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EFFECT OF DIETARY SUPPLEMENTATION OF ASCORBIC ACID ON PHYSIOLOGICAL PARAMETERS, BODY WEIGHT CHANGES AND REPRODUCTIVE PERFORMANCE OF SWAMP BUFFALOES

Dipanjali Konwar*[1,4], T.K. Amonge[1], D.J. Dutta[2], A.K. Gogoi[1], R.S. Borah[1], G.Ch. Das[3], R. Bhuyan[4] and R. Roychoudhury[1]

[1]Department of LPM, [2]Department of Veterinary Physiology, [3]Network Project on Swamp Buffalo Improvement, Department of Animal Genetics & Breeding, [4]Department of Animal Nutrition, College of Veterinary Sciences, Khanapara, Guwahati – 781 022, Assam (India)
[4]Division of LPM, Faculty of Veterinary Sciences & Animal Husbandry, SK University of Agricultural Sciences & Technology of Jammu, R.S. Pura, Jammu & Kashmir
*e-mail: *dipanjali1@rediffmail.com*

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ABSTRACT

To assess the effect of ascorbic acid supplementation on physiological parameters in buffalo *viz.*, changes in body weight, dry matter intake and reproductive performances, eighteen Swamp buffaloes were divided into three groups. One group was fed with normal diet and other two groups supplemented with 10 and 15 g ascorbic acid animal^{-1} day^{-1}. The group supplemented with 15 g ascorbic acid animal^{-1} day^{-1} had significantly lower respiration rate, pulse rate and rectal temperature as compared to the group supplemented with 10 or 0 g ascorbic acid animal^{-1} day^{-1}. The dry matter intake kg^{-1} body weight, DMI per kg $W^{0.75}$ was not affected by ascorbic acid supplementation. The body weight losses during winter were relatively less and the reproductive traits *viz..*, per cent of animals coming into oestrus, conception rate and number of services per conception improved at higher level of ascorbic acid supplementation. It may be concluded that dietary supplementation of ascorbic acid has favourable impact in maintaining the physiological parameters, body weight and improved reproductive traits of swamp buffaloes.

Key words: Ascorbic acid, physiology, reproductive traits, swamp buffalo

INTRODUCTION

Ascorbic acid, also termed as vitamin C, is an antioxidant that is essential for numerous cellular functions. It is most abundant and important water-soluble antioxidant in mammals. Supplementation of vitamin C in ruminants is not a required dietary nutrient but under certain adverse environmental conditions the metabolic need for this vitamin may exceed the inherent biosynthetic ability of ascorbic acid. Ascorbic acid has numerous biological functions; it acts as antioxidant (Weiss, 2006) that prevents the oxidation of protein, DNA and nitric oxide; acts as regulator of iron uptake (Hacisevki, 2009) and shows 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 in comparison to other farm animals are more prone to physical distress during summer because of scarce distribution of sweat glands and dark body colour. The swamp buffaloes are less productive and also inefficient breeders (have long gestation period, long calving interval, silent heat and low conception rate) as compared to river buffaloes due to their inherent susceptibility to environmental stress, which causes anestrus and sub-estrus (Das and Khan, 2010). There are several reports of supplementation of ascorbic acid 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, perusal of literature reveals that the information pertaining to the effect of ascorbic acid on physiological parameters and reproductive performance of swamp buffaloes is lacking. Therefore, the present study was aimed to investigate the effect of ascorbic acid supplementation on physiological parameters, body weight changes and reproductive performances of swamp buffaloes during the period from June to February.

MATERIALS AND METHODS

A total of eighteen healthy pre-pubertal swamp buffaloes of 3.5 years of average age and having an average body weight of 336.2 kg were randomly divided into three groups of six each. The animals in first group were maintained as control and fed diet without supplementation whereas the animal in other two groups were fed diet supplemented with ascorbic acid @ 10 and 15 g animal^{-1} day^{-1}. The animals were housed in semi-open shed with cemented anti-slippery floor and asbestos roof. The animals were fed as per Kearl feeding standard (Kearl, 1982). All the groups were fed on concentrate mixture @ 2 kg head^{-1} day^{-1} and mixed green roughages (maize, sorghum & Napier grass as per their availability) @ 25 kg animal^{-1} day^{-1}. The ingredients in concentrate mixture were maize 20%, wheat bran (37%), rice polish (20%), ground nut cake (13%), mustard oil cake (7.5%), mineral mixture (2%) and common salt (0.5%). Fresh water was made available throughout the day to all the animals. Paddy straw was fed to the animals during winter when there was scarcity of green fodders.

