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Effects of Long-term Heat Exposure on Adaptive Mechanism of Blood Acid-base in Buffalo Calves

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ABSTRACT : In order to investigate the mechanism of adaptation to long-term heat stress, six female buffalo calves of about 7 to 8 months age, were exposed to the cool-comfort environment (THI 65) for 21 days to obtain normal values of blood acid-base. An adaptive response of acid-base regulation was determined to long term (21 days) exposure of buffalo calves to hot-dry (THI 80) and hot-humid (THI 84) conditions. Higher rectal temperature and respiratory rate was recorded under hot-humid exposure compared to hot-dry. Significant reduction in the rectal temperature and respiratory rate on day 21 of hot-dry exposure indicated early thermal adaptation compared to hot-humid. Decreasing rectal temperature and respiratory rate from day 1 to 21 was associated with concurrent decrease in blood pH and pCO_2. Increased plasma chloride concentration with low base excess in blood and in extracellular fluid suggested compensatory response to respiratory alkalosis. Reduced fractional excretion of sodium with increased fractional excretion of potassium and urine flow rate indicated renal adaptive response to heat stress. (**Key Words :** Blood Acid-base, Fractional Excretion, Renal Clearance, Long-term Heat Stress, Buffalo Calves)

INTRODUCTION

Mechanism of heat stress adaptation in buffaloes were mostly studied with respect to cutaneous characteristics, respiratory frequency, rectal temperature, sweating and panting abilities as the parameters of heat stress adaptation. The basic concept of neuro-endocrine (Alam and Dobson, 1986; Minton and Blecha, 1990) and renal function shifts (Bianca and Findley, 1962) during heat stress has been studied in cattle and dogs. Chaiyabutr et al. (1983, 1990) studied the effects on water turnover and renal functions in heat stressed buffaloes. The acid-base changes with increased environmental temperature have been studied in cattle calves (Bianca and Findlay, 1962) and in pigs (Taskar, 1980). Characterization of the shifts in blood acid-base and the process of its adaptation by body systems have not been studied extensively in heat stressed buffaloes. Therefore, it is of utmost importance to provide an approach to understand the regulation of blood electrolytes metabolism and clinico-physiological status in buffaloes exposed to heat stress. Considering the dearth in the literature, present study was conducted to understand the mechanism of acid-base regulation during long-term heat stress in buffalo calves.

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MATERIALS AND METHODS

The experiment was performed on six female Murrah type buffalo calves of 7 to 8 months age (65.1 to 76.6 kg body weight) vaccinated and dewormed at specified schedule. They were maintained in the psychrometric chamber, where the climate can be varied as desired over a wide range and maintained constant within narrow limits. The size of the chamber was 7.5×7.5 meters, equipped with individual tie stall, feeders and waterers. Photoperiod schedule of L14:D10 was maintained at a constant during entire trial. Animals were fed on wheat straw and concentrate mixture (Pathak and Verma, 1993). They were acclimatized to climatic chamber for 10 days and trained to stand in animal chute. During this period they were also acclimatized for 2 to 3 h to retain Foley's catheter for renal function studies. Experiment was conducted in three parts. First part included exposure of animals to cool comfort environment at 23 to 25°C air temperature (AT) and 50 to 55% relative humidity (RH) and temperature-humidity index (THI) 65 for 21 days. In the second part, animals were exposed to hot-dry (40 to 42°C AT and 35 to 40% RH,

Table 1. Changes in rectal temperature, respiratory and pulse rate during cool-comfort and heat stress conditions

