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Dietary Supplementation of Ascorbic Acid on Hemato-Biochemical and Hormonal Parameters in Swamp Buffaloes

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ABSTRACT

Effect of ascorbic acid on hemato-biochemical and hormonal profile of swamp buffaloes were investigated during summer and post summer months. Eighteen swamp buffaloes (Avg. b.wt.336.24±10.27kg, age 3.5 years) were divided randomly into three groups of six each. The animals were supplemented with ascorbic acid (AA) at the rate of 0, 10 and 15 g/animal/day for 150 days and designated as T_1, T_2 and T_3. Blood was collected on 0, 30th, 60th, 90th, 120th and 150th day of the experiment and analysed. Results indicated that the value of haemoglobin (Hb), Packed Cell Volume (PCV) and thyroxin was higher ($p<0.05$) in T_3 as compared to T_1 and T_2 groups. The value of Mean Corpuscular Haemoglobin Concentration (MCHC) was significantly ($p>0.05$) higher while total leucocytic count (TLC), neutrophil, eosinophil, total protein, AST (Aspertate aminotransferase) and ALT (Alanine aminotransferase) was lower in T_2 and T_3 as compared to T_1. Across the treatment T_2 and T_3 showed significantly ($p<0.05$) higher tri-iodothyronine and lymphocytes and ($p<0.05$) lower serum glucose and cortisol concentration as compared to T_1. From this study, it is concluded that dietary supplementation of AA modulated hemato-biochemical and hormonal parameters in beneficial ways in swamp buffaloes to cope up thermal stress during summer and post summer months. The dose rate of AA 15 g/day/animal found to be more effective than 10 g/d/animal.

Keywords: Swamp buffaloes, Ascorbic acid, Hemato-biochemical, Hormonal parameters, Summer and Post summer

Ruminants can synthesize ascorbic acid (AA) in liver, however, several studies have shown that vitamin C level in the blood decreases during stress and diseases (Ranjan *et al*., 2005; Sivakumar *et al*., 2010; Kim *et al*., 2012). This may be the end result of decreased endogenous synthesis or increased demand or a combination of both. It is reported that AA has numerous biological functions such as antioxidant (Weiss, 2006) that prevents the oxidation of protein, DNA and nitric oxide; acts as regulator of iron uptake (Hacisevki, 2009), has beneficial effect on reproduction (Yassein *et al*., 2008), growth (Seifi *et al*., 2010; Abdel-Monem *et al*., 2013) and immunity (Sahinduran and Albay, 2004; Kumar and Kataria, 2011).

Buffaloes are more prone to physical distress during summer in comparison to other farm animals, because of scarce distribution of sweat glands and dark body colour. The swamp buffaloes are less productive and also inefficient breeders (long gestation period, long calving interval, silent heat, and low conception rate) compared to river buffaloes due to the inherent susceptibility to environmental stress, which causes anestrus and sub-estrus (Das and Khan, 2010). There are several reports of supplementation of AA to attenuate the negative effects of environmental stress in ruminants (Khan and Konwar, 2010; Kumar *et al*., 2012; Abd-Allah and Zanouny, 2014; Kassab and Mohammed, 2014). However, literature

pertaining to AA supplementation in swamp buffaloes is lacking. Therefore, in the present study an attempt was made to investigate the effect of AA supplementation on hemato-biochemical and hormonal profile of swamp buffaloes during summer and post-summer heat stress.

