



Effect of Vitamin C on Some Oxidative Stress Parameters in Water Deprived Goats

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ABSTRACT

The effect of vitamin C on physio-biochemical and hormonal parameters in water deprived goats was studied. Total 18 numbers of adult male goats were selected for the experiment during summer and winter seasons. In control group (n=6, for each season), water was offered *ad lib* throughout the experimental period; 2 treatment groups (T1 and T2) consisting of 6 animals each. In T1 group, water was restricted and subdivided into 3 experimental periods: in period one (day 1 to 7), animals were adapted to the water restriction regime by limiting access to water gradually from 15 to 3 hrs/day. In period two (day 8 to 14), animals of the treatment groups had access to water for 3 hrs/day. In period three (day 15 to 22), animals had access to water only every second day for 6 hours. In T2 group, the same protocol as mentioned above was followed with addition of vitamin C supplementation at a dose rate of 180 mg/kg b. wt./animal/day. Blood and urine was collected on 1, 7, 14 and 22 day for the estimation of different parameters. In T1 group, increasing trend ($P<0.05$) was seen in SOD, GPx, GST, catalase and LPO in blood. In T2 group, SOD, GPx, catalase and LPO levels increased upto day 14 and then decreased on day 22. T1 value of oxidative enzymes (SOD, GPx, GST, catalase and LPO) was significantly higher ($P<0.05$) than T2. Overall summer value of SOD, GPx and LPO were higher as compared to overall winter values. Water deprivation had negative impact on oxidative parameters, which can be ameliorated by supplementation of ascorbic acid at the rate of @180mg/kg b.wt./day.

Keywords: Oxidative stress, goat, water deprivation, vitamin C

Livestock undergo various kinds of stress such as physical, nutritional, chemical, psychological and thermal. Among all, thermal stress is the most concerning now a days in the ever changing climatic scenario. Thermal stress redistributes the body resources including protein and energy at the cost of decreased growth, reproduction, production and health. The state of Jammu and Kashmir is divided into three geographical regions namely the Jammu region, Kashmir valley and the Ladakh region. The total area of Jammu province is 26293 Sq. Kms, out of which forest area occupies 12066 sq. km, which is 45.89% of total area. Latitude and longitude of Jammu are 32.40°N and 74.50°E, respectively. Jammu region is located 366m above mean sea level and has two different climatic zones depending primarily on altitude. The summer monsoon comes around the middle of July and fading away in

early September, which is followed by a dry spell from September to November. Winter is mild and temperature seldom touches freezing point. Climate change is expected to account for about 20 percent of the global increase in water scarcity this century. The Intergovernmental Panel on Climate Change (IPCC) predicts that global warming will alter precipitation patterns around the world, melt mountain glaciers and worsen the extremes of droughts and floods. A study by the UK Meteorological Office predicts that if measures are not taken to mitigate climate change, severe droughts now occurring once every 50 years could occur every other year by 2050. Countries such as India are likely to be hit hard by global warming, which will lead to serious water shortages and will affect the overall animal production. Water is one of the most important nutrients, being the most abundant molecule in

all living cells (NRC, 2007); it is involved virtually in all physiological functions of the animal. Water restriction affects animal's physiological homeostasis (electrolyte imbalance, changes of osmolarity and blood pH, hormonal changes, haemato-biochemical changes) leading to behavioral changes, loss of body weight, low reproductive rates and decreased resistance to diseases.