Variables	Day	1		7		14		21		Significance
	Cool-comfort	Hot-dry	Hot-humid	Hot-dry	Hot-humid	Hot-dry	Hot-humid	Hot-dry	Hot-humid	
Rectal temperature (°C)	37.90 ±0.372	40.85 ±0.207	41.20 ±0.253	40.58 ±0.194	40.90 ±0.268	40.48 ±0.099	40.78 ±0.52	40.20 ±0.21	40.68 ±0.24	p<0.001
Respiratory rate/min	19.20 ±2.48	171.8 ±11.01	195.5 ±9.31	168.8 ±8.08	181.8 ±7.57	167.7 ±12.37	166.8 ±8.03	138.0 ±3.74	150.3 ±9.73	p<0.001
Pulse rate/min	53.00 ±5.40	102.9 ±6.68	110.0 ±10.28	95.70 ±2.95	98.60 ±6.91	92.10 ±5.08	86.40 ±9.28	82.83 ±2.92	77.90 ±10.14	p<0.001

THI 80) and in third, to hot-humid (40 to 42°C AT and 55 to 60% RH, THI 84) environment for 21 days each. THI was calculated using formula reported by Kelly and Bond (1971). Rectal temperature was recorded at regular interval and respiratory rate and pulse rate were recorded at the peak of the rectal temperature on day 1, 7, 14 and 21. Blood samples were collected at highest rectal temperature on respective days for blood gas analysis. About 1 h before the experiment, a polyethylene catheter (i.d. 1.0 mm; o.d. 1.5 mm) was inserted into jugular vein to facilitate both infusion and blood sampling. Patency was maintained by irrigating the catheter with 1-2 ml of heparinized (50-100 IU) sterile saline solution (0.9%). For blood gas analysis, blood samples (1 ml) were collected into heparinized tuberculin syringe, capped, placed on ice and were analyzed within 5 minutes. Urine was collected using two way Foley's catheter (14 to 16 size) simultaneously with collection of blood and analyzed for creatinine (Cr), para-amino hippuric acid (PAH), Na^-, K^+, Cl^- and inorganic phosphorus and stored at -20°C until analysis. Glomerular filtration rate (GFR) was determined by clearance of Cr (Sigma) as described by Rastogi (1998) and concentration of Cr in plasma and urine was analyzed using alkaline picrate (Jaffe kinetic) method described by Faulkner and King (1970). Plasma creatinine levels were compared with the results reported by Sikka et al. (1994). Renal plasma flow (RPF) was determined by PAH clearance and concentration of PAH in plasma and urine was analyzed by Bratton-Marshal reaction described by Richterich (1969) with partial modification described by Rastogi (1998). Renal clearances, filtration fraction (FF) and fractional excretion (FE) was calculated using standard clearance formula (Swenson and Reece, 1993). Analysis of whole blood pH, blood gas, hemoglobin and PCV were analyzed using stat profile PHOX reagent pack A cata kit employed on ABG analyzer (Nova Biomedicals, Waltham, USA). All values were corrected for the rectal temperature. Concentration of Na^+ and K^+ analyzed using flame photometer (Modiflame 127, Systronics, Naroda, Ahmedabad) as described by Oser (1965) and chloride by ferric thiocynate method. Inorganic phosphorus was determined using trichloroacetic acid and molybdate followed by reduction with methyl-p-aminophenol sulphate. Plasma protein was analyzed by the method described by Lowry et al. (1951). Plasma volume was measured by dilution of Evan's blue (T-1824) dye (Sigma). As described by Gregersen and Rawson (1959). The total blood volume was calculated as (100×PV)/(100-H) as described by Anderson et al. (1969). Total body water was determined using antipyrine (Sigma) and concentration in the plasma was determined by the precipitation method described by Soberman, et al. (1949).

The data obtained from different exposures to environmental conditions was subjected to analysis of variance as per the method described in Snedecor and Cochran (1994). For multiple comparisons, the Turkey's HSD method based upon the standardized-range statistics was used. Nonlinear regression analysis for concentration versus time data for antipyrine, evan's blue dye, Cr and PAH in each animal was performed using a computer program (STATIS 3) at one compartment open model.

