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**Scientific Expert Panel**

# Evaluation of the impact of nutrition on antioxidative status to support animal health and welfare

## 1. Introduction

This recommendation document is part of a set of recommendation documents developed by the IFIF Scientific Expert Panel and the IFIF Working Group (WG) on' Nutritional Innovation to Promote Animal Health.'

The IFIF WG was launched in 2017 with the objective of having animal nutrition solutions contributing to animal health and animal wellbeing **scientifically recognized, clearly understood,** and benefit from a proper regulatory **framework** to be valorized and implemented'.

Nutritional solutions, now called nutritional strategies, are aimed at supporting the development of adequate animal nutrition.

Adequate nutrition is defined as 'the oral intake of animals of adequate levels of nutrients, substances, microorganisms, and other feed constituents, considering their combination and presentation, necessary to fulfill functions related to their physiological states, including the expression of most normal behavior, and their resilience capabilities to cope with stressors of various type encountered in appropriate husbandry conditions.' Furthermore, the way to achieve adequate nutrition is described as follows:

- Optimization of feed composition, manufacturing, presentation, and delivery to animals,
- Minimization of the exposure of animals to stressors in feeds,
- Coverage of the animal's requirements for maintenance, activity, growth, production, and reproduction,
- Support of digestion and physiological functions, body systems, and behavioral expression.

The purposes of these recommendation documents are to provide:

- The developers of nutritional strategies with information on the way to evaluate the effectiveness of their strategy for a given purpose.

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- The evaluation bodies in the different jurisdictions with an approach for the evaluation of the effectiveness of nutritional strategies for a given purpose.

Each recommendation document will focus on a specific purpose, in relation to microbiome, gut function, exposure control, immunity, physiology, and others.

The present recommendation document is focusing on the evaluation of the role of nutrition on the antioxidative status to support animal health and welfare. Antioxidative status depends on various activities, and their evaluation requires the measurements of various endpoints to verify the effect of a specific nutritional strategy on the antioxidative status. Furthermore, antioxidative status is important for maintaining the resilience of animals to various stressors.

## 2. Scope

Oxidative stress has emerged as a central factor affecting the health, productivity, and welfare of livestock. It results from an imbalance between reactive oxygen species (ROS) and the animal's antioxidative defense mechanisms. Particularly during physiologically challenging phases such as weaning, parturition, rapid growth, diseases or environmental stress (e.g., heat), oxidative damage can impair immune function, reduce growth efficiency, and compromise reproductive performance (Lykkesfeldt and Svendsen 2007). Antioxidative status measurements have emerged as critical tools in modern livestock health management, providing insights into animal welfare, disease susceptibility, and production performance (Ponnampalam et al. 2022). These measurements assess the balance between ROS production and antioxidant defense mechanisms, offering valuable information for preventive healthcare strategies and production optimization (Salami et al. 2016). To date, several biomarkers have been used to assess antioxidative status and oxidative damage in livestock. Among the most frequently applied parameters are glutathione peroxidase (GPx) activity, total antioxidant capacity (TAC), superoxide dismutase (SOD), and malondialdehyde (MDA) levels (Ayemele et al. 2021, Babington et al. 2024, Nenova et al. 2024). These indicators are chosen for their relevance to the biological antioxidant defence system and their relative ease of measurement using commercially available assay kits. GPx and SOD reflect enzymatic antioxidant activity, while TAC gives a cumulative estimate of all antioxidants present in a biological sample. MDA, on the other hand, serves as a marker of lipid peroxidation and oxidative damage. Advanced biomarkers include microRNAs, advanced oxidation protein products, and tissue-specific markers that provide more detailed insights into oxidative status (Miretti et al. 2020).

Despite their utility, each of these biomarkers comes with limitations. Additionally, the lack of universally accepted reference ranges and inter-laboratory variability limits the comparability and field utility of these measurements. Thus, while the potential of antioxidative biomarkers for guiding livestock health and nutrition strategies is well-recognized, their broader application requires further validation, standardization, and adaptation to field-friendly formats.

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In this document, we aim to bridge the gap between research and practice by evaluating the feasibility, relevance, and implementation strategies for antioxidative status monitoring in livestock production systems.

