Influence of refrigeration on blood gas analysis of caprine venous blood

Influência da refrigeração na análise hemogasométrica de sangue venoso caprino

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Abstract

Blood samples collected from 14 healthy, four month-old, male goats of mixed breed, weighting from 30 to 45 kg, were analyzed in order to evaluate the effect of refrigeration on blood gas analysis. Blood samples to be used in the blood gas analysis were collected in duplicates, using disposable needles and plastic syringes containing around 1,000 IU of sodium heparin. Unpreserved samples were kept at room temperature, between 23 and 25 ºC, and those to be kept in refrigeration temperatures were placed in styrofoam coolers containing three liters of cold water and three kilograms of ice, in order to keep temperatures between zero and 4 ºC. Blood gas analyses were carried out immediately after collection and after 1, 2, 3, 4, 5, 6, 8, 10, 12 and 24 hours. Storage at room temperature affected significantly the group of variables studied, except blood concentration of HCO_3. When samples were kept under refrigeration, partial pressure of O_2, CO_2 and SO_2 were significantly affected. The significant variation in mean values of these variables when compared with initial mean values was greater in samples kept at room temperature. Results for pH, HCO_3^- and ABE in the refrigerated samples were stable for up to 24 hours of blood collection. It was concluded, therefore, that blood gas analysis of caprine venous blood may be safely carried out in up to six hours of blood collection, provided that samples are kept under adequate refrigeration.

Keywords: Blood gas. Caprine. Venous blood.

Resumo

Objetivando-se avaliar o efeito da refrigeração sobre o exame hemogasométrico, foram utilizados 14 caprinos machos, hígidos, mestiços, com cerca de quatro meses de idade e peso variando entre 30 e 45 kg. As amostras de sangue destinadas ao exame hemogasométrico foram coletadas em duplicata, utilizando-se agulhas descartáveis acopladas a seringas plásticas contendo cerca de 1000 UI de heparina sódica. As amostras não conservadas foram mantidas a temperatura ambiente, entre 23 e 25 ºC e aquelas destinadas à refrigeração foram acondicionadas em isopor contendo três litros de água gelada e três quilos de gelo, mantendo-se assim uma temperatura entre zero e 4 ºC. As análises hemogasométricas foram determinadas imediatamente após coleta e 1, 2, 3, 4, 5, 6, 8, 10, 12 e 24 horas após. Verificou-se efeito significativo da temperatura ambiente sobre o conjunto de variáveis, com exceção da concentração sanguínea de HCO_3. Quanto às amostras mantidas sob refrigeração, verificou-se efeito significativo sobre a tensão parcial de O_2 e CO_2 e SO_2; sendo que a variação significativa dos valores médios destas variáveis com os valores médios iniciais foram mais tardias em relação às amostras mantidas a temperatura ambiente. Os valores de pH, HCO_3 e ABE das amostras refrigeradas mantiveram-se estáveis até 24 horas após a colheita de sangue. Conclui-se, portanto, o exame hemogasométrico de sangue venoso de caprinos pode ser efetivado com segurança até seis horas após sua colheita, desde que mantidos sob refrigeração adequada.

Palavras-chave: Hemogasometria. Caprino. Sangue venoso.

Introduction

Blood gas analysis, which includes the analysis of blood gases and other variables required for the evaluation of acid-base balance, is an important tool in the diagnosis and treatment of diseases that affect different animal species, mainly ruminants[1,2,3]. Metabolic aci-

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dosis, characterized by decreases in pH and bicarbonate levels, is one of the important changes detected by blood gas analysis. This alteration is mainly observed in cases of ruminal lactic acidosis, pregnancy toxemia, diarrhea, renal and respiratory insufficiency[4,5].

The length of transportation and storage of biological samples considerably influence results of blood gas analyses. In veterinary medicine, immediate analysis may not be feasible, mainly for professionals working in field conditions[2,3,6].

Results of blood gas analyses in blood samples kept under refrigeration were reported by some authors: in bovines[2,7,8], in sheep[3,9,10], in horses[11]; in swine[6] in dogs[12] and goat[13].

The conclusions of these reports showed that there were differences in opinion on how long samples were viable. Indications ranged from 6 to 24 hours after collection, when samples were kept from 0 to 4º C[2,3,14].

As for the blood gas analysis in goats, there are no Brazilian reports on the subject. Due to this lack of information in the literature, the objective of the present trial was to evaluate blood gas analysis during 24 hours in samples of goat venous blood kept at room temperature (23 to 25 ºC) and between 0 and 4 ºC.