MATERIALS AND METHODS

A total of eighteen apparently healthy pre-pubertal female swamp buffaloes (3.5 years of age and average body weight of about 336.24 ±10.27 kg) were randomly divided into three groups of six each. The protocol for the animal research has been approved by the Institute Animal Ethics committee (Approval No. 770/ac/CPCSEA/FVSc/AAU/IAEC/12-13/142). The animals were housed in semi-open shed with cemented anti-slippery floor and asbestos roof maintained by the Department of Animal Genetics and Breeding, College of Veterinary Science, Khanapara, Guwahati. The animals were dewormed and vaccinated against foot and mouth diseases, black quarter and haemorrhagic septicaemia before the start of the experiment. Ascorbic acid was supplemented to the animals at the rate of 0, 10 and 15 g/day and designated as T_1, T_2 and T_3. The required quantity of ascorbic acid was mixed with 100g concentrate feed and fed before the morning feeding for the period of 150 days during summer and post summer months (mid June to mid November). All animals were offered concentrate feed at the rate of 2 kg/animal/day comprising of maize (20%), wheat bran (37%), rice polish (20%), deoiled GNC (13%), deoiled MOC (7.5%), mineral mixture (2%), common salt (0.5%). Mixed green fodders comprising of maize, sorghum and Napier were fed as per their availability at the rate of 25-30 kg/animal/day and in addition the animal were allowed to graze for 4 hrs daily. Provision for fresh drinking water was made available to all the animals throughout the day. The minimum and maximum temperatures and relative humidity (RH) were recorded inside the barn twice per day (at 06:00 h and 14:00 h) using thermo-hygrometers. The temperature-humidity index (THI) was calculated as per U.S. Weather Bureau, by using the equation THI= 0.72 (Dry bulb temp. °C + Wet bulb temp. °C) + 40.6 where, 0.72 and 40.6 are constants. Blood samples (10 ml/animal) were collected on days 0, 30th, 60th, 90th, 120th and 150th day of the experiment before feeding. The hematological parameters viz. hemoglobin, packed cell volume (PCV), total leucocyte count (TLC), differential leucocyte count (DLC), mean corpuscular hemoglobin concentration (MCHC), were estimated using routine hematological methods. Serum glucose, AST, ALT were estimated using kits (M/S KEE GAD Biogen Pvt. Ltd, New Delhi, India; total protein (M/S Aspen Laboratories, New Delhi, India). The level of tri-iodothyronine, thyroxine were estimated by ELISA kits (Diagnostic Automation/ Cortez Diagnostic, Inc, 23961 Craftman road, California 91302, USA) and cortisol was also estimated by using

Table 1: Chemical composition of concentrate, green fodder and grasses

Feed ingredients	DM	OM	CP	EE	CF	NFE	Ash	NDF	ADF	C	HC	Lignin
Concentrate	89.87 ±0.31	90.77 ±0.44	16.76 ±0.16	4.06 ±0.10	15.89 ±0.14	54.05 ±0.92	9.23 ±0.26	33.11 ±0.18	27.27 ±0.18	10.21 ±0.15	32.83 ±0.16	4.54 ±0.18
Napier	17.05 ±0.27	85.12 ±0.12	9.70 ±0.74	2.64 ±0.16	31.47 ±0.62	41.31 ±0.71	14.87 ±0.17	70.56 ±0.19	38.21 ±0.17	23.99 ±0.23	32.35 ±0.21	6.88 ±0.22
Maize fodder	19.26 ±0.72	88.06 ±0.17	12.92 ±0.16	1.69 ±0.13	27.26 ±0.25	47.49 ±0.34	11.93 ±0.35	54.81 ±0.31	32.41 ±0.13	11.19 ±0.14	22.39 ±0.17	5.59 ±0.21
Sorghum fodder	23.08 ±0.61	87.56 ±0.19	8.07 ±0.31	1.3 ±0.11	18.61 ±0.57	59.57 ±0.27	12.43 ±0.31	63.37 ±0.17	31.56 ±0.41	20.03 ±0.32	31.81 ±0.19	4.1 ±0.16
Grasses	20.18 ±0.23	88.47 ±0.28	13.30 ±0.14	1.72 ±0.13	21.48 ±0.67	51.97 ±0.42	11.53 ±0.21	59.14 ±0.27	34.56 ±0.15	27.65 ±0.17	24.58 ±0.18	6.91 ±0.15

DM=Dry matter, OM=Organic Matter, CP=Crude protein, CF=Crude fiber, NFE=Nitrogen free extract, NDF=Neutral detergent fiber ADF=Acid detergent fiber, C=Cellulose, HC=Hemicellulose, Hemicellulose was calculated by subtracting ADF from NDF and Cellulose was calculated by subtracting lignin+silica from ADF.