Proximate composition of feeds and fodders were done as per the methods of AOAC (1990). In addition, the animals were allowed to graze daily for 4 h in the morning from 8:00 to 12:00 h. The ascorbic acid was supplemented individually to the animals by mixing it with 100 g concentrate before morning feeding for 150 days from mid June to mid November. The experiment was carried out from mid June to February. The minimum and maximum temperatures and relative humidity (RH) were recorded inside the barn twice per day (at 06:00 and 14:00 h) using thermo-hygrometers. The temperature-humidity index (THI) was calculated by using the equation:

$$\text{THI} = 0.72\ (\text{dry bulb temperature} + \text{wet bulb temperature}) + 40.6$$

where 0.72 and 40.6 are constants. The RT, RR and PR were determined twice daily (at 06:00 and 14:00 h) once a week. The animal body weights were recorded at the beginning of the experiment and thereafter at monthly interval. Dry matter intake of the individual animal was recorded at every fortnight interval. The amounts of feed were weighed before offering and residue were collected the next day morning. The feed intake was calculated (on dry matter basis) by subtracting the amount of residue left from the amount of total feed offered. The dry matter intake during grazing was not recorded as grazing was additional. Reproductive parameters were recorded during breeding season. The per cent of animals coming in oestrus was determined by observing behavioural oestrus (off-fed, bellowing, mounting on herd-mate female, acceptance of bull, frequent micturation, erection of tail) and the genital status (oedema, wetness of vulva and mucus discharges) were monitored in morning and evening daily during the breeding season. The data were analyzed statistically by using SAS software (2009).

RESULTS AND DISCUSSION

The highest and lowest ambient temperature was recorded in June and January, respectively; while highest relative humidity was noticed in February at 6:00 h and lowest in June at 14:00 h (Table 1). This is in accordance to Abu-Heakal *et al*. (2014). The average temperature-humidity index (THI) >72 was observed at 6.00 h from August to October and at 14.00 h in December. The average THI > 78 was recorded from June to November at 6.00 h and throughout the experimental period at 14.00 h. The values of THI indicated that the experimental animals were in moderate to severe heat stress. The THI>72 is considered stressful and THI >78 is considered as very severe heat stress in buffaloes (Ganaie *et al*., 2012).

The overall mean respiration rate min^{-1} was subnormal from October to February at 6:00 h and above normal range from June to August at 14:00 h (Table 1). The increase or decrease in respiration rate is an adaptive response of animal to maintain homeothermy (Sabuncuoglu, 2004). The overall mean respiration rate was significantly high from June to August than December and January at both 6:00 and 14:00 h. This might be an attempt to increase the evaporative cooling in response to higher THI. Similar results have been reported by Dhenge *et al*. (2014) and Chaudhary *et al*. (2015). The result revealed that ascorbic acid supplementation reduced respiration rate as compared to the control group both at 6:00 and 14:00 h. Decrease in respiration rate was found proportional to the decrease in dose of ascorbic acid. This is in agreement with Sivakumar *et al.* (2010) and Kumar *et al.* (2012). It is obvious that respiratory rates decrease as a result of ameliorative effect of ascorbic acid supplementation.

The overall mean pulse rate min^{-1} was slightly lower than the normal range in November at 6:00 h and above the normal range from June to October at 14:00 h (Table 2). There was month-wise variation in pulse rate with higher overall averages in June and July and lower averages from October to February both at 6:00 and 14:00 h. The results are in line with Vaidya *et al*. (2010) in cattle and buffalo. The environmental temperature has significant relation with variation in pulse rate. The average values of pulse rate were higher in summer and lower in winter (Gangwar, 1988). In present study, the animal group supplemented with 15 g ascorbic acid $animal^{-1}$ day^{-1} had significantly low overall mean pulse rate as compared to control or low ascorbic acid dose group animals at 6:00 and 14:00 h. The lower pulse rate in ascorbic acid supplemented groups is similar to the Murrah buffaloes (Gade *et al.,* 2010; Ganaie *et al.,* 2012) and goats (Kumar *et al.,* 2012).

