



# The Potential Impacts of Antioxidant Micronutrients on Productive and Reproductive Performances of Mammalian Species during Stressful Conditions

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## ABSTRACT

**Background:** The antioxidant micronutrients supplementation to mammalian species during stressful condition have gained great attention of nutritionists to alleviate stressful peri parturient factors, which negatively effect on productive and reproductive performances in subtropics.

**Methods:** The antioxidant micronutrients given to mammalian species refer to minerals (copper, zinc and selenium) and vitamins (vitamins A, C and E). The balanced diet needs an adequate amount of antioxidant micronutrients during stressful conditions for mammalian species.

**Result:** The antioxidant micronutrients are played pivotal roles for body functions especially during transitional period in ruminants. They are given to improve quality of feed, cell functions, carbohydrates, protein and metabolism. Changes in animals' production, reproduction and therapeutic performances have been confirmed over antioxidant micronutrients supplementations. The antioxidant micronutrients change feed intake, nutrient digestibility, rumen fermentation and energy production. Such increases in feed digestibility and growth performance resulted in improvement of oocyte, embryo and fetus qualities. In addition, blood cells and plasma biochemistry, immunity and therapeutic performances were altered due to antioxidant micronutrient supplementation. Hence, the review article was designed to collect, consolidate and discuss the effects of antioxidant micronutrient supplementation on productive, reproductive and therapeutic performances on ruminant species during transitional period in subtropics.

**Key words:** Antioxidant, Blood, Embryos, Growth, Immunity, Micronutrients, Oocytes, Reproduction.

## INTRODUCTION

The antioxidant micronutrients refers to vitamins (vitamins A, C and E) and minerals (copper, zinc and selenium) (Adjepong *et al.*, 2016; Mohammed *et al.*, 2024a). Significant strides of development has made in antioxidant micronutrients functions and supplementations over the past couple of decades for the purposes of increasing productive and reproductive performances or treatment of dysfunctions during normal and stressful conditions of ruminant species (Senosy *et al.*, 2017; Rathor *et al.*, 2023) (Fig 1).

Supplementation of ruminant species with antioxidant micronutrients during the stressful transitional period presents a vital role to overcome the negative effects in reproduction and production (Zhou *et al.*, 2023). Restoration and early resumption of the oestrus cycle, maintaining and maximizing lactation, body weight gain of the newborns remain a demand after parturition (Mohammed and Al-Hozab 2020; Mohammed *et al.*, 2020; Al-Mufarji and Mohammed 2022; Al-Mufarji *et al.*, 2022a,b,c; Mohammed and Al-Suweigh 2023; Mohammed *et al.*, 2024b, 2024c). The antioxidant micronutrients are involved in several functions including nutrient metabolism, protecting cells from damage, nervous system and endocrine normal functions, immune functions, growth and reproduction.

Many aspects of cellular metabolism have been controlled and modified through the antioxidant

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micronutrients in addition to protecting cells from damage due to reactive oxygen species (McGrath *et al.*, 2018). In addition, the importance of antioxidant micronutrients in ovarian follicle development, semen quality and pregnancy

were confirmed in several studies (Luddi *et al.*, 2016). Therefore, this review article was designed to collect, consolidate and discuss the effects of antioxidant micronutrient supplementation on productive, reproductive and therapeutic performances on ruminant species during transitional period in subtropics.

## MATERIALS AND METHODS

The current study was carried out according to the procedure approved by Deanship of Scientific Research, King Faisal University of KSA from August to February 2024. The requirements for supplementing vitamins and minerals to mammalian species, depending on the species, the environmental conditions and the physiological conditions. The methods of supplementing antioxidant micronutrients are including fortified diets and fortified liquid through direct feeding of supplements, drinking liquids or injection of supplements (Alhussien *et al.*, 2021).