RESULTS AND DISCUSSION

An adaptive response to thermal stress was determined by recording rectal temperature (RT), respiratory rate (RR) and pulse rate (PR) and presented in Table 1. RT decreased significantly ($p<0.05$) by 2.7% and 1.6% on day 21 compared to day 1 of hot-dry and hot-humid exposures, respectively. Higher RT during hot-humid exposure indicates insufficient evaporative heat loss from body. Guyton (1986) suggested that the rise in body temperature is relatively more at high ambient temperature with high humidity. The RR decreased significantly ($p<0.001$) by 33.72% and 31.3% on day 21 compared to day 1 of hot-dry and hot-humid exposures, respectively. This result suggests that the change in RR is directly proportional to RT. More reduction in RR on day 21 of hot-dry exposure indicates early adjustment of respiratory functions, compared to hot-humid exposure. PR also decreased significantly ($p<0.001$) by 21% and 31% on day 21 compared to day 1 of hot-dry and hot-humid exposures, respectively. More reduction in the PR from day 14 to 21 was surprising during hot-humid exposure. Guyton (2001) reported that the cardiac output/pulse rate increases due to high blood volume, however this mechanism lasts for few minutes or days

Table 2. Changes in blood gas and acid-base observed during cool-comfort and heat stress conditions

Variables	Day	1		7		14		21		Significance
	Cool-comfort	Hot-dry	Hot-humid	Hot-dry	Hot-humid	Hot-dry	Hot-humid	Hot-dry	Hot-humid	
Blood pH	7.368	7.401	7.388	7.38	7.379	7.400	7.396	7.388	7.385	p<0.05
	±0.01	±0.013	±0.016	±0.01	±0.018	±0.011	±0.014	±0.016	±0.016	
pCO_2 (mmHg)	53.38	44.6	46.4	44.8	47.6	47.2	47.8	48.6	50.4	p<0.05
	±2.76	±1.15	±1.46	±2.6	±1.1	±1.96	±1.45	±1.56	±1.96	
TCO_2 (mmol/L)	33.1	30.28	29.29	33.73	31.31	32.2	31.8	34.7	32.33	Non
	±1.73	±1.21	±1.24	±2.36	±2.43	±1.33	±1.75	±1.09	±2.76	significant
HCO_3 (mmol/L)	32.85	28.3	27.7	30.7	29.05	30.1	28.8	31.3	29.2	p<0.01
	±3.09	±3.13	±2.18	±2.76	±2.33	±2.67	±1.97	±2.34	±3.79	
SBC (mmol/L)	29.41	22.6	21.9	26.6	24.41	25.53	24.33	26.25	25.18	p<0.05
	±2.84	±3.05	±1.19	±2.51	±1.93	±2.12	±1.19	±3.69	±2.31	
Base excess	8.76	5.68	6.16	2.48	2.68	5.85	1.86	4.78	2.26	p<0.01
in ECF (mmol/L)	±0.91	±2.24	±2.5	±1.98	±1.00	±2.48	±1.02	±1.4	±0.776	
BE in blood	7.35	5.61	6.4	2.5	2.86	3.51	0.65	3.48	1.25	p<0.01
(mmol/L)	±1.52	±2.62	±2.29	±1.67	±1.81	±1.14	±0.561	±1.12	±0.485	

because several compensatory mechanisms comes into play causing (i) transudation of fluid out of capillaries into the tissues, (ii) distention of veins called stress-relaxation, thus reducing mean systemic pressure and (iii) atuoregulatory increase in peripheral resistance, thus increasing resistance to venous return. These factors cause mean systemic filling pressure to return back towards normal. Therefore, gradually over a period of time cardiac output returns almost to normal. However, reports are lacking on the changes in blood volume and cardiac output during long-term studies in buffaloes.