The overall mean rectal temperature were higher in June and August both at 6:00 and 14:00 h than November to February at 6:00 h and January, February at 14:00 h (Table 2). The seasonal variation in rectal temperature observed was similar to Verma *et al.* (2000). The climatic data (Table 1) showed that THI values were above the comfort zone from June to September both at 6:00 h (78.95-80.90) and 14:00 h (85.08-85.92). However, the rectal temperatures were within the

Table 1: Average ambient temperature, relative humidity (%), temperature humidity index (THI) and day light of the micro-climate (barn)

Months	Ambient temperature (^{0}C)		Relative humidity (%)		Temperature humidity index (THI)		Daylight (hours)
	Maximum	Minimum	6:00 h	14:00 h	6:00 h	14 h	
June	35.39±0.40	27.36±0.18	83.75±2.29	65.42±2.44	80.90±0.38	85.92±0.49	13.73±0.00
July	33.26±0.40	27.82±0.20	88.84±0.67	70.35±1.48	80.11±0.17	84.55±0.36	13.59±0.02
August	33.82±0.37	27.45±0.30	88.18±0.85	70.47±2.43	79.72±0.16	85.44±0.55	13.02±0.04
September	33.45±0.41	26.72±0.15	88.97±0.73	77.81±2.71	78.95±0.21	85.08±0.59	12.30±0.04
October	31.11±0.38	23.90±0.30	88.62±0.69	67.19±1.53	74.64±0.36	81.81±0.44	11.54±0.04
November	27.67±0.15	17.02±0.27	86.23±1.91	64.58±1.12	64.91±0.49	78.30±0.25	10.87±0.03
December	24.18±0.45	14.32±0.43	88.40±0.57	81.37±1.04	61.21±0.60	72.40±1.66	10.54±0.01
January	24.19±0.35	13.08±0.26	89.77±0.45	80.61±0.82	59.85±0.36	74.68±0.54	10.71±0.02
February	24.76±0.57	13.57±0.37	91.24±0.57	88.74±0.61	60.54±0.58	76.26±0.91	11.27±0.04

RH = Relative humidity; THI = Temperature humidity index

Table 2: Physiological parameters of control and ascorbic acid supplemented swamp buffaloes

