



Effect of treated waste water intake on physiological, growth and hematological parameters in Karan-Fries, Tharparkar and Murrah female calves

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ABSTRACT

The present study was conducted to study the effect of treated effluent waste water over fresh water supply served for drinking purpose to calves on dry matter intake, water intake, growth, physiological and hematological parameters. For the study, 6-8 months old female calves (Karan-Fries (n=12), Tharparkar (n=12) and Murrah (n=12)) were selected from Livestock Research Center of ICAR-National Dairy Research Institute, Karnal. These experimental calves of each breed were distributed equally into two groups. The control group was offered fresh water and treatment group was offered effluent treated waste water for drinking purpose. The results of present study revealed that physio-chemical and microbial properties of water that were in accordance with the different drinking water standards such as Dairy NRC (2001), APHA EPA (2002) and BIS (1991). Karan-Fries, Tharparkar and Murrah experimental calves showed no significant difference in the water and dry matter intake when compared between treatment and control group calves. Non-significant differences were observed between control and treated groups of calves of all the three breeds with respect to physiological parameters (rectal temperature, respiration rate and heart rate) and hematological parameters (TEC, TLC, DLC, Hb and PCV). The observations of this study revealed that treated waste water did not show any deleterious effect on the physiological and health status of the calves. Therefore, treated effluent water can be recommended as drinking water to the animals in the water scarce areas.

Keywords: Female calves, Livestock, Treated effluent water, Water intake

Climate change is an emerging issue which is likely to influence the demand of water, its availability and quality. The rise in the temperature may affect the quality, quantity, distribution of rainfall, melting of glaciers, levels of rivers and groundwater (IPCC 2007). Due to this high rise in temperature, there is an increased evapo-transpiration and reduction in runoff which will be the principal causes of intrusion and contamination to water resources, and also the increased abstraction of ground water can lead to salinity intrusion making water unsuitable for the purpose of agriculture, livestock and mankind.

Quality of water is considered as an important factor which affects the intake and water nutrition of livestock (Schlink *et al.* 2010). It is essential for increase in growth rate and maintaining resistance against diseases (Bagley *et al.* 1997). Previous parasitic infestation studies on livestock by using treated effluent water intake reported that in calves (both male and female), no significant change was observed (Rautela *et al.* 2020).

The National Commission on Integrated Water Resources Development had suggested that the direct

watering requirement of livestock is about 5 billion cubic meters a year based on a norm of 18-30 litres per capita per day (Verma and Phansalkar 2005).

Drinking water is an important requirement for livestock and the lack of a sufficient source of water can become critically limiting factor in animal physiology and productivity of an animal (Alamer 2010). Metabolic water fails to fulfill the requirement and hence is an insignificant source as compared with the water taken freely or through feed and fodder (NRC 2001). Ruminants consume water in large quantities which in future can increase stress over the water resources. A lactating cow can drink up to 95 litres of water in a day. The water requirement per unit of body mass of a high-producing dairy cow is greater than that of any other land-based mammal (Woodford *et al.* 1985), this is because of the high yield of milk which contain nearly 87% water.

Along with drinking, livestock needs water for fodders and service purpose. Water is consumed in considerably larger quantities as compared to other nutrients so quantity and quality of water is important for animal. They meet this requirement via three sources viz. drinking or free water intake (FWI), ingestion of water contained in feed, and water produced by the body's metabolism of nutrients (Umar *et al.* 2014).

Keeping in view the above facts, recycling and

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reutilization of waste water may be the best available option if its use may not have any adverse effect on livestock as a whole.

MATERIALS AND METHODS

The present study was conducted on Karan-Fries, Tharparkar breeds of cows and Murrah breed of buffalo to study the effect of treated effluent water intake on their physiological status and hematological parameters.

Experimental location: The study was conducted in the Livestock Research Centre (LRC) at ICAR-NDRI, Karnal, Haryana. It lies at an altitude of 240 meters above the mean sea level and at 29°42'3" N latitude and 76°59'6" E longitude. The maximum recorded temperature goes beyond 45°C in summers while minimum recorded temperature is 2°C in winters. The average rainfall in the city is about 766 mm.

