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Acerola berry to reduce transportation stress in Broiler chicken

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Abstract
The present study was carried out to investigate the application potential of acerola berry (*Malpighia glabra*) as an anti-stressor to alleviate transportation stress in Vencobb strain of broiler chicken. A total of 45 chicks of five weeks age, randomly selected from a flock of 1000 birds were equally divided into three groups (G I, G II and GIII) and reared with *ad libitum* feed and water as well as identical photoperiod. The GIII group of birds were supplemented with 25 ml of aqueous extract of acerola berry fruit in drinking water for one week before transportation. At sixth week of age, GI group of birds were sacrificed without transportation. Birds of G II and G III groups were subjected to 180 km of transportation. Cage density during transportation was 0.25 sq. ft/bird (Ministry of Environment and Forest 2001). The average vehicle speed during transportation was 60 km/h. Mean temperature during transportation was 25±0.27 °C and relative humidity was 74.87±3.32%. The GII and GIII birds were sacrificed immediately after transportation. The study revealed that the heterophil count, Heterophil: Lymphocyte (H/L)ratio, levels of blood glucose, total cholesterol, uric acid and malondialdehyde (MDA) were higher and lymphocyte count was lower in GII birds when compared to GI birds. The GIII birds had a Heterophil: Lymphocyte (H/L) ratio, blood glucose and cholesterol concentrations and MDA levels comparable with that of GI birds. The serum protein concentration was not affected by the transportation. The present finding indicates that acerola berry (*Malpighia glabra*) can be used as supplement to reduce the transportation stress of birds.

Keywords: Acerola berry, stress, broiler chicken

1. Introduction
Broiler industry in India is one of the fastest growing sectors in past decades growing faster than country GDP. Total broiler market size is estimated at 4.2 million tons (carcass weight), translating into volume growth of 7% year-on-year during calendar year 2017. As per ICRA's estimate, per capita meat consumption is around 3.6 kg p.a. which puts total broiler meat market size at Rs. 730 billion in terms of retail price. India remains predominantly a live bird market with close to 90% of broiler sales being done at traditional retail outlets given consumer preference for freshly cut broiler (https://economictimes.indiatimes.com/news/economy/agriculture/icra-predicts-decent-growth-for-domestic-poultry-industry/articleshow/63613459.cms). In order to reduce the cost of production, rearing of poultry is more concentrated in rural areas. However, the demand for chicken meat is high in urban areas. Hence, transportation of these birds is inevitable to meet the requirements of the consumers. The stressors acting on livestock and poultry during transportation are numerous and the responses of the animal to these stressors are complex, non-specific and often detrimental to their health, production and ultimately the profitability of the farm. Stress of transportation not only affects the bird's well-being but also reduces the quality of meat (Minka and Ayo, 2010) [19]. Anti-stress agents are widely used to reduce stress effects especially during unavoidable stressful conditions like transportation. Herbal products are widely used in broiler and layer chickens to reduce stresses, but are costly. There are many plants with high antioxidant and vitamin content in its fruits which can act as a cheap and efficient anti-stress agent in poultry. Acerola (*Malpighia glabra*) is one such plant, is a bushy shrub that grows well in tropical climate. It can be used to provide shade to poultry houses and also yields four to five kilogram of fruits per plant per year. The fruits of acerola berry (A. berry) have high vitamin 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. The preliminary study conducted by Luveena (2009) [14] showed that aqueous extract of Acerola berry was effective to ameliorate the free radical induced hepatotoxicity in rats. No study has been conducted to estimate the anti-stress effect of this fruit. Hence the present study was envisaged to find out the potential of A. berry to reduce transportation stress in broiler chicken.

Materials and Methods

The study was conducted in five weeks old Vencobb strain of broiler chicken for a period of one week with the approval of College level ethical committee. Forty five chicks were randomly selected from a flock of 1000 birds and were equally divided into three groups (G I, G II and GIII) and reared separately and provided with *ad libitum* feed and water and identical photoperiod. The GIII group of birds were supplemented with 25 ml of aqueous extract of acerola berry fruit in drinking water for one week before transportation. At sixth week of age, GI group of birds were sacrificed without transportation. G II and G III group of birds were subjected to 180 km of transportation. Cage density during transportation was according to the norms given by Ministry of Environment and Forest (2001) [17] and it was 0.25 sq.ft/bird (cage of 55×50×35 for 12 birds). The average vehicle speed during transportation was 60 km/h. The birds were transported during the night. The RH and temperature at one hour interval during the transportation were also recorded (Table 1). The GII and GIII birds were sacrificed immediately after transportation. The blood and serum samples were collected and analysed for haematological and biochemical parameters such as Haemoglobin (Hb), Volume of packed red cells (VPRC), heterophil count, lymphocyte count, Heterophil: Lymphocyte (H/L) ratio, concentrations of total protein, blood glucose, total cholesterol, uric acid, Malondialdehyde (MDA) and superoxide dismutase using standard procedures.

