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Changes in Blood Gas Composition and Acid-Base Equilibriums in Cattle Blood Samples Kept under Different Temperature Regimens and Times

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Abstract: Changes in venous blood gas composition and acid-base equilibrium in Holstein cattle blood samples kept under different temperatures at various time points were investigated for 24 h. The blood samples were collected from 3 healthy multiparous Holstein cows. A total of 9 blood samples obtained from the animals were allocated into three groups and kept at +4°C for group 1 (n = 3), at room temperature, 22°C, for group 2 (n = 3) and in an incubator at 37°C for group 3 (n = 3). Blood gases were analyzed at 0, 1, 3, 6, 12 and 24 h after the storage. The analyses of the blood samples indicated no change at pH and pCO_2 at 4 and 22°C for the 1st h. Similarly, in addition to O_2SAT at 4°C for 3 h and at 22°C for 6 h; O_2CT at 4°C and at 22°C for 24 h, HCO_3 and BB values at 4, 22 and 37°C for 24 h remained unchanged. By contrast, PO_2, BE and glucose levels in the samples were markedly altered within the 1st h.

Key words: Bovine, blood gas, pH, delay time, temparature

INTRODUCTION

Blood gas composition and acid-base equilibrium in cattle alter during many of the respiratory system and metabolic diseases (Carlson, 1996). The precise measurement of the blood parameters is essential in the diagnosis of the diseases and the determination of their prognosis for veterinarians and researches. However, several factors including sampling method, measurement time and storage conditions are reported to affect blood gas composition and acid-base balance in cattle. The time passed between the blood sampling and its analyses is revealed to affect blood acid-base concentrations due to continuing anaerobic and aerobic activities and byproducts in humans, cattle and dogs (Haskins, 1977; Krokavec *et al*., 1987; Szenci and Besser, 1990; Boink *et al*., 1991; Liss and Payne, 1993; Gokce *et al*., 2004).

CO_2 is generated during aerobic metabolism, while lactic acid is produced all through aerobic activity in blood *in vitro*. The use of O_2 is altered depending on leukocytes, cytochrom in reticulocytes and citric acid enzymatic activities, all of which are directly regulated by the number of the cells. Moreover, glucolysis is a predominant function of the mature erythrocytes. The temperature is shown to be key-factor in the regulation of these metabolic changes in blood (Liss and Payne, 1993). Leucocytosis and anemia are also reported to yield changes in acid-base balance in blood (Haskins, 1977; Liss and Payne, 1993).

At the present study, we aimed to investigate the effect of discrete temperature points and different measurement times on venous blood gas composition and acid-base equilibrium in cattle blood samples.

MATERIALS AND METHODS

Animals and blood sampling: In this study, 3 healthy 4 years old multiparous Holstein cows were used for blood sampling and the analyses of several parameters in blood. Three blood samples obtained from the jugular vein and pulled into 10 mL injectors containing 0.08 mL heparin were collected from each animal. Before tightly capping with plastic air tight covers, air bubbles were carefully removed from the syringes. The blood samples were divided into 3 groups and were kept at refrigerator (4°C) for group 1, at room temperature (22°C) for group 2 and at an incubator (37°C) for group 3. In all of the blood samples, pH, pCO_2, pO_2, TCO_2, O_2SAT, O_2CT, HCO_3, SBC, BE, BB, Hb, Hct and glucose levels were measured at 0, 1, 3, 6, 12 and 24 h after the storage.

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Blood analyses: In the samples, using portative blood gas system measuring several blood parameters automatically and with temperature option adjustable to subject's body temperature (GASTAT-mini, Techno Medica, Japan), blood pH, partial CO_2 pressure (pCO_2), partial O_2 pressure (pO_2), total CO_2 (TCO_2), oxyhaemoglobin saturation (O_2SAT), O_2 saturation (O_2CT), actual bicarbonate (HCO_3), Standard Bicarbonate (SBC), Base Excess levels (BE), the sum of the total negative ion Buffer in Blood (BB), Hemoglobin (Hb), Heamatocrite (Hct) and glucose levels were measured. After adjusting the temperature of GASTAT-mini to the animal's body temperature, pH, pCO_2 and pO_2 were analyzed.