The areas of J&K are characterized by water scarcity and fluctuating precipitation, under the effect of global warming and unpredictable weather, rainfall is becoming even more irregular and water availability become more limited. Annual rainfall in Jammu province in the year 2015 was 1909.7 mms with an average of 914.4 mm/year. Along the water accessibility, feed and other resources will be markedly affected by climate change. Keeping view on that, strategies have to be developed to use water efficiently and conservation for a diversified production system in different locations. Blood metabolites are considered to be the most accurate measure for assessing the physiological status of goats compared to other measures used such as body condition scoring and body weight gain. They are a direct measure that considers blood parameters giving the utmost possible accuracy (Madziga *et al.*, 2013) which include glucose, blood urea, creatinine and cholesterol concentrations (Grunwaldt *et al.*, 2005; Ndlovu *et al.*, 2007). The response of animals to water restriction or water deprivation depends mainly on restriction rate, deprivation duration, animal species and breeds, physiological stage and diet composition. Exposure to thermal stress has been related to morphological and physiological modifications. Thermal stress (both heat and cold) induce denaturation and mis-aggregation of protein slowing of progression through the cell cycle, inhibit translation and transcription, disrupt cellular cyto-skeleton elements and change membrane permeability. Animal producer and researchers have looked for ways to alleviate the negative effects of common stressors. Stress alleviation strategies are numerous, and their availability to producers depend on the access to water and energy, the price they are able to pay and the adopted farming system. These strategies vary from simple on farm practices such as modifying the feeding pattern such as feeding of vitamin C, sodium betain, electrolyte therapy etc. Very few studies have been undertaken to see the effect of vitamin C in water stressed pigs, Japanese quails, rabbits and broilers. Vitamin C is known for the function as an antioxidant mainly it act as

a free radical scavenger in numerous cellular oxidation processes. The results obtained on the effect of vitamin C on blood electrolytes are not very clear and need more elaborated work. No studies have been conducted on effect of vitamin C supplementation on cortisol level. Serum cortisol is a better marker for acute stress than for chronic stress. While vitamin C and cortisol interact, the anti-cortisol role of vitamin C is still unclear and needs more research.

Goat (*Capra hircus*) is the important economic livestock in the world. They are relatively resistant to harsh environmental conditions. They have the ability to minimize water loss through various water-saving mechanisms (Alamer *et al.*, 2009). One of the water saving mechanism is that they can store water in the rumen and maintain a large extracellular volume when fully hydrated (Mengistu *et al.*, 2007). Estimated goat population of the state as per latest available integrated sample survey (2011-12) is 18.136 lakh. There is paucity of information on the effect of water deprivation stress on the hematological, biochemical and hormonal profile of goats with supplementation of vitamin C and the alleviating effects. So the present study is aimed at examining in detail the changes in these parameters in water restricted goats during extreme climates and the likely beneficial effects, if any, produced by vitamin C supplementation.

MATERIALS AND METHODS

The study was conducted in the Division of Veterinary Physiology & Biochemistry, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology-Jammu, R. S. Pura, Jammu, J&K, India.

Animals and experimental design

The study was conducted on 18 adult male goats of 2-3 years of age. Animals were kept in the Divisional Farm of Animal Nutrition, F.V.Sc. & A.H., SKUAST-J, R. S. Pura (Jammu). All animals were provided with standard concentrate ration and maintained under standard managemental conditions during the experimental period.

Experimental procedure and chemical analyses

Total eighteen number of adult male goats were selected

for the experiment. The experiment was conducted during both summer and winter seasons. In the control group (n=6, for each season), water was offered ad libitum (24 hours) throughout the experimental period. In each season, there was two (2) treatment groups (T1 and T2) consisting of 6 animals in each group. In first treatment group (T1), water was restricted and subdivided into three experimental periods: in period 1 (experimental days 1 to 7), animals were adapted to the water restriction regime by limiting access to water gradually from 15 to 3 hour per day. During the second period of the experiment (experimental days 8 to 14), animals of the treatment groups had access to water for 3 hours/day. In the final period of the experiment (experimental days 15 to 22), animals had access to water only every second day for 6 hours. In second treatment group (T2), the same protocol as mentioned above was followed with addition of vitamin C supplementation at a dose rate of 180 mg/kg body weight/animal/day. Urine was collected by free catch/voided method from each animal on 1, 7, 14 and 22 day of experiment.

