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Comparative study on effect of acerola berry and ascorbic acid to alleviate oxidative stress status of transported broiler chicken

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Abstract
The present study was executed in Vencobb strain of broiler chicken to investigate the oxdative changes during transportation and compare the efficacy of vitamin C (Vit.C) and fruit juice of Acerola berry (*Malphigia glabra*) in alleviating the transportation stress. The experiment was carried out in 5 week old chicks, which were randomly selected and divided equally into 4 groups (G-I, G-II, G-III, G-IV). The G-I and G-II group of birds formed the control. The birds in GIII were supplemented with vitamin C in drinking water at the dose rate of 60 mg/kg body weight for one week before transportation. G-IV birds were supplemented with 25 mL of aqueous extract of fruits of Acerola berry in drinking water for one week before transportation. The birds were reared on *ad libitum* water and feed and identical photoperiod. At sixth week of age all the birds except G-I birds were transported for 180 km at a vehicle speed of 60km/h. The G-I birds were sacrificed before transportation while the birds in G-II, G-III and G-IV were sacrificed immediately after transporting. The mean temperature and relative humidity during transportation was 25±0.27 °C and 74.87±3.32% respectively. In the present study it was noticed that transportation of birds produced stress and oxidative damage as indicated by increased lipid peroxide (LPO) level, heterophil concentration and H/L ratio and low lymphocyte count in G-II birds. The total antioxidant status (TAS) and reduced glutathione (GSH) levels were statistically similar in all the groups studied. The G-III and G-IV birds had low LPO level, H/L ratio, heterophil concentration and high lymphocyte count. These results indicated that the antistress agents supplemented (acerola berry extract and vitamin C) were effective in reducing the stress.

Keywords: Vitamin C, acerola berry, transportation stress, broiler chicken

1. Introduction
Poultry industry is one of the fastest growing component of agricultural sector. In India, due to better availability of land and labour, the rearing of poultry is more concentrated in rural areas, whereas the demand is high in urban areas. Hence, transportation of these birds is inevitable to meet the requirements of the consumers. Transportation of poultry by road across various ecological and climatic zones impose stress to the birds and during stress, the natural antioxidants of the body are exhausted, exposing the cells to harmful effects of reactive oxygen species (ROS). Altan *et al.* (2003) [1] reported that heat stress (38±1 ºC for 3 h) in birds at 35 and 36 days of age could result in oxidative stress, increased red blood cell susceptibility to peroxidation and increased lipid peroxidation as a consequence of increased free radical generation, as indicated by increased malondialdehyde (MDA) concentration. Yang *et al.* (2010) [13] suggested that exposure of broilers to high temperature might depress the activity of the mitochondrial respiratory chain, which lead to over production of reactive oxygen species (ROS) which ultimately resulted in lipid peroxidation and oxidative stress.
Over the years, tranquilizers were used as anti-stress agents during transportation of poultry. Unfortunately, their immediate actions are stressful and their residual effects in meat are harmful to consumers. Currently the most widely studied supplements used to prevent stress during transport of slaughter birds are those based on vitamins, minerals and amino acids and herbal preparations. The inclusion of these substances in poultry diets could provide a practical and less expensive alternative to drugs. The use of Vit. C as an antioxidant has been well disscused and it has been established that Vit. C ameliorates the adverse effects of heat during summer and overcrowding in broilers (Karthiayini and Philomina 2011) [8]. The fruits of acerola berry (A. berry) have high Vit. C content (11-14 mg/g) and other nutrients such as flavonoids and anthocyanins which form part of polyphenolic compounds. Vitamin C, flavonoids and anthocyanins are well documented for their antioxidant property.

These fruits also contain high levels of vitamin A and B complex vitamins. They are rich in calcium, iron, phosphorus, magnesium and potassium. However the potential of these fruits to reduce oxidative stress is not yet elucidated. Hence, the present study was undertaken to evaluate the oxidative damages associated with transportation stress and compare the efficacy of vitamin C and fruit juice of A. berry (*Malphigia glabra*) in alleviating the transportation stress in broiler chicken.