The mean±SE values of blood gas analysis were well within the range as reported by Sukhija et al. (1978), Nangia et al. (1980) and Singh et al. (1991), and presented in Table 2. On day 1, increased blood pH might attributed to decreased carbonic acid created by CO_2 hyperventilation (Benjamin, 1981). With decreasing RT and RR from day 7 to 21, pH also decreased significantly ($p<0.05$) and pCO_2 increased significantly ($p<0.05$) suggesting the pH directly and pCO_2 inversely proportional to the respiratory rate. The blood total carbon dioxide content (TCO_2) increased by 10.4-14.6% during both exposures and contradicts with the results reported by Singh et al. (1991) in buffaloes. However, the results are in agreement with the findings reported by Bianca and Findley (1962) in cow calves. Increased TCO_2 may be due to significant ($p<0.05$) increase in standard bicarbonate (SBC) and actual plasma bicarbonate (HCO_3) concentration, since more than 95% of CO_2 is contributed by bicarbonate. Increased SBC and HCO_3 indicated adaptive response to bring pH to normal. No significant differences between hot-dry and hot-humid exposure was observed with respect to TCO_2, SBC, HCO_3. Base excess in extracellular fluid (BEecf) and base excess in blood (BEb) during both exposures (Table 2) were significantly ($p<0.01$) decreased compared to day 1, indicating that chronic heat exposure reduces the buffer base in body fluids. The percent decrease in buffer base was higher in hot-humid exposure compared to hot-dry indicating hot-humid exposure might cause more stress to the buffalo calves. The probable cause of reduction in BEecf and BEb may be due to significantly ($p<0.05$) low oxygen tension (pO_2) (Table 5) which might increase anaerobic glycolysis to cause over production of lactic acid, also from respiratory muscle activity (Bianca and Findlay, 1962). To counteract such fall there must be gain in extracellular bicarbonate and loss of hydrogen ions to environment (Saxton and Seldin, 1986). In the present investigation the significant increase in SBC, HCO_3 and urine flow rate (Table 4) have been observed during both heat exposures. Decreased oxygen saturation (Table 5) may be due to decreased blood pH as described in Bohr's effect. Siggaard-Anderson (1971) reported decreased blood pH associated with low pO_2. Packed cell volume (PCV) during both heat exposures was significantly ($p<0.01$) decreased by 6.23 and 13.73%, respectively compared to day 1. The fall in the PCV was significantly ($p<0.01$) higher in hot-humid exposure compared to hot-dry and may attributed to increased plasma volume and total body water (Table 5). The Hb levels on day 14 of both heat exposures were nonsignificantly decreased by 3.53% and 8.18%, respectively compared to day 1 and later remained unaltered (Table 5). The fall in O_2 capacity may also be due to the fall in Hb concentration.

The GFR and RPF values (Table 3) observed during present investigation were comparable to the values reported by Chaiyabutr et al. (1990) and Rastogi et al. (2003), whereas FF values were little low. Increased GFR and RPF concomitant with decreased FF was also reported by Chaiyabutr et al. (1990) in buffaloes and attributed to decreased resistance of afferent and efferent arterioles

Table 3. Changes in renal clearance observed during cool comfort and heat stress conditions

Variables	Day	1		7		14		21		Significance
	Cool-comfort	Hot-dry	Hot-humid	Hot-dry	Hot-humid	Hot-dry	Hot-humid	Hot-dry	Hot-humid	
GFR	39.32	41.12	42.28	43.67	42.57	45.46	44.91	43.8	43.96	Non
(ml/min)	±9.17	±4.86	±5.04	±4.43	±5.04	±4.05	±4.76	±3.59	±3.35	significant
Renal plasma	314.81	325.55	339.92	351.91	345.83	351.91	361	356.39	377.34	Non
flow (ml/min)	±65.2	±62.53	±32.07	±58.69	±36.85	±58.69	±14.02	±50.09	±45.53	significant
Filtration	0.132	0.131	0.125	0.128	0.124	0.133	0.125	0.125	0.118	Non
fraction (%)	±0.04	±0.036	±0.018	±0.032	±0.016	±0.034	±0.014	±0.023	±0.015	significant
Fractional excretion	0.932	1.51	1.37	2.953	2.74	1.61	2.41	1.345	1.3	p<0.001
of sodium (%)	±0.072	±0.207	±0.359	±0.303	±0.318	±0.393	±0.26	±0.13	±0.239	
Fractional excretion	57.44	69.01	71.2	96.04	90.21	142.1	151.2	126.2	143.3	p<0.001
of potassium (%)	±9.78	±7.42	±19.1	±6.55	±14.35	±5.32	±23.86	±12.01	±13.41	
Fractional excretion	3.69	3.641	3.621	2.146	1.958	2.431	2.083	2.82	2.61	p<0.001
of chloride (%)	±0.463	±0.383	±0.445	±0.305	±0.372	±0.357	±0.325	±0.282	±0.329	
Fractional excretion	0.557	0.504	0.502	0.448	0.420	0.421	0.38	0.400	0.35	p<0.001
of inorganic phosphorus (%)	±0.119	±0.06	±0.044	±0.034	±0.09	±0.03	±0.014	±0.009	±0.011	