ELISA kits (DSI S.r.l., Saronna (VA), Volonterio, 36a, 21047, Italy). Proximate analysis of feed samples (Table 1) was done according to the method of AOAC (1990) and fiber fractionation (neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose and hemicellulose) was done as per the method of Goering and Van-Soest (1970). Hemicellulose was calculated by subtracting ADF from NDF and Cellulose was calculated by subtracting lignin+silica from ADF. The data were analyzed by two-way ANOVA using SAS software (2009) and expressed as mean ± SE and difference among all treatments were done by Duncan's multiple range test.

Table 2: Average ambient temperature humidity index (THI) of the micro-climate (barn)

Month	Temperature humidity index (THI)	
	6:00 h	14 h
June	80.90±0.38	85.92±0.49
July	80.11±0.17	84.55±0.36
August	79.72±0.16	85.44±0.55
September	78.95±0.21	85.08±0.59
October	74.64±0.36	81.81±0.44
November	64.91±0.49	78.30±0.25
December	61.21±0.60	72.40±1.66
January	59.85±0.36	74.68±0.54
February	60.54±0.58	76.26±0.91

Table 3: Hematological parameters of control and ascorbic acid supplemented swamp buffaloes

Parameters	Groups	June	July	August	September	October	November	Overall mean
Hb (%)	T_1	11.28±0.23^{a}	11.38±0.24^{a}	11.28±0.26^{a}	10.44±0.13^{a}	9.88±0.24^{a}	9.74±0.19^{a}	10.67±0.15^{a}
	T_2	11.24±0.37^{a}	11.46±0.35^{a}	11.64±0.27^{a}	10.80±0.17^{ab}	10.26±0.11^{ab}	10.12±0.15^{ab}	10.92±0.14^{a}
	T_3	11.26±0.35^{a}	11.76±0.23^{a}	11.84±0.16^{a}	11.20±0.20^{b}	10.72±0.19^{b}	10.60±0.18^{b}	11.23±0.12^{b}
PCV (%)	T_1	35.80±0.49^{a}	36.20±0.51^{a}	36.30±0.49^{a}	34.60±0.51^{a}	33.20±0.58^{a}	32.90±0.40^{a}	34.83±0.32^{b}
	T_2	36.00±0.45^{a}	36.50±0.42^{ab}	36.90±0.51^{b}	35.20±0.58^{b}	33.60±0.75^{a}	33.00±0.55^{a}	35.20±0.34^{b}
	T_3	35.98±0.92^{a}	37.00±0.35^{b}	37.36±0.50^{b}	35.90±0.64^{c}	34.70±0.86^{c}	34.40±0.68^{b}	35.89±0.32^{a}
MCHC (g%)	T_1	31.50±0.31	31.42±0.23	31.06±0.34	30.19±0.41	29.79±0.87	29.64±0.86	30.60±0.25
	T_2	31.20±0.72	31.37±0.68	31.53±0.41	30.69±0.37	30.60±0.75	30.68±0.47	31.01±0.23
	T_3	31.29±0.38	31.78±0.54	31.69±0.23	31.20±0.25	30.92±0.32	30.82±0.12	31.28±0.14
TLC (thousands/ cmm)	T_1	8.29±0.46	8.08±0.41	7.81±0.36	7.31±0.43	6.90±0.52	6.41±0.58	7.47±0.21
	T_2	8.24±0.74	7.91±0.66	7.67±0.64	7.28±0.68	6.84±0.65	6.16±0.74	7.35±0.29
	T_3	8.28±0.52	7.69±0.42	7.38±0.43	7.11±0.43	6.75±0.47	6.14±0.51	7.23±0.21
Lymphocyte (%)	T_1	58.60±0.51^{a}	57.80±0.80^{a}	58.00±1.61^{a}	58.80±1.24^{a}	59.40±1.50^{a}	58.80±0.49^{b}	58.57±0.43^{b}
	T_2	59.00±1.18^{a}	59.20±0.37^{a}	59.40±0.24^{a}	60.00±0.55^{a}	60.00±0.89^{a}	60.60±0.51^{a}	59.70±0.28^{a}
	T_3	58.20±1.07^{a}	59.00±0.45^{a}	59.80±1.56^{a}	60.40±0.51^{a}	60.60±1.08^{a}	60.80±0.49^{a}	59.80±0.39^{a}
Neutrophil (%)	T_1	32.20±0.66	32.80±0.92	31.80±1.53	32.00±1.22	32.80±1.02	33.40±0.40	32.50±0.39
	T_2	32.00±1.34	31.60±0.93	30.60±0.40	31.00±0.32	32.80±0.49	32.00±0.71	31.67±0.32
	T_3	32.80±0.97	31.60±1.21	30.40±1.21	31.00±0.45	32.20±0.58	32.40±0.51	31.73±0.36
Eosinophil (%)	T_1	7.60±0.24	7.80±0.73	7.80±0.20	7.80±0.20	6.40±0.51	6.60±0.51	7.33± 0.22
	T_2	7.60±0.40	7.60±0.60	7.60±0.24	7.60±0.68	5.80±1.16	6.00±0.55	7.03±0.31
	T_3	7.40±0.24	7.80±0.97	7.20±0.37	7.20±0.20	5.80±0.58	5.80±0.37	6.87± 0.26
Monocyte (%)	T_1	1.60±0.51	1.60±0.24	1.40±0.24	1.40±0.24	1.40±0.24	1.20±0.58	1.43±0.14
	T_2	1.40±0.40	1.60±0.24	1.40±0.24	1.40±0.24	1.40±0.24	1.40±0.51	1.43±0.12
	T_3	2.00±0.00	1.60±0.40	1.40±0.40	1.40±0.24	1.40±0.40	1.00±0.32	1.47±0.13

