb 500 Heat stress (HS) 38 °C for 24 h.	Starter: MD-, DL1 = MD + 0.295%; DL2 = MD + 1% Grower: MD-; DL1 = MD + 0.275%; DL2 = MD + 1%	Broilers reared in HS and fed DL1 and DL2 increased expression levels of GSSG and GPx7 in both feeding phases	[54]	Acute heat stress MS increased BW, DL2 reduced FI
DL-2-hydroxy-4-methylthiobutanic acid (DL-HMTBA) HS 38 °C for 24 h at 41 day Male cobb 500	Starter MD; DL-HMTBA: = 0.45% Grower DL-HMTBA = 0.42% (MD = 0.58, and 0.54; MS = 0.88 and 0.81 in starter and grower digestible Met + Cys, respectively)	↑ TRxR1, SOD, and MsrA in starter under HS and fed DL-HMTBA diet. Interaction of the HS and MS observed In grower: HS ↑ SOD, TRxR1, and MsrA; MS also ↑ levels of expression than MD but not the interaction between temperature and diet in a grower phase.	[55]	Acute heat stress HS lowered the feed intake (FI), and BW gain MS did not influence FI and BW gain
DL-Met Male cobb 500 broilers HS 27.4 °C ± 0.3 °C, and mean relative humidity was 63.7 ± 0.6% from 21 days until day 35	Control diet = 0.50, 0.42 and 0.39% in starter, grower, and finisher DLM 1 = 0.19, 0.16 and 0.15% added in starter, grower, and finisher diets, respectively. DLM2 = 0.37, 0.32, and 0.29% added in starter grower and finisher diets, respectively.	HS: ↓ plasma tocopherol, GSH: GSSG and ↑ GSSG, ↑ hepatic GSH, GSSG and ↓ Vit C than the thermoneutral group. High Met levels ↑ GSH and GSSG levels in the liver and thigh muscle of broilers subjected to HS High Met levels also ↑ tocopherols in the liver and plasma of heat-exposed ⇆ TBARS, GSH: GSSG	[56]	Chronic heat stress HS affected broilers' performance negatively. MS did not improve the birds' performance under HS
In ovo Met –cysteine (Cys) injection HS (39.6 °C for 6 h/d) from day 10 until day 18	5.90 mg L-Met plus 3.40 mg L-cysteine	↑ TAC and GSH in serum and tissues of newly hatched chicks in Met + Cys injected group	[57]	Met-Cys injection elevated the antioxidant capacity of chicks exposed to HS during incubation. No effect on hatchability
In ovo Met-Cys injection Ross broilers HS (39.6 °C for six h daily) between 10 and 18 days of the incubation	5.90 mg L-Met plus 3.40 mg L-cysteine	↓ HSP70 and ↑GSH-Px expression in the liver, jejunum, cardiac muscles, and pectoral muscles tissues ↑ T-SOD in serum and tissues ↑ GSH-Px and GSH/GSSG ratio in serum and tissues ↓ MDA in serum and tissues ⇆CAT except in pectoral muscles	[58]	In ovo injection of Met + Cys improved the antioxidant status of the newly hatched chicks exposed to heat stress during incubation.
DLM and HMTBA Peking ducks HS (summer temperature range (mean of highest) 32.1 °C to 24.7 °C (mean of lowest)	Basal diet (BD) = 0.45 and 0.40% of Met in starter and grower period 0.05%, 0.2% and 0.35% of either DLM or HMTBA were added to the basal diet during starter and grower periods.	0.35% ↑ HSP70 mRNA expression of the intestine and liver ↑ MDA by 0.35% of DLM on day 16. 0.35% DLM ↑ MDA on day 35. 0.35% HMTBA ↑ HSP70 expression compared with other treatment groups.	[59]	HMTBA improved the antioxidant status of the liver and intestine on day 35. Growth performance was not reported.
DLM and HMTBA HS 35 °C and TN 24 °C Male Ross 308.	MS; DLM = 0.31 and 0.51% = starter, 0.26 and 0.43% for grower) HMTBA = 0.34 and 0.57% for starter, 0.29 and 0.49% for grower diet.	TGSH and GSH were higher in the HS group. DLM had lower TBARS in the acute phase. ⇆ GPx. ↓ GSSG in super-adequate digestible sulfur amino acid (DSAA). GSH: GSSG ratio was higher than inadequate DSAA.	[60]	MS did not have significant influences on the antioxidants or the bird performance