Table 4. Plasma and urinary concentration of electrolytes observed during cool-comfort and hot-dry and heat stress conditions

Variables	Day	1		7		14		21		Significance
	Cool-comfort	Hot-dry	Hot-humid	Hot-dry	Hot-humid	Hot-dry	Hot-humid	Hot-dry	Hot-humid	
Plasma sodium	137.91	137.7	137.4	137.5	137.2	137.3	136.5	137.3	136.8	Non
(mmol/L)	±2.83	±3.16	±3.16	±2.31	±2.73	±2.33	±2.78	±2.33	±4.3	significant
Plasma potassium	4.11	4.23	4.21	4.28	4.35	4.26	4.39	4.24	4.26	Non
(mmol/L)	±0.221	±0.36	±0.187	±0.234	±0.234	±0.2	±0.194	±0.248	±0.172	significant
Plasma chloride	102.3	103.5	103.3	104.1	107.7	108.7	111.8	109.0	110.8	p<0.01
(mmol/L)	±1.36	±0.71	±1.83	±1.23	±1.67	±1.15	±1.63	±2.85	±2.64	
Plasma inorganic	4.858	4.9	4.886	4.817	4.831	4.75	4.686	4.71	4.64	Non
phosphorus (mmol/L)	±0.139	±0.167	±0.15	±0.117	±0.119	±0.122	±0.171	±0.126	±0.110	significant
Urine sodium	50.9	56.83	59.15	75.6	70.81	67.64	62.10	60.10	53.40	p<0.001
(mmol/L)	±12.7	±13.39	±14.37	±16.39	±16.99	±14.69	±16.66	±9.87	±13.92	
Urine potassium	146.2	149.7	151.2	156.0	164.9	189.5	194.5	197.0	206.0	p<0.001
(mmol/L)	±10.5	±8.56	±8.21	±10.4	±7.54	±8.66	±10.7	±7.76	±13.9	
Urine chloride	149.33	150.54	153.89	125.8	115.4	130.6	114.6	141.1	132.0	p<0.001
(mmol/L)	±41.2	±19.09	±15.5	±10.32	±15.47	±11.44	±22.00	±13.58	±17.72	
Urine inorganic	0.104	0.100	0.099	0.088	0.094	0.095	0.078	0.0085	0.085	p<0.001
phosphorus (mmol/L)	±0.018	±0.014	±0.012	±0.008	±0.003	±0.008	±0.008	±0.007	±0.004	

caused by nor-epinephrine (Alverez and Johnson, 1973) in cattle exposed to prolonged heat exposure. The increased GFR may also be due to increasing concentration of plasma protein (Table 5) that rapidly increases osmotic pressure by Donnen effect.

