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AN OVERVIEW OF VITAMIN C AS AN ANTISTRESS IN POULTRY

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ABSTRACTS. Stress is commonly used to describe the detrimental effects of a variety of conditions surrounding animals on their health performance. Environmental stress causes an increase in oxidative stress and an imbalance in antioxidant status. Oxidative damage increases in stressed poultry when the plasma antioxidant vitamins and minerals such as vitamin C, E, folic acid, and zinc levels declined. Stress factors in birds involve many elements of the transport processes which may be detrimental to the birds. These factors include alteration in atmospheric temperature, poor handling by man, removal of feed and water (starvation), high ambient temperature (AT) and relative humidity (RH). Other factors include vaccination, disease conditions such as coccidiosis, novelty, confinement, motion, noise, micro-thermal core within the vehicle and the use of inappropriate vehicles. Live birds of all ages are being transported mainly by roads to their various destinations

and across different ecological zones throughout the year. Mortality increases with journey length. The adverse effects of these factors and their combinations may range from mild discomfort and aversion to death. This review was aimed at providing comprehensive information on the role of vitamin C in combating stress factors in poultry with the view of exploring its potentials for future research undertaking. Information compiled in this review were obtained from search engines such as Elsevier, Pubmed, Springer, Medline, Science Direct, Google Scholar, and a library search for articles published in peer-reviewed journals. 100 to 200 mg/kg feed of vitamin C is capable of converting stress factors in poultry and thereby improving the productivity.

Keywords: vitamin C, antistress, poultry, mortality rate, comprehensive information.

INTRODUCTION

Research studies carried out in UK by Bayliss and Hinton (1990) have demonstrated that “transport stress” attributes up to 40% of “dead on arrivals” (DOA). Stress factors act concurrently on transport animals, impair normal body functions, leading to increased morbidity and mortality. It also causes decreased productivity and poor meat quality (Fallenberg and Speisky, 2006; Franco-Jimenez and Beck, 2007; Rozenboim *et al.*, 2007; Ajakaiye *et al.*, 2010). Lin *et al.* (2006) reported that heat-stress reduces the micronutrient intake by way of reducing feed intake. These micronutrients include vitamins A, C and E, which play important roles in the performance and immune functions of poultry. Vitamin C plays an important role in the biosynthesis of corticosterone which is a hormone that enhances energy supply during stress. Heat stress in different extents severely affects laying percentage, egg size, egg shell durability, body weight gain and mortality (Sterling *et al.*, 2003; Lin *et al.*, 2004; Franco-Jimenez and Beck, 2007; Ajakaiye *et al.*, 2010). Study conducted by Ramnath *et al.*, (2008) has shown that heat stress was provoked by a combination of high AT and RH, and excessive generation of free radicals (FR) or reactive oxygen species (ROS) as a result of increased metabolism in poultry. For example, Northern Nigeria is characterised by high AT and RH during the hot dry season, with little or no water supply to the transport birds, that

aggravates transport stress factors (Ayo and Oladele, 1996). Laboratory analysis changes in hematological parameters of heterophil/lymphocyte ratio (H/L) serves as a good indicator of stress factors (Altan *et al.*, 2000; Huff *et al.*, 2005).

Vitamin C (chemical names: ascorbate and ascorbic acid [AA]) is a 6-carbon lactone that is synthesised from glucose by poultry including many animals. Ascorbic acid is synthesised in the kidney in birds and reptiles, and in the liver in some mammals. Birds have the ability to synthesise AA but this ability is insufficient under stress conditions such as high or low AT, RH, high productive rate and parasite infestation (Sykes *et al.*, 1976; McDowell *et al.*, 1989; Gursu, 01/2004). A number of studies reported by Kutlu *et al.*, (2001) has shown the beneficial effect of AA supplementation on urea, glucose, triglycerides, albumin and cholesterol protein concentrations, and alkaline phosphatase.