## RESULTS AND DISCUSSION

### Peri-partum period

Peri-partum or transitional period is the most critical for reproductive and productive performances of ruminant pregnant species and their resulting newborns (Alhussien *et al.*, 2021). This period lasts from four weeks pre-partum to four weeks post-partum in ruminant animals. Disruptions during this period were observed in feed utilization and body weight gain, blood cells and plasma metabolites, milk production and composition, quality of ovarian follicle development and their resulting oocytes and embryos (Ali *et al.*, 2021; Al Mufarji and Mohammed 2022; Al Mufarji *et al.*, 2022a,b,c). The reduction in feed utilization can be reached to 30.0-35.0% when the temperature degree exceed 45.0°C in addition to decrease of nutrient digestibility. This leads

with lack of management to severe deficiencies of productive and reproductive performances (Hussein *et al.*, 2015; Al Mufarji and Mohammed 2022; Al Mufarji *et al.*, 2022a). Alhussien *et al.* (2021) concluded that micronutrient supplementation during the prepartum period can improve the health status of dairy cattles and subsequently the well-being of their calves. The vitamins and minerals injections were found to increase the growth performance and to boost the antioxidant and immunological systems of dairy calves during the transitional period in summer season (Bordignon *et al.*, 2019). Hence, restoration of those disruptions during the transitional periods might improve productive and reproductive performances of mammalian species over antioxidant micronutrients supplementation.

### Antioxidant minerals supplementation

The antioxidant trace minerals including copper, zinc and selenium have pivotal roles for protecting body functions from damage caused by free radicals, which resulting in optimal production, reproduction and immunity function (Adjepong *et al.*, 2016). Free radicals are unstable atoms, molecules, or ions and they are highly reactive that can damage cells, leading to chronic diseases such as heart and cancer diseases.

### Copper supplementation

Copper is a trace antioxidant dietary mineral that is necessary for many vital functions in mammalian species. Copper is given in the forms of cupric sulfate, cupric oxide, copper gluconate and copper amino acid chelates through oral or parenteral routes. Copper is a cofactor for over 20 enzymes regulating body biochemical reactions (Collins 2014). Copper is involved in many functions including energy production, iron metabolism, antioxidant defense, immune function, red blood cells, brain development and function,

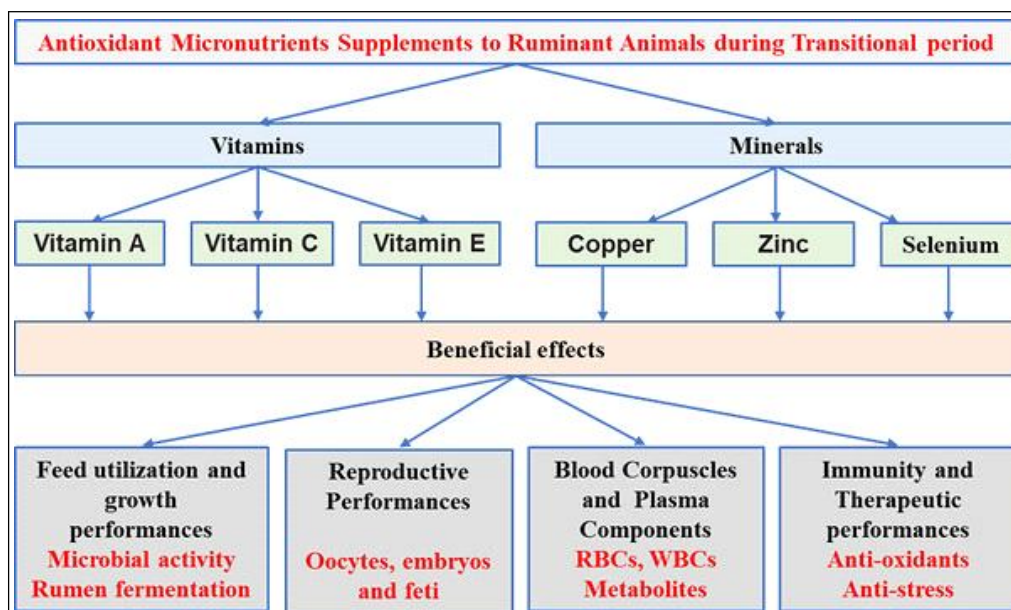


Fig 1: Effects of antioxidant micronutrient supplementation to ruminant animals during transitional period.