($p<0.05$)
Mean value with common superscript does not differ significantly
Superscript for treatment variation
T_1- Control
T_2-Ascorbic acid supplemented at the rate of 10g/animal/day
T_3- Ascorbic acid supplemented at the rate of 15g/animal/day
Basophil = Basophils were not recorded during the study period.

RESULTS AND DISCUSSION

The average THI (Table 2) above 78 was recorded at 6.00h from June to September and at 14:00h from June to November throughout the experimental period. Mean temperature humidity index (THI) above 72 was recorded in October at 6:00h. The values of THI indicated that the experimental animals were in moderate to severe heat stress. Ganaie *et al*. (2012) reported that THI>72 is considered as stressful and THI above 78 is considered as very severe heat stress to buffaloes.

Haematological parameters

Haemoglobin (g%)

The mean haemoglobin concentrations (Table 3) of animals were within the range as reported (Bernardo, 1964; Tan, 1986) for swamp buffaloes. Supplementation of AA increased the overall mean haemoglobin level in T_2 and T_3 groups and the increase was concurrent with the dose of AA. Numerically higher values were observed in T_2 group relative to T_1 group and statistically ($p<0.05$) higher values were recorded in T_3 as compared to T_1 group. The finding is in agreement with other workers (Adenkola *et al*., 2010a and Haq *et al*., 2013) which might be due to the effect of AA in protecting the membrane integrity of the erythrocyte (Adenkola *et al*., 2010b) in the T_2 and T_3 groups resulting in higher haemoglobin concentration than the control group.

Packed cell volume (PCV)

Supplementation of AA increased the PCV (Table 3) in T_2 and T_3 groups compared to the control group. The increase in the mean PCV values was concurrent to the increase in the dose rate of AA. However, the overall mean PCV value of T_3 group was non-significantly higher than T_2 group and significantly ($p<0.05$) higher than T_1 group. The rise in PCV in AA supplemented groups of buffaloes was similar with the previous reports (Sivakumar *et al*., 2010 and Haq *et al.,* 2013) and may be attributed due to the effect of AA in protecting the membrane integrity of the erythrocytes (Adenkola *et al*., 2010b).

Mean Corpuscular Haemoglobin Concentration (g %)

The values (g %) of MCHC (Table 3) were within the range reported by (Bernardo, 1964) for native Carabaos and also within the reference range of reported by Abd Ellah *et al.* (2014) for water buffaloes.

