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Ameliorative Effect of Vitamin C on Hematobiochemical and Oxidative Parameters in Ducks During Summer

Hitesh Kumar Behera[1], Geeta Rani Jena[2]*, Dhirendra Kumar[3], Surya Kumar Mishra[4], Debi Prassana Das[5], Lipismita Samal[6] and Nirupama Dalai[7]

[1]MVSc. Scholar, Department of Veterinary Clinical Medicine, College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar, Odisha, INDIA
[2]Assistant Professor, Department of Veterinary Clinical Medicine, College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar, Odisha, INDIA
[3]Scientist, In-charge, Avian Health Section, Regional Centre, Central Avian research Institute, Bhubaneswar, Odisha, INDIA
[4]Principal Scientist, Poultry Breeding Section, Regional Centre, Central Avian research Institute, Bhubaneswar, Odisha, INDIA
[5]Assistant Professor, Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar, Odisha, INDIA
[6]Assistant Professor, Department of Poultry science, College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar, Odisha, INDIA
[7]Assistant Professor, Department of Animal Physiology, College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar, Odisha, INDIA

*Corresponding Author: geetadhiru@gmail.com

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Abstract

Heat stress remains the most important stressor, especially across the tropical regions of the world. In the current study, effects of ascorbic acid were investigated for its impact on summer-induced heat-stress in ducks. A total of 60 twelve weeks old White Pekin ducks were randomly distributed into 3 experimental groups with replicates having 10 birds in each group. The ducks under the therapeutic trial groups were supplemented with vitamin C of 250mg or 500 mg per kg of feed along with a basal diet. This experimental feeding, ad libitum, continued for 7 weeks period. The maximum and minimum temperature along with relative humidity in the pen was recorded on daily basis, throughout the experiment. Blood samples were collected and analyzed for haemato-biochemical and oxidative stress parameters. Vitamin C treatment improved the haemato-biochemical and oxidative parameters alteration caused by summer-induced heat stress in ducks. Therefore, dietary supplementation by vitamin C have significant role in mitigating the summer-induced heat-stress.

Keywords: Heat stress, Vitamin C, Ducks, H/L Ratio, Biochemical and Oxidative Parameters

Introduction

Heat stress is one of the major constraints for poultry industry particularly in the hot and humid parts of the world (Rath *et al.,* 2015). Duck farming constitutes the second most important avian species, next to chickens, for table egg production in India. However, ducks are affected to heat stress at a lower threshold environmental temperature 23°C as compared to chickens (20°C) which indicates that: the thermo-neutrality zone of ducks is lower than the chickens (Shafie *et al.,* 1970). High ambient temperatures compromise performance and productivity through reduced feed intake and decreased nutrient utilization, growth rate, egg production, egg quality and feed efficiency, which lead to economic losses in poultry (Sahin *et al.,* 2009). In high ambient temperature the bird's body attempts to maintain its thermal homeostasis which leads to increase generation of reactive oxygen species (ROS) and results in the disturbance of balance between the oxidation and antioxidant defence systems, causing LPO) and oxidative damages (Droge, 2002). As such, high ambient temperature causes increased oxidative-damage and lowered plasma concentrations of antioxidant vitamins (Sahin *et al.,* 2009).

Under normal conditions, poultry can synthesize vitamin C (VC) within their body. Heat stress induces several deleterious changes including alteration in oxidative stress, which decreases plasma levels of VC, an antioxidative vitamin in poultry (Jahanian and Mirfendereski, 2015). Laying hens exposed to heat stress supplemented with VC showed the greatest production traits (Attia *et al*., 2016). In the same way, under hot conditions, birds are not able to synthesize sufficient amounts of VC (Kutlu and Forbes, 1993) and VC supplementation significantly reduces the body temperature (Orban *et al.*, 1993).

Keeping the above in view, the researchers have tried to minimize effect of heat stress by changing the environment and diets of ducks. However, it always remains expensive to cool poultry houses and hence, nutritional modifications with its low cost works out a feasible method, necessitated to combat deleterious effect of heat stress. Therefore, in the present study we investigated the effects of VC in duck pullets exposed to a prevailing higher environment temperature, as no scientific study has been carried out regarding optimisation of dosage of VC during summer stress in ducks.

