Phytochemicals, antioxidants, and oxidative stress in ruminant reproduction

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ABSTRACT

Objective: To review the importance of phytochemical compounds with antioxidant capacity in improving various reproductive processes in ruminants.
Methodology: A review of scientific literature was conducted to identify phytochemical compounds reported to positively influence ruminant reproduction, as well as to analyze the concepts of antioxidants and oxidative stress and their impact on reproductive function.
Results: Research has demonstrated that natural antioxidants found in certain plants contain various phytochemical compounds that strengthen the immune system. These compounds possess antibacterial, antiseptic, tonic, and antioxidant properties that help reduce oxidative stress, improving outcomes during gestation and parturition. Additionally, they enhance the vitality of newborns.
Limitations: Although promising results have been reported, limited research exists on certain phytochemicals with antioxidant properties that may benefit ruminant reproduction.
Conclusions: Phytochemicals with antioxidant properties neutralize free radicals, restoring balance in the endocrine, metabolic, and immune systems, and reducing oxidative stress on reproductive function.

Keywords: Oxidative stress, antioxidants, phytochemicals, reproduction.

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INTRODUCTION

In animals, the reproductive process involves the generation of free radicals (FR); however, when harmful endogenous or exogenous stimuli increase FR levels beyond the body's antioxidant capacity, oxidative stress occurs. This condition can trigger multiple cellular dysfunctions, adversely affecting fertility and reproductive performance in both females and males (Mayorga, 2015). The body possesses antioxidant defense systems composed of enzymes and other molecules whose role is to neutralize FR. Antioxidants are classified as enzymatic and non-enzymatic. The former represent the first line of endogenous defense, while the latter are primarily derived from the diet. Unlike enzymatic

antioxidants, non-enzymatic ones lose their antioxidant capacity after participating in redox reactions and must be constantly replenished. Notable examples include vitamins E and C, retinol, β-carotenes, uric acid, albumin, pyruvate, polyphenols, and flavonoids (Gumbao, 2015). In recent years, the use of natural products as additives and supplements in animal feed has increased, both to improve productive efficiency and to replace synthetic drugs and additives. Some of these products have antioxidant properties that may enhance the endogenous antioxidant system. Dietary antioxidant compounds play a crucial role in mitigating oxidative stress. The plants used to obtain these products contain active ingredients widely employed in traditional human and veterinary medicine (Carro *et al.*, 2006). These bioactive compounds, known as secondary metabolites, serve ecological functions in plants, including protection against ultraviolet radiation, adaptation to water stress, defense against herbivores, and attraction of pollinators through chemical signaling (Greathead, 2003; Silva *et al.*, 2021). It has been reported that plants may contain between 20 and 80 phytochemicals at varying concentrations, mainly monoterpenes and sesquiterpenes. However, aliphatic hydrocarbons, acids, alcohols, and esters are also present in smaller amounts (dos Santos *et al.*, 2019). These phytochemicals confer antioxidant, antibacterial, antifungal, and tonic properties (Lahlou, 2004).

Oxidative stress and reproduction

Oxidative stress is defined as an imbalance between the generation of free radicals (FR) and the body's ability to eliminate them, disrupting redox homeostasis (Sies *et al.*, 2017). Free radicals are small, unstable, and highly reactive molecules due to the presence of unpaired electrons; they diffuse easily and act as intermediaries in various biochemical reactions (Daenen *et al.*, 2019). The main types of radicals include reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Miller *et al.*, 1990). Under physiological conditions, FRs play crucial roles in host defense, cellular signaling, and biosynthetic processes. However, under internal or external adverse stimuli, their production may increase excessively. If not neutralized promptly, their accumulation can exceed physiological levels, leading to structural and functional cellular damage, such as disruptions in signaling pathways, metabolic imbalances, genetic mutations, and alterations in protein structure (Rahal *et al.*, 2014). In reproductive terms, oxidative stress can impair critical processes, including oocyte maturation, ovarian steroidogenesis, ovulation, embryonic implantation, blastocyst development, luteolysis, and corpus luteum maintenance (Leal *et al.*, 2011). It has also been linked to reproductive disorders such as infertility, spontaneous abortion, and premature membrane rupture (Mora *et al.*, 2019). In ruminants, blastocyst hatching from the zona pellucida occurs approximately seven days before implantation and is associated with increased ROS production (Aurousseau *et al.*, 2006). From conception through day 140 of gestation, a progressive increase in ROS has been observed, promoting cellular apoptosis and potentially halting embryonic development (Massman *et al.*, 1999; Moreira da Silva *et al.*, 2010). During early pregnancy, the intrauterine environment is considered hypoxic. Initially, the placenta relies on histotrophic nutrition and acquires oxygen through maternal circulation, thereby generating a concentration gradient between the mother and the fetus. The impact of oxidative stress in this context depends on the degree of oxygen tension change and the effectiveness of the placental antioxidant system. Therefore, oxidative stress may have either physiological or pathological effects depending on specific conditions (Leal *et al.*, 2011). In ruminants, maintaining an adequate antioxidant status before and during gestation is crucial for optimal placental development and function, which helps reduce embryonic mortality, facilitate uncomplicated deliveries, and ensure neonatal vitality (Chavatte-Palmer *et al.*, 2008).