The overall mean of MCHC (g %) were higher in the AA supplemented groups and the increase was concurrent with the increase in dose of AA as compared to the control group but the values were not significantly different.

Total leucocyte count (TLC)

The values were within the range (6.2-13 thousand cmm) reported by Sharma and Singh, (2005).

Lower mean TLC (Table 3) was observed in the AA supplemented groups (T_2 and T_3) as compared to the control group, but the differences were statistically non-significant.

Differential leucocyte count (DLC)

Lymphocyte (%)

All the values were found within the range (26-75%) reported by Sharma and Singh (2005).

Supplemented groups had significantly higher ($p<0.05$) overall mean values of lymphocyte (Table 3) than the control group. The result is in agreement with that of El-Bakhmy *et al*. (2007). The lower overall mean lymphocyte recorded in the T_1 group might be due to high level of glucocorticoids which decreases the secretion of cytokines IL-2 and leads to reduced proliferation of lymphocytes and also makes lymphocyte cells susceptible to apoptosis, thereby reducing its number in the circulation (Hangalapura *et al*., 2004).

Neutrophil (%)

The mean of neutrophil count did not differ significantly either among the treatments (Table 3). A downward trend in neutrophil count was observed in AA supplemented groups as compared to the control group, but the differences were non-significant.

Eosinophil (%)

Dietary supplementation of AA causes decrease in

eosinophil count (Table 3) in T_2 and T_3 groups of swamp buffaloes.

Monocyte (%)

There was no significant difference in the count of monocyte among the treatments (Table 3). The values of monocyte were relatively constant and within the normal range (1-11%) reported by Sharma and Singh (2005). According to Kannan *et al*. (2000) monocytes are not significantly affected in response to stress.

Basophil

Basophils were not recorded during the study period. No basophil found in this study did not mean there was no basophil in the circulation, but because of a very few number of basophils in the circulation. Sulong *et al*. (1980) reported that the percentage of basophil in swamp buffaloes of Malaysia was only 0.3% of the total leukocyte count.

Biochemical Parameters

Serum Glucose (mg/dl)

In the present study, the serum glucose values (Table 4) were found within the range (1.97–5.13 mmol/l) reported by Abd Ellah *et al*. (2014). Supplementation of AA significantly ($p<0.05$) reduced the overall mean serum glucose in T_2 and T_3 groups compared to T_1 group while similar values were found in T_2 and T_3. The decrease in mean serum glucose level was concurrent with increase in the dose rate of ascorbic acid. The present result is in accordance with the studies of Haq *et al.* (2013).

The decrease in serum glucose concentration in the AA supplemented groups could be due to effect of AA in increasing insulin concentration and decreasing corticosterone level as reported by Sahin *et al*. (2003) and Gursu *et al*. (2004). In contrary to the present findings, an increase in glucose concentration due to AA supplementation has been reported by Abd-Allah and Zanouny (2014) probably due to the decrease in glucose utilization.

Serum Total Protein (g/dl)

The mean of serum total protein (Table 4) were found within the range (5.6 to 8.2 g/100 ml) reported by Camba (1981) in Caraboas and the reference range (56.30 to 81.00 g/l) reported by Abd Ellah *et al*. (2014).

The overall mean of serum total protein were lower in AA supplemented groups as compared to the control which showed a clear downward trend with increase in the dose rate of AA. However, the differences among the groups were non-significant.

Serum Aspertate Amino Transferese (IU/L) and Serum Alanine Animo Transferase (IU/L)

Although the values of AST and ALT (Table 4) were found higher but it was within the normal range for healthy animals as reported by Abd Ellah *et al.* (2014).

Supplementation of AA lowered the serum concentration of AST and ALT in T_2 and T_3 but no significant difference was found between the control and the AA supplemented groups.

