Ascorbic Acid a Universal Supplement that Mitigates Road Transportation Stress in Ruminants: A Review

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ABSTRACT
This write up is aimed at proffering solution to the problem of transportation stress in ruminants in the tropics. Ascorbic acid is safe as buttressed in this write up for the improvement of meat quality in short and long terms road transportation stress. This review cover an aspect of animal physiology of stress. Veterinary pharmacological aspects of ascorbic acid, how ascorbic acid improved meat product of animals exposed to long transportation stress. Ascorbic acid had no withdrawal period and was scientifically proven to be advantageous to meat characteristics and animal welfare. This was given credence to by its advantages as an anti-oxidative agent in solving the menace of road transportation to veterinarians, animal scientists and other professionals in the livestock industry.

Key words: Ascorbic acid, Ruminants, Supplement, Transportation.

Transportation is one of the husbandry practices that is common and inevitable globally through often neglected. Animals must be moved for sale, production, slaughter, research and other uses (Ayo et al. 2006; Adenkola et al. 2009; Biobaku et al. 2018b).

It had also been scientifically established that it is not safe for humans and animals to consume meat from an animal with stress syndrome, because this could cause reproductive anomalies (Irwin et al., 2012). Ruminants are very important sources of meat (FAO 2005). Previous researches centered on transportation and alleviation of stress are those of Ayo et al. (2006), Minka and Ayo, (2007) and Adenkola et al. (2009). Transportation without good welfare exerts psychological and physical stress on the animals and could affect meat quality and result to losses (Fazio and Ferlazzo, 2003; Ayo et al., 2006). There is also a negative influence of climate change which causes heat stress and it was suggested that mitigation is important for animal welfare (Das, 2018).

The detrimental effects of long term road transportation and extreme environmental temperatures were previously studied by Sanusi et al., 2010; Biobaku et al., 2018 and Biobaku et al., 2020. Previous studies centered on the effect of temperature on animal hair coat colour in the tropics and it was found out that darker animals are more predisposed to stress than animals with lighter hair coat. In the light of this, the write up would be of value to the farmers and livestock marketers and other stakeholders in the livestock industry including the consumers. Stress has been observed to affect health and meat characteristics in food animals. The use of drugs that could help in alleviating stress of transportation would be valuable if used judiciously. But most drugs have residual effect and withdrawal periods and adherence to this could be difficult by stakeholders in the agro allied investment.

This study reviews the prospects of supplementing ascorbic acid in stress of transportation in ruminants and its scientific prospects of acceptability. This work could help to improve animal health, product quality of ruminant meat and profitability of livestock enterprise.

Physiologic considerations
Stress and handling of livestock at transportation
Animals can be stressed psychologically during restraint or handling while physical stress are exemplified by hunger, thirst, fatigue, injury or thermal extremes (Grandin, 1997). Procedures such as restraint in a squeezed chute do not usually cause significant pain, but may induce fear which is a psychological stressor in extensively raised animals (Grandin, 1997). An animal that is handled in a rough way may respond more aggressively, especially if previous handled in rough and painful way (Gregory, 2004). Rough handling would be detrimental to an excitable or temperamental animal when compared to a placid temperamental animal (Gregory, 2004; Appleby et al., 2008).

This is because animal that are aggressive have excitatory neurotransmitters that are produced at relatively higher rate than the docile ones (Appleby et al. 2008). This leads to excitement and aggression due to increased surge of adrenaline and corticosteroids thereby causing immuno-compromise and affecting the meat product (Ayo et al., 2010). This fact was buttressed in a study with Brahman cross bred cattle which showed that they had higher cortisol levels.
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When restraint in chute than English Cross bred cattle (Zavy et al., 1992).

Subordinate submissive animals are more stressed than the dominating animals. This is because subordinate animals were bullied coupled with other external stress factors such as environmental condition or transportation, which could cause more stress (Gregory, 2004). The effect of such stress could either be immediate alarm reaction or the general adaptation syndrome both of which are detrimental to the health and products from the animal (Gregory, 2004).

