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Effect of saline water on plasma Fe, Zn, Mn, Cu concentration and complete blood count in Murrah male calves

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Abstract
Twenty growing male Murrah calves, with average body weight (kg) 220 ± 5, were randomly assigned to five treatments of water containing total dissolved solids (TDS; ppm) *viz* C=500; T1=2,000; T2=4,000; T3=6,000 and T4=8,000 at National Dairy Research Institute (NDRI), Karnal India. Animals were arranged in a randomized block design with 120-day experimental period. Blood for haematological parameters and plasma mineral concentration was taken at monthly interval. Plasma Fe, Zn, Mn, Cu concentration was not affected significantly (P>0.05) in different groups consuming water of different TDS saline water. The haematological parameters like haemoglobin, red blood cells count and white blood cells count were comparable among calves in treatment groups. It may be concluded from the results that TDS concentration upto 8000 ppm has no effect on plasma Fe, Zn, Mn, Cu concentration and haematological parameters.

Keywords: Haematological parameters, Plasma mineral concentration, Male Murrah calves, Water salinity.

Introduction
Water is the most vital nutrient needed by living beings for sustaining their life and to optimize the milk production, growth rate and reproduction in livestock (Beede, 2009; Beede, 2006) [6, 5], but unfortunately the least studied component (Schlink *et al*., 2010) [28]. Water constitutes about 65-70% of adult live weight of livestock depending upon their age and physiological condition (Lardner *et al*., 2005) [17]. Milk contains about 80-90% of water and almost all the cells and organ require it. Water is used in body for different purposes (Paul *et al*., 2012) [25], including thermoregulation, lubrication, medium for chemical reactions, digestion, absorption, lactation, carrier, support, cushion, mineral balance and help for other nutrients to complete their functions (Hersom and Crawford, 2008) [13]. Total water intake by animals consists of daily drinking water intake (DWI), water through feed and metabolic water produced from the oxidation of organic nutrients ingested (NRC, 2005) [23]. Out of which drinking water is the primary source of meeting daily water requirement which mainly depends upon the environmental temperature, relative humidity, physiological state, dry matter intake, milk yield, body size, breed and disease status (Madar and Davis, 2004; Golher *et al.*, 2014) [19, 10]. Besides the above said factors, quality of drinking water ultimately determines its acceptability by the animals which in turn affects nutrient intake, utilization, animal's health and performance of animals (Makkar and Ankers, 2014; Umar, 2014) [20, 30]. Water quality mainly includes organoleptic properties (odor and taste), physiochemical properties (pH, total dissolved solids, total dissolved oxygen, and hardness), presence of toxic compounds (heavy metals, toxic minerals, organophosphates, and hydrocarbons), presence of excess minerals or compounds (nitrates, sodium, sulfates and iron) and presence of bacteria (NRC 2001) [22]. Among all these total dissolved solids (TDS), mineral compounds like Zn, Fe, S, Cu, Mn, Ca, Mg, sulphates, sulphides and chlorides affects water quality. It has been reported that sulphates make the taste of water objectionable (Lardy *et al*., 2008) [11] and disturbs the physiological parameters (Robinson *et al*., 2012) [27]. Some of the minerals like Cu, Se, and Zn are supposed to store in liver if present in excess in water (Arias and Mader, 2011) [4]. Water rich in sulphate influence on reproduction negatively and lower weight gain (Patterson *et al*., 2004) [24]. Chloride and sulphate the biologically active anions have potential to negatively influence digestion, acid-base/electrolyte balance, and milk production (Beede, 2009) [6]. Therefore,

present study was planned to see the effect of saline water mineral concentration and complete blood count of Murrah calves reared on saline water.

Materials and methods

Location

This experiment was conducted at the Dairy Unit of ICAR-National Dairy Research Institute (NDRI) Karnal, India located at 29º 42º 20º Sec N and 76º 58º 52.5º E at an altitude of 834 feet above the sea level. Duration of experiment was from 15 November 2014 to 15 March 2015.

Experimental animals and diets

Twenty growing male Murrah calves, with average body weight (kg) 220±5, were randomly assigned to five different treatments of water containing total dissolved solids (TDS; ppm) *viz* T_1=500; T_2 =2,000; T_3=4,000; T_4= 6,000 and T_5= 8,000. Animal experimentation was performed in compliance with regulations set by the cattle yard, NDRI and approved by the Institutional Animals Ethics committee. The animals were fed on ration containing chaffed green fodder (Maize, Sorghum), wheat straw and concentrate mixture to meet their nutritional requirements (ICAR, 2013) [14]. Animals were offered water twice a day, at 10:00 am and 4:00 pm. Daily feed refusals were collected at 9:30 am on next day. During the experimental period of 120 days, blood and mineral collection was done at monthly interval. All the calves were treated with Butox(R) spray (Intervet) and Panacur(R) bolus (Intervet), for ecto- and endo-parasites respectively before the start of study.