Antioxidants

Cells possess defense mechanisms that transform free radicals (FR) into less toxic or even harmless compounds. This cellular protection against FR includes the prevention of their formation, the inhibition of their propagation, and the repair of the damage they cause (González *et al.*, 2000). A second level of protection is provided by antioxidants, which neutralize FR and suppress their harmful activity within the cell. These antioxidants are classified into two major groups: enzymatic and non-enzymatic. Enzymatic antioxidants comprise three main systems: superoxide dismutase, catalase, and glutathione peroxidase. These enzymes are synthesized endogenously and can neutralize free radicals, interrupting the lipid peroxidation chain reaction due to their ability to donate hydrogen ions (Márquez, 2001) efficiently. Non-enzymatic antioxidants, on the other hand, function by binding to FR, displacing them from critical cellular sites to compartments where their effects are less damaging, or converting them into less reactive radicals. Key compounds in this group include α-tocopherol, β-carotene, ascorbic acid, glutathione, urate, flavonoids (Lawrence & Bendich, 1987), and various phenolic compounds (Burdock, 2005).

Phytochemicals

In addition to endogenous defense mechanisms, a wide variety of natural molecules with antioxidant properties can strengthen the body's antioxidant system. Among these, phytochemicals stand out —bioactive compounds found in seeds, cereals, fruits, vegetables, leaves, roots, spices, and herbs (Skerget *et al.*, 2005). These compounds play a key role in protecting plants against various biotic and abiotic stresses, including pathogens, herbivores, ultraviolet radiation, water stress, and salinity. Phytochemicals encompass a wide range of compounds, including carotenoids, phenols, flavonoids, stilbenes, coumarins, tannins, alkaloids, nitrogenous compounds, and organosulfur compounds (Hall & Cuppett, 1997) (Figure 1). Their importance in animal production has grown due to their well-established antioxidant activity. Oxidative stress induced by free radicals has been linked to numerous diseases and reproductive disorders. In this context, plant-derived products with antioxidant capacity, such as plant extracts, have been proposed as promising natural additives for use in ruminant nutrition.

Phytochemicals with antioxidant activity can act through various mechanisms, depending on the reaction system and the nature of the radical or oxidant involved (Prior *et al.*, 2005). The effectiveness of these compounds is often expressed as antiradical activity or IC50, defined as the concentration of antioxidant required to reduce the radical's absorbance by 50% relative to the initial value (Floegel *et al.*, 2011). This value is commonly used to estimate the radical-scavenging capacity against compounds such as 2,2'-azino-