Table 1. *Cont.*

Met Sources/ Poultry Species	Met Levels	Results	References	Remarks
DLM HS (38 °C for 24 h, starting on the sixth day TN (25 °C) Male meat quails.	MD, MS = 0.27% (MD = 0.57% MS = 0.84% Met + Cys digestible)	HS induced ↑ GPx, ↑UA, ↑ H_2O_2 production, ↓GSH, ↓ MAD levels on MS diet. Interaction between HS and diet on GPx and CAT	[61]	MS can mitigate ROS-induced damage by increasing the activities of antioxidants. MS did not affect feed consumption and weight gain.
DLM. Breeder Japanese quail	BD = 0.70% Met + Cys; BD + MS with DLM to provide proportions concentrations of 1.15, 1.30 and 1.45 times the quail requirements per NRC (1994) recommendations.	MS ↑ plasma and liver SOD, CAT, GPx, and ↓ MAD. 1.15 times the NRC ↑ plasma and liver SOD, CAT, GPx and ↓ MAD in HS quails. MS ↑ plasma TAC compared to HS and TN groups.	[62]	Met could improve the performance, immunity, and antioxidant status of quails by reducing the adverse effects of HS. MS improved the productive performance of birds.
DL-HMTBA Broilers. [Low (12 to 14 °C) vs. control temperature (thermoneutral, 24 to 26 °C)] from 8 to 28 days of age.	BD = 0.32% Met MS = 0.17% or 0.51% of DL-DL-HMTBA added to BD (HMTBA containing 88% of active substance)	0.51% ↑ hepatic GSH and GSH-Px and lung SOD activity of broiler. Low temperature reduces the expression of GSH synthetase and increases GSH reductase gene expression. Higher DL-HMTBA induced ↑ GST in the lung, GSH synthetase in the liver and lung, and ↓ GSH reductase in the lung at low temperatures.	[63]	At low temperatures, 0.51% MS ↑ GSH synthesis gene expression hence increases the antioxidant capacity in the liver and lung No effect of MS on the growth performance
DL-HMTBA Broilers. 12–14 and 24–26 °C for Low ambient temperature (LAT) and normal ambient temperature.NAT	BD = 0.32% Met as-fed basis MS (0.17, 0.34, 0.51, and 0.68% DL-HMTBA added to BD)	LAT ↑ serum MAD and PC on day 21; ↓ serum GSH, GSH-Px and TAC on days 21 and 28. MS ↑ serum GSH content, GSH-Px activity, TAC and SOD at day 28, and SOD and GSH-PX activities and ↓ PC at day 21 under LAT conditions.	[64]	MS improved the serum antioxidant activities in a dose-dependent manner under LAT conditions. LAT ↓ BWG and ADFI at days 7–14 and 15–21 of the experiment.
Zinc- Met Male cobb 500 The average daily temperature ranged from 33 °C to 36 °C	BD = 0.25, 0.23 and 0.15% Met for starter, grower, and finisher diets, respectively. MS = 0, 0.025, 0.05, and 0.10% ZnMet (98% ZnMet purity)	The higher level of Zn-M ↑ plasma GPx activity and ↓ muscles MDA in broilers reared in higher ambient temperature	[65]	Chronic stress ZnM supplementation increased the BWG and BW and improved FCR under HS

⇀ Represents no difference, or similar or no effect. ↑ Represents increased or high or upregulated. ↓ Represents decreased or low, or downregulated. Abbreviations: BD— basal diet, MS—methionine supplementation, MD—methionine deficient, HS—heat stress, DL—DL-methionine, MHA/HMTBA—methionine hydroxyl analogue/DL-2-hydroxy-4-(methylthio)butanoic acid; T-SOD—Total superoxide dismutase, TAC—total antioxidant capacity, GPx—glutathione peroxidase, PC—protein, MDA—malondialdehyde, GSH/GSSG—glutathione and glutathione disulfide ratio, FRAP—ferric reducing the ability of plasma, SOD—superoxide dismutase, CAT—catalase, GSH—glutathione, TRxR1—thioredoxin receptor 1, MsrA—methionine sulfoxide reductase A enzyme, HSP70—heat shock protein 70, UA— uric acid.

Met protects against oxidative stress in two ways (Figure 3): first, by indirectly producing antioxidants (precursors of cysteine and glutathione), and second, by Met residues in proteins. [53]. The latter route is during repair, where several ROS products attack Met residues in proteins to form Met sulfoxide, producing cells by scavenging the reactive species [49]. Met sulfoxide reductases (MSRs) are enzymes that reduce reactive oxygen species in all organisms, from bacteria to mammals (ROS). This is performed by oxidizing eight Met residues per protein molecule while maintaining the enzymatic integrity of the individual protein. Thioredoxin (TRx) prepares the enzyme for another catalytic cycle by reducing the oxidized MSR. NADPH is also a reactive nitrogen and oxygen species scavenger [49]. The antioxidant potential through this pathway is influenced by heat stress and Met supplementation (MS) [55], where TRxR1 and MsrA genes were highly expressed in heat-stressed broilers and supplemented with DL-HMTBA.