Ascorbic acid as an anti-stress agent in transportation of food animals

Several studies have demonstrated the effect of ascorbic acid on biochemical and other signs of various types of stress, such as in birds (Nakano and Suzuki, 1984). Stress provokes significant rise in the serum level of ascorbic acid, caused a reduction in its concentration in skin, lungs and adrenal glands. Zulkifli et al. (2000) showed that the mechanism by which ascorbic acid alleviates stress is not fully understood. However, ascorbic acid is thought to be involved in the regulation of the sympathetic and parasympathetic nervous systems, thereby increasing resistance to infection, increasing the defense mechanism and anti-oxidant status of the animal and reduce the detrimental effects of certain eicosanoids (Stilborn et al. 1988). The elimination of reactive oxygen species could likely be one of modes of ascorbic acid anti-stress activity (Minka and Ayo, 2007). Road transportation or any form of transportation followed by loading, unloading, journey to abattoir, lairage or market and other related procedures may have adverse effects of the carcass quality (Singh, 2007). Transportation coupled with other forms of stress such as sound, humidity, cold and fear may be reflected on the carcass quality. The reaction sequel to subjecting food animals to stress of transportation could be detrimental according to Singh, (2007) and Biobaku et al. (2016a) manifested alarm or emergency reaction and general adaptation syndrome (GAS) in (Fig 1) it was shown that ascorbic acid administration tends to anti oxidants and improved heart rate variability and survival. Physiology would be adjusted to normacy with the use of ascorbic acid.

Alarm reaction occurs as a result of sudden adverse stimuli that take place immediately. It is reflected in an increased activity of sympathetic nervous system which involves the involuntary muscle secreting glands and the heart (Guyton and Hall, 2006). This results in increased released catecholamines such as noradrenaline and adrenaline from the adrenal medulla which could lead to increased heart rate and force of contraction, constriction of peripheral blood vessels, elevated blood pressure, cessation of digestion and mobilization of liver glycogen and hyperglycaemia due to the release of glucagon from islets of langerhans of the pancreas (Chauhan and Agarwal, 2006). The glucose level increases as a result of mobilization of liver glycogen (Chauhan and Agarwal, 2006). The alarm reaction is followed by the GAS, which lasts for a long period. The GAS due transportation stress could be associated with the release of adrenocorticotrophic hormone by the pituitary gland and brings about the production of corticosteroids such as cortisol which mobilizes carbohydrates, protein and fats on long term basis resulting to gluconeogenesis but this adverse effect of this would be averted if therapeutic intervention and welfare is rendered to the animals (Biobaku et al., 2016a and Biobaku et al., 2016c). Also ketone bodies are produced by fats metabolized in the liver. This sequence of biochemical changes result in increased glucose level in the blood and ketone bodies as a result of ketosis (Chauhan and Agarwal, 2006). Other changes associated with GAS are hypertrophy of the adrenal gland but reduction of ascorbic acid content and cholesterol level (Biobaku et al. 2017). There would also be eosinopenia and lymphocytopenia which is accompanied by polynucleosis which is an increase in polymorphonuclear leucocytes; this would cause a general effect of increase susceptibility to disease, weight and growth reduction (Biobaku and Amid, 2018). The (Fig 2) also showed how ascorbic acid aided ameliorated short and long term road transportation stress adjustment to metabolism by improvement of electrolytes namely: sodium, potassium, calcium and chloride ions respectively.