Statistical analyses: The effect of temperature and time on blood parameters as described above was determined using SPSS 13.0 (Windows) and Anova-Tukey test.

RESULTS

The mean hemoglobin and heamatocrite values of the sampled animals were measured to be 7.97±1.83 g dL^{-1} and 27.63±0.17%, respectively. At the time of sampling, the mean body temperature for the animals was found to be 38.4±0.1°C.

Blood borne acid-base, gas and glucose concentrations measured at different time points as designated and pertaining to all 3 groups are illustrated on Table 1-11.

The parameters determined for the groups at different hours were compared to the 0 h measurement results. The results showed that pH (Fig. 1) and pCO_2 remained stable for the 1st h in group 1 and 2. Whereas, there was a statistically significant sudden decrease in pH values and swift marked increase in PCO_2 in

Table 1: Effect of storage time on pH of bovine venous blood samples at +4°C (Group 3), +22°C (Group 2) and +37°C (Group 3) (n = 3) (mean±SE)

		Time (h)						
Parameter	Group	0	1	3	6	12	24	p-value
pH	+37 (Group 1)	7.39±0.015a	7.317±0.017abc	7.197±0.057de	7.223±0.023cd	7.150±0.014de	7.100±0.011e	0.000
	+22 (Group 2)	7.39±0.015a	7.390±0.010a	7.343±0.003ab	7.327±0.015abc	7.266±0.023bcd	7.191±0.010de	
	+4 (Group 3)	7.39±0.015a	7.420±0.047a	7.353±0.027ab	7.383±0.007ab	7.360±0.014ab	7.297±0.015ab	

Table 2: Effect of storage time on pCO_2 of bovine venous blood samples at +4°C (Group 3), +22°C (Group 2) and +37°C (Group 3) (n = 3) (mean±SE)

		Time (h)						
Parameter	Group	0	1	3	6	12	24	p-value
PCO_2	+37 (Group 1)	43.03±2.14d	45.70±0.20cd	73.33±8.67ab	60.10±5.08bcd	81.70±7.01a	91.32±9.94a	0.000
	+22 (Group 2)	43.03±2.14d	41.57±1.32d	47.20±2.01cd	53.40±4.08cd	62.66±3.62bc	76.67±1.43ab	
	+4 (Group 3)	43.03±2.14d	42.23±5.16d	45.37±5.26cd	49.87±3.53cd	52.14±4.01cd	49.85±3.55cd	

Table 3: Effect of storage time on $p0_2$ of bovine venous blood samples at +4°C (Group 3), +22°C (Group 2) and +37°C (Group 3) (n = 3) (mean±SE)

		Time (h)						
Parameter	Group	0	1	3	6	12	24	p-value
PO_2	+37 (Group 1)	47.33±3.56bcdef	46.20±0.40cdef	53.83±1.33abcd	57.70±3.80abc	36.50±0.84efg	25.10±0.41g	0.000
	+22 (Group 2)	47.33±3.56bcdef	46.27±1.85cdef	63.17±9.34ab	55.07±0.15abc	36.13±0.88efg	38.37±1.13defg	
	+4 (Group 3)	47.33±3.56bcdef	51.13±0.07abcde	58.57±5.81abc	64.13±0.83a	34.33±2.64fg	36.62±1.33efg	

Table 4: Effect of storage time on TCO_2 of bovine venous blood samples at +4°C (Group 3), +22°C (Group 2) and +37°C (Group 3) (n = 3) (mean±SE)