Material and Methods

Fourteen male, healthy goats, of mixed breed, around four months of age and weighting from 30 to 45 kg were used in the study. Animals were kept in a collective enclosure and were fed with corn and soybean meal concentrate, Coast Cross hay, mineral salt and water *ad libitum*. Animals were kept in the enclosure for 30 days before the beginning of the trial.

On the day before the trial, rectal temperature of each animal was measured and hemoglobin concentration was determined by means of a blood sample collected by venopuncture of the external jugular vein using EDTA-coated vacutainer® tubes. Blood samples for the blood gas analysis were collected in duplicate, using 10 mL plastic syringes containing 1,000 IU of sodium heparin. Immediately after venopuncture, the tip of the needle was sealed with a rubber stopper in order to prevent gas from moving in or out of it.

After collection, syringes were immediately taken to the laboratory. Unpreserved samples were kept at room temperature, between 23 and 25 °C, and those to be kept under refrigeration were placed into a styrofoam coolers containing three liters of iced water and three kilograms of reusable gel ice packs, in order to keep temperature between zero and 4 °C. A thermometer was placed inside the water in order to control water temperature and avoid raising over 4 °C, during the whole study[15].

The blood gas was properly calibrated before the start of the experiment (the solutions calibration blood gas are: Calibration solution pH 7.383; calibration solutions pH 6.841 and calibration solutions Rinse). Hemoglobin concentration was determined by means of the cyanohemoglobin method, using a commercial kit (Labtest®). Reading of the samples was carried out in a spectrophotometer (E-225-D, CELM).

Blood gas analyses were carried out soon after collection and after 1, 2, 3, 4, 5, 6, 8, 10, 12, and 24 hours in all samples, using a blood pH and gas analyzer. The analyzer is able to measure pH and partial pressures of oxygen (PO_2) and carbon dioxide (PCO_2) by means of a electrode system (ABL- 5, Radiometer, Copenhagen) and to automatically calculate the other variables: plasma total carbon dioxide (TCO_2), plasma bicarbonate concentration (HCO_3^-), base excess or deficit in blood (ABE) and oxygen saturation in blood (SO_2)[15].

After the blood aliquot was placed in the blood gas analyzer, hemoglobin and rectal temperature values were corrected for each animal evaluated, once the standard values used for the device are related to humans.

Data were analyzed by the Statistical Analysis System[16] software, after assessing the normality of the residues by means of the Kolmogorov-Smirnov test. Variance analysis was used in order to assess the ef-

fects of storage on blood samples. Differences between mean values were carried out by means of the d.m.s (minimum significative difference) and the Duncan test, considering a significance level of 0.05.

Results and Discussion

Mean values for the blood gas variables are shown in table 1 and figures 1 and 2. Storage at room temperature significantly affected blood pH ($p < 0.0001$),