Experimental animals: The study was conducted on 6–8 months old healthy female calves of Karan Fries (KF), Tharparkar (TP) breed of cattle and Murrah (MU) breed of buffalo for a period of 75 days. The experimental animals (n=36) from each breed (n=12) were distributed in two groups i.e. control and treatment groups comprising of 10 animals in each experimental group. The control group animals received fresh underground tube well water supplied by tap in experimental shed *ad lib*. Whereas, treatment group animals received treated effluent waste water *ad lib*. The animals of both the groups were kept under routine management practices followed at the institute's LRC and were fed as per the standard feeding practices (NRC 2001). Concentrated mixture consisted of 20% CP, 75% TDN, 2% mineral mixture and 1% common salt, and green fodder mixture of barseem (*Trifolium alexandrinum*), maize (*Zea mays*), sorghum (*Sorghum bicolor*) and wheat (*Triticum aestivum*) straw.

Collection of blood samples: Blood samples (9 ml/animal) were collected at weekly intervals in sterile heparinized vacutainer (BD Vacutainer TM, UK) tubes by puncturing the jugular vein, imposing minimum disturbance to the animal. Immediately after collection, the vials were transported to the laboratory in ice box for further analysis and processing.

Preparation of treated water from the effluent treatment plant waste water: The waste water from institute's Effluent Treatment Plant (ETP) was collected in 1000 liters' capacity container and 1000 g (1 kg) powdered aluminium sulfate (industrial alum) was mixed thoroughly and kept for 2 h for complete coagulation and settlement. After this coagulation, 500 g powdered activated charcoal (PAC)/dung cake fresh ash was added and mixed thoroughly. This mixed water was allowed to stand 8-10 h in the same container and clear water was decanted in another container with simple muslin cloth filter and 1% sodium hypochlorite solution (250 ml) was thoroughly mixed in water before use to make it free from biological infections. The obtained purified water was analyzed for different quality parameters for use in livestock drinking purpose.

All the parameters were within the range of different national and international drinking water standards like IS: 10500, 2012; APHA and EPA standards). The underground tube well water available at Livestock Research Center (LRC), ICAR-NDRI, was used for control group animals. The underground water was also analyzed for its physical, chemical and microbiological parameters.

Physiochemical parameters of treated water: Colour, taste, odour was checked organoleptically. Turbidity, pH, total dissolved solids (TDS), alkalinity, electrical conductivity (EC), oxidative reduction potential, dissolved oxygen were studied by microprocessor soil and water analysis kit. BOD was tested by using Winkler's method, while COD was measured using a strong oxidant. Among minerals, sodium and potassium was estimated using Systronic flame photometer Type:128. Calcium and magnesium in water samples was estimated using Atomic Absorption Spectrophotometer (ZEEnit 700 P). Other minerals including macro- and micro-minerals were estimated by using multitype ICP Emission Spectrophotometer (ICPE-9000).

Microbiological parameters: Total coliform count was done by the most probable number (MPN) method and total bacterial count was done by total viable count method (TVC).

Daily water intake (litre/day/animal): Water was offered *ad lib*. three times a day at 8.30 AM, 1.00 PM and 5.30 PM using marked bucket to the animals and the leftover water was measured to determine the water intake by calculating the difference between the initial amount of water and leftover amount of water.

Daily fodder/feed intake (kg/day/animal): Weighed *ad lib*. fodder/feed was offered to animals and on the consecutive next day the left over fodder was weighed. Total intake of fodder was calculated by simple difference method.

Dry matter (DM) of feed: Weigh 50-100 g quantity of sample in a dry aluminium moisture cups or aluminium trays (for fodders). The cups/trays were placed in hot air oven and dried at 100±5°C for 24 h. The loss in moisture content after drying was estimated and the dry matter was calculated as follows:

$$DM (\%) = \frac{(\text{Wt. of moisture cup} + \text{Sample after drying} - \text{Wt. of moisture cup}) \times 100}{\text{Wt. of fresh sample}}$$

Body weight in growing animals: Body weight (kg) was measured by using weighing balance during morning hours.

Measurement of physiological responses: All the physiological parameters were recorded in the morning time nearly between 8.30 to 10.30 AM at weekly interval for 75 days. In morning hours, the environmental variables are in pleasant conditions.

Rectal temperature (RT): Rectal temperature (°C) was recorded with a digital thermometer by keeping the thermometer in contact with rectal mucosa for 2 min.