collagen and melanin production (Araya *et al.*, 2006; Senosy *et al.*, 2019). Absorption value of copper is low in ruminant species (<1.0-10.0%) in comparison to non-ruminant species (Underwood and Suttle, 1999). Trace metal imbalances including iron, zinc, sulfur and molybdenum are decreased copper bioavailability (Radostits *et al.*, 2007). In addition, copper deficiency in grazing ruminant species is a common problem in the world (Underwood and Suttle, 1999). In Egypt, copper deficiency was confirmed in western desert in soil, pasture and grazing animal species (Yousef, 2006).

Deficiency of copper has an adverse effect on growth and fertility, immune function and iron deficient anemia (Galbat *et al.*, 2015). Copper deficiency leads to abnormalities in cholesterol and glucose metabolism (Roughhead and Lukaski, 2003). It is well-known that estradiol and progesterone are steroidal hormones synthesized from cholesterol (Ačimovič and Rozman, 2013). In addition, it leads to low red blood cells and hemoglobin values and variations in RBCs size and shape. The type of anemia of the Cu-deficient animals observed in our study is macrocytic and hypochromic anemia. Therefore, the examined animals with Cu-deficiency show weakness and paleness in their mucous membranes (Senosy *et al.*, 2019).

Copper supplementation improves reproductive performance through the increase in numbers and sizes of ovarian follicles in addition to increase of ovulation rate (Senosy *et al.*, 2019). The earlier reports concluded that copper administration can stimulate the release of both GnRH (Murawski *et al.*, 2006) and LH release (Michaluk and Kochman (2007). Copper supplementation significantly improved the values of total proteins, albumin, globulin, creatinine and glucose. These positive effects might be attributed to the increase in thyroid hormone values, which had a positive effect on metabolism.

### Zinc supplementation

Zinc is a trace antioxidant dietary mineral that has proposed protective benefits in various physiological functions including cell growth and repair, reproduction, immune function, hormone production and wound healing (Duffy *et al.*, 2023). Zinc is supplemented in the forms of zinc citrate, zinc acetate, zinc glycerate, zinc picolinate and zinc monomethionine. The supportive benefits are due to its antioxidant properties, zinc-related immune signaling and integration into zinc-containing proteins. These specific functions of zinc leads to reducing inflammation, reducing the risk of certain types of cancer, improving cognitive function and protecting against age-related macular degeneration. On the other hand, zinc deficiency leads to various health problems including low growth and reproductive performance.

Zinc dietary supplementation is given in different chemical forms as nano- particles of zinc oxide, zinc sulfate. The zinc supplementation is given to animal species and human as well to give the requirements, promote the function, or repair dysfunctions. Zinc supplementation to

Zn-deficient animals presented a pivotal role in restoration of productive and reproductive performances (Shao *et al.*, 2023). It could promote body health and microbiome balance (Yusof *et al.*, 2023). Zinc supplement improves memory deficit by enhancing anti-oxidative capacity *via* Nrf 2 up-regulation in male Wistar rats (Akintoye *et al.*, 2023). Shao *et al.* (2023) found that dietary zinc supplementation at the level of 120 mg/kg for breeding pigeons improves the carcass traits and breast muscle yield of squabs through regulating antioxidant capacity in liver and breast muscle and myogenic regulatory factor expression. Zinc supplementation to *in vitro* maturation medium of ovine oocytes promotes maturation and further embryo development.