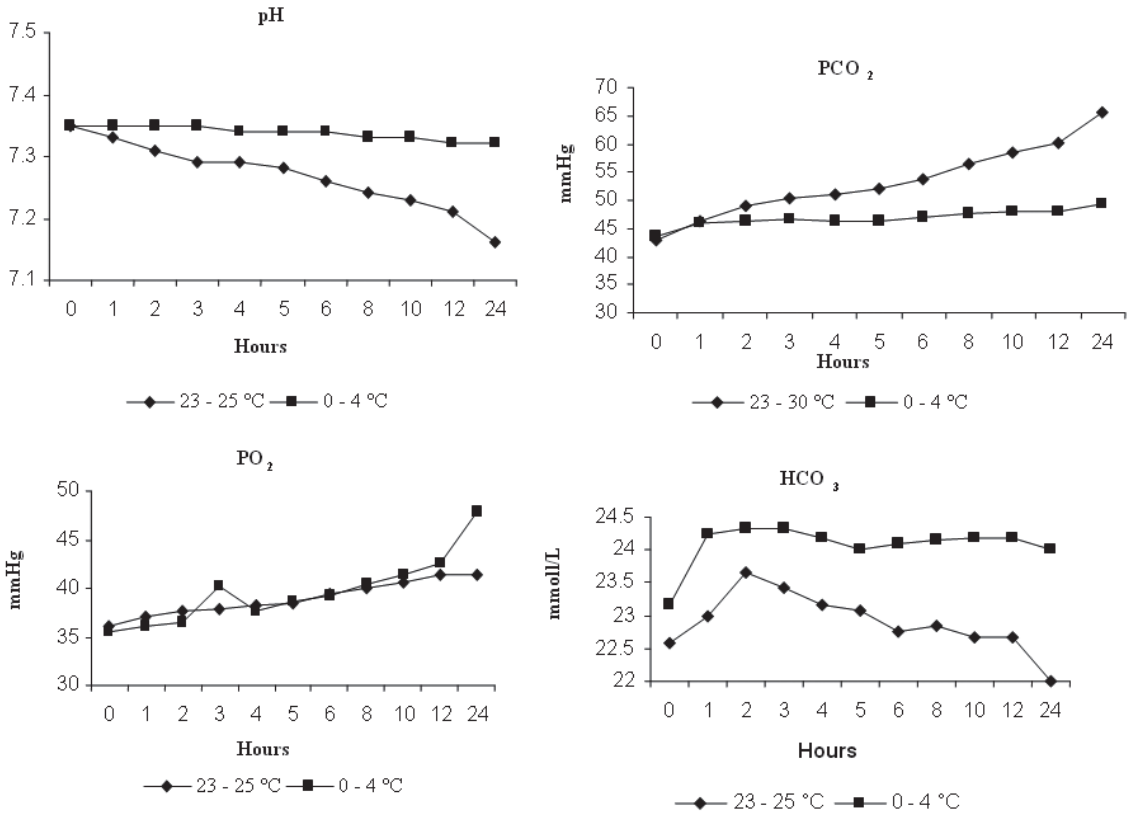

Figure 1 - Mean values from pH, partial pressures of carbon dioxide ($PC0_2$) and oxygen ($P0_2$), and bicarbonate (HCO_3^-) in caprine venous blood (n =14) kept at room temperature (23 to 25°C) and under refrigeration (0 to 4°C) during 24 hours

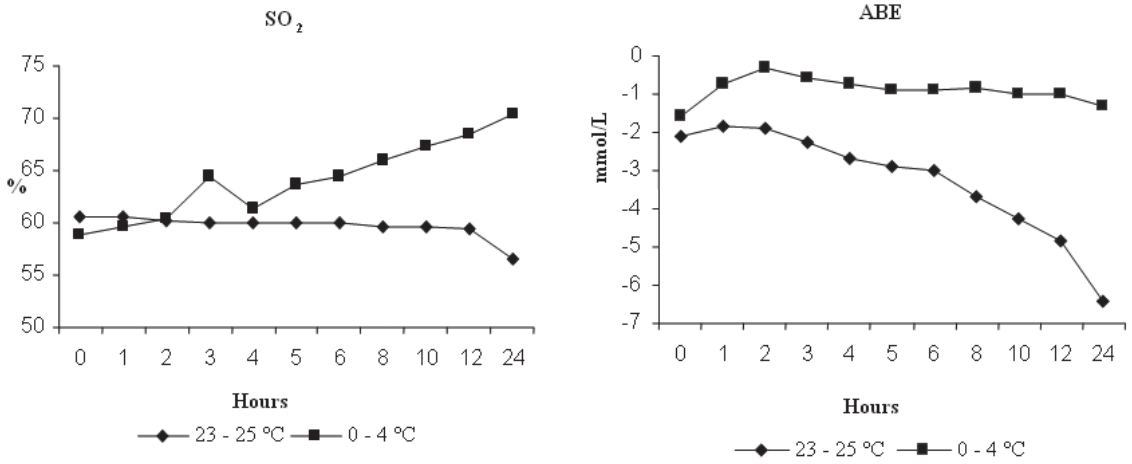

Figure 2 - Mean Values from oxygen saturation (SO_2) base excess or deficit (ABE) in caprine venous blood (n=14) kept at room temperature (23 to 25°C) and under refrigeration (0 to 4° C) during 24 hours

Table 1 – Mean values(X) and standard deviations (s) of blood gas variables in venous blood from goats (n = 14) kept at room temperature (RT: 23 ˚ to 25 ˚C) and under refrigeration (R: 0 to -4 ˚C) over the period of 24 hours