Aqueous Extract of *Malpighia glabra was prepared by using* mature fresh acerola berries. The fruit pulp was prepared in 1:5 dilutions, by diluting 25 ml of finely crushed pulp of acerola fruit in 125 ml of distilled water and sieved. The extract was prepared freshly every day.

The data obtained were analysed 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 program Statistics Package Software System (SPSS) version 17.0.

Result and Discussion

In the present study transportation for 180 kms did not produce any change in the Hb concentration (Table-2). Azeez *et al.* (2011) [14] reported an increase in Hb level in chicken transported for 200km. Ajakaiye *et al.* (2010) [1] and Minka and Ayo (2008) [18] reported a decreased Hb concentration in birds transported for six hours at a vehicle speed of 50 km/h. during the hot-dry season (mean temperature of 34.1±1.5 °C and R.H of 44.5±1.4 per cent). The contradictory results obtained in different studies indicated that haemoglobin concentration cannot be taken as a reliable indicator of stress response. The Hb concentration vary with duration of transport as well as temperature during transport. Supplementation did not produce any effect on Hb concentration of birds.

No significant difference in VPRC was noticed between transported and control birds. Minka and Ayo (2008) [18] observed a reduced VPRC in 18 week old Shika Brown pullets transported by road for 6 h at a vehicle speed of 50 km/h during the hot-dry environment. The difference in the result of present study and that of earlier workers might be because they transported the birds during hot dry season whereas in this study birds were transported at a mean temperature of 25 °C and RH of 74.87 per cent. However, the acerola supplemented birds had a significantly high VPRC value when compared to other birds. This might be due to the increased erythropoiesis and reduced erythrocyte oxidative damage by phytochemicals present in acerola. Acerola berry is rich in vitamin C, Iron, pyridoxine and also contains a number of antioxidants such as flavonoids, anthocyanins and phenolic acids which might have increased bone marrow activity for erythropoiesis.

An increase in heterophil frequencies is the simple, quick, reproducible and sensitive hematological parameter to measure stress in chicken. Among the experimental groups a significantly ($P \leq 0.05$) higher heterophil count was observed in group II birds compared to other groups (Table-2). Heterophilia observed in the present study might be due to the response of poultry to mild 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 Maxwell (1993) [16] who reported an increased heterophil count in broiler chicken on transportation. However, the supplemented birds of G-III group did not show any heterophilia which might be due to the supplementation of antistress agents. Lokhande *et al.* (2009) [13] also reported a reduced heterophil count in birds supplemented with antistress agents.

Among the experimental groups the lowest lymphocyte count was observed in G-II group of birds compared to birds from other two groups. Lymphopenia observed in the present study might be due to the effects of transportation stress. The present finding was in accordance with results of Ajakaiye *et al.* (2010) [1] who reported a reduced lymphocyte count after transportation of broiler chicken. However, the G-III group of transported birds did not show any lymphopenia which could be due to the supplementation of acerola which alleviated the stress effects and maintained their level near pre transportation values. Similarly, Lokhande *et al.* (2009) [13] showed that supplementation of polyherbal formulation Stresroak @ 1g per kg of feed to broiler chicken under overcrowding stress had lead to normalization of heterophil and lymphocyte counts.

The H/L ratio has been widely accepted in avian research as a measure of the chicken's perception of stress in its environment. The transported and non-supplemented G-II group of birds showed a significantly ($P \leq 0.05$) high H/L ratio (0.62) when compared to all other groups of birds. This indicated 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 also stressful to birds. The results of the present study is in close agreement with the result of Ghareeb *et al.,* (2008) [9] 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 (0.37) indicated the stress alleviating effect of acerola berry extract. These are in accordance with results of Amponsem *et al.* (2000) [2] and Zulkifli *et al.* (2000) [22] who observed a reduced H/L ratio in antistress agents supplemented stressed broilers.

The stress of transportation significantly ($P \leq 0.05$) increased the blood glucose concentration of the birds under study. Zhang *et al.* (2009) [23], Vosmerova *et al.* (2010) [21] and Bryer

et al. (2011) [7] also observed a significantly high blood glucose value in their experiment in transportation of gilts. The increased glucose concentration observed in the G-II group of birds during transportation might be due to the increased breakdown of glycogen, due to the increased release of corticosterone, the stress hormone in birds. The supplementation could have decreased the intensity of stress response which might be responsible for maintaining a blood glucose level similar to pre transportation levels in birds of G-III group. This stress alleviating property of acerola fruits might be attributed to the multiple phyto-chemicals with antistress activity in it, all of which would have contributed to reduce stress.