### Selenium supplementation

Selenium is an antioxidant trace mineral that plays a pivotal role in many important body functions including antioxidant defense, immune function, reproduction and muscle function and quality thyroid function resulting in physiological homeostasis. Selenium is supplemented in the forms of selenomethionine or of selenium-enriched yeast or as sodium selenite or sodium selenate (Schrauzer, 2001). Selenium deficiency leads to several health problems including reduced growth and performance, reproductive problems, muscle weakness and poor quality. Selenium supplementation is often used in animal production to improve animal productivity and health. Selenium can be supplemented through different routes either through the diet or water or injection in different chemical forms; from inorganic to organic forms as selenium-enriched yeast and selenium nanoparticles (Ferrari *et al.*, 2023).

Barcelos *et al.* (2023) concluded that inclusion of selenium reduces oxidative stress without influencing oxidative stress in neonates fed with milk from goats supplemented with selenium in their diets. Simultaneously, improvement in immunity and production performance and antioxidant capacity of laying hens over dietary supplementation with selenium polysaccharide from selenium enriched *Phellinus linteus* (Liu *et al.*, 2023). Overall, the evidences does not support the need to supplement selenium beyond the recommended dietary intake to obtain beneficial effects on immune function (Filippini *et al.*, 2023). In addition, the high cost of organic selenium limits its application in animal production (Li *et al.*, 2023).

On the other hand, selenium supplementation could be used to treat dysfunctions during normal and stressful conditions. Selenium supplementation attenuates autism-like behavior and improves inflammation, oxidative stress and related gene expression in an autism disease (Wu *et al.*, 2022). In another study, the effects of dietary organic selenium supplementation were evaluated on the immune responses and histopathology of lambs infected with *Haemonchus contortus*. (Battányi *et al.*, 2023). They concluded that organic selenium supplementation improves immune response and enhances the resistance of lambs to parasitic infection.

### **Antioxidant vitamins supplementation to mammalian species**

The antioxidant vitamins micronutrients refers to A, C and E. Vitamins A and E are fat-soluble vitamins whereas vitamin C (ascorbic acid) is a water soluble vitamin.

#### **Vitamin A and $\beta$ -carotene**

Vitamin A is a fat soluble vitamin and an essential nutrient for animals. Vitamin A is given in the form of retinyl acetate or retinyl palmitate, beta-carotene, or a combination. Vitamin A has multiple roles includes growth, embryo development and immune system functions. Carotenoid or  $\beta$ -carotene is a precursor or pro-vitamin A, acting as an antioxidant to prevent oxidative damage through scavenging free radicals. Aragona *et al.* (2021) investigated the effects of  $\beta$ -carotene supplementation to prepartum Holstein cows on colostrum quality and calf performance after parturition. The study indicated increased of fat and protein concentrations in colostrum upon  $\beta$ -carotene supplementation in addition to improved feed efficiency in calves. On the other hand, supplementing  $\beta$ -carotene negatively affects apparent efficiency of IgG absorption to cows in the prepartum period. Hye *et al.* (2020) explored two intramuscular injection of beta-carotene postpartum on fertility parameters. They found that two treatments with Carofertin postpartum increased  $\beta$ -carotene concentration in blood but had no effect on the fertility parameters.

Green algae (sources of beta-carotene) are variable in size and shape and include thousands species; unicellular and colonial flagellates. Microalgae chlorella was supplemented to support oocyte and embryo production in goats (Silva *et al.*, 2023). The microalgae Chlorella did not enhance the quality of oocytes whereas it improved the quality of embryos and stimulated their mitochondrial function. Our work on *Dunaliella salina* (Senosy *et al.*, 2017; Mohammed 2018; Ali *et al.*, 2021) resulted in improvement in reproductive hormones values, oocytes and embryo development and quality.

Ruminant animals are experienced oxidative stress during transitional period when it is difficult to meet energy demands. This occurs when free radical production exceeds antioxidant production (Goff and Horst, 1997), leading to production of reactive oxygen species (ROS). The ROS are oxygen-containing molecules created during metabolic processes that maintain homeostasis via lipid peroxidation, cell signaling, host defense and apoptosis. Within the body, the balance of ROS is a perpetual process that is affected during times of stress (Albera and Kankofer, 2010). Therefore, the  $\beta$ -carotene as an antioxidant is beneficial for such oxidative stress circumstances.