The FE of sodium was increased on day 7 while on day 14 and 21, it remained unaltered (Table 3). This indicates that renal loss of sodium might have controlled carefully to maintain optimal ECF sodium concentration. Similar results have also been reported by Chaiyabutr et al. (1990) in buffalo and El-Nouty et al. (1980) in cattle. Our results also indicate that, kidney might have sensed the low plasma volume and total body water (Table 5), thereby retention of sodium and water. Increased FE of potassium (Table 3) could be explained by the fact that, during respiratory alkalosis, the kidneys plays significant role in acid-base regulation by increased exchange of potassium ions for hydrogen ions in the renal tubular fluid (Johnson and Selkurt, 1966). Renal adaptive response to heat stress normally result in sodium retention and production of urine with low sodium concentration (Kurtz et al., 1987), which was evident in the present investigation. They further reported that the sodium retention by the kidneys do not occur unless plasma chloride is present in excess. Significant ($p<0.01$) increase in the plasma chloride concentration (Table 4) might have caused sodium retention. Decrease in the concentration of the inorganic phosphorus in the plasma (Table 4) might result in the reduction in

Table 5. Changes in various parameters during cool-comfort and hot-dry and heat stress conditions

Variables	Day	1		7		14		21		Significance
	Cool-comfort	Hot-dry	Hot-humid	Hot-dry	Hot-humid	Hot-dry	Hot-humid	Hot-dry	Hot-humid	
Oxygen tension (mmHg)	48.1 ±1.9	46.75 ±3.42	45.4 ±3.8	45.8 ±2.42	42.5 ±2.62	44.3 ±4.14	40.4 ±3.22	44.8 ±1.94	42.2 ±2.84	p<0.05
Oxygen content (ml/dl)	11.3 ±0.486	11.1 ±0.341	11.3 ±0.225	10.8 ±0.412	10.7 ±0.345	10.4 ±0.381	10 ±0.29	9.6 ±0.422	8.5 ±0.186	p<0.05
Oxygen saturation (%)	75.5 ±2.31	72.1 ±2.45	72.5 ±3.14	70.3 ±1.95	67.2 ±2.14	68.1 ±2.86	66.2 ±2.64	67.3 ±2.15	64.9 ±1.85	p<0.05
Oxygen capacity (mmol/L)	14.8 ±0.531	14.11 ±0.804	14.11 ±0.53	13.76 ±0.493	13.36 ±0.75	14.11 ±1.00	13.37 ±1.31	14.16 ±0.947	13.47 ±0.366	p<0.01
Packed cell volume (%)	31.8 ±1.16	30.5 ±1.08	30.66 ±1.06	29.16 ±1.72	28.14 ±2.94	28.16 ±2.22	27.83 ±3.12	28.55 ±2.02	26.45 ±2.6	p<0.01
Haemoglobin (g/dl)	11.5 ±0.88	11.29 ±0.383	11.36 ±0.358	10.9 ±0.389	10.73 ±0.207	10.8 ±0.25	10.4 ±0.2	10.93 ±0.414	10.43 ±0.35	p<0.05
Total plasma protein (g/dl)	6.74 ±0.335	6.82 ±0.48	7.00 ±0.32	7.78 ±0.2	7.62 ±0.68	7.35 ±0.14	8.15 ±0.59	7.39 ±0.21	7.81 ±0.44	p<0.01
Plasma volume (ml/kg)	41.1 ±0.524	41.37 ±0.444	41.78 ±0.435	41.66 ±0.23	42.82 ±0.471	42.91 ±0.467	43.64 ±0.51	41.56 ±0.298	42.42 ±0.225	p<0.05
Blood volume (ml/kg)	56.3 ±0.908	56.03 ±0.968	56.34 ±0.277	55.96 ±0.931	57.51 ±0.807	55.81 ±0.842	57.39 ±0.747	54.94 ±0.832	54.06 ±0.863	p<0.05
Total body water (Liters/100 kg)	62.1 ±3.16	62.58 ±2.89	62.76 ±2.58	61.91 ±3.16	61.40 ±3.55	60.62 ±3.05	61.26 ±3.57	57.11 ±3.54	58.48 ±2.97	p<0.05

filtered load and thereby causing decrease in FE of inorganic phosphorus as also reported by Chaiyabutr et al. (1990).