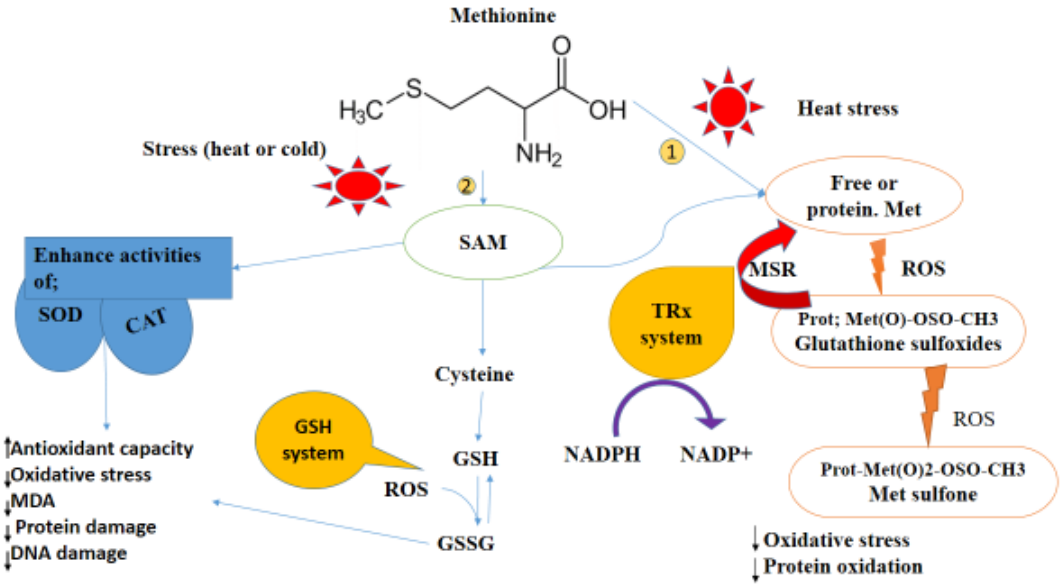

Figure 3. Role of dietary Met and free/protein-bound Met in the poultry antioxidant system under heat stress. Adapted from [66–68]. (1). Through the involvement of Met residues in proteins during ROS oxidation. Methionine's sulfur side chain is oxidized by reactive oxidative species, which may cause Met sulfoxide to undergo additional oxidation to form methionine sulfone under prolonged exposure to ROS. Methionine sulfoxide reductase can convert methionine sulfoxide back into Met, but it cannot convert methionine sulfone. (2). Activation of antioxidant enzymes (SOD and CAT) and production of GSH, which reduce oxidative stress caused by heat stress through Met metabolites.

Met participation in the synthesis of cysteine, S-adenosylmethionine, and glutathione has led to extensive studies on the effect of Met supplementation on glutathione's role in heat-stressed poultry (Table 1, Figure 3). Several studies have reported the high-level glutathione in heat-stressed broilers with Met supplementation [53,56,58]. In another study involving DL- Met on the enzymes (cystathionine β-synthase, glutathione synthetase, and glutathione peroxidase) participating in glutathione synthesis, higher expression was observed in heat-stressed broilers [54]. Increasing the expression of these enzymes results in the effective synthesis of these most potent antioxidants and, thus, a significant contribution to the scavenging system's ability to alleviate the ROS effect. [36]. In ovo Met injection into eggs exposed to heat stress from d10 to d18 increased GSH in serum and tissues of newly hatched chicks [57], GSH-Px gene expression, and activity of GSH-Px and GSH/GSSG in serum and tissues of newly hatched chicks [58]. MS has proved to provide protection not only during high temperatures but also during low ambient temperatures. The studies involving HMTBA with different levels at low temperatures (12 to 14 °C) increased the hepatic GSH and GSH-Px at high levels of MS [63,64].

Apart from TRx and glutathione systems, the influence of MS on various enzymatic antioxidant systems, which includes the action of SOD and CAT in heat-stressed poultry, has been extensively studied [50,55,58,62]. SOD is an essential enzyme for eliminating superoxide free radicals generated during a dismutation reaction. The H_2O_2 that is produced is removed through catalase-catalyzed processes or the glutathione system [69]. On the other hand, HS induces the production of H_2O_2 and MS was able to mitigate it by increasing the activity of CAT in the plasma and liver of quails [54,62]. Several studies have been carried out on the influence of the MS effects on SOD under different heat-stress conditions and life stages of poultry. Most of these studies confirmed that MS significantly increases SOD regardless of the tissues in heat-stressed poultry [7,60,61]. In addition, Met deficiency has decreased SOD and CAT even in thermoneutral-reared broilers [49]. Furthermore, other antioxidants such as total antioxidant capacity, ferric reducing the power of plasma,

and non-enzymatic antioxidants (vitamin C and tocopherols) are also influenced by MS in heat-stressed poultry [50,56,57,62].

3. Effect of Met on Poultry Oxidative Stress Underlying Health Conditions

The antioxidant potential of Met has also been investigated in different conditions, such as viral and bacterial infection and stocking density (Table 2). Both infection and stocking density could lead to drastic impacts on poultry production. High stocking density (HSD) has been seen as farmers' optimal strategy to increase broiler production in small areas. However, it has been reported to increase oxidative stress levels in broilers [70,71]. Several experiments have documented that HSD or overcrowding induced oxidative stress in broilers by increasing the level of MDA and decreasing GSH levels, SOD, and GSH-Px activities in different tissues [72–74]. A recent study indicated that HSD caused an increase in protein carbonyl oxidation and malondialdehyde (MDA) and decreased total antioxidant capacity of broiler as well as GPx of broiler, and that effect was mitigated by dietary Met supplementation [71]. In another experiment, Magnuson and his colleagues reported that an additional 30% of the Met recommendation provides partial protection against the adverse effect of HSD in age and tissue-dependent manner [8]. For example, HSD decreased hepatic GSH but not in the growers' plasma, breasts, and thighs. The 30% extra MS caused a significant reduction in MDA concentration in the thigh and breast muscles of the finishers' broiler chicken. However, the higher level of MS caused undesirable outcomes in the small intestinal wall in turkeys challenged with HEV by decreasing SOD and increasing LOOH and MDA concentration, as well as CAT activity [40]. In their experiment, Met's role in oxidative stress was tissue-dependent, with its function being impaired in the intestine but not in the plasma and liver of turkeys. In the mixed model infection study, it was discovered that broilers fed diets supplemented with 0.31% L-Met (0.61% of Met for 100% TSAA in the diet) had elevated amounts of hepatic GSH compared to those fed diets deficient in L-Met (0.30% and 0.45% of Met in the diet), reducing the negative effects of coccidiosis on the antioxidant defense system [75]. The lower availability of substrates necessary for GSH synthesis, especially Cys, which is regarded as one of the most limiting AA in this process, may cause the decreased GSH concentration in broilers fed a Met-deficient diet. Del Vesco [54] claims that adding Met to the diet at recommended or excessive levels causes broilers' expression of the genes that control the antioxidant system of GSH to rise. In another study with broilers medicated or vaccinated against coccidia under an *Eimeria tenella*-challenged situation, an increased GPx activity and total antioxidant capacity (TAC) in serum were linked to increasing dietary Met concentrations from 0.45% to 0.56% (from the addition of 0.15 to 0.27% Met) in treated chickens. However, in chickens that had received vaccinations, the rise in dietary Met concentrations had no such impact [76].