ROS such as superoxide anions, hydrogen peroxide, and hydroxyl radicals are continuously generated during cellular respiration and immune responses. Under physiological conditions, these are neutralized by endogenous antioxidants. However, excessive ROS, whether from infection, metabolic stress, toxins, or environmental stressors, can overwhelm these defenses (Lauridsen 2019, Ponnampalam et al. 2022, Abdulrahman et al. 2024). Most reactive oxygen radicals emerge in the mitochondrial respiratory chain during incomplete four-electron oxygen reduction. The electron transport system generates superoxide anion radicals when electrons leak to oxygen instead of the next acceptor in the chain (Zorov et al. 2014). Multiple papers confirm the continuous generation of key ROS species during aerobic metabolism. ROS include superoxide anion radical (O2•-), hydroxyl radical (•OH), hydrogen peroxide (H2O2), and singlet oxygen (Mukherjee et al. 2024). These species are continuously formed as side products of biological reactions involving electron transfer.

Livestock possess a huge network of antioxidative systems, enzymatic antioxidants such as GPx, SOD, catalase, non-enzymatic antioxidants such as Vitamins E and C, selenium, carotenoids, flavonoids, and other plant polyphenols (Surai 2021).

When antioxidant defenses are insufficient, oxidative damage occurs, affecting lipids, proteins, and DNA. Biomarkers of oxidative damage (e.g., MDA) and antioxidant activity (e.g., GPx, TAC) and a growing number of molecular markers can be used to evaluate the oxidative status of the animal (Ayemele et al. 2021, Tufarelli et al. 2023).

In any case, measurements should never be viewed in absolute terms but should always be compared with an untreated control group, whereby the required number of replicates must be estimated in advance based on biological variation.

## 3. Descriptions of endpoints

Table 1 provides an overview of the various methods used in animal studies.

*Table 1: Examples of measurements in animals of oxidative stress and antioxidative defense*

| Category | Marker / Assay | Sample Type | Principle | Advantages | Limitations |
|---|---|---|---|---|---|
| Oxidative damage | MDA (TBARS assay) | Plasma, serum, tissues | Reaction of MDA with thiobarbituric acid → color/fluorescence | Simple, cheap, widely used | Low specificity (other aldehydes interfere) |
| | F2-isoprostanes | Plasma, urine, tissues | LC-MS/GC-MS detection of lipid peroxidation products | High specificity, gold standard | Expensive, requires advanced equipment |
| | Protein carbonyls | Plasma, serum, tissues | DNPH reaction with oxidized proteins | Reliable marker of protein oxidation | Requires careful handling |
| | 8-OHdG | Urine, serum, tissues | ELISA or LC-MS for oxidized DNA bases | Specific for DNA oxidation | More costly, stability issues |
| | LDL Oxidation Resistance | Blood, plasma | Oxidation kinetics after isolation of LDLs | Oxidizability of LDL particles | Surrogate marker, not a direct measure |
| Enzymatic antioxidants | Superoxide dismutase (SOD) | Erythrocytes, tissues | Inhibition of superoxide-driven reactions | Sensitive, functional measure | Needs fresh samples |
| | Catalase (CAT) | Erythrocytes, tissues | Breakdown of $H_2O_2$ → $O_2 + H_2O$ | Simple, functional | Interference from hemoglobin |
| | Glutathione peroxidase (GPx) | Plasma, erythrocytes, tissues | Uses GSH to reduce peroxides | Selenium-dependent, sensitive | Requires NADPH-based assays |
| | Glutathione (GSH, GSSG) | Tissues, blood cells | HPLC, fluorometric or enzymatic | Redox-sensitive marker | Labile, requires rapid processing |

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| Category | Marker / Assay | Sample Type | Principle | Advantages | Limitations |
|---|---|---|---|---|---|
| Non-enzymatic antioxidants | Vitamin E (α-tocopherol) | Plasma, serum, tissues | HPLC detection | Gold standard for Vit. E | Needs chromatography |
| | Vitamin C, carotenoids | Plasma, serum | HPLC or colorimetric | Nutrient-specific | Sample stability |
| Global antioxidant capacity | TAC (Total Antioxidant Capacity) | Plasma, serum | General measure of radical scavenging | Simple, integrative | Non-specific |
| | FRAP (Ferric Reducing Antioxidant Power) | Plasma, serum | Reduction of $Fe^{3+}$ → $Fe^{2+}$, color change | Quick, low cost | Measures mainly hydrophilic antioxidants |
| | ORAC (Oxygen Radical Absorbance Capacity) | Plasma, serum | Antioxidant protection against peroxyl radicals (fluorescence) | Sensitive, dynamic | More complex, fluorescence needed |
| | ABTS/TEAC assay | Plasma, serum | Antioxidants quench ABTS radical cation | Widely used, flexible | End-point assay, not always physiological |
| | GSH:GSSG ratio | Blood cells, tissues | HPLC or enzymatic quantification | Strong indicator of cellular redox state | Requires careful handling |
| | Thiol:disulfide balance | Plasma, serum | Spectrophotometric or HPLC | Reflects protein oxidation status | Less common |