Statistical analyses

For all the observed data in the present experiment, the standard statistical procedures recommended by Snedecor and Cochran (2004) have been followed. The data were presented by showing mean and standard error. The significant differences of values for different parameters studied statistically analyzed by the Two Way ANOVA. Analysis of variance (ANOVA) was done with the help of Duncan's Multiple Range Test by Statistical Software Sigma Stat.

Climate conditions

Ambient temperature (°C) was recorded two times a day (morning and evening) at 10 days interval using dry and wet bulb thermometer. The psychrometric tables were used to calculate relative humidity in percentage using dry and wet bulb recording. A temperature-humidity index (THI) is a single value representing the combined effects of air temperature and humidity. The mean THI was calculated as per U.S. Weather Bureau (Wiersma and Armstrong, 1989) using the following equation:

$$THI = (1.8t + 32) - ((0.55 - 0.55RH)(1.8t - 26))$$

Mean climatological values of ambient temperature (°C), relative humidity (%) and THI for the experimental periods are shown in Table 3. A THI of 74 or less is considered normal, 75-78 is alert status, 79-83 is danger status and a THI equal to or above 84 is an emergency.

RESULTS AND DISCUSSION

Climatic condition

The environmental temperature, relative humidity and THI levels on the experimental periods are presented in Table 1. From the data we can observed that average temperature (°C) values during summer were higher than the critical temperature of 24-27°C for most species. In the present study, THI is higher during summer and is considered as danger status.

$$THI = (1.8t + 32) - [(0.55 - 0.55RH)(1.8t - 26)]$$

Table 1: Average environmental temperature, relative humidity and THI during the experimental period

Days	Temp (°C)		RH (%)		THI	
	Summer	Winter	Summer	Winter	Summer	Winter
1	34.80	16.10	22.00	64.00	78.2	60.37
2	33.15	17.85	28.50	62.00	78.43	62.85
3	33.50	11.45	26.50	95.00	78.43	52.76
4	33.35	10.80	28.50	87.00	78.65	51.91
5	31.20	11.90	53.50	79.50	80.45	53.94
6	33.35	11.20	50.50	71.00	82.98	53.09
7	31.00	09.70	53.50	73.00	80.18	50.73
8	21.30	09.65	56.50	73.50	67.39	52.36
9	33.25	10.80	28.00	74.50	78.45	52.36
10	33.85	10.20	29.50	64.00	79.39	51.87
11	33.70	09.50	54.50	72.50	83.99	50.45
12	29.40	15.60	55.50	40.50	78.33	59.37
13	32.20	10.65	49.00	84.50	81.02	51.75
14	32.80	09.35	49.00	87.00	87.04	49.49
15	33.65	11.10	49.50	86.50	82.97	52.43
16	29.45	12.55	66.50	72.00	80.03	55.11
17	26.80	10.20	80.50	80.00	77.85	51.2
18	29.95	11.30	65.00	77.00	80.54	53.06
19	29.20	15.00	58.00	77.50	78.42	58.94
20	29.90	13.30	50.50	82.00	78.25	56.14
21	33.05	13.20	54.50	76.50	83.11	56.05
22	31.55	13.20	72.00	78.00	84.05	56.03

Oxidative stress

Table 2, 3, 4, 5 and 6 depicted that oxidative stress parameter (SOD, GPx, GST, catalase and LPO) levels were showed an increasing trend in water deprived goats (T1 group) from day 1 to day 22 of experiment in both seasons. Bengoumi (1998) reported that the serum LDH activity increased from 1334 to 2227 U/L during dehydration and decreased rapidly after watering. The water restriction increases production of ROS which responsible for oxidative stress might be the reason of increased level of these enzymes in water deprived goats (T1 group). In rats, increased LPO activity was linked to enhanced oxidative metabolism (Asayma *et al.*, 1987). In contrast, low activities if SOD and catalase in dehydration was found in male rats (Das *et al.*, 2010). The SOD, LPO and GPx are well-known markers of oxidative stress (Pathan *et al.*, 2009).