VARIABLES			HOURS											*p value
			0	1	2	3	4	5	6	8	10	12	24	
pH	RT	χ	7,35^{a}	7,33^{ab}	7,31^{abc}	7,29^{bcd}	7,29^{cd}	7,28^{cde}	7,26^{de}	7,24^{ef}	7,23^{f}	7,21^{f}	7,16^{g}	**0,0001**
		s	0,0127	0,0114	0,0111	0,0112	0,0116	0,0099	0,0117	0,0127	0,0131	0,0133	0,0120	
	R	χ	7,353^{a}	7,349^{a}	7,348^{a}	7,345^{a}	7,344^{a}	7,339^{a}	7,338^{a}	7,334^{a}	7,332^{a}	7,328^{a}	7,316^{a}	**0,0939**
		s	0,0084	0,0082	0,0080	0,0089	0,0088	0,0082	0,0080	0,0082	0,0085	0,0083	0,0081	
PO_2 (mmHg)	RT	χ	36,08^{d}	37,08^{cd}	37,67^{bcd}	37,92^{bcd}	38,33^{abcd}	38,42^{abcd}	39,33^{abcd}	40,08^{abc}	40,67^{ab}	41,42^{a}	41,33^{a}	**0,0018**
		s	0,866	0,941	0,995	0,925	0,948	1,138	0,948	0,964	0,979	1,090	1,275	
	R	χ	35,50^{d}	36,00^{d}	36,50^{abcd}	40,25^{bcd}	37,58^{abcd}	38,58^{bcd}	39,25^{bcd}	40,42^{abcd}	41,42^{abcd}	42,58^{ab}	47,75^{a}	**0,0011**
		s	1 ,346	1,359	1,395	2,541	1,345	1,443	1,508	1,540	1,588	1,658	1,661	
PCO_2 (mmHg)	RT	χ	42,92^{f}	46,42^{ef}	49,00^{de}	50,33^{de}	51,17^{de}	52,20^{cd}	53,58^{cd}	56,33^{cd}	58,33^{b}	60,17^{b}	65,58^{a}	**0,0001**
		s	1,083	1,246	1,354	1,394	1,522	1,164	1,574	1,755	1,910	2,015	1,786	
	R	χ	43,67^{c}	45,83^{bc}	46,42^{abc}	46,58^{abc}	46,42^{abc}	46,41^{abc}	46,83^{ab}	47,50^{ab}	47,83^{ab}	48,00^{ab}	49,33^{a}	**0,0226**
		s	0,838	0,936	0,996	1,011	0,933	0,941	1,006	0,989	1,043	0,977	0,995	
SO_2 (%)	RT	χ	60,50^{a}	60,58^{a}	60,25^{a}	60,08^{a}	60,00^{a}	60,00^{a}	60,00^{a}	59,66^{a}	59,58^{a}	59,33^{a}	56,50^{a}	**0,9736**
		s	1,857	1,990	1,903	1,959	1,981	1,969	1,950	1,932	1,944	1,943	2,204	
	R	χ	58,91^{c}	59,66^{c}	60,42b^{c}	64,33^{abc}	61,33^{bc}	63,58^{abc}	64,33^{abc}	66,00^{abc}	67,25^{abc}	68,50^{ab}	70,42^{a}	**0,0316**
		s	2,533	2,487	2,536	3,506	2,669	2,454	2,547	2,480	2,513	2,379	2,378	
HCO_3 (mmol/L)	RT	χ	22,58^{a}	23,00^{a}	23,67^{a}	23,42^{a}	23,17^{a}	23,08^{a}	22,75^{a}	22,83^{a}	22,66^{a}	22,67^{a}	22,00^{a}	**0,7809**
		s	0,596	0,564	0,555	0,543	0,519	0,570	0,579	0,562	0,560	0,560	0,590	
	R	χ	23,17^{a}	24,25^{a}	24,33^{a}	24,33^{a}	24,17^{a}	24,00^{a}	24,08^{a}	24,16^{a}	24,17^{a}	24,17^{a}	24,00^{a}	**0,9853**
		s	0,588	0,167	0,631	0,678	0,588	0,615	0,621	0,588	0,588	0,590	0,615	
ABE (mmol/L)	RT	χ	-2,08^{a}	-1,83^{a}	-1,92^{a}	-2,25^{ab}	-2,67^{ab}	-2,92^{abc}	-3,00^{abc}	-3,67^{abc}	-4,25^{bc}	-4,83^{cd}	-6,42^{d}	**0,0001**
		s	0,690	0,672	0,645	0,591	0,607	0,645	0,651	0,568	0,629	0,649	0,657	
	R	χ	-1,58^{a}	-0,75^{a}	-0,33^{a}	-0,58^{a}	-0,75^{a}	-0,91^{a}	-0,91^{a}	-0,83^{a}	-1,00^{a}	-1,00^{a}	-1,33^{a}	**0,9849**
		s	0,633	0,617	0,632	0,609	0,617	0,668	0,668	0,672	0,651	0,651	0,632	

a, b, c, d, e – different letters in the same group line statistical difference.

which decreased with time. Therefore, the period of stability for this variable in relation to the initial value was short, ending in the second hour after blood collection. Nevertheless, the samples kept under refrigeration showed little non significant variation (p = 0.0939) until the 24th hour after collection. According to Almosny[17], blood pH is almost neutral, with a slight tendency to alkalinity (approximately 7.4), and metabolic reactions continuously shift this pH towards acid or basic values. There was a decrease in pH with time (7.35 in the beginning to 7.21 and 7.16 after 12 and 24 hours, respectively) in the blood maintained at room temperature (Table 1 and Figure 1). These changes may be justified by shifts in intraerythrocitary metabolism, as occurs in acidemia cases.