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A variety of methods have been developed to assess oxidative stress and antioxidative capacity. These differ in terms of biological relevance, practicality, and cost. The most commonly used biomarkers are summarized in Table 1 and discussed below, along with examples of their application.

## 4. Methods to measure the parameters.

### a. Malondialdehyde (MDA)/TBARS

MDA represents a well-established biomarker of oxidative damage and is widely utilized in livestock research for the assessment of oxidative stress. Its quantification is most frequently achieved through colorimetric assays, which are particularly relevant for evaluating stress responses under physiological and pathological conditions. Among these methods, thiobarbituric acid reactive substances (TBARS) assays remain the most commonly applied due to their relative simplicity and cost-effectiveness (Islayem et al. 2022). Nevertheless, the specificity of such assays is limited, as interfering reactive substances may contribute to false-positive results. To overcome these limitations, more advanced techniques have been developed. High-performance liquid chromatography (HPLC), for instance, has demonstrated superior sensitivity and specificity, with studies in bovine semen highlighting improved detection limits compared with traditional colorimetric approaches (Yonny et al. 2015).

Elevated MDA concentrations are indicative of increased lipid peroxidation, thereby reflecting a heightened state of oxidative stress. While MDA remains a reliable marker for oxidative damage, the inherent methodological limitations of colorimetric assays underscore the necessity for continued refinement of analytical techniques. The adoption of more specific and sensitive methods is therefore essential to ensure robust and accurate assessment of oxidative stress in livestock populations. Examples are given in Table 2.

*Table 2 - Examples of application of MDA/TBARS measurements in animals*

| Species | Sample Type | Purpose in Feed Additive Studies | Examples of Additives Studied | Reference |
|---|---|---|---|---|
| Pigs, sows | Serum | Reproductive performance, antioxidant, and immune functions of primiparous sows and parity-2 sows | Nano selenium and plant extracts | (Li et al. 2025) |
| Broilers | Muscle tissue | Antioxidant capacity, meat quality in broilers after lipopolysaccharide (LPS) stimulation | Gallic Acid | (Xiong et al. 2024) |
| Cattle (Calves) | Muscle tissue | Muscle quality in beef cattle | Guanidino acetic | (Yi et al. 2024) |

### b. F2-isoprostanes

F2 isoprostanes are considered robust and highly specific biomarkers of lipid peroxidation and oxidative stress, and are therefore well-suited for the assessment of oxidative status in livestock (Putman et al. 2021). Quantification of these compounds provides objective measures that may inform therapeutic interventions and contribute to the attenuation of inflammation-associated processes in veterinary medicine. Nevertheless, their utilization in veterinary species remains comparatively underexplored, underscoring the need for further research to establish their clinical applicability (Table 3).

*Table 3 - Examples of application of F2-isoprostane measurements in animals*

| Species | Sample Type | Purpose in Feed Additive Studies | Examples of Additives Studied | References |
|---|---|---|---|---|
| Pigs | Urine | Oxidized fat | -- | (Lindblom et al. 2018) |
| Calves | Plasma | Compensation of colostrum deprivation | Quercetin | (Gruse et al. 2016) |

### c. Protein carbonyls

Protein carbonyls are widely recognized as key biomarkers of protein oxidation in livestock, with particular relevance to meat quality and safety. Their formation is primarily a consequence of oxidative stress, which can compromise both the nutritional value and the sensory attributes of meat products. Accordingly, the quantification of protein carbonyls has become an essential tool for evaluating oxidative damage, with the dinitrophenylhydrazine (DNPH) assay representing the most commonly employed method in food and meat science.