Materials and Methods

The Current investigation was conducted collaboratively, at the Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Orissa University of Agriculture & Technology, Bhubaneswar and the Duck farms of Regional Centre, Central Avian Research Institute (RC-CARI), Bhubaneswar during period: covering mid-April, May and June of 2016. The daily maximum and minimum temperature along with relative humidity in the pen was recorded during experimental period using dry bulb and wet bulb thermometers.

Experimental Birds

A total of 60 (n=10) 12 weeks, White Pekin ducks were taken for the study in deep litter system of rearing, at the new farm complex at RC-CARI, Bhubaneswar. Uniform healthcare measures including routine vaccinations against duck cholera and duck plague; and uniform management and feeding were extended to all the experimental ducks with free access to swimming-water.

Experimental Design

The experimental ducks were randomly distributed into 3 groups, having two replicates each constituted with 10 ducks. The ducks of therapeutic trial groups were supplemented with VC (L- Ascorbic acid) of 250mg or 500 mg per kg of feed along with basal diet continued for a period of 7 weeks. Feeding was ad libitum for all the Ducks, with uniform access to water. The maximum and minimum temperature of the day along with relative humidity in each pen was recorded during the experimental period by dry bulb and wet bulb thermometer, on daily basis. Usual blood samples were collected from brachial vein (wing vein) of ducks using commercial vials, each coated with sodium fluoride (NaF), clot activator, whereas EDTA-treated vials were used for blood glucose, biochemical parameters and haematological study, respectively. Under biochemical estimation, the PCV estimation was done by Wintrob's haematocrit method, while the haemoglobin and Heterophil% and; Lymphocyte% were estimated by standard method (the latter was visualized by modified Giemsa stain).

Serum samples were harvested by standard methods, at 3 time points, i.e 0, 23 and 46 days of trial, and stored in deep freeze at a temperature of –20°C in properly capped and labelled glass vials for analysis. Biochemical parameters like glucose, LDH, total protein, calcium, phosphorus, BUN, cholesterol, triglycerides were estimated by spectrophotometer using commercially available kits (Coral). For Study of oxidative stress biomarkers**,** assay of erythrocyte oxidant – antioxidant status was assessed.

Regarding preparation of RBC hemolysate and RBC suspension, about 3mL of heparinized blood samples were collected RBCs were harvested and used for reduced glutathione (GSH) estimation, as per standard protocols. Using the RBCs, 10% hemolysate was used for the estimation of super oxide dismutase (SOD), lipid peroxidation (LPO) and catalase. Haemoglobin concentration of hemolysate was estimated by cyanohemoglobin method (Tentori and Salvati, 1981). From prepared hemolysate the following oxidative stress biomarkers were studied LPO was measured in terms of production of malondialdehyde (MDA), SOD, nitro blue tetrazolium (NBT) assay activity, Catalase, GSH and GPx. GSH was estimated by DTNB method as per Prins and Loos (1969). GPx activity in hemolysate was measured by the method of Rotruck *et al.* (1973) using H_2O_2 as substrate in the presence of GSH. MDA level in the RBC hemolysate was determined by the method of Placer (1966). Catalase activity in hemolysate was estimated by using H_2O_2 as a substrate as per the method of Bergmeyer (1983). SOD was estimated as per the method described by Madesh and Balasubramanian (1998). The respiratory burst activity was measured by the reduction of NBT by intracellular superoxide radicals. All experimental protocols were carried out with approval of Local Ethics Committee.

Table 1: Ingredient composition of control diet methods

Feed Ingredients	**Percentage**
Wheat (kg)	53
Deoiled rice bran(kg)	32
Soybean (kg)	12
Fish meal (kg)	2
DCP(kg)	0.5
Calcite(kg)	0.5
Trace Min.(kg)	0.33
DL-Methionine (g)	50
L-Lysine (g)	65
Vit.A,D3,B2 & K(kg)	15
B complex (g)	20
Toxin Binder(g)	150
Ch. Chloride(g)	100
Calculated values	
Crude protein	18%
ME	2650 Kcal/ kg

Statistical Analysis

The data obtained were analyzed by applying two-way analysis of variance (ANOVA) followed by Bonferroni's post test using the Graph Pad Prism v4.03 software program (San Diego, CA, USA), and the differences between the experimental and control groups were considered statistically significant at $P \leq 0.05$ or lower.