Vitamin C Combats the Effects of Heat-Stress

Ascorbic acid improves performance associated with the suppressed stress responses indicated by lowering of the plasma corticosterone level and adrenocorticotrophic hormone (Mckee and Hurrison, 1995; Sahin, 2003; Mahmoud *et al.*, 2004, Lin *et al.*, 2006). Under heat-stress condition, AA supplementation in poultry feeds has been reported to have positive effects such as weight

gain and improved immune response (Puthongsiriporn *et al.*, 2001; Lin *et al.*, 2003). Vitamin C also promotes resistance to some infectious and contagious diseases (Gross, 1988). Attia (1976) and Kutlu (1993) observed reduction in rectal and skin temperatures in broilers and layers given AA and also reduced panting rates. This suggests that AA may decrease the heat load of birds, increase heat loss or improve the birds' tolerance to high AT. Peebles and Brake (1984) also reported that supplemental AA holds promise for increased production during high AT or for nutritionally marginal diets. When AA was used at levels of 100 mg/kg (45 mg/lb) of feed or less for commercial layers, there was improvement in egg production, egg shell quality and livability. Perek and Kendler (1963) conducted two different experiments in Jordan Valley, where hens were subjected to hot temperatures, and reported an increase in egg production of 11.2% and 23% with birds given supplemental AA. Increased egg weights and decreased culls and mortality were also reported, but the shell quality remains the same.

The use of AA and vitamin E as antioxidants in combating heat stress has been documented in pullets (Sinkalu and Ayo, 2008) and in Japanese quails (Sahin *et al.*, 2003a, b; Ciftci *et al.*, 2005). Demir *et al.* (1995) reported that AA supplementation in the diet (200 mg/kg) during heat stress increased feed intake and thickness of the eggshell. Mckee and Harrison (1995) also detected an

improvement in feed conversion ratio of broilers as a result of AA supplementation during heat stresses. However, Miraei-Ashtiani (2004) reported that AA inclusion in broiler feed did not result in any difference in cloacal temperature compared to chickens receiving diet without AA supplementation.

Vitamin C Combats the Effects of Cold-stress and Disease Stress Factors

Pardue and Williams (1990) reported that plasma AA levels in poultts were significantly depressed by cold stress, injection at one and 14 days of age, and beak trimming. However, a study by Douglas *et al.* (2004) has shown that individuals under stress, such as those exposed to cold weather or strenuous physical exercise, may experience therapeutic benefit. Supplemental vitamin C (150 mg/kg or 68.2 mg/lb diet) enhanced performance of broiler chicks exposed to multiple concurrent environmental stress factors (McKee and Harrison, 1995). Many reports documented the beneficial effect of supplementing the diet with AA for both stressed newly hatched and mature poultry. The benefits include; improved egg production, growth rate, egg shell strength and thickness, spermatozoa production and fertility. It also counteracts the adverse effects of unfavourable weather and housing conditions, and in case of intoxications or diseases (McDonald *et al.*, 1981; Bashir *et al.*, 1998).

Satterfield *et al.*, (1940) reported that birds infected with fowl typhoid had decreased levels of AA in their blood and administration of AA at 1,000 mg/kg (454 mg/lb) in the diet resulted in decreased early mortality due to the typhoid infection. Chickens fed with a diet containing AA supplement showed more resistance to a combined *Mycoplasma gallisepticum* and Newcastle disease virus infection, and to primary as well as secondary *E. coli* challenges (Takahashi *et al.*, 1991). Frikke-Schmidt and Lykkesfeldt (2009) and Versari *et al.* (2009) reported that several randomised, double-blind, placebo-controlled studies had shown that treatment with AA consistently results in improved vasodilation in individuals with coronary heart disease as well as congestive heart failure, high cholesterol, and high blood pressure.