Reversing anxiety of transportation stress by ascorbic acid and its advantages

Vitamin C is an antioxidant with high reducing potential. It donates one electron to a variety of oxidants, including oxygen free radical and peroxides. It also appears to have an important function in the reduction of oxidized vitamin E within red blood cells membrane (May, 1998). The single electron-oxidized formed is a stable free radical that can either donate or accept electrons. The dehydroascorbate and the two electron-oxidized forms can be reversed back its reduced form to an ascorbate non-enzymatically by glutathione transferase and enzymatically via the glutaredoxin reaction. The use of Vitamin C in the alleviation of stress was suggested to be due to its antioxidative property that is its ability to mop up oxygen radicals effect of stress was suggested to be due to its antioxidative property that is its ability to mop up oxygen radicals effect of stress was suggested to be due to its antioxidative property that is its ability to mop up oxygen radicals effect of stress was suggested to be due to its antioxidative property that is its ability to mop up oxygen radicals effect of stress was suggested to be due to its antioxidative property that is its ability to mop up oxygen radicals effect of stress was suggested to be due to its antioxidative property that is its ability to mop up oxygen radicals effect of stress was suggested to be due to its antioxidative property that is its ability to mop up oxygen radicals effect of stress was suggested to be due to its antioxidative property that is its ability to mop up oxygen radicals effect of stress was suggested 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Biobaku, 2015a; Biobaku et al. 2018). It could be administered virtually in all food animals with relative safety due to its wide therapeutic window. It improves animals shelf life and colour of the meat, it improves carbohydrates and intermediary pathways of metabolism, improves the energy expenditure in muscles of transported or stressed animal, administration is easy, possess wide safety margin with less tendency to cause toxicity (Biobaku et al. 2017). Ascorbic acid is among the cheapest supplements used in animal welfare due to the aforementioned reasons why ascorbic acid is the supplement of choice in rendering transportation welfare (Biobaku et al. 2017; Biobaku et al. 2018).

Figure 1: Diagram to show ascorbic acid the ameliorating stress of transportation in food animals.

Physiological role of ascorbic acid

Ascorbic acid functions as a co-factor in a number of hydroxylation and amidation reactions by transferring electrons to enzymes that provide reducing equivalents (Levine et al. 1993). Thus, it is required for facilitation of conversion of proline and lysine residues in procollagen to hydroxyproline and hydroxylysine. The role of ascorbic acid hydroxylation of proline and lysine amino acid residues in the stabilization of collagen molecule had been studied extensively by previously and it was linked with scurvy. In the beginning of last decade (Levine et al. 1993) mentioned that at least 27 collagen types with at least 42 distinct polypeptide chains and more than 20 more similar to collagen domains were identified in his previous study. In collagens that have similar to collagen domains were associated with 4-hydroxyprolines were previously found to be ubiquitous (Chauhan and Agarwal, 2006; Levine et al. 1993). It was therefore; found that proline hydroxylation has a very vital role in interaction between proteins in the organism and the maintenance of physiologic mechanisms. In the course of collagen synthesis, the oxidation of lysine side chains in protein to provide hydroxylated lysine for carnitine synthesis, the conversion of folic acid to folinic acid, microsomal drug metabolism, and hydroxylation of dopamine to form norepinephrine. Ascorbic acid promotes the activity of an amidating enzyme thought to be involved in the processing of certain peptide hormones, such as oxytocin, anti-diuretic hormone and to cholecystokinin (Levine et al. 1993). This could also be linked with the reason of ascorbic acid modulating effect on serum electrolytes such as sodium, potassium, chloride, calcium and phosphorous. In previous studies (Ayo et al. 2009; Biobaku et al. 2016b and Biobaku et al. 2018) gave insight of the amelioration of electrolytes...
deficit by ascorbic supplementation. Trace elements are also of importance to fertility in domestic animals this is due to the fact electrolytes are of utmost importance to the physiology of animals Talukdar et al. (2016). The Fig 2 gives a simplified schematic diagram to show the adjustment of ascorbic acid when administered in animals subjected to long and short term transportation stress in animals. The physiologic roles of ascorbic acid its synthesis in the biological system and other questions concerning its biochemical such molecular transport, metabolism and antioxidative role were attended to by Mandi et al. (2009) Kumar et al. (2010) Majekodunmi et al. (2015) and Biobaku et al. (2018).

Veterinary Pharmacological Considerations

The ease of selection of administrative route of ascorbic acid

In food animals, ascorbic acid could be administered either through the parenteral as previously adopted by Biobaku et al. (2016) or enteric routes Ayo et al. (2006). The ease for the selection of the routes of administration is due to its wide safety margin in food animals species most importantly ruminants. The safest and the most convenient route of administration in rendering welfare in transported animals is the oral route. Adenkola et al. (2011) administered ascorbic acid orally, while previous researchers adopted the intramuscular route (Biobaku et al. 2016c); Fazeli et al. (2010) administered subcutaneously in goats. In another study by Biobaku et al. (2016b) it was administered orally in bucks in a study conducted to improve animal welfare in long term transportation stress and for improvement of chevon. Most recently in a study in bucks ascorbic acid was administered orally in improvement of animal welfare at long term road transportation and at different stocking rates (Biobaku et al. 2020).