Results and Discussion

The mean maximum and minimum temperature recorded were: 39.16^o C and 26.57^o C, the mean maximum and minimum relative humidity was: 82.98 % & 46.13% respectively. Heat stress continues to be a major concern for poultry operations, especially in the hot regions of the world for its impacts entailing poor growth performance, oxidative stress and immunosuppression (Tawfeek *et al*., 2014; El-Deep *et al.,* 2019). External factors such as heat, infection, radiation, hyperoxia, toxins and exercise can lead to increased free radicals and other ROS (Halliwell

et al., 1992).

In the present study, Hb level was significantly decreased by supplementation VC at higher dose i.e. 500mg/ kg wt on day 23 and 46 ($p<0.05$; $p<0.01$) respectively with compare to control group. Heat stress or high environmental temperature causes an increase in Hb level due to dehydration and haemoconcentration (Maxwell *et al.*, 1992). VC has a protective effect on the haemoglobin levels and that it can reduce the prevalence of anaemia (Yang *et al.*, 2014). The PCV value was increased under both the doses of VC fed ducks exposed to heat stress compared to control group significantly on day 23 and 46 ($p<0.05$; $p<0.01$) respectively. There was an increase in PCV value during the stress is an indication of tissue hypoxia due to suppressed respiration rate and subsequent increase in the erythropoiesis (Deaton *et al.*, 1969). Heat stress causes increase in heterophil percentage and decrease in lymphocyte percentage leading to increase H/L ratio (Gross and Siegel, 1997). Increase in H/L ratio is good indicator of stress in poultry and its ratio of 0.5 is observed to be optimum level of stress (Gross *et al.*, 1983). Our findings revealed that there was optimum level of stress in control group as indicated by increase in H/L ratio which was significantly reduced at higher dose of VC on day 23 ($p<0.05$) and in both the doses of VC on day 46 ($p<0.001$). Mashaly *et al.* (2004) reported that heat exposure resulted in an increased H/L ratio which is in agreement with our study. Heat stress caused a reduction in haematocrit and an increase in H/L ratio apparently associated with hemodilution, an adaptive response enabling water loss by evaporation without compromising plasma volume with most of the evaporative water loss coming from the extra cellular compartment (Anwar and Aslam, 2013).

Table 2: Effect of vitamin C on haematological parameters in heat stressed ducks

Haematological parameters	Group	(in days)		
		0	**23**	**46**
Haemoglobin gm%	Control	10.29±0.49	11.31±0.30	13.31±0.37
	Vitamin C @250 mg/kg feed)	10.66±0.42	10.79±0.30	11.69±0.33
	Vitamin C @ 500mg/kg feed)	10.31±0.32	10.41±0.31*	10.93±0.35**
PCV %	Control	30.9±1.10	33.7±0.98	35.3± 1.12
	vitamin C @250 mg/kg feed)	31±1.12	32.8±1.34	34.5±1.11*
	vitamin C @ 500mg/kg feed)	30.2±1.00	31.9±1.33	33.4±1.32**
Heterophil %	Control	18.8±1.10	25.2±1.07	28.7±0.83
	vitamin C @250 mg/kg feed)	19.2±1.16	24.1±0.91*	20.1±1.96**
	vitamin C @ 500mg/kg feed)	18.2±1.25	21.8±1.19***	18.2±0.90***
Lymphocyte %	Control	71.6±1.45	65.7±1.57	64.7±2.49
	vitamin C @250 mg/kg feed)	70.4±1.15	67.3±0.88	70.4±1.39**
	vitamin C @ 500mg/kg feed)	71.1±1.21	74.4±1.34*	73.9±1.76***
H/L%	Control	0.26±0.02	0.39±0.02	0.45±0.03
	vitamin C @250 mg/kg feed)	0.27±0.02	0.36±0.02	0.29±0.03*
	vitamin C @ 500mg/kg feed)	0.26±0.02^{a}	0.29±0.02***	0.24±0.02***