Vitamin C Alleviates Nutritional Stress

Vitamin C can also alleviate nutritional stress. Balnave *et al.* (1994) showed that poor egg shell quality of birds given saline drinking water could be overcome by addition of AA to the water (1 g/L). Supplemental AA provided definite improvements in egg production, interior egg quality and egg shell strength (El-Boushy and Van Albada, 1970; Cheng *et al.*, 1990).

Vitamin C Acts as an Anti-Oxidant and as Photoprotectant

Vitamin C plays major roles in cellular anti-oxidant defenses. Seven (2008) reported that AA acts as an anti-oxidant not through reaction with all oxygen species but by formation of dehydroascorbyl (a particular inert radical), and also by transferring radical equivalents from lipid phases too. In complement, AA participates in the regeneration of reduced glutathione from oxidised form in the cytoplasm and allows tocopherol regeneration through non-enzymatic reactions (Luadicina and Marnett, 1990).

Vitamin C limits the damage induced by ultraviolet (UV) light exposure. Vitamin C is not a sunscreen because it does not absorb light in the UVA or UVB spectrum. Rather, the antioxidant activity of AA protects against UV-induced damage caused by free radicals (Darr *et al.*, 1992). Vitamin C transport proteins are elevated in keratinocytes in response to UV light, suggesting an increased need for AA uptake for adequate protection (Kang *et al.*, 2007; Steiling *et al.*, 2007). UV light reduces AA content of skin, an effect that is dependent on the intensity and duration of UV exposure (Shindo *et al.*, 1993; Shindo *et al.*, 1994; Podda *et al.*, 1998). In cultured keratinocytes, the addition of AA decreases lipid peroxidation and UV-related DNA damage, limits the release of pro-inflammatory cytokines, and protects against apoptosis (Stewart *et al.*, 1996; Tebbe *et al.*, 1997). Nakamura

et al. (1997) and Savini *et al.* (1999) study indicated that AA modulates redox-sensitive cells signaling in cultured skin cells and consequently increased cells survival following UV exposure. Cosgrove *et al.* (2007) reported that higher intakes of dietary AA have been correlated with a decreased risk of dry skin, suggesting that it may have effects on trans-epidermal water loss. Studies by Ponec *et al.* (1997) and Pasonen-Seppanen *et al.* (2001) in cell culture models, showed that addition of AA increases the synthesis of barrier lipids, that established a functioning stratum corneum with decreased water permeability. However, Campos *et al.* (2008) suggested that AA increases trans-epidermal water loss when applied to the skin.

Vitamin C Acts as Pro-oxidant and Improves Resistance to Infections

McGregor *et al.* (2006) reported that AA also behaves as pro-oxidant. Satoh and Sakagami (1997) has shown that AA reduces transition metals, such as cupric ions (Cu^{2+}) to cuprous (Cu^{1+}), and ferric ions (Fe^{3+}) to ferrous (Fe^{2+}) during the conversion of ascorbate to dehydroascorbate *in vitro*. This reaction can generate superoxide and other reactive oxygen species (ROS). Copper (Cu) and iron (Fe) are bound to diverse proteins, though; free transition elements are unlikely to be present in the body (McGregor *et al.*, 2006). Reduction of Fe^{3+} into Fe^{2+} improves iron assimilation by

intestine and thereby improves resistance to infections. Hallberg (1987) reported that 25 mg and upwards amount of dietary AA is required to increase iron absorption and depends largely on the amount of inhibitors, such as polyphenols and phytates, present in the meal. Locally, oxidative lesions leading to conformational modifications of proteins could induce pancreatic enzyme inhibition and/or dietary protein resistance to digestion. Consequently, the presence of anti-oxidants (vitamin E and/or C) could partially interfere with oxidative protein denaturation and would improve digestibility of nutrients and feed efficiency. Mühlhöfer *et al.* (2004), however, reported that the intravenous use of AA does not appear to improve pro-oxidant activity. Thus, AA as a pro-oxidant is unlikely to convert metals to create ROS *in vivo*.