		Time (h)						
Parameter	Group	0	1	3	6	12	24	p-value
TCO_2	+37 (Group 1)	27.30±1.21ab	24.77±0.97b	30.40±0.10ab	28.43±0.96ab	31.20±0.11ab	31.40±0.28a	0.004
	+22 (Group 2)	27.30±1.21ab	26.43±1.21ab	27.03±1.35ab	29.74±2.76ab	30.17±0.60ab	31.41±0.10a	
	+4 (Group 3)	27.30±1.21ab	28.04±0.62ab	26.37±1.60ab	31.50±2.60a	30.80±0.74ab	28.82±0.91ab	

Table 5: Effect of storage time on O_2 SAT of bovine venous blood samples at +4°C (Group 3), +22°C (Group 2) and +37°C (Group 3) (n = 3) (mean±SE)

		Time (h)						
Parameter	Group	0	1	3	6	12	24	p-value
O_2SAT	+37 (Group 1)	81.37±4.17ab	76.87±0.47bcd	77.50±4.30abc	80.37±4.37ab	52.00±3.54e	28.91±1.91f	0.000
	+22 (Group 2)	81.37±4.17ab	80.87±2.13ab	88.40±4.11ab	85.70±0.68ab	58.77±0.98e	57.20±1.10e	
	+4 (Group 3)	81.37±4.17ab	89.70±5.19ab	86.93±5.17ab	92.13±0.13a	61.92±0.97de	65.20±3.58cde	

Table 6: Effect of storage time on O_2 CT of bovine venous blood samples at +4°C (Group 3), +22°C (Group 2) and +37°C (Group 3) (n = 3) (mean±SE)

		Time (h)						
Parameter	Group	0	1	3	6	12	24	p-value
O_2 CT	+37 (Group 1)	11.94±0.17ab	10.37±0.44ab	17.07±6.67ab	18.35±6.08a	11.20±1.21ab	5.44±0.90b	0.086
	+22 (Group2)	11.94±0.17ab	10.73±0.74ab	12.07±1.08ab	11.20±0.87ab	10.60±1.10ab	8.23±0.57ab	
	+4 (Group3)	11.94±0.17ab	9.80±1.67ab	10.13±1.56ab	10.74±0.33ab	7.70±0.40ab	8.75±0.54ab	

Table 7: Effect of storage time on HCO_3 of bovine venous blood samples at +4°C (Group 3), +22°C (Group 2) and +37°C (Group 3) (n = 3) (mean±SE)

		Time (h)						
Parameter	Group	0	1	3	6	12	24	p-value
HCO_3	+37 (Group1)	25.97±1.84ab	23.37±0.97b	28.07±0.16ab	26.40±0.80ab	28.60±0.92ab	28.50±0.84ab	0.017
	+22 (Group2)	25.97±1.84ab	25.17±1.18ab	25.53±1.29ab	28.10±2.63ab	28.27±0.52ab	29.03±0.13ab	
	+4 (Group3)	25.97±1.84ab	26.77±0.48ab	24.97±1.47ab	29.90±2.50a	29.20±1.99ab	27.20±1.45ab	

Table 8: Effect of storage time on SBC of bovine venous blood samples at +4°C (Group 3), +22°C (Group 2) and +37°C (Group 3) (n = 3) (mean±SE)

		Time (h)						
Parameter	Group	0	1	3	6	12	24	p-value
SBC	+37 (Group1)	25.75±1.08abc	22.17±1.07bcd	22.73±0.64bcd	22.57±0.13bcd	20.81±0.41cd	19.90±0.87d	0.017
	+22 (Group2)	25.75±1.08abc	24.90±1.07abcd	24.43±1.01abcd	26.17±2.17ab	23.70±0.62abcd	23.37±0.47bcd	
	+4 (Group3)	25.75±1.08abc	26.57±0.51ab	24.37±0.94abcd	28.90±2.10a	27.21±0.28ab	25.43±0.45abc	

S.D.: HCO_3=SBC

Table 9: Effect of storage time on BE of bovine venous blood samples at +4°C (Group 3), +22°C (Group 2) and +37°C (Group 3) (n = 3) (mean±SE)