Six hours road transportation for a distance of 180 kms at a mean temperature of 25 °C and RH of 74.87 per cent did not significantly affect the protein level in six week old broiler chicken. Supplementation of extract also did not make any change in the serum protein concentration. Azeez *et al.* (2014) [15] did not find any significant differences in the total serum protein levels in adult female chicken of Nera black strain transported through a distance of 200km. Vosmerova *et al.* (2010) [21] in their study in 42 day old broiler chicken observed a decreased ($P \leq 0.01$) serum protein concentration after 70 km transport. Exposure to acute sound stress (100 dB intensities for 10 min) significantly ($P \leq 0.05$) increased the TP level of 42 days old broiler chicks (Chloupek *et al.*, 2008) [8]. The variations in results indicate that serum protein concentration is not a reliable indicator of stress response in birds.

In the present study transportation of broiler chicken significantly increased the blood uric acid concentration. This increase in blood uric acid level might be due to the release of stress hormone corticosterone as a stress adaptive mechanism during transportation which might have increased gluconeogenesis and therefore uric acid concentration in blood of stressed birds. The present result is in accordance with Malherios *et al.* (2003) [15] who reported an increased blood uric acid concentration during stress. Uric acid is a major antioxidant in birds and has been used as a biomarker for physiological processes such as oxidative stress and tubular function (Hartman *et al.* 2006) [10]. However, the levels of uric acid in G-III group of birds were lower than that of G-II group of birds. This might be due to the antioxidant property of Acerola berry which might have decreased the stress effects in supplemented group of birds and prevented increase in uric acid level. This is in accordance with the results of Harsini *et al.* (2012) [11] who reported a reduced blood uric acid concentration in broiler chicken under heat stress after administering antistress agents.

The transported G-II group of birds showed a significantly increased level of lipid peroxidation indicated by high level of MDA. Tekelioglu *et al.* (2010) [20] observed an increased MDA level in Japanese quails subjected to 2 or 5 h of road transportation. Lipid peroxidation is an autocatalytic process which is a common consequence of cell death. It is a molecular mechanism of cell injury, during which oxidative deterioration of poly unsaturated lipids takes place leading to the generation of peroxide and lipid hydro peroxides. These can further decompose to yield a wide range of cytotoxic products, which are thiobarbituric acid reactive substances (TBARS), such as malondialdehyde (MDA). Lipid peroxidation reduces the cell membrane fluidity, thus altering the function of intrinsic proteins. Under physiological conditions, low concentrations of lipid peroxides are found in tissues and cells. However, in the presence of oxidative stress, more lipid peroxides are formed and released into the serum due to cell damage. The stress of transport resulted in lipid peroxidation in G II group of birds indicated by high MDA levels in them. The acerola berry extract might have alleviated the lipid peroxidation, which might be due to the presence of high vitamin C content and flavanoids that protected 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].

Table 1: Climatic data recorded at one hour interval during the transportation of broiler chicken.

Time (am)	Temperature (°C)	RH (%)
12 (Midnight)	24	70
1	26	55
2	26	71
3	25	80
4	25	77
5	25	82
6	24	81
At abattoir	25	83
Mean ±SE	25±0.27 °C	74.87±3.32%

Table 2: Effect of transportation stress and supplemented Acerola berry extract on haematological parameter Mean ± SE (n=15)

Groups Parameters	G-I	G-II	G-III
Hb concentration (G%)	8.30^{a} ±0.58	9.08^{a} ±0.34	9.04^{a} ±0.30
VPRC (%)	22.60^{a} ±1.95	21.80^{a} ±1.25	29.83^{b}±2.01
Heterophil Count (%)	29.60^{a} ±0.15	38.40^{b} ±0.21	29.03^{a} ±0.15
Lymphocyte count (%)	67.32^{a} ±1.61	59.80^{b}±2.10	69.10^{a} ±1.89
H/L Ratio	0.44^{a} ±0.04	0.62^{b}±0.06	0.37^{a} ±0.03

Within a row values with at least one common superscript did not differ at 5% level.

Table 2: Effect of transportation stress and supplemented acerola berry extract on biochemical and oxidative stress parameters Mean ± SE (n=15).

Groups Parameters	G-I	G-II	G-III
Glucose (mg/dl)	211.18^{a} ±12.96	262.45^{b} ±8.29	216.97^{a} ±11.97
Total cholesterol (mg/dl)	105.29^{a} ±16.50	172.92^{b} ±7.11	111.35^{a} ±8.58
Uric acid (mg/dl)	4.68^{a} ±0.97	7.14^{b} ±0.55	5.82^{ab} ±0.32
Total Proteins (g/dl)	3.57^{a} ±0.30	3.38^{a} ±0.38	3.80^{a} ±0.42
MDA (nM/ml)	1.03^{a}±0.01	1.25^{b}±0.07	1.06^{a}±0.02

Within a row values with at least one common superscript did not differ at 5% level.

Conclusion

The present study revealed that supplementation of 25 ml of aqueous extract of acerola berry fruit in drinking water for one week can reduce stress of transportation in six week old broiler chicken. This is the first study to report the antistress potential of acerola (*Malpighia glabra*) fruits. These fruits and their products can be used as a very cheap and efficient antistress agent in birds.

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