Protein carbonylation occurs through oxidative modifications of amino acid residues, often mediated by radical-driven processes as well as reactions associated with the Maillard reaction (Mario et al. 2021). Specific oxidation products, such as α-aminoadipic semialdehyde (AAS) and lysinenorlucine (LNL), have been identified as reliable markers for assessing the extent of protein carbonylation and thereby the degree of oxidative damage (Wenhui et al. 2023). These structural alterations impair protein functionality and digestibility, which in turn negatively influence technological and nutritional aspects of meat (Mario et al. 2021). Furthermore, experimental evidence indicates a strong correlation between carbonyl content and the intensity of oxidation, with direct consequences for the sensory properties of meat, including texture, flavor, and overall consumer acceptability (Wenhui et al. 2023). On a biological level, protein carbonyls are not only passive indicators of oxidative stress but may also function as signaling molecules that modulate antioxidant defenses and influence immune responses (Estévez et al. 2022). However, despite their broad acceptance as markers, the heterogeneous mechanisms underlying protein carbonyl formation, together with variability in measurement techniques, complicate the interpretation of their role in both livestock health and meat quality (Edyta et al. 2015). Examples for measurements in livestock are included in Table 4.

*Table 41 - Examples of application of protein carbonyl measurements in animals*

| Species | Sample Type | Purpose in Feed Additive Studies | Examples of Additives Studied | Reference |
|---|---|---|---|---|
| Sows | Plasma | Antioxidative status after mycotoxin challenge | Mycotoxin binder | (Papatsiros et al. 2023) |
| Broilers | Muscle | Protection against cold stress | Rutin | (Zhang et al. 2025) |
| Cows | Plasma | Antioxidant status of Jersey cows during the transition period | Green tea and oregano extracts | (Vizzotto et al. 2021) |

### d. 8-hydroxy-2'-deoxyguanosine (8-OHdG)

8-OHdG is regarded as a sensitive and specific biomarker of oxidative DNA damage resulting from ROS-mediated stress. Elevated concentrations of 8-OHdG have been associated with a range of pathological conditions, including cancer, diabetes mellitus, and chronic inflammatory diseases. Measurement of 8-OHdG in biological fluids such as urine, blood, and saliva enables the assessment of systemic oxidative stress and may support both diagnostic and prognostic evaluations. Despite its established relevance in human medicine, the application of 8-OHdG in veterinary species remains limited, highlighting the potential for further clinical and translational research. Examples for studies in livestock are listed in Table 5.

*Table 52 - Examples of application of 8-hydroxy-2'-deoxyguanosine measurements in animals*

| Species | Sample Type | Purpose in Feed Additive Studies | Examples of Additives Studied | Reference |
|---|---|---|---|---|
| Sows | Plasma | Mammary gland development, oxidative stress in gestating gilts | Silymarin | (Farmer et al. 2014) |
| Broilers | Serum | Mycotoxin mitigation | *Vernonia amygdalina* leaf extract | (Oloruntola et al. 2025) |
| Cows | Serum | Mammary Oxidative Stress in Dairy Cows | Dandelion extract | (Zhang et al. 2024) |

### e. Low-Density Lipoproteins (LDL) Oxidation Resistance

The susceptibility of LDL to oxidative damage is a key factor in the development of atherosclerosis in humans, and it is influenced by both dietary components and biochemical processes. Oxidation of LDL is a complex process that can be evaluated using different methodologies, each offering specific

insights into the oxidative resistance of LDL particles. For example, pigs fed soybean oil, which is rich in polyunsaturated fatty acids, exhibit a higher susceptibility of LDL to oxidation than pigs fed olive oil or beef tallow, which contain mainly monounsaturated and saturated fats. (Eder and Kirchgessner 1997). Different methods are used to assess LDL oxidation, with the conjugated diene (CD) assay being one of the most common. In this approach, LDL is exposed to copper ions to initiate oxidation, and the lag time before oxidation begins serves as a key indicator of oxidative resistance (Scheffer et al. 2000).

Antioxidants also play an important role in modulating LDL oxidation. Vitamin E, for instance, can enhance LDL resistance to oxidative stress, although its effectiveness depends on the concentration of prooxidants such as copper ions used in the assays (Ziouzenkova et al. 1996). Furthermore, dietary antioxidants, can improve LDL's oxidative resistance independently of the antioxidant content within the lipoprotein itself (Bonanome et al. 1992).

Examples for measurements in livestock are included in Table 6.