Data are expressed as mean ± SE; n=10; Means (within a parameter) with a different asterisk differ significantly on the same day; ($^{*}P\leq0.05$, $^{**}P\leq0.01$, $^{***}P\leq0.001$) from the corresponding control group

The blood glucose value was decreased significantly at higher dose of VC on day 23 ($p<0.01$) and in both the doses of VC on day 46 ($p<0.001$) in comparison of control group. A decrease in blood glucose level was observed in broiler chickens supplemented with VC during summer months (Hazim *et al*., 2001; Oke *et al*., 2016). The total serum protein was at significantly higher level in birds supplemented with higher dose of VC on day 23 and 46 ($p\leq0.05$), as compared to control. This increase in protein could be due to amelioration properties of VC in reducing in catabolic activity of stress hormones (Borges *et al.*, 2007). Heat stress inversely affects the metabolic activity of hepatocytes (Jahanian and Mirfendereski 2015), which is consequently cause reduction in protein synthesis which is partially alleviated by VC feeding (Seven *et al.*, 2012). BUN level was decreased significantly ($P<0.01$) at higher dose of VC on day 46 in comparison to control group. VC has been reported to decrease synthesis and secretion of catabolic corticosteroids during heat stress (Abdel-Latif, 2018). Ward and Peterson (1973) observed that there was significant increase in LDH and ALP level in heat stressed birds which was in agreement with our findings. ALP

was significantly lower in birds supplemented with higher dose of VC (P<0.001) on day 23 and day 46 in comparison to control group. The birds treated with VC showed significant decrease in LDH at higher dose on 23 day (P<0.01) and at both doses on day 46 (P<0.01; P<0.001).

Serum calcium and serum phosphorous value increased significantly in higher dose VC treated group (P<0.05) on 46 day, as compare to control group. Similarly, in heat stressed layer hens supplemented with VC, higher serum concentrations of calcium and phosphorus compared to control birds are reported (Torki *et al*., 2014). VC is a necessary cofactor for the bioconversion of vitamin D3 to its active form 1,25-$(OH)_2D_3$. Volker and Weiser (1993) have reported that VC in the diet of poultry increased plasma concentrations of 1,25- $(OH)_2D_3$, which led to elevated activities of duodenal calcium-binding protein to increase serum Ca. The serum triglyceride level in our study was significantly (p<0.001) decreased in higher doses on both the day 23 and 46 but, birds fed with lower doses showed significant decreases on day-23 (p<0.05) and day-46 (p<0.001), in comparison to control group. This indicates that: higher doses of VC are more effective in reducing lipolytic activity of stress hormones. Zhigang *et al.* (2013) investigated the plasma concentrations of triglyceride, which was significantly increased (P<0.05) in Cherry Valley ducks when exposed to high ambient temperature. In our experiment, cholesterol level was decreased significantly (P<0.01) under both doses of VC on day 46 in comparision to control group. High temperature reduced feed intake and broilers compensated their need to energy by lipolysis of body lipid which leads to increase in the blood cholesterol and triglycerides (Rashidi *et al*., 2010). Zeng *et al.* (2014) indicated that there is a potential relationship between heat stress and the antioxidant defence system. Exposure to heat stress lead to manifestation of oxidative stress in birds (Akbarian *et al*., 2016). In the present study, there was high lipid peroxidation and increased superoxide generation as indicated by increased MDA level and NBT levels respectively, in erythrocytes in heat stressed birds.