Respiration rate (RR): Respiration rate of the experimental animals was recorded by flank movement method. One complete outward and inward movement of flank was counted as one respiration. Respiration rate was counted for 2-3 min and expressed as breaths per min.

Pulse rate (PR): Pulse rate (PR) of the animals was counted by palpating the pulsation of middle coccygeal artery at the base of the tail. The pulse rate was counted for 2-3 min and expressed as pulse rate per min.

Hematological parameters: Whole blood samples were collected from the jugular vein of the animals in EDTA vacutainer tubes and then immediately placed in the icepacks and brought to the laboratory. The parameters were estimated within an hour of blood collection. Hemoglobin (g/dl) was measured by using Sahli's hemoglobin meter, total erythrocyte Count (TEC) ($\times 10^6/\text{ml}$) and total leukocyte count (TLC) ($\times 10^3/\text{ml}$) was done using Neubauer's (Haemocytometer) method, packed cell volume (%) was measured by micro-hematocrit method and differential leukocyte count (DLC) was performed using direct slide method and stained using Leishman stain. A total of 100 cells were counted and different types of leukocyte were expressed in percentage.

RESULTS AND DISCUSSION

For both control and treated water groups, the different physio-chemical properties of water such as temperature, salinity, dissolved oxygen, pH, total dissolved solids, oxidative reduction potential and turbidity were recorded. All the parameters were within the permissible limits of different drinking water standards of Dairy NRC (2001).

The results of average water intake, dry matter intake, physiological responses and growth of female calves served with control and treated water are presented in Table 1. Non-significant differences were observed between control and

treated groups for all the parameters. Dry matter intake was highest in buffalo calves with no difference among both the groups. Maximum water intake (ltr./d./animal) was recorded in afternoon hours in all the breeds in both the groups. The KF calves exhibited maximum water intake during noon hours and minimum in evening hours, followed by buffalo calves and Tharparkar cows. The pulse rate (pulse/min) of Karan Fries and Tharparkar female calves for control and treatment group were recorded.

KF calves recorded the highest pulse rate, followed by buffalo calves and then by Tharparkar calves. Respiration rate (breaths/min) and rectal temperature ($^{\circ}\text{C}$) also varied non-significantly between the three groups. The final body weight (Kg) was found to be higher in all the breeds of calves as compared to the initial weight taken.

The decreased total water intake by treatment group is in agreement with the studies conducted by Singh *et al.* (2014) and Debbarma (2019) in Murrah calves during summer season. Sharma *et al.* (2017) studied water intake with different TDS levels and found that with increase in TDS level in water, the water intake decreases. At 557 TDS, water intake was 7.1 ± 1.3 litre per 100 kg body weight.

Physiological responses are the immediate response by animal to any change in normal condition. Animals being homeotherms try to maintain their body condition by changing their physiological parameters. The rectal temperature rises with the increase in ambient temperature. A slight increase in rectal temperature was observed in all the three breeds but the increase was non-significant in both the groups. The rectal temperature in treatment group in Murrah calves, was in agreement with the findings of Haque *et al.* (2013) in growing and adult buffaloes. RT at 22°C were 38.5°C and 37.5°C in young and adult Murrah buffaloes, which increased when temperature increased to 40°C . The values obtained in the present study are almost

Table 1. Mean $\pm$ SEM values of dry matter, water intake, physiological responses, growth of Karan-Fries, Tharparkar and Murrah female calves