Hormonal parameters

Triiodothyronine (nmol/l)

The mean serum concentration of T_3 (Table 5) were within the normal range. Similar result was also reported by Ingole *et al*. (2012) for buffalo calves (1.58 ± 0.16 ng/ml). The overall mean serum triiodothyronine level of T_1 group was significantly ($p<0.05$) lower compared to T_2 and T_3 groups, while similar values were found in T_2 group and T_3 group. This might be due to the reason that vitamin C is an important antioxidant within aqueous phase of tissue and thus prevents the increased production of reactive oxygen metabolites and H_2O_2 within the cells which were responsible for reduced level of triiodothyronine in the control group (Ganaie *et al.,* 2012). Similar results were also reported in buffalo calves (El-Bakhmy *et al.,* 2007), goats (Sivakumar *et al.,* 2010) and Murrah buffaloes (Ganaie *et al*., 2012).

Thyroxin (nmol/l)

The values (nmol/l) of mean serum T_4 concentration (Table 5) were within the similar range reported by Micu (1979)

Table 4: Biochemical parameters of control and ascorbic acid supplemented swamp buffaloes

Parameters	Groups	June	July	August	September	October	November	Overall mean
Glucose	T_1	62.46±1.36^a	62.97±2.11^a	60.25±1.97^a	59.43±2.18^a	57.74±2.26^a	54.98±2.65^a	59.64±0.94^a
(mg/dl)	T_2	61.82±2.95^a	58.70±2.23^b	58.12±1.17^b	56.75±1.76^b	51.11±0.65^a	49.67±1.77^b	56.03±1.06^b
	T_3	61.66±1.62^a	57.46±1.67^b	57.56±1.62^b	54.39±2.58^c	51.56±3.10^a	49.26±3.12^b	55.31±1.17^b
Total protein	T_1	6.69±0.30	6.86±0.12	6.87±0.41	7.78±0.23	7.77±0.19	7.89±0.09	7.29±0.13
(g/dl)	T_2	6.71±0.41	6.86±0.12	6.78±0.22	7.31±0.29	7.17±0.46	7.42±0.33	7.00±0.14
	T_3	6.61±0.38	6.44±0.26	6.66±0.29	7.10±0.45	7.19±0.52	7.24±0.47	6.87±0.16
AST	T_1	104.76 ±11.92	103.89± 9.20	103.54± 7.12	98.82±8.59	90.44±9.46	85.80±10.06	97.87±3.78
(IU/L)	T_2	105.63±4.21	100.29 ±.11	101.27± 6.10	96.41±2.72	85.55±4.74	84.50±7.06	95.61±2.35
	T_3	104.58±11.53	99.70±13.45	93.4±8.63	95.68±4.03	86.95±2.93	80.12±7.48	93.41±3.61
ALT	T_1	26.25±3.39	26.08±1.81	26.99±1.85	26.07±2.41	25.35±3.32	24.91±3.05	25.94±0.30
(IU/L)	T_2	26.17±3.81	25.17±0.41	25.00±1.42	24.07±2.13	23.91±0.69	22.38±1.53	24.45±0.53
	T_3	26.30±1.41	24.72±2.72	24.31±3.14	23.41±2.05	23.33±1.57	22.30±1.52	24.06±0.56

($p<0.05$)
Mean value with common superscript does not differ significantly
Superscript for treatment variation
T_1- Control
T_2- Ascorbic acid supplemented at the rate of 10g/animal/day
T_3- Ascorbic acid supplemented at the rate of 15g/animal/day

Table 5: Hormonal parameters of control and ascorbic acid supplemented swamp buffaloes