Table 2. Effect of Met on poultry oxidative stress/antioxidant status at different stocking densities and health conditions.

Stocking Densities/ Poultry Species	Met Source/ Met Levels	Antioxidants Studied	Results	References	Remarks
Male Cornish Cross cockerels SD: (9 and 12 birds/m^2)	MS (DLM: (grower: 0.29 or 0.377% and finisher: 0.260 or 0.338%) representing 100% and 130% of the recommendations.	GSH, GSSG, MDA, FRAP, GPx, SOD, glutathione S-transferase (GST), and glutathione reductase (GR).	HSD ↑ GSH in the liver but ↓ GSH in other tissues. In finisher: HSD ↓ GSSG in plasma, liver, breast, and GSH in the thigh. 130% ↓ GSSG in the plasma but ↑ GSH and GSSG 130% MS ↓ hepatic SOD, MDA in the breast and thigh muscles ⇆ FRAP, PC, and corticosterone HSD ↓ thigh GST in both phases.	[8]	30% extra MS partially attenuated the adverse effects caused by stocking density.

Table 2. *Cont.*

Stocking Densities/ Poultry Species	Met Source/ Met Levels	Antioxidants Studied	Results	References	Remarks
MHA and DLM Female Hybrid Converter turkey Stress inducer: hemorrhagic enteritis virus (HEV)	BD = 0.40 and 0.35% for weeks 1–4 and weeks 5–8) MS = 0.15 and 0.38% in weeks 1–4 of age, and 0.10 and 0.30% in weeks 5–8 of age, respectively)	MDA, Lipid peroxides (LOOH), GSH + GSSG, FRAP, vitamin C, SOD and CAT.	In blood redox, High-level MS ↑ of GSH + GSSG, LOOH, and ↓ MDA MHA ↓ Vitamin C, GPx, FRAP, LOOH and ↑ SOD, and CAT than DLM. In the intestinal wall: Higher level ↑ CAT, LOOH, MDA, and lower SOD.\ DLM ↑ CAT and ↓ MDA than MHA In liver: ↑ Met; ↑ CAT, SOD LOOH, and ↓ MDA. DLM ↑vitamin C and ↓ SOD	[40]	DLM had more effects on the redox status in the small intestine, blood, and liver of turkeys than MHA
Arbor Acres Broiler: (14 and 20 broilers per m^2) in the finisher phase (from 21 to 42 days)	Basal diet (BD) = 0.29% Met MS (DLM = 0.05, 0.1, 0.15, or 0.2% added to BD (99% DLM purity used)	Plasma T-SOD, Total antioxidant capacity (TAC), GPx, PCO, MDA, GSH/GSSG	HSD ↓ TAC, GPx, ↑ PCO, and MDA of plasma. ⇆ T-SOD and GSH/GSSG in HSD as compared with LSD. 0.2% MS ↑ GPx, GSH/GSSG ratio in the plasma. 0.15% MS ↑ T-SOD and ↓ GPx in the liver under HSD ⇆ SOD and GPx mRNA expression. 0.15–0.2% ↓ MDA and ↑ T-SOD in the jejunum.	[71]	MS mitigates the oxidative stress caused by HSD (0.1% to 0.15% should be supplemented.
DLM Male partridge shank broilers medicated or vaccinated against coccidia under EImeria tenella-challenged situation	BD = 0.30% Met MS (DLM = 0.15 0.27 and 0.39% from 22 to 42 days of life.	GSH-GPx, TAC, MDA	The serum antioxidative of chicken were not consistent ⇆ serum MDA by anticoccidial programs Interaction of anticoccidial program and Met level on TAC and GPx. High Met levels ↑ MDA in medicated and vaccinated chickens High Met levels ↑ GSH-GPx and TAC in medicated chickens only	[76]	Increased MS levels could not alleviate the oxidative stress effect in the mixed infection model.

⇆ Represents no difference. ↑ Represents increased or high or upregulated. ↓ Represents decreased or low, or downregulated. Abbreviations: DL—DL-methionine, T-SOD—Total superoxide dismutase, TAC—total antioxidant capacity, GPx—glutathione peroxidase, PCO—protein carbonyl oxidation, MDA—malondialdehyde, GSH/GSSG—glutathione and glutathione disulfide ratio, FRAP—ferric reducing the ability of plasma, SOD—superoxide dismutase, CAT—catalase, GSH—glutathione, LOOH—lipid peroxides.

4. Influence of Met Sources and Levels on the Antioxidants Defense System in Poultry (MDA SOD, CAT, GPx, and GSH)

4.1. Effects of Met Sources on Oxidative Stress/Antioxidants of poultry

Several parameters related to redox capacity or antioxidant status, which are markers of oxidative stress, have been studied as the responses to dietary Met in broilers [8,36,63,76,77], turkeys [25–27,33,40], quail [78], ducks [79], and piglets [80]. The most common and studied antioxidants in different tissues of poultry include total antioxidant capacity (TAC), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione (GSH). According to research, antioxidant status varies depending on the Met source, with HMTBA having a higher antioxidant status than DLM in broilers [36,63] and turkeys [23,33]. Regarding Met, LM is expected to provide immediate benefit in oxidative stress conditions or with antioxidant status because it is directly available for use in the synthesis of GSH. This is evidenced when the three Met sources are compared on GSH, with LM being more effective in improving GSH regardless of the tissue and level, followed by MHA and then DLM [20,23]. In addition, in some experi-

ments, MHA has been shown to have better antioxidant status by reducing the rate of lipid peroxidation and increasing the hepatic concentration of total and reduced GSH in broilers and turkeys compared to DLM [25,36,50].