		Time (h)						
Parameter	Group	0	1	3	6	12	24	p-value
BE	+37 (Group1)	3.39±0.72a	0.33±0.12bc	-1.10±0.09cd	-1.64±0.37cde	-3.14±0.12de	-3.85±0.13e	0.000
	+22 (Group2)	3.39±0.72a	1.57±0.69ab	0.10±0.56bc	-3.81±0.76e	0.14±0.65bc	0.78±0.11abc	
	+4 (Group3)	3.39±0.72a	2.50±0.45ab	0.33±0.72bc	0.10±0.59bc	2.48±0.78ab	1.57±0.35ab	

Table 10: Effect of storage time on BB of bovine venous blood samples at +4°C (Group 3), +22°C (Group 2) and +37°C (Group 3) (n=3) (mean±S.E.)

		Time (h)						
Parameter	Group	0	1	3	6	12	24	p-value
BB	+37 (Group1)	46.73±0.86a	43.40±1.03a	46.43±2.93a	44.13±0.67a	45.10±0.21a	43.52±1.02a	0.055
	+22 (Group2)	46.73±0.86a	46.37±1.32a	45.84±1.30a	47.60±2.57a	47.13±0.74a	45.47±0.27a	
	+4 (Group3)	46.73±0.86 a	47.20±0.56a	45.10±0.78a	50.07±2.23a	49.18±0.54a	47.30±0.69a	

Table 11: Effect of storage time on glucose of bovine venous blood samples at +4°C (Group 3), +22°C (Group 2) and +37°C (Group 3) (n = 3) (mean±SE)

		Time (h)						
Parameter	Group	0	1	3	6	12	24	p-value
Glucose	+37 (Group1)	61.30±1.41	40.93±0.67	22.94±3.97	13.50±0.28	NM	NM	0.000
	+22 (Group2)	61.30±1.41	52.60±1.66	40.47±3.64	34.41±3.64	27.07±3.37	NM	
	+4 (Group3)	61.30±1.41	60.87±3.32	56.03±3.60	54.00±0.21	31.69±2.24	NM	

NM = Not measured

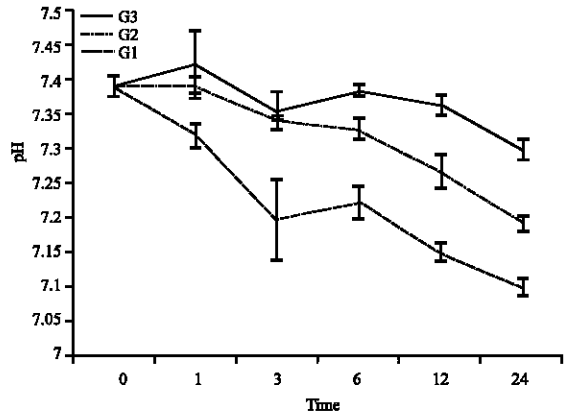

Fig. 1: Alterations of pH levels dependent on storage time

group 1. Moreover, pO_2 in the entire groups was also markedly and promptly increased.

The decrease in TCO_2 was meaningful and fast in group 1 but it stayed constant for 12 h in group 2 and for 3 h in group 3. We also determined that O_2SAT showed sudden change in group 1 but remained steady for 6 h in group 2 and for 3 h in group 3. O_2 CT maintained its level for 3 h in group 1 and for 24 h in group 2 and 3; by contrast, HCO_3 concentration had a rapid change in group 1 but stayed constant for 24 h in group 2 and for 3 h for group 4. Furthermore, SBC and BE amounts showed sudden alteration in the groups; in contrast, BB remained stable in all groups for 24 h. Similar to SBC and BE, augmentation in glucose levels was swift and statistically consequential.

DISCUSSION

In living beings, the maintenance of blood buffering system at pH between 7.35-7.45 is required for normal metabolic activities. Nearly, all of the enzyme systems in the body affect the H^+ ion concentration and therefore, the sustenance of H^+ ion concentration at physiological levels is a very delicate balance in the body (Di Bartola *et al*., 1994).