Para-meters	Time		June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Overall
RR	6:00	T_1	17.67 ±0.60^{a}_{A}	16.83 ±0.25^{a}_{A}	18.17 ±1.24^{a}_{A}	16.33 ±1.01^{a}_{AB}	14.83 ±0.59^{a}_{B}	11.17 ±0.56^{a}_{C}	7.33 ±0.38^{a}_{D}	7.17 ±0.61^{a}_{D}	8.50 ±0.24^{a}_{D}	13.11 ±0.63^{a}
		T_2	16.50 ±0.27^{a}_{A}	15.33 ±0.44^{b}_{AB}	16.51 ±0.45^{ab}_{A}	14.67 ±0.41^{a}_{B}	11.67 ±0.37^{b}_{C}	9.83 ±0.33^{ab}_{D}	7.33 ±0.35^{a}_{E}	6.83 ±0.45^{a}_{E}	8.67 ±0.52^{a}_{D}	11.93 ±0.52^{b}
		T_3	15.17 ±0.42^{b}_{A}	14.67 ±0.15^{b}_{A}	15.20 ±1.02^{b}_{A}	14.17 ±0.7^{a}_{A}	11.33 ±0.43^{b}_{B}	9.17 ±0.41^{b}_{C}	7.33 ±0.21^{a}_{D}	5.67 ±0.46^{a}_{E}	8.00 ±0.3^{a}_{CD}	11.11 ±0.51^{c}
		Mean	16.44 ±0.36_{AB}	15.61 ±0.27_{BC}	16.60 ±0.57_{A}	15.06 ±0.46_{C}	12.61 ±0.46_{D}	10.06 ±0.31_{E}	7.33 ±0.18_{G}	6.56 ±0.32_{G}	8.39 ±0.21_{F}	
	14:00	T_1	27.50 ±0.82^{a}_{A}	26.77 ±0.50^{a}_{A}	27.46 ±1.43^{a}_{A}	21.26 ±0.88^{a}_{B}	20.08 ±0.81^{a}_{B}	17.60 ±0.46^{a}_{C}	12.67 ±0.44^{a}_{D}	11.38 ±0.71^{a}_{D}	13.75 ±0.75^{a}_{D}	19.83 ±0.87^{a}
		T_2	26.61 ±0.67^{ab}_{A}	24.60 ±0.48^{b}_{A}	24.75 ±2.21^{a}_{A}	17.80 ±0.83^{b}_{B}	16.33 ±0.78^{b}_{B}	15.67 ±0.53^{b}_{B}	12.33 ±0.55^{a}_{C}	10.88 ±0.45^{a}_{C}	12.38 ±0.82^{a}_{C}	17.93 ±0.83^{b}
		T_3	24.94 ±0.55^{b}_{A}	23.00 ±0.22^{c}_{B}	23.71 ±1.15^{a}_{AB}	17.17 ±0.62^{b}_{C}	16.00 ±0.46^{b}_{CD}	14.63 ±0.76^{b}_{D}	12.00 ±0.49^{a}_{E}	10.25 ±0.38^{a}_{E}	11.79 ±0.53^{a}_{CD}	17.05 ±0.75^{c}
		Mean	26.35 ±0.45_{A}	24.79 ±0.44_{B}	25.31 ±0.98_{AB}	18.74 ±0.61_{C}	17.47 ±0.59_{C}	15.97 ±0.44_{D}	12.33 ±0.28_{E}	10.83 ±0.31_{F}	12.64 ±0.43_{E}	
PR	6:00	T_1	50.27 ±0.59_{AB}^{a}	51.87 ±0.41_{A}^{a}	48.76 ±1.26_{AB}^{a}	49.20 ±1.67_{B}^{a}	43.17 ±0.79_{C}^{a}	40.93 ±1.23_{C}^{a}	41.79 ±0.42_{C}^{a}	40.42 ±0.96_{C}^{a}	40.96 ±0.65_{C}^{a}	45.26 ±0.67^{a}
		T_2	47.05 ±0.65_{AB}^{b}	47.77 ±0.98_{AB}^{b}	47.91 ±1.55_{A}^{a}	45.00 ±1.70_{B}^{a}	42.25 ±0.96_{CD}^{a}	39.43 ±0.54_{D}^{a}	41.33 ±0.28_{CD}^{ab}	40.71 ±1.06_{CD}^{a}	42.88 ±1.11_{C}^{a}	43.81 ±0.56^{b}
		T_3	46.45 ±1.11_{A}^{b}	46.90 ±1.03_{A}^{b}	43.48 ±2.01_{AB}^{a}	41.60 ±1.30_{BC}^{b}	39.71 ±1.98_{C}^{a}	38.77 ±1.43_{C}^{a}	40.71 ±0.31_{C}^{b}	41.42 ±0.97_{BC}^{a}	41.17 ±1.62_{BC}^{a}	42.24 ±0.58^{c}
		Mean	47.93 ±0.60_{A}	48.85 ±0.70_{A}	46.72 ±1.01_{C}	45.27 ±1.07_{B}	41.71 ±0.81_{D}	39.71 ±0.65_{D}	41.28 ±0.21_{D}	40.85 ±0.55_{D}	41.67 ±0.68_{D}	