2. Materials and Methods

The study was conducted in 60 Vencobb broiler chicks of five weeks of age randomly selected from a flock of 1000 birds. The selected sixty birds were randomly divided into four groups (G-I to G-IV) of 15 birds each. All the birds were fed with basal diet according to BIS 1992. The environmental conditions were equal for all groups and all the chickens had free access to feed and water during the experiment. The Group I birds were kept as control group and were not subjected to transportation. The remaining three groups were subjected to transportation as follows: Group II birds were transported without any supplementation. Group III birds were supplemented with Vit. C in drinking water (60 mg/kg body weight) for one week before transportation. Group IV birds were supplemented with aqueous extract (25 ml) of fruits of A. berry in drinking water for one week before transportation. Mature fresh acerola berries were cleaned and aqueous extract of the fruit pulp was prepared in 1:5 dilutions, by diluting 25 ml of finely crushed pulp of acerola in 125 ml of distilled water and sieved. The extract was prepared freshly every day. The Vitamin C concentration of the extract was assessed by redox titration using Iodine solution and the birds were supplemented with an amount of extract that will supply 60 mg of vitamin C per kg body weight. The birds were transported for a distance of 180 km at a vehicle speed of 60 km/h. Cage density during transportation was according to the norms given by Ministry of Environment and Forest (2001) [11] and it was 0.25 sq.ft/bird (cage of 55×50×35 for 12 birds). The birds were transported during the night hours at a mean temperature of 25 °C and a relative humidity (RH) of 74.87 percent.

The birds of G-I group were sacrificed at the farm and the birds of other groups (G II to G-IV) were sacrificed immediately after transportation. Blood samples were collected with (Sodium EDTA at a dose rate of 2mg/ml of blood) and without anticoagulant. Blood smears prepared using fresh blood were stained with Leishman-Giemsa stain solution and different leukocytes were counted and their percentages and H: L ratio were calculated. The TAS was estimated by using the method of Benzie and Strain (1996) [5]. The LPO in serum were estimated by the method of Fraga *et al.* (1988) [6]. Levels of reduced glutathione (GSH) in serum were determined by the method of Moron *et al.* (1997) [10].

Data collected on various parameters were statistically analyzed as per the method of Snedecor and Cochran (1994). The results obtained were analyzed using one way Analysis of Variance (ANOVA) method followed by Duncan's multiple range tests at the levels of significance $p \leq 0.01$ and $p \leq 0.05$ for comparison between groups using the Statistics Package Software System (SPSS) version 17.0.

3. Results and Discussion

Table 1: Effect of transportation stress and supplemented antistress agents on Differential leukocyte count (DLC) of broiler chicken. Mean±SE (n=15)

Parameters Groups	DLC %					H/L Ratio
	Heterophil count	Eosinophil count	Basophil count	Lymphocyte count	Monocyte count	
G-I	29.60^{a}±0.15	1.13 ^{a}±0.40	1.05 ^{a}±0.08	67.32 ^{a}±1.61	0.90 ^{a}±0.23	0.44^{a}±0.04
G-II	38.40^{b}±0.21	0.70 ^{a}±0.30	0.60 ^{a}±0.06	59.80 ^{b}±2.10	0.50 ^{a}±0.16	0.62^{b}±0.06
G-III	25.40^{a}±0.16	2.20 ^{a}±0.44	1.10 ^{a}±0.08	71.10 ^{a}±1.71	0.20 ^{a}±0.13	0.42^{a}±0.03
G-IV	29.03$^{a\pm}$0.15	1.40 ^{a}±0.30	0.70 ^{a}±0.07	69.10 ^{a}±1.89	0.40^{a}±0.16	0.37$^{a\pm}$0.03

Table 2: Effect of transportation stress and supplemented antistress agents on total antioxidant status (TAS), lipid peroxidation (LPO) and reduced glutathione (GSH) of broiler chicken. Mean±SE (n=15)

Parameters Groups	TAS (mM/L)	LPO (nM/ml)	GSH (µg/ml)
G I	0.11±0.02 ^{a}	1.03±0.01 ^{a}	0.63±0.07 ^{a}
G-II	0.07±0.01 ^{a}	1.25±0.07 ^{b}	0.72±0.08 ^{a}
G-III	0.07±0.01 ^{a}	1.09±0.07 ^{a}	0.50±0.09 ^{a}
G-IV	0.08±0.01 ^{a}	1.06±0.02 ^{a}	0.63±0.06 ^{a}

The values within a column with no common superscript are significantly different at 5% level.