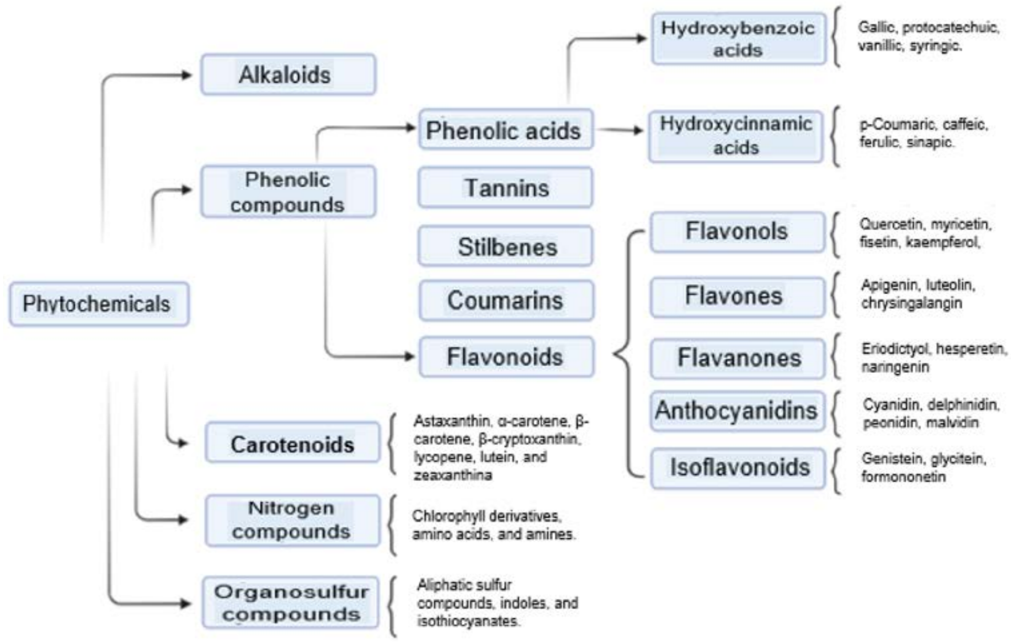

Figure 1. Leading groups of natural phytochemicals (Liu, 2003).

bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) or 2,2-diphenyl-1-picrylhydrazyl (DPPH•), comparing it to standard antioxidants. However, the antioxidant capacity of plant products also depends on the extraction method and the concentration of phytochemicals present (Castillo *et al*., 2007). León *et al.* (2015) reported higher antioxidant activity in the essential oil of *Plectranthus amboinicus* (French oregano) obtained through conventional hydrodistillation compared to that obtained through microwave-assisted hydrodistillation. When evaluating three methods to estimate antioxidant capacity (DPPH•, ABTS•+, and ORAC), the lipophilic ORAC method yielded the highest antioxidant activity values, highlighting a result of 682,952.60 μmol trolox/100 g of sample, significantly higher than that of many oils, fruits, and vegetables (Apak *et al.*, 2007; Rautenbach & Venter, 2010). Similarly, Muñoz *et al.* (2007) (Table 1) analyzed the chemical composition and antioxidant capacity of six plant species: thyme, common oregano, wild oregano, Castilian oregano, creeping oregano, and marjoram. All essential oils evaluated exhibited high levels of thymol and carvacrol, with antioxidant capacities comparable to those of standard synthetic antioxidants, such as BHA, BHT, and α-tocopherol, as measured by the ABTS•+ radical cation scavenging assay.

Arango *et al.* (2012) evaluated the antioxidant activity of the essential oil of *Lippia origanoides* (EO) using spectrophotometric methods based on DPPH• and ABTS•+ radicals. The effective concentrations (EC50) at 50% values obtained for the EO and BHT (butylated hydroxytoluene, a reference synthetic antioxidant) were 5.58 and 1.33 mg/mL, respectively. In contrast, the Trolox Equivalent Antioxidant Capacity (TEAC) values were 0.33 mg/ml for the EO and 0.68 mg/ml for BHT. These results suggest that wild EO exhibits a significant ability to stabilize free radicals, comparable to that of synthetic antioxidants and even superior to other essential oils from the same species. Plant-based

Table 1. Total Antioxidant Activity (TAA) values for reference substances and essential oils with high thymol and carvacrol content, based on ABTS radical cation scavenging capacity.

Substance	TAA (mmol Trolox kg^{-1} SE) Averages
α-Tocopherol	2590±33
BHT	990±31
BHA	6900±487
Wild orégano	2040±21
Castile orégano	670±24
Thyme	890±32
Common orégano	800±24

Total Antioxidant Activity (TAA); mmol Trolox kg^{-1} - Test substance (Muñoz *et al.*, 2007).

products rich in phenylpropanoid-type compounds, such as those found in species of the *Lippia* genus, have demonstrated antioxidant performance superior to many conventional essential oils. Various assessment methods indicate that their antioxidant capacity may be up to five times greater than that of commonly used synthetic compounds in the food and pharmaceutical industries, such as BHT and α-tocopherol (Stashenko *et al.*, 2014).