Table 3: Effect of vitamin C on biochemical parameters in heat stressed ducks

Biochemical parameters	Group	(in days)		
		0	23	46
Glucose (mg/dl)	Control	218.6±6.01	260.6±5.11	306.1±5.75
	Vitamin C @250 mg/kg feed)	216.5±6.36	238.8±5.67	261.7±7.10***
	Vitamin C @ 500mg/kg feed)	215.6±6.71	232.5±7.31**	249.3±7.43***
ALP(U/L)	Control	209.7± 4.46	293.5± 7.78	360.8± 6.06
	Vitamin C @250 mg/kg feed)	210.1± 3.50	266.4±5.21	321.5±7.33
	Vitamin C @ 500mg/kg feed	210.9± 3.78	261.5±4.78***	291.5± 8.20***
Total protein(gm/dl)	Control	4.952± 0.14	4.489± 0.08	3.791± 0.11
	Vitamin C @250 mg/kg feed)	5.082± 0.12	4.839± 0.09	3.915±0.09
	Vitamin C @ 500mg/kg feed)	5.034± 0.10	4.905± 0.13*	4.232± 0.06*
Triglyceride (mg/dl)	Control	100.6± 2.50	132.3± 3.17	164.2± 3.46
	Vitamin C @250 mg/kg feed)	98.8±2.85	120.2± 2.58*	143.2±4.75***
	vitamin C @ 500mg/kg feed)	102.6±2.77	115.1±2.80***	133.2±3.37***
Cholesterol (mg/dl)	Control	127.3± 5.35	150.7± 4.32	171.8± 5.02
	Vitamin C @250 mg/kg feed)	125.4± 4.47	141.6±2.9	158±3.73
	vitamin C @ 500mg/kg feed)	129.1± 4.88	138.9± 3.08	148±4.73**
BUN (mg/dl)	Positive control	5.10±0.13	5.92±0.13	6.21±0.14
	Vitamin C @250 mg/kg feed)	5.12±0.27	5.66±0.09	5.71±0.15
	Vitamin C @ 500mg/kg feed)	5.05±0.23	5.46±0.11	5.37±0.3**
Serum Calcium (mg/dl)	Control	9.9±0.20	9.1±0.15	8.877± 0.28
	Vitamin C @250 mg/kg feed)	9.8±0.21	9.561±0.36	9.491±0.39
	Vitamin C @ 500mg/kg feed)	10.2±0.20	9.788±0.43	9.884±0.22*
Serum Phosphorus (mg/dl)	Control	5.29±0.14	4.813± 0.09	4.64±0.08
	Vitamin C @250 mg/kg feed)	5.28±0.19	5.114± 0.05	4.979± 0.10
	Vitamin C @ 500mg/kg feed)	5.29±0.18	5.347± 0.08*	5.205± 0.09*
LDH (U/L)	Control	326.1±9.13	395.6±11.85	437.1±10.76
	Vitamin C @250 mg/kg feed)	332±9.20	360.5±10.79	378.9±11.80**
	Vitamin C @ 500mg/kg feed)	329.9±9.15	348.3±10.97**	350.2±13.59***

*Data are expressed as mean ± SE; n=10; Means (within a parameter) with a different asterisk differ significantly on the same day; (*P≤0.05, ** P≤0.01, *** P≤0.001) from the corresponding control group*

Zeng *et al*. (2014) found that there was increased lipid peroxidation as a consequence of increased free radical

generation in heat stress. LPO and NBT value was decreased significantly in VC supplemented birds at lower dose ($p<0.01$) on day-46 and at higher doses on day-23 and 46 ($p<0.001$) indicating that: higher dose of VC is more effective in alleviating detrimental effects of oxidative stress caused by high ambient temperature. Jahanian and Mirfendereski (2015) reported that VC ameliorated the adverse effect of stresses via increasing antioxidant capacity of birds. In addition, it was observed that VC reduced the increased MDA in tissues of birds exposed to heat stress (Akbarian *et al.*, 2016).

GSH level was increased significantly in VC treated groups at both doses ($p<0.01$, $p<0.001$) on day 23 and 46 in comparison to control. Activities of SOD and GPx was increased significantly at both doses of VC supplementation at both doses on day 23($p<0.01$, $p<0.001$) and day 46($p<0.001$, $p<0.001$) as compared to control birds. Kumar *et al.*, (2010) observed that supplementation of VC to the heat stressed buffalo increases the serum SOD level which corroborate our findings. Further, Rafiee *et al.* (2016) have reported that the effect of VC supplementation increased GPx activity in broilers. Catalase level was also significantly increased at both doses on day 23 ($p<0.05$, $p<0.001$) and 46 ($p<0.001$, $p<0.001$). Panda *et al.* (2008) reported that VC led to significant increase in catalase activity in heat stressed chicks.