#### **Vitamin C (ascorbic acid) supplementation**

Vitamin C (ascorbic acid) is a water soluble vitamin and is known as antioxidant agent. Vitamin C is given in the form of ascorbic acid powder added to water or liposomal vitamin C. This encapsulated vitamin C in a liposome is protected

from being destroyed by stomach acid and allows it to absorb easily to bloodstream. In human, ascorbic acid is supplemented when the amount of ascorbic acid in the diet is not enough or intestinal malabsorption problem from cancer or kidney disease. In animals, ascorbic acid has been widely used to alleviate environmental and physiological stressful conditions (Kassab and Mohammed, 2014a). The amount of ascorbic acid produced by the animals under specific environment and physiological conditions might be insufficient to meet their requirements (Pardue *et al.*, 1985). Free radicals under heat stress are generated in a large quantity that the antioxidant defense system are overwhelmed. Consequently, antioxidant agent may provide a pivotal role against heat stress (Sen, 2001). Ascorbic acid supplementation in different kind of stresses and diseases in animals and human have been explored (Kassab and Mohammed, 2014a; Tarzaali *et al.*, 2023).

In human, Xiaoqin *et al.* (2022) reported that dietary ascorbic acid intake is associated with improved glucose metabolism and liver function. They suggested that dietary ascorbic acid might play a pivotal role in reducing the risk of non-alcoholic fatty liver disease (NAFLD). In addition, Tarzaali *et al.* (2023) concluded that ascorbic acid administration and adjuvant thiamine in out-of-hospital cardiac arrest (OHCA) survivors with targeted temperature management (TTM) did not improve their neurologic outcome after one month. Üstündağ *et al.* (2023) explored a new treatment approach of ascorbic acid and melatonin synergy shields against sepsis-induced kidney and heart damage in male rat. The synergistic effect of ascorbic acid and melatonin may provide stronger protection against oxidative stress, tissue damage and inflammation.

#### **Vitamin E supplementation**

Vitamin E is a fat-soluble vitamin is known as antioxidant agent that is essential for animal production and health (Luan *et al.*, 2023). Vitamin E family consists of four tocotrienols and tocopherols (Jiang, 2014). Vitamin E is rare to deficient, which is usually occurred due to digesting dietary fat problem rather than from a diet low in vitamin E. It is an antioxidant important agent for protecting cells from damage caused by free radicals, reproduction and muscle development and immune function (Salles *et al.*, 2022a,b). Vitamin E plays pivotal roles in animal and human reproduction concerning the development of the female and male reproductive organs and protecting the oocytes and spermatozoa from damage. In female, it is necessary for the growth and development and maturation of oocytes and the resulting corpora lutea. In addition, it is improved pregnancy rates and reduced the incidence of early embryonic death as in dairy cattle. In male, it is necessary for production of good quality spermatozoa. It is improved the morphology and motility parameters of spermatozoa and protecting them from free radical damage as in beef bulls. Vitamin E is important for animal production concerning improvement of feed utilization and digestibility, body weight gain and carcass quality (Ali *et al.*, 2004; Kassab

and Mohammed, 2014a). Vitamin E helps to protect cell membrane integrity and the health status. Therefore, it is helpful to reduce incidence of diseases such as udder mastitis and metritis. The animal requirements of vitamin E is depending on the species, age and productive stage. It is generally recommended to provide vitamin E to animal with a diet containing 20-50 IU/kg body weight, which can be taken from feedstuffs including forages and grains. Therefore, vitamin E supplementation is necessary for animals that are not receiving enough vitamin E from their diet including animals that are grazing on poor-quality forages, animals that are being fed high-grain diets, or animals that are under stress. Collectively, the various sources of vitamin E supplementation is a cost-effective way to improve animal production and reproduction. It is a safe and effective nutrient that can be used to improve the performance of all types of livestock and human in addition to repair of dysfunctions under stressful or diseases conditions (Tolba *et al.*, 2023).