Temperature is reported to be one of the leading factors affecting most of the biological reactions in blood. At their study on the measurement of the blood gases in dogs, Di Bartola *et al*. (1994) report that the determination of blood gases should be done within in 15-30 min at the room temperature or within 2 h at 4°C after withdrawing. In contrast to dogs, controversies exist among the studies performed to elucidate the effect of temperature on acid-base balance in cow blood samples stored at different temperatures. According to the work of Szenci and Basser (1990), blood samples can be used within 24 h for diagnostic purposes. Other researches indicate that the pH determined in blood samples kept at 4°C for 5-6 h (Paulsen and Surynek, 1977) or stored at 4°C for 48 h (Gokce *et al*., 2004) can be used in clinical applications; in addition, they show that storage time and temperature can change pH and bicarbonate values. At the present study, in contrast to earlier studies, pH values clearly decreased after 1 h in blood samples kept at 4°C but BE values, similar to other reports, were lower 1 h after storage. It appears that ongoing anaerobic activities and lactic acid formation during storage can alter BE and SBC via reducing pH. Rapid changes in pH values 1 h after storage at 22°C or immediately at 37°C that we measured in this study, further support our observation.

Anaerobic, aerobic metabolisms and their byproducts are the most prevailing factor in the increase of pCO_2 in dog and cow blood owing to storage (Haskins, 1977; Poulsen and Surynek, 1977). In addition, oxygen infiltration through plastic syringe wall is shown to cause increase in pO_2 in blood sample (Gokce *et al*., 2004). A recent study reported by Knowles *et al*. (2006) suggests that measurements of blood gases in the samples kept in plastic syringes should be done as soon as possible. In the present study, we found sudden noticeable increase in PO_2 at all temperatures studied, in PCO_2 when compared to 0 h measurements, at 37°C and 1 h after the storage in group 2 and 3. Rapid and marked increases in PCO_2 levels measured in this study, seem to be owing to anaerobic and aerobic activities of blood cells (Szenci and Beser, 1990; Beaulieu *et al*., 1999). Moreover, in addition to anaerobic and aerobic activities of blood cells, oxygen infiltration through plastic syringe wall appeared to be responsible for the increase in PO_2 (Gokce *et al*., 2004; Knowles *et al*., 2006).

Glucolysis is a predominant metabolism of mature erythrocytes and it is strictly regulated by temperature (Liss and Payne, 1993). In the present study, initiate of sudden decrease in glucose levels at different temperatures tested and the presence of little or no glucose at 12th h at 37°C and 24th h at 22°C indicate the consumption of glucose during erythrocyte metabolism.

Most of the circulating oxygen is bound to hemoglobin and only a small portion of it exists in dissolved form. The amount of circulating O_2 bound to hemoglobin is the oxygen saturation (O_2SAT) (Carlson, 1996). Our observation of sudden decrease in O_2SAT at +37°C is in line with the study of Gokce *et al*. (2004). In their interpretation, they reason that low pH induces separation of O_2 from hemoglobin thereby reducing O_2SAT. Our present data are similar and further support their view.

CONCLUSION

Moreover, the present study results suggest that changes in blood parameters are time and temperature dependent therefore, all parameter should be measured and combined carefully prior to interpretation for precise diagnosis. For instance, we observed no change in blood pH and PCO_2 values at 4 and +22°C' for the 1st h; O_2SAT at 4°C for 3 h and 22°C 6 h; O_2CT at 4 and 22°C for 24 h in addition to HCO_3 and BB values at 4, 22 and 37°C 24 h, while PO_2, BE and glucose levels showed marked changes within first hour. Therefore, acid-base and blood gases should not be evaluated separately but rather combined for proper diagnosis. Moreover, current study also indicates that the storage of samples at 4°C or 22°C extends sample stability for the measurement of blood gas composition and acid-base balance in the diagnoses of diseases. Measurements should be preferably done right after or within 1 h after sampling; for this purpose, portative blood gas system can be used for the measurement at the site.

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