Water formulation

In order to formulate the water for the different treatments, Saline water was procured from the Central Soil Salinity Research Institute, Karnal farm located at a village Naine in Panipat District (Haryana). The water was brought in mobile water tank and stored in tanks at experimental site. The TDS of collected water was 8000 ppm. It was further diluted with fresh farm water to prepare treatment water of 2000 ppm, 4000 ppm, 6000 ppm and 8000 ppm. Water offered in different treatment groups was tested for TDS daily by digital TDS meter.

Experimental measures and sample analysis

Feed analysis: Feed samples of maize fodder, sorghum fodder, concentrate mixture and wheat straw were analyzed for their proximate principles as per standard procedures of Association of Official Analytical Chemists (AOAC, 2005) [3]. Cell wall constituent's viz. neutral detergent fibre (NDF) and acid detergent fibre (ADF) were estimated as per methods described by (Van Soest *et al*., 1991) [31].

Collection and processing of blood

Blood (10 ml/calves) was drawn in sterile K2E (EDTA) coated BD vaccutainer (Greiner bio-one) tube from jugular vein puncture after sterilizing the area to be punctured, posing minimum disturbance to the animal during collection. The blood from each calf was collected at day 0,30,60,90 and 120. Immediately after collection the tubes were transported to the laboratory in ice for further processing. Half of the blood was centrifuged at 3500 rpm for 10 min at 4^0C in a centrifuge and plasma was separated and was stored at -20ºC until further analysis and the remaining whole blood was utilized for cell count by auto analyzer.

Plasma mineral estimation

The modified method of closed system of acid digestion of samples (EPA, 2001) was adopted. Plasma minerals were estimated with the help of Atomic absorption Spectrophotometer (Model Z-5000, Polarized Zeeman Atomic absorption Spectrophotometer, Hitachi High-Technologies Corporation, Tokyo, Japan). The procedure described in AAS (1988) [1] manual for preparation of stock and standard solutions and choice of instrumental conditions were followed. Concentration of minerals was expressed as ppm in samples depending upon the amount of a particular mineral present.

Statistical analysis

The data obtained during this study were presented as mean ± standard error and subjected to two way analysis of variance (ANOVA) using software package SPSS version 16.0, 2010. The post-hoc comparision of means was done for the significant difference ($P < 0.05$) by Tukey's b test.

Results and discussion

The chemical composition of feedstuffs is presented in Table 1.

Table 1: Chemical composition of feeds and fodders (% DM basis)

Attribute	Maize fodder	Sorghum fodder	Oat fodder	Concentrate mixture	Wheat Straw
DM	18.58	28.78	16.24	90.17	92.19
OM	89.71	89.90	90.4	91.54	88.55
Total Ash	10.29	10.10	9.60	8.46	11.45
Ether Extract	1.73	1.60	2.70	4.73	1.56
Crude Protein	7.88	9.80	13.60	21.34	3.27
NDICP	4.59	4.40	6.56	2.46	1.27
ADICP	1.75	1.97	1.51	0.89	0.75
NDF	54.54	68.49	31.11	23.89	72.77
ADF	31.72	44.24	27.54	14.56	48.95
Hemicellulose	27.82	24.25	24.14	11.33	23.82
Cellulose	32.26	39.46	25.14	12	41.29
ADL	4.46	4.78	2.40	2.56	7.66
td CP	0.77	1.27	0.88	20.98	0.76
tdNFC	19.75	14.12	23.45	43.25	11.98
td FA	1.73	1.60	2.70	4.73	1.56
td NDF	34.26	36.61	31.11	10.66	37.09
TDN	51.67	48.60	54.51	78.54	46.33

NDICP- Neutral Detergent Insoluble Crude Protein; ADICP- Acid Detergent Insoluble Crude Protein; td CP- truely digestible Crude Protein; td NFC- truely digestible Non Fibrous Carbohydrate; td FA- truely digestible Fatty Acids; td NDF- truely digestible Neutral Detergent Fibre

Mean plasma Fe (ppm) and Zn (ppm) concentration was 1.97±0.27, 2.10±0.16, 1.90±0.16, 2.09±0.41, 2.09±0.41(Table 2) and 2.24±0.14, 2.21±0.15, 2.22±0.15, 2.18±0.23, 2.25±0.15 in C, T1, T2, T3 and T4 respectively (Table 3) at the start of experiment. With the subsequent monthly estimation of Fe (ppm) and Zn (ppm) concentration, no significant ($P>0.05$) difference was observed. The overall average values (ppm) of Fe in C, T1, T2, T3 and T4 were 2.16, 2.15, 2.09, 2.17, 2.17and of Zn were 2.09, 2.16, 2.10, 2.08 and 2.11 respectively. Plasma Fe and Zn concentration did not differ significantly ($P>0.05$) among different groups as well as within the group. There was no effect of saline water on plasma concentration of Fe (ppm) and Zn (ppm) between treatment and days. Plasma Mn and Cu concentration also did not differ significantly ($P>0.05$) among different groups as well as within the group. Mean values of Mn (ppm)