*Table 6 - Examples of application of LDL Oxidation Resistance measurements in animals*

| Species | Sample Type | Purpose in Feed Additive Studies | Examples of Additives Studied | Reference |
|---|---|---|---|---|
| Pigs | Blood | Resistance against oxidation of porcine LDL subfractions | Tocopherols | (Knipping et al. 1990) |
| Poultry, broilers | -- | -- | -- | -- |
| Goats | Plasma | Anti-peroxidation effects on low density lipoprotein and milk fat globule membrane | Vitamin E | (Yang et al. 2004) |

## f. Superoxide Dismutase (SOD)

SOD is a key antioxidant enzyme that catalyzes the dismutation of superoxide anions ($O_2^{-}\bullet$) into molecular oxygen ($O_2$) and hydrogen peroxide ($H_2O_2$). Superoxide anions are ROS generated as byproducts of normal cellular respiration, particularly within the mitochondrial electron transport chain (Islam et al. 2022). Due to their high reactivity, these radicals can initiate lipid peroxidation, protein oxidation, and DNA damage if not adequately neutralized. SOD represents the first enzymatic line of defense in the cellular antioxidant system. Its activity is essential in mitigating oxidative stress by reducing superoxide concentrations and thereby limiting the formation of secondary ROS. The resultant hydrogen peroxide is subsequently detoxified by catalase (CAT) and GPx, further reducing potential oxidative damage.

Multiple isoforms of SOD exist in mammalian systems, each with distinct localization and metal cofactors, with: Cu/Zn-SOD (SOD1): Predominantly cytosolic, using copper and zinc as cofactors; Mn-SOD (SOD2): Mitochondrial isoform, utilizing manganese; EC-SOD (SOD3): Found in extracellular fluids and tissues.

Measurement of SOD activity serves as a widely accepted biomarker for assessing oxidative stress and antioxidant defense mechanisms in both physiological and pathological conditions. Variations in SOD activity levels can reflect the organism's capacity to respond to oxidative challenges or the presence of oxidative damage (Table 7).

*Table 7 - Examples of application of Superoxide Dismutase measurements in animals*

| Species | Sample Type | Purpose in Feed Additive Studies | Examples of Additives Studied | Reference |
|---|---|---|---|---|
| Piglets | Liver | Antioxidant function in porcine epidemic diarrhoea virus-infected piglets | Monolaurin | (Wang et al. 2024) |
| Broilers | Ileum | Oxidative Stress by Heat | Organic Zinc Salts | (Adam et al. 2024) |
| Calves | Serum and milk | Effects on oxidative status, immune response, and intestinal mucosa development | Nucleotides | (Dinardo et al. 2022) |

## g. Catalase

Catalase is a key antioxidant enzyme in livestock, responsible for protecting cells from oxidative injury by catalyzing the decomposition of hydrogen peroxide into water and oxygen. Assessment of catalase activity provides valuable information on antioxidant capacity, microbial contamination, and overall health status. Several analytical approaches have been developed to quantify catalase activity, each with specific methodological advantages and applications in livestock research and management. Spectrophotometric assays remain widely used in clinical and research laboratories, relying on the decomposition of hydrogen peroxide and subsequent colorimetric quantification, thereby providing precise and reproducible measurements of catalase activity (Thulfeqar & Mahmoud, 2020). Fluorimetric techniques offer enhanced sensitivity and speed, using microplate-based systems to enable high-throughput and accurate catalase quantification, making them a viable alternative to conventional spectrophotometric approaches (Naazeri et al., 2014). Example for applications in livestock are summarized in Table 8.

*Table 8 - Examples of application catalase measurements in animals*

| Species | Sample Type | Purpose in Feed Additive Studies | Examples of Additives Studied | Reference |
|---|---|---|---|---|
| Pigs | Muscle | Performance, Antioxidant Capacity, excretion | Amino Acid-Chelated Trace Minerals | (Xiong et al. 2025) |
| Broilers | Serum | Performance, antioxidant capacity, intestinal microbiota | *Lactobacillus gallinarum* | (Zhou et al. 2025) |
| Calves | Whole blood | Performance, metabolism, rumen volatile fatty acid profile | *Yucca schidigera* Extract | (Dos Santos et al. 2025) |

## h. Glutathione peroxidase (GPx)

Enzyme activity is measured in blood or tissue. It reflects selenium-dependent antioxidant defense (Ullrey 1987). Selenocysteine (SeCys) is the essential amino acid incorporated into selenoproteins, such as GPXs, which play a key role in preventing free radical formation and protecting tissues from oxidative damage. The major GPX isoforms include: GPX1 (cytosolic), GPX2 (gastrointestinal-specific), GPX3 (plasma/extracellular), GPX4 (phospholipid hydroperoxide/intracellular), and GPX5 (mitochondrial capsule of spermatozoa). Examples of measurements in different animal species are shown in Table 9.