Vitamin C Facilitates Wound Healing and Involves in Maintenance

One of the distinctive features of scurvy is poor wound healing (Lind, 1751). Kim *et al.* (1993) and Shukla *et al.* (1997) reported rapid decrease of vitamin C levels at a site of injury. Inflammatory responses often increase free radicals at the wound site. Studies by Shukla *et al.* (1997) and Sorensen *et al.* (2005) have shown that the presence of AA may limit free radical damage and free radicals may play a complex role in the healing response that is not yet understood. However, in wound healing, synthesis of more dermal collagen

may elevate the utilisation of AA. Vitamin C facilitate wound healing by promoting keratinocyte differentiation (Savini *et al.*, 2002; Duarte *et al.*, 2009), stimulating the formation of the epidermal barrier (Boyce *et al.*, 2002), and re-establishing the stratum corneum (Ponec *et al.*, 1997). It helps in the maintenance of collagen which is a structural protein for cartilage, bones, muscles and blood vessels. It plays roles in shell's organic matrix formation thereby improves eggshell quality. Geesin *et al.* (1988) reported that AA stabilises collagen mRNA, thus increasing collagen protein synthesis for repair of the damaged skin. This occurs concurrently with a decrease in elastin production; the elastin protein is often overproduced in response to photodamage (Davidson *et al.*, 1997). Phillips *et al.* (1994) indicated that AA elevates rate of proliferation of fibroblasts, an ability that decreases with age. Duarte *et al.* (2009) reported AA stimulates DNA repair in cultured fibroblasts. Vitamin C is included in oral therapies for pressure ulcers and burns, along with vitamin E, zinc and other nutritive factors (Boyce *et al.*, 2002; Desneves *et al.*, 2005; Ellinger *et al.*, 2009).

Vitamin C Boosts 'Immune System'

A high concentration of AA are found in immune cells and is consumed readily during infections. However, it is not certain how AA interacts with the immune system; but it has been hypothesised to modulate the activities of phagocytes, the

production of lymphocytes and cytokines, and the number of cell adhesion molecules in monocytes (Preedy *et al.*, 2010).

Usage of Vitamin C as 'Antihistamine'

Vitamin C is a natural antihistamine. It both improves the detoxification of histamine and prevents its release. Low concentrations of serum AA has also been correlated with increased serum histamine levels (Clemetson, 1980; Johnston *et al.*, 1996).

Vitamin C Plays Important Roles in Sperm Production

Vitamin C supplementation favoured male reproduction. Monsi and Onitchi (1991) supplemented the diets of heat-stressed broiler breeders with 0, 125, 250 or 500 ppm of AA. The result showed significant increase in semen volume, motile sperm per ejaculate and total sperm per ejaculate. Dobrescu (1987) found that sperm concentration and semen volume of tom turkeys increased by 28% when 150 mg/kg (68.2 mg/lb) supplement of AA was added to breeder ration. Noll (1993) reported 16% improved semen volume and 18% increased sperm concentration when 200 mg/kg (90.0 mg/lb) of AA was supplemented for eight weeks in male breeder turkeys ration. Noll (1997) also reported improved sperm cell concentrations in males and more eggs per hen when turkey breeder diets were supplemented with 200 mg/kg (90.9 mg/lb) of AA. Vitamin C therefore improved