Antioxidant phytochemicals in reproduction

Naturally, the body has developed an antioxidant system to protect itself when there is an imbalance between free radicals (FR) and antioxidants. When this balance shifts toward an excess of reactive oxygen species (ROS), oxidative stress occurs. Free radicals play a significant role in pathological processes that affect the female reproductive system, influencing various physiological functions —from oocyte maturation and fertilization to embryonic development and gestation (Agarwal *et al.*, 2005). The fetus and placenta are protected by an antioxidant defense system that prevents lethal effects caused by high rates of FR production. Glutathione (GSH) production and metabolism are crucial for preventing gestational pathologies, not only due to its ability to scavenge free radicals but also for its role in maintaining intracellular redox balance. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) form a group of specialized enzymes that defend against ROS (Aurousseau *et al.*, 2006). However, when external factors weaken the antioxidant system, reproductive performance may be negatively affected. Atlagich (2006) administered antioxidant vitamins C and E to pregnant ewes as a preventive measure against hypobaric hypoxia. A hypoxic environment triggers oxidative stress, evidenced by increased oxidative damage and reduced antioxidant capacity. Morphological changes observed in the placenta revealed it as the most affected organ, resulting in reduced nutrient transport efficiency to the fetus, growth retardation, and impaired postnatal development. Supplementation with vitamins C and E improved redox balance and reversed fetal growth and placental morphological differences. Furthermore, supplementation during lactation enhanced lamb development.

According to Lizárraga *et al.* (2017), supplementation with minerals (Cu: 50 mg, Zn: 200 mg, Mn: 50 mg, and Se: 25 mg) along with vitamins A and E (3,150 IU and 250 IU, respectively), administered to cows during intravaginal device placement, prevented a drop in antioxidant capacity and the oxidative damage typically associated with the physiological state. This strategy proved useful in mitigating the detrimental effects of oxidative stress on fertility and preventing reduced conception rates in reproductive protocols. Nieto (2009) evaluated the effects of supplementing 10% and 20% distilled rosemary (DRL) and thyme leaves (DTL), as well as 3.7% and 7.5% fresh thyme leaves (FTL), to pregnant ewes during gestation, parturition, and lactation. The quality of fresh lamb meat was analyzed under modified atmosphere packaging and aerobic cooking conditions, with storage at 4 °C. Diets containing FTL reduced lipid and pigment oxidation, improved the sensory attributes of the meat, and limited the growth of spoilage microorganisms in fresh cuts. Regarding the role of β-carotene in the endocrine and reproductive axis of goats, it has been shown that this molecule regulates ovarian activity both during the reproductive season and in anestrus, and modulates the secretion of growth hormone (GH) in adult females and luteinizing hormone (LH) in yearlings (López, 2021). In synchronization protocols, antioxidant phytochemical supplementation in bovine females has been shown to have positive effects, enhancing reproductive potential and improving outcomes in reproductive biotechnologies such as follicular aspiration and in vitro embryo production (Brem *et al.*, 2001; Agudelo & Medina, 2022). Madureira *et al.* (2020) found that Holstein cows with higher plasma concentrations of beta-carotene exhibited greater fertility and fewer gestational issues. β-carotene, as an antioxidant phytochemical, helps protect uterine and embryonic cells from lipid peroxidation, promoting embryo implantation and survival. It also prevents inactivation of the cytochrome P450 enzyme, which is responsible for converting cholesterol into hormones, thereby enhancing corpus luteum size and increasing serum progesterone levels. Overall, phytochemicals with antioxidant potential have proven to be an excellent alternative for stabilizing abrupt changes in metabolic, endocrine, and immune systems during reproduction in both females and males, significantly reducing oxidative stress across various reproductive stages.

CONCLUSIONS

The use of plant-based products, such as phytochemicals with antioxidant potential, in ruminant nutrition represents a promising alternative for reducing oxidative stress conditions that occur during conception, gestation, and parturition, affecting both the dam and the offspring. Studies on the antioxidant activity of these compounds have demonstrated their potential as powerful natural antioxidants, with the ability to enhance various reproductive aspects in ruminant animals.

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