Parameter	KF calves		TP calves		Murrah calves	
	Control	Treatment	Control	Treatment	Control	Treatment
	Mean	Mean	Mean	Mean	Mean	Mean
DMI (Kg/animal/day)	1.96 $\pm$ 0.04	1.91 $\pm$ 0.09	2.28 $\pm$ 0.03	2.29 $\pm$ 0.06	2.76 $\pm$ 0.07	2.76 $\pm$ 0.07
Water intake (litre/animal/day)						
Morning (WIM)	3.36 $\pm$ 0.22	2.66 $\pm$ 0.13	0.92 $\pm$ 0.04	1.16 $\pm$ 0.08	2.05 $\pm$ 0.11	1.89 $\pm$ 0.88
Noon (WIN)	4.59 $\pm$ 0.48	4.22 $\pm$ 0.14	2.68 $\pm$ 0.07	2.67 $\pm$ 0.68	2.30 $\pm$ 0.11	2.04 $\pm$ 0.10
Evening (WIE)	1.78 $\pm$ 0.08	1.91 $\pm$ 0.09	1.41 $\pm$ 0.06	1.92 $\pm$ 0.06	2.05 $\pm$ 0.11	1.89 $\pm$ 0.88
Physiological responses						
PR (pulse/min)	88.67 $\pm$ 3.52	86.00 $\pm$ 2.10	69.91 $\pm$ 2.80	68.89 $\pm$ 1.85	70.25 $\pm$ 0.41	71.20 $\pm$ 0.35
RR (breaths/min)	29.35 $\pm$ 1.34	27.30 $\pm$ 1.36	21.20 $\pm$ 0.40	21.25 $\pm$ 0.39	20.84 $\pm$ 0.75	21.25 $\pm$ 0.53
RT ($^{\circ}\text{C}$)	38.36 $\pm$ 0.19	38.65 $\pm$ 0.15	38.51 $\pm$ 0.11	38.70 $\pm$ 0.13	37.22 $\pm$ 0.05	37.25 $\pm$ 0.07
Body weight (Kg)						
Initial	93.53 $\pm$ 8.98	96.38 $\pm$ 10.85	64.21 $\pm$ 13.14	75.47 $\pm$ 8.56	110.40 $\pm$ 11.51	106.82 $\pm$ 11.51
Final	108.91 $\pm$ 10.51	112.25 $\pm$ 9.71	77.76 $\pm$ 13.28	89.05 $\pm$ 8.54	124.25 $\pm$ 11.27	120.53 $\pm$ 11.73

Dry matter (DM), Water intake (WI), Pulse rate (PR), Rectal temperature (RT), Body weight (BW), Total erythrocyte count (TEC), Total leukocyte count (TLC), Packed cell volume (PCV), Erythrocyte sedimentation rate (ESR). *No significant differences were observed between control and treatment groups ($P < 0.05$).

consistent with this observation that clearly indicates that RT of the experimental animals was in normal range. Similar findings were also observed by Vaidya *et al.* (2012).

The first and foremost visible sign when animals are exposed to ambient temperature above the thermo neutrality zone causes an increment in respiration rate. It is more in summer as compared to winter which may be due to the more demand of oxygen by the tissues, enabling dissipation of excess body heat through evaporative cooling. RR increases with increase in temperature or with stress. Young and growing animals have a more respiration rate than adults. Under similar kind of environmental conditions, in forenoon, in control group and in experimental group using fan cum mist system, the values of RR are in agreement with the observations recorded by Singh *et al.* (2014) in Murrah heifers of 18-24 months age during summer. Similarly, in Murrah buffaloes under high pressure fogger system, average counts were reported by Ambulkar *et al.* (2011). Aggarwal and Singh (2008) in lactating Murrah buffaloes, reported high RR in morning time in showering group, which are in accordance with the results of present study.

Rise in pulse rate is also considered as stress indicator. The observations recorded in treatment group animals were in agreement with the records reported by Aggarwal and Singh (2008). Joshi *et al.* (1982) reported that pulse rate increased moderately during exposure to hot environment in buffaloes. Moreover, with the values falling in the range as reported by other researchers, the treatment group animals showed no variation in physiological changes from control group animals.

The results of average hematological parameters of all three breeds of calves served with control and treated effluent water are given in Table 2. Non-significant variations were observed between both the groups in all the three breeds. KF calves showed lower values for many of the hematological parameters (TEC, Basophils). Tharparkar calves showed lower values of TLC, lymphocytes, Hb and PCV whereas Murrah calves had lowered neutrophils, monocytes and eosinophils. The differences were non-significant.

Hematological profile can serve as baseline information for evaluating physiology and general health status

of an animal. Under stress or diseased conditions, the physiological, biochemical and behavioural responses change as per the genetic makeup and environment (Mohammed *et al.* 2007).