Table 4: Effect of vitamin C on erythrocytic oxidative stress indices in heat stressed ducks

Oxidative parameters	Group	(in days)		
		0	**23**	**46**
LPO (nmolMDA/mg Hb)	Control	1.936±0.036	2.886±0.057	3.079±0.034
	vitamin C @250 mg/kg feed)	1.948±0.039	2.599±0.05**	2.419±0.055**
	vitamin C @ 500mg/kg feed)	1.943±0.041	2.443±0.064***	2.423±0.047***
NBT(nmol/min/mg Hb)	Control	0.122±0.004	0.151±0.005	0.229±0.0079
	vitamin C @250 mg/kg feed)	0.118±0.003	0.117±0.003**	0.11±0.005**
	vitamin C @ 500mg/kg feed)	0.121±0.004	0.107±0.006***	0.093±0.006***
GSH (µmol/mL of packed RBC)	Control	2.83±0.038	2.22±0.068	1.95±0.051
	vitamin C @250 mg/kg feed)	2.84±0.046	2.52±0.027**	2.52±0.046**
	vitamin C @ 500mg/kg feed)	2.845±0.060	2.67±0.027***	2.628±0.051***
SOD (U/gHb)	Control	47.975±1.83	37.478±1.89	32.632±1.586
	vitamin C @250 mg/kg feed)	46.864±1.75	55.148±1.91**	63.033±1.76***
	vitamin C @ 500mg/kg feed)	47.223±2.01	66.39±2.16***	71.83±2.57***
Catalase (µmol H_2O_2decomposed/min/mg Hb)	Control	0.32± 0.014	0.283±0.014	0.243±0.02
	vitamin C @250 mg/kg feed)	0.323±0.011	0.348±0.019*	0.376±0.02***
	vitamin C @ 500mg/kg feed)	0.333±0.010	0.406±0.022***	0.509±0.021***
GPx (U/gHg)	Control	16.104±1.12	11.254±0.59	9.66±0.41
	vitamin C @250 mg/kg feed)	16.082±1.18	19.692±0.49**	25.079±0.50***
	vitamin C @ 500mg/kg feed)	15.056±0.42	24.321±0.56***	30.655±0.30***

*Data are expressed as mean ± SE; n=10; Means (within a parameter) with a different asterisk differ significantly on the same day; (*P≤0.05, ** P≤0.01, *** P≤0.001) from the corresponding control group*

Seven *et al.* (2009) have found decreased GPx activity in blood, liver, kidney, and heart from constant chronic HS exposed broilers (1-day-old, at 34 °C for 41 d). Furthermore, Sahin *et al.* (2010) observed a reduced hepatic SOD, GPx, and Catalase in chronic heat stressed quail. The increase in lipid peroxidation reduces antioxidant levels such

as VC and vitamin E in tissues during heat stress which increases the requirement of these vitamins during stress condition in poultry (Seven *et al*., 2008). VC in hydrophilic environment is considered as major chain-breaking antioxidants (Halliwell and Gutteridge, 1999). VC could alleviate the adverse effect of stresses via increasing antioxidant capacity of birds (Jahanian, and Mirfendereski, 2015). Sahin *et al.* (2003) reported that dietary supplementation of VC decreased MDA values in serum, liver, heart, and kidney of heat stress-subjected Japanese quails. VC is important nutrient involved as protective agent in different stressful conditions (Puthpongsiriporn *et al*., 2001).

Eshginia and Marjani (2013) reported that VC has a protective effect on the superoxide dismutase activity and contributes to counteract oxidative stress via transcriptional and post-translational mechanisms. VC act directly by scavenging reactive oxygen species (ROS) generated by stressors and prevent ROS-mediated cell damage by modulating gene expression (Catani *et al*., 2014).

Conclusion

Based on these investigations, it is concluded that dietary supplementation with VC @ 500mg/kg feed has significant ameliorative potential in mitigating the summer-induced heat-stress in ducks and therefore, when used judiciously, VC supplementation can contribute to salvage bulk of stress-induced losses in duck-production particularly, in summer months.

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Conflict of Interests

There is no conflict of interest.

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