Parameters	Groups	June	July	August	September	October	November	Overall mean
Triiodothy-ronone	T_1	2.53± 0.03^a	2.49 ± 0.02^a	2.47 ± 0.02^b	2.45 ± 0.01^b	2.43 ± 0.01^b	2.43 ± 0.02^b	2.47 ± 0.01^b
	T_2	2.53± 0.03^a	2.53 ± 0.02^a	2.55 ± 0.02^a	2.54 ± 0.01^a	2.53 ± 0.01^a	2.54 ±0.01^a	2.54 ± 0.01^a
	T_3	2.54± 0.02^a	2.55 ± 0.02^a	2.57 ± 0.02^a	2.56 ± 0.02^a	2.56 ± 0.02^a	2.55 ± 0.03^a	2.55 ± 0.01^a
Thyroxin	T_1	182.75± 3.13^a	170.14±2.92^a	162.16±1.63^a	158.30±7.65^a	142.60±7.39^b	141.57±8.27^b	159.59±6.50^b
	T_2	180.44±6.11^a	177.09±6.83^a	169.76±11.45^a	164.22±11.76^a	154.83±0.69^{ab}	156.76±10.88^{ab}	167.18±4.28^b
	T_3	181.98±4.69^a	179.92±4.89^a	176.45± 3.75^a	176.83± 2.02^a	174.00± 7.53^a	173.10± 9.40^a	177.05±1.39^a
Cortisol	T_1	71.60±0.51^a	73.00±0.32^a	72.00±0.55^a	70.80±0.80^a	68.00±0.55^a	68.20±0.86^a	70.60±0.42^a
	T_2	72.40±0.24^a	65.60±0.81^b	65.80±1.39^b	65.00±1.67^b	63.40±0.93^b	63.60±0.68^b	65.97±0.69^b
	T_3	72.00±0.45^a	65.20±0.66^b	65.00±1.22^b	63.60±0.87^b	61.80±0.66^b	62.20±0.66^b	64.97±0.70^c

($p<0.05$)
Mean value with common superscript does not differ significantly
Superscript for treatment variation
T_1- Control
T_2- Ascorbic acid supplemented at the rate of 10g/animal/day
T_3- Ascorbic acid supplemented at the rate of 15g/animal/day

for the Philippine Carabaos. The overall mean of serum thyroxin was significantly ($p<0.05$) higher in T_3 group and non-significantly higher in T_2 group as compared to T_1 group. The result revealing higher concentration of serum thyroxin in AA supplemented groups is in agreement with those reported by other workers (El-Bakhmy *et al*., 2007; Sivakumar *et al*., 2010; Ganaie *et al*., 2012) which might be due to the reason that vitamin C is an important antioxidant within aqueous phase of tissue and thus prevents the increased production of reactive oxygen metabolites and H_2O_2 within the cells. Free radical H_2O_2 serves as a substrate for the thyroperoxidase enzyme which catalyzes the synthesis of thyroid hormone, namely T_3 and T_4. Production of more H_2O_2 under stress condition might have reduced the levels of thyroid hormone (Usha *et al*., 2002) in the T_1 group. The efficiency of feed utilisation might also have been better in AA supplemented groups as vitamin C scavenges free O_2 radicals, so preventing the oxidative stress of the cell membrane of the digestive system and restoring efficient feed utilization (Abou-Zeid *et al*., 2000) and thereby there was no marked reduction in T_4 concentration in the serum.

Serum Cortisol

The mean values of serum cortisol (Table 5 of experimental swamp buffaloes were higher than the normal range (1.4-1.6 µg/dL) reported by Khan *et al.* (2003). The micro-climatic data (Table 1) revealed temperature humidity index values above comfort zone (THI>74) from June to October at 6:00 h and from June to November at 14:00 h, which indicated that the climate of the region caused thermal discomfort to the animals and thereby increased the serum cortisol level. Serum cortisol values above normal range were also reported by Silva *et al*. (2014) due to climatic stress in swamp buffaloes.

The overall mean cortisol level of T_1 group was higher in comparison to T_2 and T_3 groups and, T_3 group showed significantly lower value than other two groups. The result is in consistent to the findings reported by other researchers (Kumar *et al*., 2010; Kumar *et al*., 2011and Ganaie *et al.,* 2012) indicating that AA supplementation reduces stress in animals. The reduction in cortisol level by vitamin C may be achieved by reducing the synthesis and/or secretion of cortisol or by breaking it down (Orth, 1992 and Webel *et al*., 1998). Reduction in cortisol levels may help in keeping the animal healthy and stronger to fight immuno-suppression generally observed due to stress.

CONCLUSION

From the study it is concluded that the dietary supplementation of AA modulate the hemato-biochemical and hormonal profile (haemoglobin, PCV, serum glucose, triiodothyronine, thyroxin and cortisol in swamp buffaloes to cope up thermal stress during summer and post summer months. The dose at the rate of 15 g/day/animal was found to be more effective in maintaining the physiological, hemato-biochemical and hormonal profiles.

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