reproductive performance in spite of environmental temperature fluctuations

Absorption, Transport And Excretion

Vitamin C is absorbed in the body by both simple diffusion and active transport. Sodium-Dependent Active Transport Sodium-Ascorbate Co-Transporters (SVCTs) and Hexose transporters (GLUTs) are the two transport systems required for absorption. SVCT1 and SVCT2 import the reduced form of AA across the plasma membrane (Savini *et al.*, 2008). Sotiriou *et al.* (2002) reported GLUT1 and GLUT3 as the two glucose transporters and transfer only the dehydroascorbic acid form of AA. Although dehydroascorbic acid is absorbed in higher rates than AA, the amount of dehydroascorbic acid found in plasma and tissues under normal conditions is low, as cells rapidly reduce dehydroascorbic acid to AA (Packer, 1997; May *et al.*, 2003). Thus, SVCTs appear to be the predominant system for AA transport in the body. SVCT2 is involved in AA transport in almost every tissue (May *et al.*, 2007), the notable exception being red blood cells, which lose SVCT proteins during maturation (Savini *et al.*, 2008). With regular intake, the absorption rate varies between 70 to 95%. However, the degree of absorption decreases as intake increases.

Vitamin C Storage Organs

The renal threshold largely determines body's maximal store of vitamin C

for blood. Vitamin C concentration is maintained by many tissues far higher than in blood. Hediger (2002) reported that the biological tissues which accumulate over 100 times the level of AA in blood plasma are the adrenal glands, thymus, pituitary, retina and corpus luteum. Those tissues with 10 to 50 times the concentration present in blood plasma include the brain, lung, spleen, testicle, liver, lymph nodes, thyroid, pancreas, small intestinal mucosa, leukocytes, kidney and salivary glands.

Relationship of Vitamin C to Other Vitamins

Vitamin C is active in altering folic acid to tetrahydro derivate, a reduced form, and deficiency of AA impairs use of folic acid. McDowel *et al.* (1989) reported greater antioxidant ability against oxidative damage when a combination of antioxidant vitamins and minerals are utilised. It is involved in the regeneration of vitamin E, and these two vitamins appear to work together in their antioxidant effect. Vitamin C reduces the toxic effects, due to excessive intake of vitamin A. Vitamin C serves as a cofactor for the bioconversion of vitamin D3 to its active form 1,25-(OH)2D3. Weiser *et al.* (1990) reported that 100 mg per kg of AA in the chick feed elevated plasma concentrations of 1,25-(OH)2D3. The led to increased activities of duodenal calcium-binding protein and greater weights and breaking strength of bones. Farquharson *et al.* (1998) has shown that AA influences the

developmental processes in the growth plate for bone growth.

VITAMIN C INSUFFICIENCY AND PATHOLOGY

Disease conditions have been found to affect AA metabolism in poultry. When chicks were infected with fowl typhoid, intestinal coccidiosis, their plasma AA concentrations were reduced (Hill and Garren, 1958; Kechik and Sykes, 1979). The dietary addition of AA to broiler diets significantly reduced the ascites mortality while it had no effect on performance parameters (Ladmakhi *et al.*, 1997). A common feature of AA deficiency is anemia. The anti-oxidant properties of AA may stabilise folate in feed and plasma, and increased excretion of oxidised folate derivatives in human scurvy was reported (Stokes, 1975).

Padayatty *et al.* (2003) showed that deficiency symptoms of AA (known as scurvy) appear once plasma concentrations drop below 10 micro-molar, a level that can be prevented by consuming as little as 10 mg of AA daily. Declines in collagen synthesis result in cutaneous manifestations of scurvy, leading to fragility of blood vessels and disruption of connective tissue. Early symptoms in the skin include spots of small subcutaneous bleeding and a thickening of the stratum corneum (Hodges *et al.*, 1969; Hodges *et al.*, 1971). Wound healing is impaired as scurvy progresses, due to the loss of mature collagen that allows wounds to remain open (Lind *et al.*, 1953; Ross

et al., 1962). Skin lesions caused by AA deficiency are remediated by an adequate intake of AA.

Dietary Sources Of Vitamin C

Haytowitz, (1995) reported vitamin C found in many vegetables and fruits. Citrus fruits and juices are particularly rich sources of AA, but other fruits such as mangoes, papaya, strawberries, watermelon, and tomatoes also contain variable amounts of AA. Vegetables such as cabbage, Brussels sprouts, bean sprouts, cauliflower, red and green peppers, peas, and tomatoes may be more important sources of AA than fruits. This is often true because vegetable supply extend for longer periods during the year than fruit supply.