Experiments have been carried out regarding the influence of Met sources on antioxidant enzymes, but only a few studies have included all the sources of Met (Table 3). In a study on turkey, SOD and CAT were influenced by sources differently; SOD activity was observed to be increased in turkey fed with DLM diets, while CAT was higher in LM, followed by DLM, and last MHA in the breast muscles [24]. In another experiment on turkeys, the SOD activity in the small intestinal homogenates was influenced by MET sources (DLM > LM > MHA). At the same time, the same pattern was not observed in the liver and plasma [23]. This indicates the dependence of the tissues being analyzed. For example, the activities of SOD and CAT in the liver were affected differently by the dietary Met level and sources, and the difference was not clear and hard to interpret.

Table 3. Met sources influence poultry oxidative stress and antioxidant status.

Met Sources/Poultry Species	Met Levels	Main Results	References	Remarks
DLM and LM and HMTBA/male cobb 500 broiler	MS = 0.22% DLM, 0.22% LM or 0.31% HMTBA	In breast muscles: ↑ TGSH and rGSH in LM and HMTBA were observed. ⇆ by met source for MAD, FRAP, and the ratio of rGSH: GSSG and GSSG: TGSH.	[20]	Met + Cys deficiency did not compromise the antioxidant capacity of chickens
LM, DLM, and MHA Hybrid Converter turkey	BD = 0.40, 0.34, 0.29 and 0.26% Met for 1–4, 5–8, 9–12 and 13–16 weeks respectively; MS = NRC 1994 –Low and 40% extra NRC recommendation-High	Higher Met content ↑ SOD; CAT activity, Vit C concentration, and ↑ plasma FRAP, TGSH, and ↓ MDA ⇆ SOD, Vit C, MDA, or LOOH in the small intestinal or liver of turkey-fed diets with different Met levels. MHA ↑ TGSH and ↓ SOD, and MDA in the plasma compared with DLM. LM ↑ TGSH compared with DLM In the small intestine, SOD; DLM > LM > MHA ⇆ MDA and ↑ LOOH in LM compared with other Met sources. In liver: ⇆ Vit C, LOOH, and SOD but interaction for SOD, CAT, and LOOH.	[23]	A higher Met level improved the indicators of redox status MHA lowered plasma, and intestinal SOD more than DLM or M. LMH and LM decreased plasma and hepatic MDA and increased plasma glutathione levels.
DLM, LM, and MHA Hybrid converter turkey	Low = 100% and High = 150% of NRC (1994) recommendations for each feeding phase	Met source and Met level; ⇆ vit C, LOOH, and MDA Higher Met Level ↓ CAT, ↑ SOD DLM: ↑ SOD CAT: LM > DLM > MHA	[24]	MHA reduced the CAT activity in the breast meat compared with LM and DLM sources.
DLM and L-Met Hatched turkey.	basal diet (BD), the BD + 0.17 or 0.33% DL-Met or L-Met (60, 75, and 90% for SAA of NRC)	L-Met ↑ GSH and ↓ MAD in the liver than DLM during 28 days. MS regardless of the source ↓ MAD in the duodenal mucosa. ⇆ PC, TAC on day 7 but day 28 MS regardless of the source ↓ PC and ↑ TAC in the duodenum than BD	[25]	L-Met was more effective in reducing oxidative stress and improving glutathione in the liver than DL-Met.
MHA and DLM Female Hybrid converter turkey	0.15% and 0.37% in weeks 1–4 of age, and 0.0% and 0.1% in weeks 5–8 of age added to BD (0.40 and 0.35% Met at 1–4 and 5–8 weeks of age, respectively)	Higher Met level ↑ SOD, GSH + GSSG, and FRAP values in the blood. ⇆ MAD, GPx, Vitamin C, and CAT. MHA ↓ SOD, CAT, and GSH + GSSG in the blood and ↑ MDA	[33]	DLM improved the activities of SOD, CAT, and GSH + GSSG levels

Table 3. *Cont.*

Met Sources/Poultry Species	Met Levels	Main Results	References	Remarks
DLM and HMTBA Male ROss 308	0.25% of either DLM or HMTBA was added to either 18.3 or 23.2% of CP diets.	DLM ↑ plasma TBARS and FRAP compared with HMTBA at 4 weeks of age. At 6 weeks of age: interaction of protein and met source for FRAP and TBARS MHA ↑ SOD and reduced GSH but ⇀↽ GSH synthetase, glutathione reductase, or Msr-A gene expression in the liver at 6 weeks old chicken.	[36]	MHA presented a more pronounced antioxidants effect than DLM
DLM and LM 1-d-old Ross 308	basal diet (BD), the BD + 0.095% LM or DLM, the BD + 0.190% LM or DLM, and the BD + 0.285% LM or DLM (representing 60, 70, 80, and 90% of the Met + Cys requirement)	0.285% of LM ↑ GSH and TAC but ↓ PC in the duodenum compared to DLM at the same level.	[44]	L-Met served better in reducing protein oxidation and increasing antioxidant status in the duodenum/gut of chicks than DLM.
DLM and MHA Cherry Valley ducks	0.04, 0.12, 0.16, and 0.20% of Met equivalents for the grower phase	Booth dietary Met source and level affected the TAC < GPx, GSSG, and MDA in pectoralis major muscles on day 42. MHA ↑ TAC and GPx in pectoralis major muscles and GSH in the breast muscles compared with DLM. ↑ Met level; ↑ GSH, GSSG, and MDA regardless of the Met source.	[81]	MHA improved the antioxidant status of cherry valley pectoralis major muscles compared with DLM (increased antioxidants capacity markers (TAC, GPX, and GSH)