G-I Negative control, G-II Positive control, G-III Stressed + vitamin C supplemented group, G-IV Stressed + acerola extract supplemented group.

In the present study, it was noticed that the transportation of six week old chicken for six hours at a vehicle speed of 60 km/h and temperature of 25 °C is stressful to birds as indicated by a significantly high H/L ratio (0.62) when compared to other groups of birds. The results of the present study is in close agreement with the result of Ghareeb *et al.*, (2008) [7] and Ayo and Ojo (2010) [4] who reported a higher H/L ratio (1.18 and 0.81 respectively) during transportation of chicken. The lower values of H/L ratio in groups G-III (0.42) and G-IV (0.37) indicated the stress alleviating effect of Vit. C and A. berry extract. These are in accordance with the results of Amponsem *et al.* (2000) [2] and Zulkifli *et al.* (2000) [14]. They observed a reduced H/L ratio when Vitamin E and ascorbic acid was supplemented in feed and drinking water respectively to stressed broilers. Among the experimental groups a significantly higher heterophil count and lymphopenia was observed in group II birds (table no:1) compared to other groups. Heterophilia observed in the present study might be due to the response of poultry to moderate stress; as heterophils clearly respond to early phase of hormonal surge associated with acute stress. The present finding is in accordance with the findings of Karthiayini and Philomina (2011) [8] who reported an increased heterophil count and lymphopenia in birds under overcrowding stress. However, the birds of G-III and G-IV groups did not show any heterophilia and lymphopenia which might be due to the supplementation of antistress agents which could have reduced the stress response. Lokhande *et al.* (2009) [9] also reported that birds supplemented with antistress agent, Stresroak at a dose rate of one gram per kilogram of feed, had

lower H/L. The study revealed that the transportation of chicks for 180 kms at a vehicle speed of 60 km/h did not produce any significant change in the eosinophil count, monocyte count and basophil count.

In the present study, no significant difference in TAS was observed between the different groups of birds. This indicated that transportation at sixth week, for a distance of 180 kms at a mean temperature of 25 °C and RH of 74.87 percent did not affect the TAS of birds.

Lipid peroxidation is an autocatalytic process, during which oxidative deterioration of poly unsaturated lipids leads to the generation of peroxide and lipid hydro peroxides which further decompose to yield a wide range of cytotoxic products like malondialdehyde (MDA). Lipid peroxidation reduces the cell membrane fluidity, thus altering the function of intrinsic proteins. In the present study, G-II group of birds showed a significantly increased level of lipid peroxidation (LPO), which was in accordance with the reports of Altan *et al.* (2003) [1]. The high LPO level noticed in G -II group of birds might be due to some impairment in the pathway for defense against the oxidative damage or increase in ROS production. Further research is needed to elucidate the molecular level mechanism of oxidative damage. The birds of G-III and G-IV had lesser levels of lipid peroxidation compared to G-I and this might be due to the presence of high Vit. C content which could reduce the malondialdehyde concentration and protect the cell membrane and cytosolic component of cells against oxidative damage by removing singlet oxygen, hydroxyl, hydroperoxyl, superoxide and lipid peroxyl radicals (Aydemir *et al.* 2000) [3]. In the present study, road transportation for six hours covering a distance of 180 kms at 25 °C mean temperature and RH of74.87 percent did not produce any significant change in GSH level in six weeks old broiler chicken

4. Conclusion

The results of the present study revealed that transporting birds even under standard transporting conditions are stressful to them indicated by high H/L ratio in G-II birds. Transportation did not affect the overall antioxidant status. However it produced oxidative changes as indicated by increased LPO level. The low H/L ratio and LPO levels in G-III and G-IV groups of birds indicate that the supplemented antistress agents (vitamin C and aqueous extract of A. berry) were effective in reducing the stress as well as oxidative damage. The effect of the oxidative stress on meat quality has to be elucidated further. Acerola is a bushy plant that grow well in tropical country like India and its berry can be better utilizedas a cheap source for reducing stress of transportation in broiler chicken.

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