#### **Combination of antioxidant mineral and vitamin supplementation**

There are many forms of combination antioxidant mineral and vitamin supplements available, which have unique of ingredients as tablets and capsules, powders and liquid supplements. Several studies were investigated combination of antioxidant mineral and vitamin supplementation for productive, reproductive and treatment of dysfunctions purposes (Kassab and Mohammed 2014a,b; Salles *et al.*, 2022a; Li *et al.*, 2023). Vitamin A ( $\beta$ -carotene), vitamin C and vitamin E are dietary antioxidants increased the antioxidant capacity of body fluids, cells and tissues. The benefits of those vitamins are demonstrated (Li *et al.*, 2023). Collectively, those vitamins can benefit body health through modifying the metabolic activities of the gut microbiota, improving intestinal barrier and maintaining the immune functions. In addition, antioxidant mineral and vitamin as antioxidant agents (vitamin E and selenium) have complementary role in the protection of body cells from the damaging effects of lipid peroxidation and free radicals. The multiple functions of both nutrients extend over antioxidant protection, as their inclusion is associated with improvements productive, reproductive performances and immune competence (Bilbis *et al.*, 2014). Barcelos *et al.*, (2023) concluded that inclusion of vitamin E and selenium reduces oxidative stress without influencing oxidative stress in neonates fed with milk from goats supplemented with selenium and vitamin E in their diets. Simultaneously, vitamin E and selenium inclusion ameliorates the oxidative stress of lactating cattle (Salles *et al.*, 2022a). Furthermore, milk bio fortification was explored through selenium and vitamin E dietary supplementation and sunflower oil (Salles *et al.*, 2022b). They found that the most desirable nutritional profiles of milk was produced by cows without affecting animal productivity.

#### **Antioxidant micronutrients on reproductive performance and fertility**

Antioxidant micronutrients are played crucial roles in reproduction and fertility in mammalian species (Kassab and Mohammed 2014a,b; Senosy *et al.*, 2017, 2019). Firstly, they effect on ovarian follicle development, oocyte maturation, embryo development, pregnancy and offspring (Kassab and Mohammed 2014a,b; Senosy *et al.*, 2017). They change the oxidative stress in follicular fluid of aged women undergoing IVF, which increased the number of good quality oocytes recovered at the pick-up (Luddi *et al.*, 2016). In addition, antioxidant micronutrients play a role in protecting sperm and oocyte from damage, improving hormone production and supporting the development of the embryo and fetus (Al-Gubory *et al.*, 2010).

It has been found that vitamin C is important for placenta and uterus development (Lo *et al.*, 2015). Zinc and vitamin E are important for the production of progesterone and testosterone hormones in addition to sperm development and functions (Weiss, 2022). Furthermore, selenium is known to support maximal expression of the selenoenzymes, sperm development and functions (Zhou *et al.*, 2023). Beta-carotene, vitamins C and E supplementation and to women during pregnancy resulted in a higher pregnancy rate (Menard, 1997). In addition, a higher sperm quality was found over supplementation of zinc, selenium, vitamins C before assisted reproductive technology (ARTs) (Khalil *et al.*, 2019). Therefore, supplementing antioxidant micronutrients is required to maintain body functions, specially reproduction and fertility.

#### **CONCLUSION**

The antioxidant micronutrients' supplementation changes rumen micro-organism, nutrient digestibility, body weight gain, blood and plasma profiles. Such enhancement over antioxidant micronutrient supplementation lead to increase in ovarian follicle numbers and quality of their oocytes, embryos and newborns in addition to alleviation of impairment in body functions. The continuous progress in antioxidant micronutrient supplementation is necessitated for both animals and human as well. Therefore, the future approaches of beneficial antioxidant multiple micronutrient supplementation during different physiological stages are targets for improving productive and reproductive performances and body health.

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#### **Conflict of interest**

There is no conflict of interest for authors to declare.

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