Table 2, 3, 5 and 6 revealed that in T2 group, SOD, GPx, catalase and LPO activities also showed an increasing trend from day 1 to day 14, but the levels decreased on day 22 during summer and winter seasons. GST activity showed similar trend during winter, but in summer the levels increased from day 1 to 7, then declined on day 14 and 22 of experiment (Table 4). Between groups, values for all the oxidative stress enzymes in water deprived goats (T1 group) during summer and winter seasons found significantly higher ($P < 0.05$) than control values from one week after the starting (middle) of experiment to the end (day 7 to 22). When compared between the treatment groups (T1 and T2), T1 activities of most of the enzymes were significantly higher ($P < 0.05$) on day 14 and 22. Lower level of the oxidative enzymes in vitamin C supplemented group indicated less oxidative stress than water deprived goats. Low level of physiological

Table 2: Mean±SE values of SOD level (U/mg of protein) in control and treatment groups of goats during summer and winter seasons

Season		Summer					Winter				
Days	Group	1	7	14	22	Total	1	7	14	22	Total
Control		57.35 ± 1.64	58.14 ^A ± 1.05	56.60 ^A ± 1.56	57.70 ^A ± 1.26	57.45 ^{Aq} ± 0.66	54.92 ± 1.01	54.47 ^A ± 0.40	53.20 ^A ± 0.61	56.09 ^A ± 1.03	54.67 ^{Ap} ± 0.44
	T1	57.35 ^a ± 1.64	63.43 ^{Bb} ± 1.53	71.50 ^{Cc} ± 1.34	82.29 ^{Bd} ± 1.35	68.64 ^B ± 2.07	54.16 ^a ± 1.11	60.56 ^{Bab} ± 2.05	67.13 ^{Cb} ± 2.25	77.16 ^{Bc} ± 1.87	64.75 ^B ± 1.98
T2		56.91 ^a ± 1.09	59.84 ^{ABab} ± 0.96	62.82 ^{Bb} ± 0.81	60.55 ^{Ab} ± 0.67	60.03 ^{Aq} ± 0.61	53.41 ^a ± 0.69	56.80 ^{ABb} ± 0.76	60.77 ^{Bc} ± 0.54	59.15 ^{Abc} ± 0.38	57.54 ^{Ap} ± 0.64

a, b, c, d describe significant differences within groups; A, B, C describe significant differences between groups; p, q describe significant differences between seasons.

Table 3: Mean±SE values of GPx level (U/mg of Hb) in control and treatment groups of goats during summer and winter seasons

Season		Summer					Winter				
Days	Group	1	7	14	22	Total	1	7	14	22	Total
Control		41.41 ± 1.57	42.31 ± 0.88	41.67 ^A ± 1.65	39.85 ^A ± 2.30	41.31 ^{Aq} ± 0.80	36.46 ^{Aa} ± 0.91	41.10 ^{Ab} ± 0.76	38.63 ^{Aab} ± 0.49	40.98 ^{Ab} ± 0.45	39.29 ^{Ap} ± 0.51
	T1	40.21 ^a ± 1.19	44.38 ^b ± 0.93	51.43 ^{Bc} ± 0.60	62.25 ^{Bd} ± 0.35	49.57 ^B ± 1.78	39.85 ^{Ba} ± 0.58	44.56 ^{Bb} ± 0.45	50.57 ^{Bd} ± 0.36	60.04 ^{Bd} ± 0.39	48.75 ^B ± 1.59
T2		41.65 ^a ± 0.40	42.92 ^a ± 0.30	45.28 ^{Ab} ± 0.33	42.01 ^{Aa} ± 0.33	42.96 ^A ± 0.34	38.84 ^{ABa} ± 0.81	41.73 ^{Ab} ± 0.62	44.82 ^{Bc} ± 0.66	41.47 ^{Ab} ± 0.55	41.71 ^A ± 0.54

a, b, c, d describe significant differences within groups; A, B, C describe significant differences between groups; p, q describe significant differences between seasons.