Vitamin C Overdose/Adverse Effects

Vitamin C is water soluble, with dietary excesses not absorbed. An excess of AA in the blood are rapidly excreted and thereby, exhibits remarkably low toxicity. The LD50 (the dose that will kill 50% of a population) in rats is accepted to be 11.9 g/kg body weight when given by forced gavages (orally). The mechanism of death from such doses is unknown but may be more mechanical than chemical (Safety (MSDS) data for ascorbic acid, 2005).

Relatively large doses of AA may cause indigestion, particularly when taken on an empty gizzard. However, taking AA in the form of calcium ascorbate and sodium ascorbate may lower this effect

(Pauling, 1976). Taken large doses of AA causes diarrhea, nausea and fatigue in healthy subjects. As AA enhances Fe absorption (Fleming *et al.*, 2002), Fe poisoning can become an issue in subjects with rare Fe overload disorders, such as hemochromatosis. A genetic condition that results in decrease levels of enzyme glucose-6-phosphate dehydrogenase (G6PD) which can cause sufferers to develop hemolytic anemia after ingesting specific oxidising substances, such as very large dosages of AA (Cook and Reddy, 2001).

Vitamin C, at concentrations above the renal re-absorption threshold, is passed freely and excreted. At high dietary doses, AA is accumulated in the body until the plasma levels reach the renal re-absorption threshold. Traxer *et al.* (2003) and Massey *et al.* (2005) reported that a high intake could increase the risk of oxalate (an AA metabolite) kidney stones. Some studies have reported that supplemental AA increases urinary oxalate levels (Wandzilak *et al.*, 1994; Liebman *et al.*, 1997; Auer *et al.*, 1998). Choi *et al.* (2009) found that total daily AA intake was inversely associated with risk of gout, with higher intakes being associated with greater risk reductions. High doses of AA have also been found to interfere with the interpretation of certain laboratory examination such as serum creatinine, serum bilirubin, and the guaiac assay for occult blood (Hendler *et al.*, 2001).

Testing of Vitamin C in the Body

Simple tests use dichlorophenolindophenol a redox indicator, to measure the levels of AA in the serum or blood plasma and the urine. However, these reflect recent dietary intake rather than the level of AA in body stores (*Food Standards Agency*, 2007). Reverse-phase high-performance liquid chromatography is used for determination of storage levels of AA within a tissue and lymphocytes. It has been observed that serum or blood plasma levels follow the circadian rhythm or short-term dietary changes. Those within tissues themselves are more stable and give a better view of the availability of AA within the organism (Yamada *et al.*, 2004; Emadi-Konjin *et al.*, 2005).

CONCLUSION

Vitamin C plays an important role in the biosynthesis of corticosterone, a hormone that enhances energy supply during stress. It is actively transported into tissues. The utilisation and demand for AA increase during periods of heat-stress but its synthesis is limited. The improved performance resulting from the use of AA is associated with the suppressed stress responses indicated by reduction in plasma corticosterone level (Mckee and Hurrison, 1995; Mahmoud *et al.*, 2004) and adrenocorticotrophic hormone (Sahin, 2003). Vitamin C plays major roles in cellular anti-oxidant defence where it participates in the regeneration of

reduced glutathione from its oxidised form in the cytoplasm and allows tocopherol regeneration through a non-enzymatic reaction (Luadicina and Marnett, 1990). It also acts as a pro-oxidant and thereby improves iron assimilation by reduction of Fe^{3+} into Fe^{2+} which is more assimilated by intestine and thereby, AA improves resistance to infections. It also plays important roles in sperm production, photoprotection, wound healing, boost the immune system and prevents dry skin, histamine release and increases the detoxification of histamine. The dosage of AA required to achieve antistress activities usually ranges from 100 to 200 mg/kg feed.

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