in respective groups were 0.132, 0.134, 0.133, 0.133 and 0.128 (Table 4) and Cu (ppm) was 0.37, 0.41, 0.39, 0.40 and 0.38 (Table 5). These valued showed that there was no change in the values of these minerals. There was no effect on interaction between treatment and days. Normal plasma Cu values reported by (Akhtar *et al.*, 2009) [2] were 0.34 to 2.01 ppm in buffaloes. Ward and Spears (1999) [32] suggest that cattle undergoing stressful periods have increased blood levels of Cu and ceruloplasmin (Cp). Excess of iron can cause toxicity in livestock. Recommended iron level in drinking water is 0.3- 2.4 ppm (Beede, 2009) [6]. High iron level in drinking water cause cellular oxidative stress and block zinc and copper absorption resulting in detrimental effects on healing and performance (Beede, 2009; Genther and Beede, 2013) [6, 13]. Normal plasma Zinc values reported by (Akhtar *et al.*, 2009) [2] were 1.92 to 2.43 ppm in buffaloes. The present findings are in agreement with Halder *et al*., (2009) [12] who reported that serum Zn (0.48 to 0.60 µg/ml) concentration remains unaffected by supplementation of saline water of different TDS. The present findings are also in agreement with Biswas *et al*., (2006) [7] who reported that plasma Mn level were 0.11 to 0.24 µg/ml and remained unaffected by high TDS water in treatment groups. Thus, the feeding of saline water did not have any adverse effect on these trace minerals concentration.

Table 2: Effect of different levels of saline water on plasma Fe (ppm) concentration in Murrah buffalo calves

Day	C	T1	T2	T3	T4
0	1.97±0.27	2.10±0.16	1.90±0.16	2.09±0.41	2.09±0.41
30	2.10±0.07	1.88±0.07	1.98±0.07	1.97±0.16	1.97±0.16
60	2.21±0.10	2.22±0.12	2.12±0.12	2.24±0.12	2.24±0.12
90	2.30±0.28	2.31±0.19	2.21±0.19	2.32±0.23	2.32±0.23
120	2.26±0.19	2.28±0.23	2.28±0.23	2.26±0.16	2.26±0.16
Avg	2.16±0.02	2.15±0.02	2.09±0.01	2.17±0.01	2.17±0.01

Table 3: Effect of different levels of saline water on plasma Zn (ppm) concentration in Murrah buffalo calves

Day	C	T1	T2	T3	T4
0	2.24±0.14	2.21±0.15	2.22±0.15	2.18±0.23	2.25±0.15
30	2.18±0.20	2.28±0.13	2.17±0.29	2.10±0.21	2.16±0.29
60	2.12±0.08	2.13±0.09	2.02±0.06	2.16±0.16	2.11±0.06
90	2.09±0.03	1.97±0.15	1.97±0.15	2.09±0.09	1.96±0.09
120	2.20±0.04	1.91±0.10	2.02±0.09	2.02±0.04	2.01±0.12
Avg	2.16±0.02	2.10±0.03	2.08±0.01	2.11±0.02	2.09±0.03

Table 4: Effect of different levels of saline water on plasma Mn (ppm) concentration in Murrah buffalo calves

Day	C	T1	T2	T3	T4
0	0.127±0.02	0.131±0.03	0.133±0.02	0.131±0.02	0.127±0.02
30	0.125±0.02	0.133±0.02	0.132±0.02	0.134±0.02	0.130±0.02
60	0.132±0.04	0.128±0.02	0.133±0.02	0.133±0.02	0.128±0.02
90	0.134±0.02	0.129±0.02	0.134±0.02	0.136±0.02	0.127±0.02
120	0.136±0.01	0.132±0.01	0.135±0.01	0.132±0.01	0.131±0.02
Avg	0.132±0.02	0.134±0.02	0.133±0.03	0.133±0.02	0.128±0.02

Table 5: Effect of different levels of saline water on plasma Cu (ppm) concentration in Murrah buffalo calves

Day	C	T1	T2	T3	T4
0	0.36±0.01	0.42±0.02	0.39±0.01	0.44±0.01	0.38±0.02
30	0.37±0.02	0.41±0.03	0.40±0.01	0.37±0.02	0.34±0.01
60	0.38±0.02	0.40±0.02	0.41±0.04	0.39±0.02	0.42±0.03
90	0.35±0.02	0.41±0.01	0.42±0.02	0.40±0.02	0.39±0.02
120	0.41±0.02	0.42±0.02	0.37±0.01	0.43±0.03	0.41±0.02
Avg	0.37± 0.02	0.41±0.03	0.39±0.01	0.40±0.02	0.38±.03