Johnson and Selkurt (1966) reported that increased plasma chloride concentration is associated with low base in blood and ECF, as also observed in the present investigation. Chaiyabutr et al. (1990) suggested increased plasma chloride concentration in nonshaded buffaloes could be due to respiratory alkalosis and possibly due to renal excretion of base. The changes in chloride concentration are normally associated with roughly proportional changes in sodium concentration, total body water and plasma volume (Saxton and Seldin, 1986) and inversely associated with plasma bicarbonate, as also observed in the present investigation. Divers et al. (1986) reported that disproportionate increase in chloride level is associated with normal or low anion gap, hyperchloremic metabolic acidosis and may observed in compensatory response for primary respiratory alkalosis. The percent decrease in plasma inorganic phosphorus concentration was non-significantly higher in both heat exposures. Knochel and Caskey (1977) suggest that respiratory alkalosis may be responsible for the reduction of inorganic phosphorus as it is related to the cellular trapping of phosphorus utilized as phosphorylate glycolytic intermediates to meet metabolic demand in heat stress animals.

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REFERENCES

Alam, M. G. S. and H. Dobson. 1986. Effect of various veterinary procedures on plasma concentrations of cortisol, L.H., PGF2 α metabolite in the cows. Vet. Res. 118:7-10.

Alvarez, M. B. and H. D. Johnson. 1973. Environmental exposure in cattle: Plasma catecholamines and glucocorticoids. J. Dairy. Sci. 56:189-194.

Anderson, D. M., I. McDonald and F. W. H. Elsley. 1969. The estimation of plasma and red cell volumes in pigs. J. Agric. Sci. Camb. 73:501-505.

Benjamin, M. M. 1981. Fluid and electrolytes. In Outline of Veterinary Clinical Pathology. Iowa State Univ. Press, Ames., pp. 213-228.

Bianca, W. and J. D. Findlay. 1962. The effect of thermally induced hyperpnoea on the acid-base status of the blood of calves. Res. Vet. Sci. 3:38-49.

Chaiyabutr, N., C. Buranakarl, S. Chanpongsang and P. Loypetjra. 1990. Changes in renal function of swamp buffaloes during short-term exposure to the sun. Int. J. Anim. Sci. 5:175-180.

Chaiyabutr, N., S. Chanpongsang, P. Loypetjra and A. Pichaicharnarong. 1983. Renal function studies in normal and heat stressed swamp buffaloes. Proceeding of V World Conference in Animal Production, Tokyo, pp. 763-764.

Divers, T. J., D. E. Freeman, E. L. Ziemer and J. L. Becht. 1986.

Proc. Annu. Meet. An. Assoc. Equine Pract., 32nd, Nashville, Tennessee, pp 69-80. Cited In Clinical biochemistry of Domestic Animals. (Ed. J. J. Kaneko, J. W. Harvey and M. L. Bruss), 5th Harcourt Brace and Co. Asia PTE LTD, p. 512.

El-Nouty, F. D., I. M. Elbanna, T. P. Davis and H. D. Johnson. 1980. Aldosterone and ADH response to heat and dehydration in cattle. J. Appl. Physiol. 48:249-255.

Faulkner, W. R. and J. W. King. 1970. Renal Function Tests. In Fundamentals of clinical Chemistry. (Ed. N. W. Tietz), W.B. Saunders Company, Philadelphia. pp. 722-725.

Gregresen, M. L. and R. Rawson. 1959. Blood volume. Physiol. Rev. 39:307-342.

Guyton, A. C. 1986. Textbook of Medical Physiology. (7th Ed) W. B. Saunders Company, Philadelphia. pp. 849-851.

Guyton, A. C. 2001. Textbook of Medical Physiology. (7th Ed) W. B. Saunders Company, Philadelphia. p. 230.

Jonhson, P. C. and E. E. Selkurt. 1966. Respiratory and renal regulation of acid-base balance. In Physiology. (Ed. E. E. Selkurt). Little, Brown, Boston, Mass. pp. 537-538.