⇀↽ Represents no difference. ↑ Represents increased or high or upregulated. ↓ Represents decreased or low, or downregulated. DLM—DL-methionine, LM—L-methionine, MHA/HMTBA—methionine hydroxyl analogue/DL-2-hydroxy-4-(methylthio)butanoic acid; TGSH—total glutathione, rGSH—reduced glutathione, GSSG, GPx—glutathione peroxidase, MDA—malondialdehyde, GSH/GSSG—glutathione and glutathione disulfide ratio, FRAP—ferric reducing the ability of plasma, TBARS—thiobarbituric acid reactive substances, MsrA—methionine sulfur reductase A, SODsuperoxide peroxidase, CAT—catalase, LOOD—lipid peroxides, Vit C—vitamin C, TAC—total antioxidant capacity, PC—protein carbonyls.

Lipid peroxidation is most studied as one of the indicators of oxidative stress in poultry because one of its products is malondialdehyde (MDA), which is easily detectable. This section will discuss the role of different Met sources on MDA as poultry's oxidative stress index.

Numerous studies have indicated that dietary Met sources influence the concentration of MDA in poultry differently. According to studies on turkey, different Met sources significantly affect oxidative stress [33]. Lipid peroxidation (measured as plasma thiobarbituric acid reactive substances (TBARS) levels) in the plasma of 4-week-old broilers was significantly affected by Met sources as it was highest in DLM supplemented than in HMTBA [36]. However, contradictory results were reported by Jankowski et al. [23]. They found lower MDA concentrations were observed in the small intestinal walls of turkeys receiving DLM-supplemented diets compared to those fed MHA-supplemented diets.

Furthermore, LM-supplemented diets were more effective in reducing oxidative stress (MDA) in the liver of turkeys during the first 28 days of age than DLM. This indicates that LM is more beneficial than DLM [25]. However, Jankowski et al. [23] showed no significant difference in MDA concentration in the small intestinal walls of birds- fed diets supplemented with LM and DLM. In another experiment, Jankowski et al. [38] reported that MDA concentrations in the small intestine were affected by both Met sources and levels (lower in DLM-supplemented diets compared to MHA-supplemented diets, as well as high met levels, decreased MDA concentrations) in an experiment on young turkeys infected with the hemorrhagic enteritis virus. However, it was further demonstrated that dietary Met levels did not affect MDA content in newly hatched ducklings' livers or brains [79]. Kalvandi et al. [62] reported that the plasma and liver MDA concentrations were higher in the heat-stressed group breeder quails than in the thermoneutral group. They further

showed that adding 1.15 times the NRC [27] (recommended Met requirement to the diet) reduced MDA concentrations in heat-stressed quails to a level similar to those under thermoneutral conditions. In addition, another study showed that *Eimeria spp.* challenged broilers had high levels of oxidative substances such as nitrite and TBARS with no effect of Met supplementation on the same [77]. Zhao et al. [81] observed that MDA concentrations were increasing with increasing Met levels, and they further noted that there was no significant difference between DLM and DL-HMTBA.

4.2. Effect of Dietary Met on Antioxidants/Oxidative Stress of Poultry at Different Supplementation Levels

The effects of Met supplementation at different dietary levels in poultry are shown in Table 4. Numerous studies have shown the antioxidant effect of Met in poultry and its potential for ameliorating oxidative stress, as reviewed by [68]. However, the effects of Met on the antioxidant status are concentration-dependent and age-dependent. The increased Met level from 0.49% to 0.59% led to higher SOD activity in the serum, lower hepatic MDA at 7 days of age, and a reduced hepatic GSH: GSSG ratio at 21 days in broilers [82]. In another experiment with young turkeys (16 weeks old), similar results were reported; the diet supplemented with 0.47% of Met improved the total antioxidant potential of the serum [83]. Met is known to serve as the precursor for synthesizing GSH and taurine, which greatly protect against oxidative stress. The glutathione antioxidant system comprises the enzymes glutathione peroxidase (GPx) and GSH reductase, which moderate the activities of the whole glutathione system [84]. According to some experiments, feeding 0.5% Met proved to promote the synthesis of GSH in the liver of growing broilers when supplemented with increased unsaturated fats [85].

Table 4. Effects of dietary Met supplementation at different levels on the antioxidant status of poultry.