	14:00	T_1	59.44 ±1.43^{a}_{A}	59.63 ±1.06^{a}_{A}	55.65 ±0.89^{a}_{A}	55.44 ±1.51^{b}_{B}	52.43 ±0.48^{bc}_{BC}	50.87 ±0.66^{bcd}_{BCD}	50.21 ±0.73^{cd}_{CD}	46.74 ±1.52^{e}_{E}	48.75 ±1.16^{de}_{DE}	53.24 ±0.70^{a}
		T_2	57.22 ±0.83^{a}_{A}	56.97 ±1.27^{a}_{A}	52.51 ±0.82^{ab}_{AB}	52.00 ±1.86^{b}_{B}	50.00 ±0.47^{b}_{B}	49.10 ±2.47^{b}_{B}	49.08 ±0.86^{b}_{B}	50.00 ±0.81^{b}_{B}	49.00 ±2.71^{b}_{B}	51.76 ±0.64^{ab}
		T_3	55.89 ±1.72^{a}_{A}	54.53 ±1.58^{ab}_{AB}	50.30 ±1.70^{bc}_{BC}	53.20 ±1.58^{c}_{C}	47.38 ±0.60^{c}_{C}	47.97 ±1.70^{c}_{C}	50.13 ±1.46^{c}_{C}	48.58 ±0.79^{c}_{C}	49.46 ±0.90^{c}_{C}	50.83 ±0.57^{b}
		Mean	57.52 ±0.68_{A}	57.04 ±0.71_{A}	52.82 ±0.72_{B}	53.55 ±0.72_{BC}	49.94 ±0.51_{CD}	49.31 ±0.74_{D}	49.80 ±0.48_{CD}	48.44 ±0.51_{D}	49.07 ±0.72_{D}	
RT	6:00	T_1	38.01 ±0.00^{a}_{A}	37.98 ±0.01^{a}_{A}	38.19 ±0.01^{a}_{A}	37.97 ±0.01^{a}_{A}	37.68 ±0.01^{a}_{B}	37.04 ±0.03^{a}_{C}	36.89 ±0.01^{a}_{CD}	36.74 ±0.00^{a}_{D}	36.86 ±0.01^{a}_{CD}	37.48 ±0.09^{a}
		T_2	37.84 ±0.00^{b}_{A}	37.71 ±0.01^{b}_{AB}	37.97 ±0.00^{b}_{A}	37.72 ±0.01^{b}_{AB}	37.54 ±0.01^{ab}_{B}	36.99 ±0.02^{a}_{C}	36.97 ±0.03^{a}_{C}	36.94 ±0.01^{ab}_{C}	36.94 ±0.03^{a}_{C}	37.40 ±0.06^{b}
		T_3	37.81 ±0.00^{b}_{A}	37.62 ±0.01^{b}_{A}	37.69 ±0.00^{c}_{A}	37.60 ±0.01^{b}_{AB}	37.47 ±0.02^{b}_{B}	36.96 ±0.02^{a}_{C}	37.01 ±0.00^{a}_{C}	37.01 ±0.01^{b}_{C}	37.06 ±0.01^{a}_{C}	37.36 ±0.04^{b}
		Mean	37.89 ±0.00_{AB}	37.77 ±0.00_{B}	37.95 ±0.01_{A}	37.76 ±0.01_{B}	37.56 ±0.01_{C}	36.99 ±0.01_{D}	36.96 ±0.00_{D}	36.90 ±0.00_{D}	36.95 ±0.01_{D}	
	14:00	T_1	38.62 ±0.02^{a}_{AB}	38.56 ±0.02^{a}_{AB}	38.71 ±0.04^{a}_{A}	38.50 ±0.09^{a}_{AB}	38.49 ±0.09^{a}_{AB}	38.41 ±0.08^{a}_{B}	38.07 ±0.15^{a}_{C}	37.45 ±0.31^{a}_{D}	37.61 ±0.12^{a}_{D}	38.27 ±0.11^{a}
		T_2	38.50 ±0.02^{b}_{A}	38.26 ±0.05^{b}_{ABC}	38.46 ±0.06^{b}_{AB}	38.22 ±0.06^{b}_{ABC}	38.08 ±0.24^{b}_{C}	38.14 ±0.15^{b}_{BC}	38.12 ±0.21^{a}_{C}	37.70 ±0.30^{a}_{D}	37.72 ±0.36^{a}_{D}	38.13 ±0.09^{b}
		T_3	38.28 ±0.04^{c}_{A}	38.08 ±0.02^{c}_{ABC}	38.08 ±0.02^{c}_{AB}	38.02 ±0.10^{c}_{ABC}	37.94 ±0.20^{b}_{BC}	38.06 ±0.15^{b}_{ABC}	38.19 ±0.21^{a}_{ABC}	37.92 ±0.24^{a}_{BC}	37.89 ±0.34^{a}_{C}	38.05 ±0.06^{b}
		Mean	38.47 ±0.06_{A}	38.30 ±0.09_{B}	38.42 ±0.09_{A}	38.25 ±0.10_{BC}	38.17 ±0.14_{BC}	38.20 ±0.10_{BC}	38.13 ±0.11_{C}	37.69 ±0.18_{D}	37.74 ±0.17_{D}	