Table 4: Mean±SE values of GST level ($\mu\text{mol}/\text{min}/\text{mg}$ of Hb) in control and treatment groups of goats during summer and winter seasons

Season		Summer					Winter				
Days Group	1	7	14	22	Total	1	7	14	22	Total	
	Control	58.86 ±3.00	60.24 ^A ±1.58	57.27 ^A ±0.83	62.61 ^B ±0.93	59.74 ^{Aq} ±0.94	51.78 ^{Aa} ±0.73	56.36 ^{Ab} ±0.67	51.94 ^{Aa} ±0.56	53.61 ^{Aab} ±1.30	53.42 ^{Ap} ±0.56
T1	59.77 ^a ±0.33	66.73 ^{Bb} ±0.31	74.78 ^{Cc} ±0.42	84.29 ^{Cd} ±0.73	71.39 ^{Bq} ±1.92	53.59 ^{ABa} ±0.70	60.21 ^{Bb} ±0.71	66.06 ^{Cc} ±0.73	74.77 ^{Cd} ±0.79	63.66 ^{Cp} ±1.66	
T2	58.59 ^a ±0.99	63.49 ^{ABb} ±1.15	61.28 ^{Bab} ±0.80	59.11 ^{Aa} ±0.75	60.62 ^A ±0.60	55.33 ^{Ba} ±1.15	59.18 ^{ABab} ±0.93	62.81 ^{Bb} ±0.62	58.19 ^{Ba} ±1.51	58.88 ^B ±0.76	

a, b, c, d describe significant differences within groups; A, B, C describe significant differences between groups; p, q describe significant differences between seasons.

Table 5: Mean±SE values of Catalase level ($\mu\text{mol H}_2\text{O}_2$ utilized/min/mg of protein) in control and treatment groups of goats during summer and winter seasons

Season		Summer					Winter				
Days Group	1	7	14	22	Total	1	7	14	22	Total	
	Control	42.18 ±1.55	42.22 ±0.75	42.51 ^A ±0.77	43.41 ^A ±1.40	42.58 ^{Aq} ±0.56	37.82 ±1.27	40.33 ^A ±0.59	38.88 ^A ±0.48	41.35 ^A ±1.14	39.60 ^{Ap} ±0.52
T1	40.10 ^a ±0.54	44.27 ^b ±0.40	49.27 ^{Bc} ±0.46	55.93 ^{Bd} ±0.42	47.39 ^B ±1.25	38.64 ^a ±0.66	43.15 ^{Bb} ±0.81	47.58 ^{Cc} ±0.96	52.88 ^{Bd} ±0.51	45.56 ^B ±1.16	
T2	39.80 ±1.36	42.08 ±1.15	43.26 ^A ±0.89	41.10 ^A ±0.87	41.56 ^A ±0.57	40.31 ^a ±0.60	42.75 ^{Bb} ±0.51	44.16 ^{Bb} ±0.31	40.29 ^{Aa} ±0.27	41.88 ^A ±0.40	

a, b, c, d describe significant differences within groups; A, B, C describe significant differences between groups; p, q describe significant differences between seasons.