Sources	Dietary Met Levels	Main Observation and Conclusion (Oxidative Stress Responses)
[8] Broilers	100 and 130% of the recommendations and stocking density	The 130% of DL Met decreased the content of GSSG in the plasma but improved/enhanced both GSH and GSSG in the thigh of the growers compared to 100%. The 130% DL-Met decreased the MDA concentration in the breast and thigh of the finisher.
[32] Egg-laying ducks	0.0, 0.5, 1.0, 1.5, 2.0, or 2.5 g Met/kg	Met supplementation had no effects on the plasma concentrations of GSH, GSSG, GPx, and MDA
[54] Broilers	Starter: MD-, DL1 = MD + 0.295%; DL2 = MD + 1% Grower: MD-; DL1 = MD + 0.275%; DL2 = MD + 1%	MD-without Met supplementation; The results indicated that heat stress (38 °C for 24 h) resulted in a higher expression of oxidative stress-related genes (CBS, GSS, and GPx7) levels in broilers supplemented with DL1 and DL2 diets.
[62] Breeder Japanese quail	1.00-, 1.15-, 1.30-, and 1.45-times NRC 1994 quails recommended in heat stress (34 °C)	The dietary supplementation of Met increased the serum and liver activity of antioxidant enzymes (SOD, CAT, and HGPx) and reduced MDA concentration in heat-stressed quails.
[78] Quail breeders	(0, 0.5, 1.5, 2.5, and 3.5 g/kg) quail breeders	DL-Met supplementation (0.5 to 1.5 g/kg) increased the SOD, TAC, and CAT, quadratically reduced GSH and quadratically decreased the MDA levels compared to the control diet.
[79] Laying duck breeders	2.00, 2.75, 3.50, 4.25, 5.00, and 5.75 g/kg	The dietary Met levels improved the hepatic levels of GSH and activities of GPx and TAC in laying duck breeders at 43 weeks and reduced MDA levels in a quadratic manner.
[82] Broilers	4.9 and 5.9 g/kg (0.49% and 0.59%)	Broilers supplemented with 5.9 g/kg had enhanced serum SOD activity and lowered hepatic MDA at 7 days of age. Moreover, the hepatic GSH: GSSG ratio was small/reduced by high levels of Met on day 21.

Table 4. *Cont.*

Sources	Dietary Met Levels	Main Observation and Conclusion (Oxidative Stress Responses)
[83] Turkeys	T1 2.86/2.44, T2 3.24/2.83, T3 4.01/3.44, T4 4.67/4.15, T5 5.55/4.67, T6 6.10/5.46 (9–12 weeks/13–16 weeks)	Increasing the amount of Met in the diet did not affect CAT and SOD activities or GSH + GSSG concentrations. Plasma FRAP values increased with increasing met levels. Low dietary Met levels had little effect; however, a significant rise in the Met content of turkey feeds accelerated the oxidation processes in the liver, as indicated by increased MDA levels.
[86] Langshan layer hens (dual purpose -indigenous breed of China)-52 wk old	3.2, 4.0, and 5.4 g/kg (0.32%, 0.4%, and 0.54% Met) Se: 0, 0.3, and 0.6 mg/kg (0, 0.3 and 0.6% Se).	The high level of Met supplementation in maternal diets (5.4 g/kg) decreased the concentration of GSH, GPX, and MDA in the yolks of eggs. However, the same level seems to decrease the activity/level of GPX and carbonyl group but not the GSH in the albumen of eggs. Met supplementation at 4.0 and 5.4 reduced the carbonyl concentration compared with 3.2 g of Met/kg.
[87] Broilers	0.26 and 0.50% starter 0.28 and 0.40% grower	Met deficiency diet (0.26 and 0.28%) resulted in a significantly lower serum level and splenic SOD and GPx activities. Still, it markedly increased the MDA contents of the same tissues in broilers at 28 and 42 days.
[88] Laying hens	20, 40, 60, 80, and 100 mg of Zn/kg as Zn-Met (basal diet 80 mg of Zn/Kg as Zn-Sulphate)	The serum MDA level decreased linearly with increasing Zn-Met level. The liver CAT and serum GSH-Px activities had quadratic effects. The CAT activity in the liver increased with increasing Zn-Met levels. The 60 mg/kg Zn-Met increased the TAC and GSH-Px activity in the serum and liver of the laying hen compared to the control.
[89] Meat ducks	0, 30, 60, 90, 120, or 150 mg/kg Zn-Met for 35 d	Dietary Zn-Met supplementation improved the intestinal antioxidant status in meat ducks by increasing the activity of SOD, CAT, and GSH and decreasing the level of MDA in the jejunum.
[90] Turkey	Dietary ratios of arginine and Met were relative to lysine. Arg90Met30, Arg90Met45, Arg100Met30, Arg100Met45, Arg110Met30, and Arg110Met45	An increase in Met level from 30 to 45 of Lys content improved the antioxidants capacity of turkey regardless of the Arg levels by decreasing the plasma protein carbonyl concentration as well as CAT and SOD activity in the breast muscles and liver, respectively.
[91] Turkeys	0.6, 1.5, 2.0, 2.7, 3.4 g/kg for 1–4 weeks 0.4, 0.9, 1.3, 2.8, 3.7 g/kg from 5–8 weeks	The redox status in the blood of turkeys is observed to deteriorate in unsupplemented diets. However, the highest and lowest dietary Met levels showed similar effects on antioxidant parameters. Therefore, their results were inconclusive and ambiguous.
[92] Arbor Acres and partridge shank (AA and PS)	MS (Low = LW, adequate AM and High HM) LW 0.0/0.0%, AM 0.15/0.13%, HM 0.30/0.26% (1–21/22–42 d, respectively)	The HM diets indicated strain-dependent changes in oxidative muscle status, AA broilers had high TAC, while PS broilers had increased MDA and GPx activity, but the SOD was high in both strains.
[93] Geese	In ovo injection of disaccharide (DS) and or Met DS injection (25 g/L maltose + 25 g/L sucrose + 7.5 g/L NaCl), Met injection (5 g/L Met + 7.5 g/L NaCl), or DS plus Met injection (25 g/L maltose + 25 g/L sucrose + 5 g/L Met + 7.5 g/L NaCl)	In ovo injection of Met enhanced serum uric acid, GSH, and glutathione peroxidase concentrations as well as lower GSSG/GSH ratio, serum glutathione disulfide (GSSG), and malondialdehyde (MDA) concentration than the noninjected group on the day of hatch.