Mean value with same superscript and subscript does not differ significantly ($p<0.05$); Superscript represent treatment variation and subscript month-wise variation; T_1 - Control; T_2 - Ascorbic acid supplemented @ 10 g animal^{-1} day^{-1}; T_3- Ascorbic acid supplemented @ 15 g animal^{-1} day^{-1}; RR = respiration rate; PR = pulse rate; RT = rectal temperature

normal range as the buffaloes were in shed at the time of recording the rectal temperature. This might be due to the fact that buffaloes maintained their rectal temperature within normal range at the expense of higher respiration rate inside the barn (Gudev *et al*., 2007).

During the period of ascorbic acid supplementation the mean rectal temperature in ascorbic acid supplemented groups were significantly ($p<0.05$) lower than control group both at 6:00 and 14:00 h. The decrease in rectal temperature corresponded to the decrease in the dose of ascorbic acid. The decrease in rectal temperature by ascorbic acid indicated that it has hypothermic effect subjected to high ambient temperature (Fayomi *et al.*, 2008). Similar results have been reported in Murrah buffaloes (Gade *et al*., 2010) and goats (Sivakumar *et al*., 2010; Kumar *et al*., 2012). Thus the supplementation with ascorbic acid may be beneficial in maintaining low rectal temperature in buffaloes during summer. In contrast, during the post period of ascorbic acid supplementation, ascorbic acid supplemented groups showed an increasing trend in retal temperature as compared to control group both at 6:00 and 14:00 h which was significant ($p<0.05$) in January in high ascorbic acid fed group at 6:00 h during the period of lowest THI (59.85). Similar results have earlier been reported by Purkayastha and Lazar (1992) in rats. This might be due to the effect of ascorbic acid, as it enhances resistance to the cold by raising body temperature and basal metabolism (Nakamura *et al.,* 1967). The higher rectal temperature in 15 g ascorbic acid supplemented group can also be attributed to better body conditions as feed utilisation in this group might have been better during the period of ascorbic acid supplementation. Abou-Zeid *et al.* (2000) reported that ascorbic acid scavenges free O_2 radicals so prevents the oxidative stress of cell membrane of digestive system thereby restore efficient feed utilization. Heat in body is continuously generated as a by-product of metabolism, and a healthy animal has significant internal sources of heat from the metabolic processes (Aleksandrova, 2013). Thus it appears that administration of Vitamin C for a prolonged period before cold exposure is beneficial for significant improvement in cold tolerance.

Chemical composition of feeds and fodders

The protein and NFE content of experimental concentrate mixture was 16.84±0.11 and 54.70±0.37%, respectively. The protein content of fodders ranged from 8.29±0.19 in sorghum to 10.12±0.32% in maize fodder while that of grasses grazed by experimental animal was 13.70±0.12%. The NFE content of fodders used for feeding the experimental animal ranged from 42.32±0.17 in hybrid Napier to 59.83±0.16% in sorghum fodder; while the value of same in mixed grasses grazed by experimental animals was 51.37±0.16% (Table 4).

Table 4: Chemical composition of concentrate, green fodder and grasses

Feed ingredients	DM	OM	CP	EE	CF	NFE	Ash	NDF	ADF	C	HC	Lignin
Concentrate	90.87 ±0.12	90.02 ±0.15	16.84 ±0.11	4.21 ±0.08	15.92 ±0.61	54.70 ±0.37	8.33 ±0.16	60.11 ±0.25	28.17 ±0.13	11.26 ±0.22	31.94 ±0.25	4.54 ±0.15
Napier grass	18.13 ±0.21	85.13 ±0.15	9.34 ±0.14	2.83 ±0.12	32.27 ±0.11	42.32 ±0.17	13.24 ±0.11	70.44 ±0.61	38.63 ±0.13	23.99 ±0.19	31.81 ±0.21	6.78 ±0.18
Maize fodder	19.19 ±0.10	88.17 ±0.19	10.12 ±0.32	1.87 ±0.10	27.31 ±0.34	49.53 ±0.18	11.17 ±0.16	55.21 ±0.18	32.22 ±0.27	11.21 ±0.13	22.99 ±0.11	5.69 ±0.19
Sorghum fodder	23.28 ±0.11	88.46 ±0.15	8.29 ±0.19	1.65 ±0.08	18.11 ±0.18	59.83 ±0.16	12.12 ±0.17	63.48 ±0.54	31.18 ±0.27	21.12 ±0.21	32.30 ±0.29	4.18 ±0.15
Grasses	20.87 ±0.17	88.91 ±0.21	13.70 ±0.12	1.91 ±0.10	21.58 ±0.13	51.37 ±0.16	11.44 ±0.21	59.84 ±0.31	34.19 ±0.15	27.15 ±0.18	25.65 ±0.27	6.39 ±0.16

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. The hemicellulose was calculated by subtracting ADF from NDF, and cellulose by subtracting lignin + silica from ADF.