Kelly, C. F. and T. E. Bond. 1971. In Biocalimatic factors and their measurement: A guide to environmental research on animals. Washington DC: National Academy of Sciences.

Knochel, J. P. and J. H. Caskey. 1977. The mechanism of hypophosphatemia in acute heat stroke. JAMA. 238:425-426.

Kurtz, T. W., H. A. Al-Bander and R. C. Morris. 1987. New Engl. J. Med. 317: 1043-1048. Cited In Clinical biochemistry of Domestic Aniamls. (Ed. J. J. Kaneko, J. W. Harvey and M. L. Bruss), 5th Harcourt Brace and Co. Asia PTE LTD, p. 512.

Lowry, D. H., N. I. Rosenburgh, A. L. Farr and R. S. Randall. 1951. Protein measurement with folin phenol reagent. J. Biol. Chem. 193:265-275.

Minton, J. E. and F. Blecha. 1990. Effect of acute stress on endocrinological and immunological functions in lambs. J. Anim. Sci. 68:3145.

Nangia, O. P., N. Singh and S. S. Sukhija. 1980. Effect of exercise on thermal and acid-base balance in buffaloes. Trop. Anim. Hlth. Prod. 12:185-188.

Oser, B. L. 1965. Hawk's physiological chemistry (14th Ed) Tata McGraw-Hill Publishing Company Ltd. NY.

Pathak, N. N. and D. N. Verma. 1993. In Nutrient requirements of Buffalo. International Book Distributing Company, Lucknow (India). pp. 12-24.

Rastogi, S. K. 1998. Basal and applied renal function tests in buffalo calves. Ph.D. Thesis. G.B. Pant Univ. of Agril.and Tech. Pantnagar, India.

Rastogi, S. K., M. Mohsin, V. K. Sharma, L. D. Sharma and H. Pandey. 2003. Renal clearance of inulin, para-aminohippuric acid, endogenous creatinin and urea in male and female buffalo calves. Ind. J. Anim. Sci. 73(9):1002-1005.

Richterich, R. 1969. In Clinical Chemistry, Theory and Practice, (2nd Ed) S. Kargar, Basal (Switzerland), NY Acad. Press. New York. pp. 479-468.

Saxton, D. R. and D. W. Seldin. 1986. In Fluids and Electrolytes (Ed. J. P. Kokko and R. L. Tannen), Saunders, Philadelphia, Pennsylvania, pp. 3-62.

Siggaard-Anderson. 1971. An acid-base chart for arterial blood with normal and pathophyiological reference areas. Scandinavia J. Clinical Laboratory Investigation. 27:239-245.

Sikka, P., R. K. Sethi, A. K. S. Tomar and S. C. Chopra. 1994. Blood metabolities level in relation to age and live weight in young buffalo calves. Asian-Aust. J. Anim. Sci. 7(2):201-205.

Singh, R., S. S. Randhawa, M. S. Setia and S. P. S. Singha. 1991. Changes in acid-base status and blood gas dynamics in relation to season and pregnancy in buffaloes- A longitudinal study. Ind. J. Anim. Sci. 61(6):584-589.

Snedecor, G. W. and W. G. Cochran. 1994. Statistical methods. 8th edition, Iowa State University Press, Ames, Iowa.

Soberman, R., B. B. Brodie, B. B. Levy, J. Axeloood, V. Hollander and J. Steele. 1949. The use of antipyrine in the measurement of total body water in man. J. Biol. Chem. 179:31.

Sukhija, S. S., N. Singh and O. P. Nangia. 1978. Ind. J. Expt. Biol. 17:843-844.

Swenson, M. J. and W. O. Reece. 1993. Duke's Physiology of Domestic Animals. 11th edn. Cornell University Press, London. p. 585.

Taskar, J. B. 1980. Clinical Biochemistry of Domestic Animals (Ed. J. J. Kaneko). 2nd edn. Academic Press, New York. pp. 402-447.