Table 6: Mean±SE values of LPO level (n mol MDA/ ml) in control and treatment groups of goats during summer and winter seasons

Season		Summer					Winter				
Days Group	1	7	14	22	Total	1	7	14	22	Total	
	Control	4.22 ^{Aa} ±0.07	4.23 ^{Aa} ±0.09	4.99 ^{Ab} ±0.08	4.70 ^{Ab} ±0.05	4.54 ^A ±0.08	4.20 ^{Aa} ±0.10	4.37 ^{Aab} ±0.15	4.86 ^{Ab} ±0.16	4.71 ^{Aab} ±0.15	4.54 ^A ±0.09
T1	4.93 ^{Ba} ±0.06	6.09 ^{Cb} ±0.06	7.30 ^{Cc} ±0.09	9.72 ^{Bd} ±0.22	7.01 ^C ±0.38	4.83 ^{Ba} ±0.08	5.10 ^{Ba} ±0.11	6.27 ^{Cb} ±0.10	8.23 ^{Bc} ±0.08	6.11 ^B ±0.28	
T2	4.74 ^{Ba} ±0.03	5.60 ^{Bc} ±0.03	6.06 ^{Bd} ±0.06	5.14 ^{Ab} ±0.04	5.38 ^{Bq} ±0.11	4.64 ^{Ba} ±0.10	4.84 ^{Bab} ±0.10	5.50 ^{Bb} ±0.22	4.88 ^{Aab} ±0.11	4.96 ^{Ap} ±0.10	

a, b, c, d describe significant differences within groups; A, B, C describe significant differences between groups; p, q describe significant differences between seasons.

concentration of reactive oxygen species (ROS) are very much required to cater the diverse physiological functions including cellular signalling involved in cell proliferation and differentiation. Excess production of ROS induces oxidative stress in cellular micro environment that damaged lipid, proteins and DNA of cells. Vitamin C acts as a non-enzymatic antioxidant which catalyse ROS activity. Nutritional antioxidants help to augment the normal antioxidant protection level and prevent damage to cellular components from ROS. The role of vitamin C in preventing water-induced oxidative stress in animals are very limited. Vitamin C supplementation can attenuate exercise-induced protein oxidation in a dose-dependent manner with no effect on lipid peroxidation and glutathione status. An increase in oxidative stress markers has been reported with aerobic exercise in human (Sastre *et al.*, 1992; Sen, *et al.*, 2000). Oxidative stress has been reported in response to exercise based on changes in antioxidant enzyme activity (Ji and Fu, 1992; Khassaf *et al.*, 2003), glutathione status (Gohil *et al.*, 1986; Ji and Fu, 1992; Chung *et al.*, 2009), increased lipid peroxidation (Bazzare and Yuhas, 1983; Ji and Fu, 1992; Alessio *et al.*, 1997), and increased protein oxidation (Reznick *et al.*, 1992; Radak *et al.*, 1998; Lee *et al.*, 2002). These results suggest that the normal defense mechanisms in the body can be insufficient to adequately handle the increased production of ROS. Nutritional antioxidants have been proposed to help augment the normal antioxidant protection level and help prevent damage to cellular components from ROS. In contrast, Thompson *et al.* (2001) reported that acute vitamin C supplementation (0.4 gm for 1 day) had no effect on oxidative stress. The up-regulation of the antioxidant enzyme. SOD during pregnancy related oxidative stress and the positive effect of diet-acquired antioxidant molecules for enabling efficient oxidative shielding and avoiding oxidative damage was reported by Stier *et al.* (2017).

Overall oxidative stress parameters in summer were significantly higher ($P < 0.05$) in all the groups (control, T1 and T2) (Table 2, 3, 4, 5 and 6). Superoxide dismutase (SOD), glutathione peroxidase (GPx) and lipid peroxidase (LPO) antioxidants increased significantly during summer (Rathwa *et al.*, 2017). Heat stress is also responsible for increased production of free radicals, which cause oxidative stress (Ghosh *et al.*, 2013). Other studies also revealed that the higher concentration of SOD, LPO, and GPx during

summer season due to the heat stress conditions (Ghosh *et al.*, 2013, Maan *et al.* 2013; Chaudhary *et al.* 2015).

CONCLUSION

The biochemical parameters were changed with environmental stress. THI was higher during summer and was considered as danger status. Water deprivation had negative impact on biochemical parameters, which can be ameliorated by supplementation of ascorbic acid at the rate of @180mg/kg b.wt./day.

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