Dry matter intake

The month-wise overall mean dry matter intake showed significantly ($p<0.05$) lower dry matter intake from June to October and significantly ($P<0.05$) higher dry matter intake in the months of January and February (Table 5). The results are in consistent to Verma *et al*. (2000) which reported that dry matter intake per 100 kg body weight was significantly varied ($p<0.01$) between seasons, being highest during winter (3.63±0.03) and lowest in summer (2.83±0.03) and rainy (2.69±0.11) seasons. The ascorbic acid supplementation had no effect on dry matter intake in buffaloes. This is in variance with the finding of Abdel-Monem *et al*. (2013) who reported increased daily feed intake in ascorbic acid supplemented kids.

Body weight

The average body weight of the animals did not differ within the groups (Table 5). The body weight of all the animals across the treatments increased up to August and then showed a decrease in body weight gain and the lowest body weights were recorded in January. The loss in body weight might be due to the low ambient temperature. Warriss (1993) reported that animals can lose weight when they are subjected to greater energy demands, such as those needed to maintain balance or to thermo-regulate. Ascorbic acid supplementation had no significant effect on body weight changes in ascorbic acid supplemented groups (loss in body weight 0.23 and 0.18%) as compared to control group (0.03%). This may be attributed to the efficient feed utilization (Abou-Zeid *et al.*, 2000). The present results are in partial agreement with Karnib (2009) who reported that a daily dose of vitamin C caused decrease in weight loss in adult female Awassi sheep subjected to a water-restricted regime and that the pre-transportation administration of ascorbic acid significantly decreased weight loss caused by transportation under unfavourable thermal conditions to goats (Minka and Ayo, 2007) and to sheep (Kassab and Mohammed, 2014).

Table 5: Average dry matter intake (kg) and body weight (kg) of swamp buffaloes in different treatment groups

Treatments	Control		Ascorbic acid @ 10 g day^{-1}		Ascorbic acid @ 10 g day^{-1}		Overall mean	
Months	DMI	Body weight	DMI	Body weight	DMI	Body weight	DMI	Body weight
June	$6.81_A\pm0.07$	336.2±10.06	$6.91_B\pm0.07$	336.1±18.21	$7.06_A\pm0.14$	336.5±25.36	$6.93_B\pm0.06$	336.2±10.27
July	$6.81_A\pm0.02$	337.2±9.58	$6.95_B\pm0.06$	336.5±17.94	$6.94_A\pm0.10$	338.4±25.58	$6.90_B\pm0.04$	337.4±10.23
August	$6.82_A\pm0.03$	338.0±9.87	$6.93_B\pm0.07$	338.4±17.89	$6.93_A\pm0.07$	339.6±25.58	$6.89_B\pm0.03$	338.7±10.25
September	$6.83_A\pm0.06$	338.0±10.16	$6.95_B\pm0.10$	338.0±17.68	$6.94_A\pm0.10$	339.1±25.17	$6.91_B\pm0.05$	338.4±10.14
October	$6.85_A\pm0.02$	337.4±10.08	$6.84_B\pm0.06$	336.9±17.69	$6.84_A\pm0.03$	338.7±25.50	$6.84_B\pm0.02$	337.7±10.22
November	$6.93_A\pm0.16$	336.9±10.34	$7.08_{AB}\pm0.13$	336.5±17.65	$7.10_A\pm0.15$	338.4±25.25	$7.04_{AB}\pm0.08$	337.3 ±10.18
December	$7.07_A\pm0.13$	336.5±10.21	$7.12_{AB}\pm0.08$	336.3±17.36	$7.16_A\pm0.12$	337.3±25.24	$7.12_{AB}\pm0.06$	336.7±10.11
January	$7.21_A\pm0.27$	335.3±10.23	$7.51_A\pm0.39$	335.1±17.29	$7.16_A\pm0.36$	336.3±25.34	$7.29_A\pm0.19$	335.6±10.13
February	$7.14_A\pm0.19$	335.4±10.69	$7.28_{AB}\pm0.26$	335.5±17.82	$7.17_A\pm0.18$	336.3±24.87	$7.20_A\pm0.12$	335.7±10.15
Mean body wt.	-	336.8±3.12	-	336.6±5.45	-	337.9±7.78	-	-
Net DMI animal^{-1}	6.94±0.08	-	7.06±0.06	-	7.03±0.06	-	-	-
DMI as %BW	2.06±0.02	-	2.10±0.02	-	2.09±0.02	-	-	-
DMI kg^{-1} W$^{0.75}$	78.58±0.54	-	77.12±1.63	-	78.57±1.34	-	-	-

Mean values with common subscript do not differ significantly ($p<0.05$), Subscript represent month-wise variation.