*Table 9 - Examples of application of glutathione peroxidase measurements in animals*

| Species | Sample Type | Purpose in Feed Additive Studies | Examples of Additives Studied | References |
|---|---|---|---|---|
| Pigs (weaners, growers, sows) | Whole blood, plasma, erythrocytes, liver | Biomarker of oxidative stress, selenium status, antioxidant capacity | Organic/inorganic selenium (Se yeast, Se-Met, sodium selenite) | (Surai and Fisinin 2016, Falk et al. 2020, Zhou et al. 2021) |
| Poultry (broilers, layers, turkeys) | Plasma, erythrocytes, liver, breast muscle | Meat oxidative stability, stress response, antioxidant defense | Selenium/different sources | (Zepeda-Velazquez et al. 2021, Huang et al. 2024, Liu et al. 2025) |
| Ruminants (cattle, sheep, goats) | Whole blood, plasma, milk, liver biopsy | Selenium bioavailability, antioxidant capacity, milk quality | Se-fertilized forages, Se yeast, trace mineral premixes | (Ranches et al. 2017, Nedelkov et al. 2020, da Silva et al. 2021) |

### i. Glutathione (GSH, GSSG)

Glutathione, present in reduced (GSH) and oxidized (GSSG) forms, represents a central redox regulator and is one of the most widely studied markers of oxidative stress. The GSH:GSSG ratio provides a sensitive measure of cellular redox status and has been applied to a range of livestock species. Analytical approaches include spectrophotometric enzymatic assays (Necasova et al. 2021), HPLC (Begic et al. 2017), and liquid chromatography–tandem mass spectrometry (LC–MS/MS) (Florholmen-Kjær et al. 2014), each offering specific advantages in terms of sensitivity and precision. Studies in cattle and pigs have shown that glutathione levels are influenced by lactation stage, diet, heat stress, and production phase, reflecting both environmental and physiological determinants of redox balance. Importantly, disruptions in glutathione homeostasis are linked to oxidative damage and disease development, underlining its dual role as both a biomarker and a regulator of cellular function.

Examples of measurements in different animal species are shown in Table 10.

*Table 10 - Examples of application of glutathione measurements in animals*

| Species | Sample Type | Purpose in Feed Additive Studies | Examples of Additives Studied | References |
|---|---|---|---|---|
| Gilts | Plasma | Requirements for gilts | Methionine | (Munoz Alfonso et al. 2025) |
| Broilers | Liver | Substituting ethoxyquine | Tea polyphenols, propyl gallate | (Xiao et al. 2024) |
| Calves | Blood | Heat stress reduction | Phytogenic additive | (Wickramasinghe et al. 2023) |

### j. Vitamins E and C

In livestock, the measurement of vitamins E and C is a useful approach to study antioxidative effects. Vitamin E (mainly α-tocopherol) is the most important lipid-soluble antioxidant, protecting lipids in lipoproteins, cellular membranes, and tissues against peroxidation. Its concentration in plasma, LDL, and muscle strongly is diet-responsive and linked to oxidative protection of blood lipoproteins as well as meat and milk. For this reason, plasma or tissue vitamin E is widely used as a biomarker of antioxidant status and should ideally be expressed relative to plasma cholesterol or total lipids to account for lipoprotein variability. Vitamin C is water-soluble and contributes to antioxidative protection by regenerating oxidized vitamin E. While it can provide valuable information, especially under stress conditions such as heat load, transport, or infection, its measurement in livestock is technically more demanding because of its instability and the fact that most species can synthesize it endogenously. Both vitamins, however, complement functional assays. Evaluating dietary or physiological effects on antioxidant status in livestock is supported by the vitamin measurements but should ideally be combined with additional markers such as selenium, GPx activity, or direct oxidation resistance tests for a comprehensive assessment. There are a large number of studies published in the literature and the methods for measurement are established in many laboratories.