As for partial pressures of O_2 and CO_2, it was observed that both treatments significantly affected blood samples as well as it increased continuously along the time. The difference in PO_2 and PCO_2 mean values was smaller in refrigerated samples, with a significant difference starting at 10th hour on (p = 0.0226) for PO_2 and at the 6th hour on (p = 0.0011) for PCO_2, differently from samples kept at room temperature, which showed differences in the 6th hour for PO_2 (p = 0.0018) and in the 1st hour for PCO_2 (p = 0.0001) (Table 1 and Figure 1).

When the samples were kept at room temperature there was a progressive decrease in the values of SO_2 (p = 0.0316) (Table 1 and Figure 2). Under refrigeration, SO_2 in venous blood was stable until the 10th

hour, being significantly greater 12 and 24 hours after collection (Table 1 and Figure 2).

HCO_3^- concentration in venous blood was stable until the 24th hour after collection, both at room temperature ($p = 0.7809$) and under refrigeration ($p = 0.9853$) (Table 1 and Figure 2). These results are similar to those observed in blood samples from cattle[2], sheep[3,13] and goats[13] under the same conditions of storage.

Mean values observed for ABE decreased with time at room temperature ($p < 0.0001$), and were stable until the 8th hour when compared with the initial analysis. ABE´s decrease in this group was twice the initial value after that moment, whereas at the 24th hour the decrease was three times the initial value. Stability of ABE in refrigerated blood was maintained up to the 24th hour ($p = 0.9849$).

As it may be observed (Table 1 and Figures1 and 2), blood samples kept at room temperature were significantly affected. Samples kept for 24 hours at room temperature showed changes in blood gas analysis that are due to blood metabolic activity. This *in vitro* activity includes aerobic metabolism, with consumption of O_2 and production of CO_2 in the tricarboxylic acid cycle, and anaerobic glycolysis together with production of acidic metabolites, mainly lactic and pyruvic acids[18]. The continuous accumulation of acids in blood may, therefore, support the changes observed in blood gas parameters. A delay in erythrocyte metabolism was observed in the refrigerated blood samples, supporting the behavior of the studied variables.

Lisboa et al.[2] determined critical moments for some variables in bovine blood samples kept under refrigeration: four hours for pH, six hours for PCO_2 and ABE and ten hours for PO_2. The only similar result obtained in the present study was related to PCO_2 (critical time six hours). Different values were observed for pH, HCO_3 and ABE, which showed to be stable for up to 24 h after collection in goat blood (Table 1 and Figures 1 and 2). In one study conducted to evaluate the performance of blood gas variables of goats kept at room temperature and under refrigeration[13], the authors might not determine the critical time for the use of chilled samples, because only the variables measured before and after 24 hours, impossible to compare these results with those obtained in this study.

Longer PO_2 stability in samples kept under refrigeration may be explained by the release of oxygen from hemoglobin as a consequence of pH decrease, which diminishes hemoglobin affinity to oxygen (Bohr effect)[19]. This would counterbalance the loss of oxygen attributed to the aerobic metabolism, leading to discreet changes in PO_2 once the metabolism of goat blood, as observed in the present trial, occurs in a less intense manner when compared to that of dogs, horses, pigs and bovines[2,6,11,12].

Keeping goat venous blood in cold water drastically reduced cell metabolism in a way that there were no significant changes in blood gas variables during the 24 hours of the study. Similar results were obtained by Leal et al.[3], studying ovine venous blood. Results of the present trial were different from those obtained by Szenci e Besser[18] and Lisboa et al.[2], who observed significant pH variations in bovine venous blood beginning at six and four hours of storage, respectively, together with later changes in other blood gas variables. This discrepancy may be attributed to differences in the methodology of each trial, besides the erythrocytic metabolic profile of the different species. This observation should incentive further discussions on this profile in other ruminant species, such as buffaloes.

Conclusions

The results indicated that samples obtained from healthy goats, concerning the venous blood gas analysis, may remain viable for the diagnosis up to six hours when properly kept in ice water bath.

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