The pharmacokinetics of ascorbic acid was found to be in consonance with the first order kinetics (Hickey et al. 2008) in humans. In studies in humans various route had been exploited in the administration of ascorbic acid (Hickey et al. 2008) used the oral route of administration of ascorbic acid, while Kazemi et al. (2014) explored the intravenous route in their study on gene expression. Stephenson et al. (2013) used the intravenous route at a high dose in patients with cancer. The study of Stephenson et al. (2013) gives credence to the safety in spite the clinical condition of the patients. Hidroglou et al. (1997) administered it orally in sheep. The decision for selection for the route of administration in critical cases and for animal welfare in animals is the duty of the veterinarian to ensure appropriate

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**Fig 2:** Diagram to show the ameliorative effect of ascorbic acid on Serum Electrolytes.
choice for the management of the case at hand. In most studies in Sub-Saharan Africa oral route is the most exploited route of administration of ascorbic acid in amelioration of stress. In goats the parental and enteral route had no significant influence on the surge of cortisol and interleukin-6 and enzymatic antioxidative markers of stress (Biobaku et al. 2018b).

Dose consideration of ascorbic acid in road transportation stress

Ascorbic acid is a supplement with broad safety margin and could be given at high dose range. Ascorbic acid could be given at hundred of milligram. Ayo et al. (2006) administered ascorbic acid at 200 mg/kg in Sokoto red; Adenkola et al. (2009); Minka and Ayo (2009) administered ascorbic acid at 200 mg/kg. Biobaku, (2015a) Biobaku et al. (2016b) Biobaku (2017) administered it at 150 mg/kg, 200mg/kg, 300 mg/kg and 400 mg/kg respectively. Experience had shown that it is safe to give up to 2000 mg/kg daily in ruminants because their urine is alkaline in nature. Veterinary clinical experience of close to two decade had shown that ascorbic acid had been proven in the management of endemic contagious ecthyma, PPR and improved healing of mange and other common in small ruminants in the tropics when administered intramuscularly. It should however be given with caution deep intramuscularly at a lower dose in one site because it could predispose to irritation. Dose consideration of ascorbic acid in transportation could be determined based on the following factors age, distance traveled or terms of transportation; it means long term would require higher doses while short term transport would require low doses, goats could tolerate higher doses than other ruminants due to their metabolism than other ruminants. Larger or heavier animals and breeds such as Kalahari in goats and the Sokoto gudali cattle would tolerate higher doses. It is important to be aware of the differences between species due to the metabolic differences and the different levels of stress they are subjected to.

Absorption of ascorbic acid

Ascorbic acid is a highly labile chemical substance that is oxidized by dehydroascorbate reductase (Bender, 1992). Its biotransformation to metabolites occurs readily in blood and plasma in the body. The reverse metabolism of vitamin C could also occur. The addition of metaphosphoric acid to the plasma prevents the breakdown of the ascorbic acid prior to analysis. This would therefore give a more accurate result in the assessment of vitamin C (Mandi et al. 2009). There are various levels of ascorbic acid in plasma and these were ascribed to the method of analysis. Macpherson, (1983) reported 4-8 µg/ml in clinically healthy sheep while in another study Macpherson and Moon, (1976) gave the concentration as 5.79±0.60 µg/ml in copper supplemented sheep. In previous studies bioavailability of ascorbic acid administered by different routes tends to differ in animals. Higher values were observed in animals administered intramuscularly than the intravenous route in sheep (Black and Hidiroglou, 1994; Hidiroglou et al. 1997). Animals that are not deficient would have higher elimination rates than animals that are deficient which would have low elimination rates. This indicates that ascorbic acid moves quickly and easily into the extravascular space (Black and Hidiroglou, 1994). The area under curve parameter can be used as a measure of total exposure of an individual to the drug. The bioavailability of IM administration appeared to be much higher than that after intravenous administration. The reason for greater area under curve after IM administration and thus greater exposure to ascorbic acid was suggested by Black and Hidiroglou, (1994) to be due to the slower disappearance of ascorbic acid from the plasma. There was however decrease of concentration with in the first 24-48 hrs and this depends on the kinetics, dose administered and the extent of endogenous synthesis of ascorbate and the elimination rate (Hidiroglou et al. 1997). The intramuscular route is of greater benefit than the intravenous route to attain high area under curve and plasma concentration. It was suggested that this could be due non-uniform demand of the vitamin by different tissues especially if the animal is subjected to unpredictable stress factors and different factors predisposing to changes in metabolic dynamics (Hidiroglou et al. 1997). The bioavailability of ascorbic acid in sheep
using different formulations of ascorbic acid was also previously assessed by Hidiroglou et al. (1997).