Furthermore, Met supplement of the maternal diet with 0.30% increased GSH concentration in egg albumen [86]. The GSH-PX activity was significantly influenced by Met supplementation along with Se yeast in maternal diets. The higher levels resulted in re-

duced GSH-Px activity in egg yolk, with 0.3 mg of Se/kg and 3.2 g of Met/kg resulting in the highest GSH-Px activity in both egg yolk and albumen [86]. All these experiments suggest that a higher dietary Met level enhances the antioxidant status in poultry.

However, there is a growing number of experiments on the effect of Met restriction on mammalian oxidative stress and immune system function. Some studies have found that restricting Met increases GSH synthesis and thus reduces the adverse effects of oxidative stress in animals [45]. However, according to research by Maddineni et al. [94], mice with restricted dietary Met consumption show less oxidative stress but maintain the same level of antioxidant enzyme activity. The role of Met restriction on oxidative stress is thought to be through Met metabolites, which in turn participate in different pathways responsible for maintaining the normal equilibrium in the cell. Met restriction was observed to facilitate a transsulfuration pathway which leads to Met catabolism and remethylation through homocysteine in broilers [95].

4.3. Superoxide Dismutase (SOD)

SOD is involved in the first-line defense of poultry. SOD is a metalloenzyme that dismutates the superoxide anion (formed as a by-product of respiration metabolism in the cell's mitochondria) into peroxide and oxygen [96]. There is a growing body of information on the influence of MS on the SOD in poultry under normal conditions at different supplementation levels (Table 4). Experiments in broilers have shown that SOD activity is greatly influenced by the supplementation levels, with Met deficiency negatively influencing SOD [87]. In contrast, a high level positively influences the activity of SOD in different tissues [82]. SAM is a significant methyl donor that can improve SOD activity [17]. However, the activity of SOD has also been influenced by other factors such as nutrition, environment, age, and diseases. For example, one experiment showed that the SOD activities of broilers supplemented with 20% CP were affected by varying Met levels in almost all tissues [97]. Most studies examine total SOD activity without distinguishing between manganese SOD and copper, iron, or zinc SOD. Since manganese SOD is an inducible enzyme, stress may have caused its activity to increase [98].

4.4. Catalase CAT

Superoxide dismutases and catalases are essential enzymes that catalyze the dismutation of hydrogen peroxide and superoxide anion to control the number of reduced oxygen species in living systems. Several scholars have investigated Met levels' effect on poultry's CAT activity. The findings from these studies are, however, contradictory. Some experiments indicated that increased dietary Met led to increased CAT activities [24,62], while some showed that increased Met concentration does not affect CAT activities [23,83]. The difference in the CAT activity may be attributed to the magnitude of the Met levels used in the particular study and the poultry species studied. In the experiment with turkeys, with an increased Met level of 50% above the NRC recommendation [27], the antioxidant defense system was improved [23]. Similar results were reported in Japanese quail breeders receiving diets of 1.30 and 1.45 times the recommended Met levels, and they had higher plasma and liver CAT activities [62]. In another study, which involved six Met levels (0, 0.5, 1.5, 2.5, and 3.5 g/kg), the CAT activity in quails was increased quadratically [78]. However, in another study with turkeys, increasing the Met level to 50% of the NRC recommendation did not improve antioxidant status and decreased CAT activity in the breast muscles [24]. In addition, the increased dietary Met (2, 2.75, 3.50, 4.25, 5.00, and 5.75 g/kg) in duck breeders at 38 weeks did not influence the relative hepatic and cerebral genes in the hatchling related to CAT [79]. The latter experiment indicated that dietary Met levels improved the expression of the CAT gene linearly in laying ducks at 43 weeks.

Moreover, few studies have investigated the role of Met levels on CAT activities in poultry supplemented with other compounds, with inconsistent results. For example, the CAT activity was improved with increasing concentration in the experiments involving dietary Zn-Met supplemented at different levels in laying hens [88] and meat ducks [89]. In

contrast, Jankowski and his collaborators [90] studied the effects of increasing Arg and Met to lysine ratios and found that the CAT activity in turkey breast muscles was decreased by increasing the Met level from 30 to 45% regardless of the Arg content in the diet.

5. Conclusions

Met has an increasing role in antioxidant status. Both dietary, free, and protein-bound Met have antioxidant potential in poultry and can alleviate oxidative stress in poultry in different environmental stressors and internal stress conditions. In general, the antioxidant value of Met was assessed in relation to the NRC recommendations based on the summary of the existing studies. Most findings showed that increasing Met inclusion ratios over the NRC standards did not affect growth or production performance, although it did improve antioxidant status. However, there is scanty information to estimate the optimal level of Met at which the antioxidants are activated in response to oxidative stress. Therefore, further studies should be conducted to determine the optimal level of Met for the essential antioxidants such as GSH, SOD, CAT, and GPX, which are involved in the first line of antioxidant defenses in poultry. Thus, it appears critical to identify whether Met (or Total sulfur AAs) concentrations in poultry diets should be increased without assessing the antioxidant system network. Due to significant changes in antioxidant status as the recommended Met levels in their diets increased and the lack of studies evaluating the antioxidant responses of turkeys to varying inclusion rates of Met, special consideration should be given to turkeys.

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