The values obtained in the treatment group animals, were in accordance with the normal hematological range as mentioned in Merck Manual (2012). The average range observed in treatment group of calves i.e TEC (million/ μ l), TLC (thousand/ μ l), DLC (%), Hemoglobin (g/dl) and PCV (%) of Murrah calves are in agreement with the reports of Khan *et al.* (2016). Similar range is also reported by Haque *et al.* (2013) in young and adult Murrah buffaloes under controlled environment condition. As per the reports when both young and adult Murrah buffaloes were exposed to high ambient temperature i.e 40, 42 and 45°C, the values of TEC, PCV, Hb rose with the temperature in both young and adult animals.

In treatment group of Murrah buffalo calves, the TEC values are in agreement with the findings of Khadjeh and Papahn (2002) in Iranian native buffaloes and Parmar *et al.* (2013). Parmar *et al.* (2013) reported similar values of TLC in treatment group of Murrah calves. However, non significant differences in blood variables in summer and winter were observed. The eosinophil value reported were more than observed in this study.

At high environmental temperature, the PCV value was high which was consistent with the observations of Fagiolo *et al.* (2004) who reported that the PCV was higher at the higher environmental temperature (summer-40.75% vs winter-32.63%). They also reported higher Hb in lactating buffaloes during the summer season than in the winter season. This increase may be due to hemo-concentration of plasma due to increase in temperature or may be due to increase in RBC and Hb concentration. The change or increment in values of hematological variables may be due to ambient temperature. Similar values for hematological parameters were obtained for control group animals in the same environment and no significant change was observed between the values as stated in the results above.

Based on the experimental data, it may be concluded that the intake of purified waste water had no deleterious

Table 2. Mean $\pm$ SEM values of hematological parameters of female calves served with control and treated effluent water

Breed	TEC (million/ μ l)	TLC (thousand/ μ l)	Neutrophil (%)	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)	Basophil (%)	Hb (g/dl)	PCV (%)
<i>Murrah female calves</i>									
Control	8.50 $\pm$ 0.33	13.18 $\pm$ 0.45	25.45 $\pm$ 1.51	66.05 $\pm$ 2.50	4.30 $\pm$ 0.50	2.30 $\pm$ 0.87	0.75 $\pm$ 0.20	11.01 $\pm$ 0.36	33.83 $\pm$ 0.46
Treatment	8.75 $\pm$ 0.21	12.98 $\pm$ 0.85	24.30 $\pm$ 1.90	67.53 $\pm$ 2.23	4.32 $\pm$ 0.68	2.25 $\pm$ 0.83	0.76 $\pm$ 0.23	11.00 $\pm$ 0.50	34.02 $\pm$ 0.50
<i>Karan Fries female calves</i>									
Control	7.01 $\pm$ 0.09	10.89 $\pm$ 0.43	27.24 $\pm$ 1.05	62.52 $\pm$ 3.30	5.50 $\pm$ 0.50	3.20 $\pm$ 0.82	0.36 $\pm$ 0.11	10.30 $\pm$ 0.53	31.52 $\pm$ 0.56
Treatment	7.20 $\pm$ 0.10	11.00 $\pm$ 0.46	26.50 $\pm$ 1.10	63.32 $\pm$ 2.30	5.89 $\pm$ 0.75	3.50 $\pm$ 0.83	0.33 $\pm$ 0.12	10.50 $\pm$ 0.83	31.83 $\pm$ 0.68
<i>Tharparkar female calves</i>									
Control	8.96 $\pm$ 0.59	10.31 $\pm$ 0.87	31.24 $\pm$ 2.32	58.20 $\pm$ 1.11	4.20 $\pm$ 0.30	3.98 $\pm$ 0.23	0.82 $\pm$ 0.10	9.80 $\pm$ 0.56	30.85 $\pm$ 1.56
Treatment	8.95 $\pm$ 0.55	10.42 $\pm$ 0.83	30.98 $\pm$ 2.65	59.22 $\pm$ 1.10	4.50 $\pm$ 0.32	4.01 $\pm$ 0.22	0.81 $\pm$ 0.11	9.95 $\pm$ 0.53	31.01 $\pm$ 1.68

Total erythrocyte count (TEC); Total leukocyte count (TLC); Packed cell volume (PCV); Erythrocyte sedimentation rate (ESR). The values with same superscripts between rows did not differ significantly ($p>0.05$).

effect on growth and physiological status of experimental female calves. Hence, the purified/effluent treated waste water may be used for drinking purpose in female calves.

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