The concentration of ascorbic acid in plasma was assessed by the area under the curve above the basal concentration. In a formulation of ascorbic acid increased the concentration of ascorbic acid above the normal basal concentration. Further more in a previous study of Hidiroglou et al. (1997) observed that all formulations of ascorbic acid when administered significantly increased the area under curve when administered for 28 days, compared to the animals in the control group. The difference between single and multiple dosing regime was suggested by Hidiroglou et al. (1997) to be due to differences in cellular uptake of ascorbic acid by the tissue. The multiple oral suppletions was found by Hidiroglou et al. (1997) to cause elevation of plasma concentration due to the saturation of ascorbic acid in tissues. The concentration of the ascorbic acid in the control was found to be significantly lower than that of animals administered with the multiple dose of vitamin C.

Some therapeutic uses of ascorbic acid in veterinary medicine

Ascorbic acid is a dietary requirement in many animals such as includes primates, guinea pig and various laboratory animals. The supplement is used in chronic diseases, and healing of soft tissue in horses. Guinea pig cannot synthesize vitamin C hence supplementation is paramount in this specie and up 200mg daily is the recommended dose in this species. In horses vitamin C is used as an antidote against poisoning with Red maple. Vitamin C is used as an immunomodulator in goats during transportation (Minka and Ayo, 2010). Ascorbic acid is also used to improve electrolytes balance in long term transportation in goats (Ayo et al. 2009; Biobaku et al. 2016b).

Table 1: Some studies conducted in animals and findings at a glance.

| Findings                                                                 | Animal Model | Author(s)                                      |
|--------------------------------------------------------------------------|--------------|------------------------------------------------|
| Ascorbic acid improve biochemical parameters of birds and rodents due to stress | Rats         | Nakano and Suzuki, 1984                        |
| Ascorbic acid ameliorated fear behaviour before slaughter                | Broilers     | Minka and Ayo, 2008                            |
| Ascorbic acid reduced the detrimental effect of ecosanoids               | Broilers     | Stillborn et al. 1988                          |
| Ascorbic acid improved heterophils to lymphocyte ratio at tonic immobility due to pre-slaughter handling | Broilers     | Zulkilli et al. 2000                           |
| Ascorbic acid improved excitability score and ameliorated depression due to stress of transportation | Goats        | Ayo et al. 2006                                |
| Ascorbic acid improved electrolyte deficit due to 12 hours of road transportation | Goats        | Ayo et al. 2009                                |
| Ascorbic acid improved welfare in harmattan                             | Pigs         | Adenkola et al. 2009                           |
| Ascorbic acid improved haematological parameters in 8 hours road transportation | Pigs         | Adenkola et al. 2009                           |
| Ascorbic acid improved physiological responses at hot dry season         | Goats        | Minka and Ayo, 2007                            |
| Ascorbic acid improved biochemical activities and protected against muscular soreness of 12 hours road transportation at hot dry season | Goats        | Minka and Ayo, 2010                            |
| Ascorbic acid improved meat characteristics in animals subjected to stress alone and when combined with xylazine | Goats        | Biobaku, 2015b                                |
| Ascorbic acid supplementation improved meat quality in long term road transportation | Goats        | Biobaku et al. 2016                           |
| Ascorbic acid intramuscularly and orally did not influence interleukin-6 and cortisol at short term transportation. | Goats        | Biobaku et al. 2018a                          |