Reproductive performances

Only 66.7% animals came into oestrus during breeding season in control (Fig. 1) as compared to 83.3% in ascorbic acid supplemented groups. The present result showed favourable influence of ascorbic acid on the onset of oestrus which is in agreement with Chris and Nieske (2006) who reported enhanced ovulation due to the ascorbic acid supplementation. Similarly, Yassein *et al.* (2008) also reported that vitamin C supplementation significantly ($p<0.05$) improved the overall mean receptivity in rabbit does. The conception rate in control group was less (50%) as compared to 60% observed in ascorbic acid supplemented groups (Fig. 2). The results are in agreement with Yassein *et al.* (2008) in rabbits and Mehta *et al.* (1981) in buffaloes. The number of service required for conception was higher in control group (1.5%) as compared to ascorbic acid supplemented groups (1.3 and 1.0%) [Fig. 3]. The markedly lower number of service per conception in supplemented group as compared to control could be due to the higher level of plasma ascorbic acid

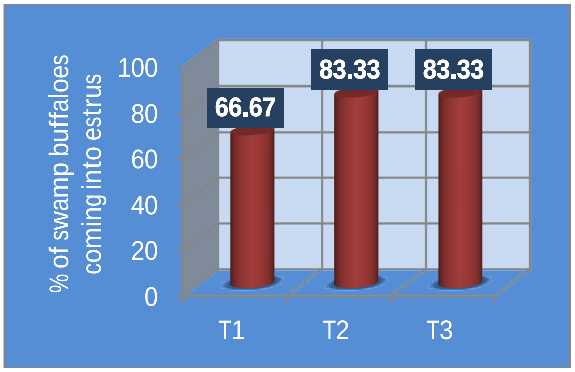

Fig. 1: Percentage of swamp buffaloes coming into estrus in different treatment groups

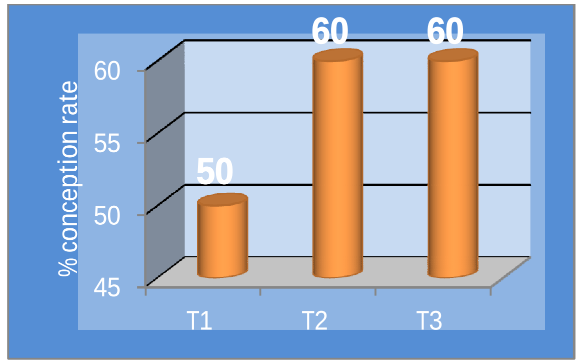

Fig. 2: Percentage of conception rate of the swamp buffaloes in different treatment groups

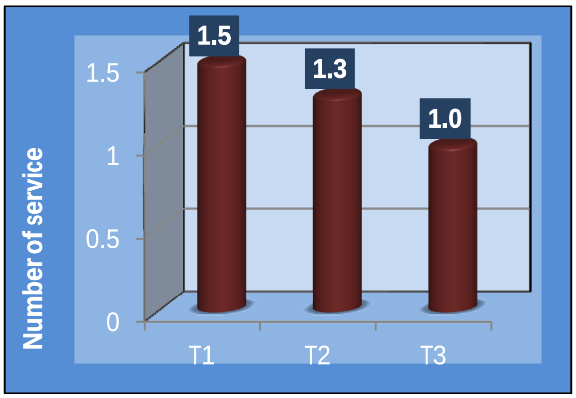

Fig. 3: Number of services per conception of swamp buffaloes in different treatment groups.

In all the three figures T_1 - Control treatment; T_2 - Ascorbic acid supplemented @ 10 g animal^{-1} day^{-1} and T_3 - Ascorbic acid supplemented @ 15 g animal^{-1} day^{-1}

following supplementation of ascorbic acid (Mehta *et al*., 1981).

Conclusions: Ascorbic acid can be supplemented during stressful period of summer in order to maintain body temperature, body weight and to improve the reproductive traits in swamp buffaloes. Future studies with large sample size are warranted to establish the reproductive potential of the female swamp buffaloes. The level of ascorbic acid that showed better performances was 15 g ascorbic acid animal^{-1} day^{-1} in comparison to 10 g ascorbic acid animal^{-1} day^{-1}.

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