Table 6, 7 and 8 shows the effect of different levels of saline water on hematological parameters i.e. Hb, RBC and WBC respectively. There was no significant (P>0.05) effect observed in the mean concentration Hb, RBC and WBC on introducing different TDS water in Murrah buffalo calves with mean Hb concentration (%) of 10.03, 9.95, 9.82, 9.71 and 9.52 (Table 6); RBC concentration (10^6/µL**)** 5.36, 5.33, 5.34, 5.29 and 4.79 (Table 7) and WBC (10^6/µL**)** were 18.85, 17.40, 18.70, 18.43 and 18.70 (Table 7) in C, T1, T2, T3 and T4 groups, respectively. No significant (P>0.05) difference was observed in haemoglobin level, RBC and WBC between the periods as well as between the groups. This showed that animals were able to maintain themselves in normal physiological state and no inflammatory response was there inside the body of the animal. Haematological parameters are indicators of erythrocytic normalcy and general well-being of the farm animals (Radositits *et al*., 2007) [26]. The mean haemoglobin level of the experimental Murrah calves was within the normal range (8–12 g/dl) as per Kaneko *et al*. (1997) [15]. Kattnig *et al* (1992) [16] reported a numerical decrease in Hb level and RBC concentration by feeding high saline water. At the same time there was a rise in WBC level but that was not significant (P>0.05) in high saline water fed steers than regular low TDS fed steers (P>0.10). Results showed that animals were able to maintain its normal physiological state and no inflammatory response was there inside the body of the animal by the salinity of water. Thus, the lack of treatment effect on total leucocyte count and erythrocyte count suggests no inflammatory effect of saline water in Murrach calves. The studies conducted by Shaker (2014) [29] on sheep fed salt tolerant fodder, observed no significant (P>0.05) effect on haematological parameters. Contrary to this, Moustafa *et al*. (2004) [21] found that there was increase in the total leucocyte count in the blood of experimental rabbits showing significant (P<0.05) with drinking natural saline well water.

Table 6: Effect of different levels of saline water on blood Hb in murrah buffalo calves

Day	C	T1	T2	T3	T4
0	10.88±0.40	10.52±1.12	10.26±0.85	10.00±0.47	9.88±0.32
30	9.71±0.48	10.03±0.51	10.28±0.31	9.94±0.19	9.93±0.31
60	9.38±1.18	9.89±0.79	9.46±0.52	9.66±0.09	8.93±0.21
90	10.53±1.74	9.49±0.47	9.7±0.48	9.46±0.12	9.16±0.06
120	9.67±0.65	9.15±0.13	9.43±0.47	9.70±0.31	9.63±0.06
Avg	10.03±0.45	9.95±0.49	9.82±0.33	9.75±0.29	9.52±0.26

Table 7: Effect of different levels of saline water on red blood cells in murrah buffalo calves

Day	C	T1	T2	T3	T4
0	5.54±0.17	5.37±0.07	5.30±0.10	5.36±0.18	5.35±0.17
30	5.23±0.12	5.27±0.21	5.38±0.19	5.35±0.22	5.23±0.16
60	5.30±0.10	5.38±0.26	5.29±0.28	5.33±0.14	5.25±0.10
90	5.37±0.07	5.31±0.14	5.33±0.16	5.25±0.10	5.29±0.06
120	5.36±0.18	5.35±0.10	5.43±0.14	5.19±0.04	5.15±0.03
Avg	5.36±0.06	5.33±0.09	5.34±0.10	5.29±0.10	4.79±0.14

Table 8: Effect of different levels of saline water on white blood cells in murrah buffalo calves

Day	C	T1	T2	T3	T4
0	18.85±0.51	17.4±0.64	18.70±1.14	18.43±0.96	18.7±0.88
30	18.23±0.29	18.26±0.48	17.70±0.65	19.31±1.00	18.45±0.09
60	17.53±0.42	19.4±1.05	18.13±0.93	17.58±1.26	17.20±0.50
90	17.33±2.18	18.1± 1.41	17.28±1.23	18.20±1.77	18.28±0.83
120	18.70±1.16	18.17±1.22	18.78±1.34	18.33±2.16	19.63±0.66
Avg	18.85±0.51	17.4±0.64	18.70±1.14	18.43±0.96	18.7±0.88

Conclusions

It may be concluded that no doubt the water salinity in water over acceptance level causes number of problems including toxicity, electrolyte balance, acid/base balance and interfere with many physiological parameters and in other ways by reducing palatability and acceptance by animal but TDS concentration upto 8000 ppm has no adverse effect on plasma Fe, Zn, Mn, Cu concentration and haematological parameters.

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