The oral supplementation of ascorbic acid was reported to prevent muscular soreness and improved biochemical parameters following transportation in goats (Minka and Ayo, 2010). The same researchers also found that supplementation with vitamin C could be of utmost economic importance in the meat industries and to consumers especially in countries where standard abattoirs and slaughter houses with designated lairages for resting transported animals before slaughter are either lacking or the laws are not enforced. In dogs and cats, vitamin C is recommended twice daily to sustain the physiologic level to enhance health in stress of an infection. Vitamin C could be used in chronic skin diseases in calves (Hidiroglou et al. 1997) it had been reported to increase plasma and seminal ascorbate which also improved fertility in rams and goats (Hidiroglou et al. 1997). The administration of ascorbic acid at 20mg/kg and 40mg/kg improved the fertility in Markhouz bucks when administered subcutaneously (Fazeli et al. 2010). Nutrition was also found to support the animal wellbeing and confer immunity to parasites (Ingale et al. 2010). Ascorbic acid could be used as a supportive supplement in trypanosomosis (Biobaku et al. 2016b). It is also used in the management of immunosuppression due to environmental factors such as diseases predisposing in Nigeria amongst which is exposure to pesticides, drugs and other toxicities (Biobaku and Amid, 2018). Effects and advantages of ascorbic acid is presented in Table 1.

Effect of stress on meat quality and its improvement by ascorbic acid

The pH is a measurement of relative acidity or alkalinity of meat and it could be affected by transportation stress (Singh, 2007; Biobaku et al. 2016a). The most common method of determination involves physical measurement with a suitable meter equipped with glass electrodes (Buncic,
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2006). Changes in meat quality are mainly associated with the fluctuations in the levels of glycogen. In a stress condition, the glycogen level decreases due to contraction and further loss of glucose. This further affects the water holding capacity and the electrolyte balance. This leads to a higher pH and poor shelf-life of the meat (Singh 2007; Minka and Ayo, 2010). In the same vein, if there is increase in the level of glucose and this by implication would decrease in pH immediately after death while temperature of carcass is still high, then there is a reduced ability of muscle protein to hold which would result in the solubility of some protein water and some proteins become soluble in acid tissue fluid, with the production of pale soft exudative meat (Minka and Ayo, 2010). The stressful condition sequel to transportation and prior to slaughter may also cause dark firm dry condition (Bunicb, 2006; Singh, 2007). Minka and Ayo, (2010) observed that Ascorbic acid and vitamin E improved growth performance, intestinal morphology and meat quality in broilers in the tropics. Biobaku et al. (2018b) also found that ascorbic acid when co-administered with xylazine improved the water holding capacity, color, shrinkage adverse effect and ultimate pH in buck’s subjected long road transportation. In the correlation study involving the supplementation of ascorbic acid and xylazine it was observed that the combination of xylazine and ascorbic acid improved the electrolytes and triiodothyronine and tetraidothyronine which aided physiological compensation for recovery from the long road transportation stress. Appropriate stocking density should be ensured, rational supplementation with ascorbic acid when rendering welfare to animals. It is also important that some authors suggest that strict adherence to drug withdrawal should be ensured if the ascorbic acid is to be co-administered with other drugs (Biobaku et al. 2018b). Ascorbic acid was also found not to affect the taste and organoleptic property of chevon of bucks subjected to long term transportation (Biobaku et al. 2020).

CONCLUSION

Ascorbic acid should be considered the most universally accepted supplement considering its pharmacological disposition, safety, availability and low cost. It could be used in ruminants with no fear of toxicity and has no residual effect. It improves meat quality of ruminants if pretreated prior to the transportation. Ascorbic acid administration should be considered by policy makers in the livestock industry, farmers and marketers under veterinary supervision and advice. Ascorbic acid when used would improve welfare, quality and ultimate goal of profitability.

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