### k. Total Antioxidant Capacity (TAC)

TAC is a functional parameter that reflects the cumulative action of all antioxidants present in a biological or nutritional sample and their ability to neutralize ROS or other oxidants. TAC provides an

integrated measure of the overall redox potential and thus offers valuable insight into the balance between oxidant challenge and antioxidant defense. A variety of assays are available for the determination of TAC, each based on different chemical principles. The ABTS radical cation decolorization assay is widely applied and expresses results as Trolox equivalent antioxidant capacity (TEAC). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is based on the reduction of the stable DPPH radical, while the ferric reducing antioxidant power (FRAP) assay quantifies the reduction of $Fe^{3+}$ to $Fe^{2+}$ in the presence of 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ). The oxygen radical absorbance capacity (ORAC) assay measures the inhibition of peroxyl radical–induced oxidation using a fluorescence-based approach, and the cupric reducing antioxidant capacity (CUPRAC) assay relies on the reduction of $Cu^{2+}$ to $Cu^{+}$. Depending on the methodology and reference compound employed, TAC is commonly expressed in micromoles of Trolox equivalents per liter of plasma or per gram of sample, but alternative standards such as ascorbic acid or gallic acid equivalents are also in use. Although methodological differences may lead to variations in absolute values, TAC assessment provides a useful comparative index for evaluating antioxidant potential in biological systems, foods, and feedstuffs (Manuelian et al. 2021, Nuamah et al. 2024) (Table 10). Due to the large number of possible measurements, not all TAC analyses were carried out in the various studies.

*Table 11 - Examples of application of Total Antioxidant Capacity measurements in animals*

| Species | Sample Type | Purpose in Feed Additive Studies | Examples of Additives Studied | Reference |
|---|---|---|---|---|
| Sows, piglets | Blood and liver | Antioxidative status in sows and piglets | Sodium butyrate | (Lu et al. 2025) |
| Poultry | Liver | Alternative antioxidants in broilers | Lycopene | (Cao et al. 2025) |
| Cattle | Serum and milk | Health and oxidative response in heifers | Grape seed oil | (Signor et al. 2024) |

# 5. Conclusions

Measurement of antioxidative status provides an objective, biologically meaningful approach to evaluate the impact of nutrition and feed additives on animal resilience. For the feed industry, integrating these biomarkers into product development pipelines and field trials can enhance scientific credibility and market competitiveness.

As tools for antioxidative assessment become more accessible, feed additive companies are encouraged to adopt a biomarker-driven strategy for innovation and quality assurance. In doing so, the gap between scientific insight and practical livestock nutrition can be effectively bridged—from bench to barn.

## 6. Abbreviations

| | |
|---|---|
| 8-OHdG | 8-hydroxy-2'-deoxyguanosine |
| AAS | α-AminoAdipic Semialdehyde |
| CUPRAC | Cupric Reducing Antioxidant Capacity |
| DNPH | DiNitroPhenylHydrazine |
| DPPH | 2,2-diphenyl-1-picrylhydrazyl |
| FRAP | Ferric Reducing Antioxidant Power |
| GPx | Glutathione Peroxidase |
| GSH | Glutathione, reduced |
| GSSG | Glutathione, oxidized |
| HPLC | High-Performance Liquid Chromatography |
| LC–MS/MS | Liquid Chromatography–Tandem Mass Spectrometry |
| LDL | Low-Density Lipoproteins |
| LNL | LysineNorLucine |
| MDA | MalonDiAldehyde |
| ORAC | Oxygen Radical Absorbance Capacity |
| ROS | Reactive Oxygen Species |
| SeCys | Selenocysteine |
| SOD | SuperOxide Dismutase |
| TAC | Total Antioxidant Capacity |
| TBARS | Thiobarbituric Acid Reactive Substances |
| TEAC | Trolox equivalent antioxidant capacity |
| TPTZ | 2,4,6-Tris(2-pyridyl)-s-triazine |
| WG | Working Group |

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# 8. Glossary of Terms

**Endpoints:** The measurable impact of a nutritional strategy on the animal, its physiology, or its microbiome.

**Health:** The state of normally functioning animal, especially the state of being sound, free from physical disease, pain or (symptom of) stress.

**Welfare:** The physical and mental state of an animal in relation to the conditions in which it lives and dies.