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*Original article*

# Acid-base balance parameters and a value of anion gap of arterial and venous blood in Małopolski horses

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## Abstract

The comparative study of the acid-base balance (ABB) parameters has been performed on 20 clinically healthy mature Małopolski horses. An arterial blood sample from the facial artery and a sample of venous blood from the external cervical vein were collected from each animal. In the samples tested, the blood pH, pCO<sub>2</sub>, tCO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup>, concentration of Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, and a value of the anion gap were determined. The difference among pCO<sub>2</sub>, tCO<sub>2</sub>, and HCO<sub>3</sub><sup>-</sup> in both samples tested was statistically significant, whereas the pH of the arterial blood and the pH of the venous blood did not differ significantly. The anion gap in both types of blood did not differ significantly. Conclusions: 1) ABB parameters such as pCO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup>, and tCO<sub>2</sub> determined in the arterial and venous blood of the Małopolski horses differ from each other significantly. 2) In spite of the lack of the differences between pH of the arterial and venous blood, the ABB parameters in horses should be determined in the arterial blood, because the comparative study performed proves that the analysis of the ABB parameters determined for the venous blood of a healthy horse may lead to a wrong diagnosis of the compensated respiratory acidosis. 3) The mean value of anion gap in horses aged 8-12 years amounts to 20.9 mmol/l for the arterial blood and 19.93 for the venous blood; the difference between the two values is not statistically significant.

**Key words:** acid-base balance, horses, anion gap

## Introduction

Basic acid-base balance parameters (ABB): blood pH, hydrogen carbonate concentration (HCO<sub>3</sub><sup>-</sup>), and carbon dioxide molecule pressure (pCO<sub>2</sub>) are used to diagnose: metabolic disorders – metabolic acidosis and alkalosis, the respiratory system diseases – respir-

atory acidosis and alkalosis, calf respiratory syndrome, the digestive system diseases – liver diseases, vomiting, diarrhoea, and the circulatory system diseases in people and animals (Di Bartola 2006, Sławuta et al. 2007). Their diagnostic suitability results from the fact that, in accordance with the Henderson-Hasselbach equation which describes ABB, the blood pH is the

resultant of the metabolic component expressed by  $\text{HCO}_3^-$  and the respiratory component expressed by  $\text{pCO}_2$  (Brobst 1975).

In the veterinary medicine, the ABB parameters are used mainly in sport horses for the assessment of the impact of training on the oxygen content and the carbon dioxide content in the arterial and venous blood (Nolte et al. 1982, Greene et al. 1991, Aguilera-Tejero et al. 2000, Fenger et al. 2000, Bayly et al. 2006) and the impact of high-fat and high-protein feed on maintaining proper blood pH in the course of training (Ferrante et al. 1994, Taylor et al. 1995, Zeyner et al. 2002, Connysson et al. 2006). In sport horses, the ABB should be regularly checked during a sodium bicarbonate therapy. This medicine is applied for treatment of striated muscles diseases resulting from physical effort and may cause metabolic alkalosis (Freestone et al. 1989, Rivas et al. 1997). In the horseracing environment, sodium bicarbonate is used as an illegal alkalizing agent (Waller and Lindinger 2007). ABB is also monitored during anaesthesia (Matthews et al. 1992). The ABB parameters can be determined in the arterial, venous and arterialized capillary blood. It is a well-known fact that examination of the arterial blood enables the most credible insight into current condition of the acid-base balance as a venous blood test often gives a falsified result of acidosis due to the necessary pressure of a vessel (Stainsby and Eitzman 1988, Brechue and Stainsby 1994). What results from the review of the available literature is that, in practice, the ABB parameters are determined both in the arterial and venous blood, and the capillary blood (Nolte et al. 1982, Matthews et al. 1992, Looney et al. 1998, Fenger et al. 2000). In the field practice, the arterial blood is rarely taken from horses. This results from the fact that a sample of venous blood is sufficient for the basic haematological and biochemical examination and it is also generally believed that arterial blood is hard to be drawn. In the case in which it is necessary to draw blood frequently from the same horse, an arterial vessel requires surgical preparation. This refers, first of all, to the carotid artery (Foreman et al. 1996, Fenger et al. 2000). In medical-veterinary practice, horse blood is also drawn from little peripheral arterial vessels i.e. the mandible artery and the lateral metatarsus (Matthews et al. 1992), and, as described in this paper, from the facial artery (Nolte et al. 1982).

A concept of anion gap (AG) is closely related to the acid-base balance. In order to maintain electrical inertness of body fluids, concentration of cations and anions must be equal to each other. However, when we compare the density of main ions –  $\text{Na}^+$  and  $\text{K}^+$  with the density of main anions –  $\text{Cl}^-$  and  $\text{HCO}_3^-$  in blood serum, the so-called anion gap is observed. Its value mainly consists of the negatively charged blood proteins, phosphates and sulphates. Calculation of

AG in mature horses is useful for the diagnosis of metabolic acidosis (Gossett et al. 1987, Constable et al. 1998); a change in the AG value is also used as a prognostic indicator of colic affections (Bristol 1982). In foals, the anion gap is used as a survival ratio for animals undergoing intensive care (Hoffman et al. 1992, Saulez et al. 2007) and in the case of pulmonary diseases (Bedenice et al. 2003). The anion gap in horses changes with age (Gossett and French 1983) and depends on the physiological condition of the animal e.g. it is significantly higher in nursing mares (Harvey et al. 2005).

So far, it has been examined in horses how the values of ABB determined for arterial blood have changed during anaesthesia (Nolte et al. 1982, Mathews et al. 1992) and physical exercise (Greene et al. 1999, Fenger et al. 2000). However, the following question has not been clearly answered: whether and to what extent the basic ABB parameters determined in the arterial and venous blood of horses differ from each other as no comparative study of ABB determined in the arterial and venous blood has been performed so far in study horses of one breed that have not been subject to training, premedication or anaesthesia. The available literature describes diagnostic possibilities offered by the calculation of the anion gap (Bristol 1982, Gossett et al. 1987, Bedenice et al. 2003) yet, the values given by the authors as reference often substantially differ from each other. The comparative study of the anion gap calculated for the arterial and venous blood of horses has not been carried out either.

## Materials and Methods

The study has been performed on 20 clinically normal mature Małopolski horses (M) aged 8-12 years. An arterial blood sample from the facial artery and a sample of venous blood from the external cervical vein were drawn from each animal. Haematological holders manufactured by IDEXX VetStat™, equipped with heparinized syringes into which blood was drawn were used for the research. The facial artery was localized palpably. After skin disinfection and pulse localization, a  $\varnothing$  0.7 mm needle was inserted into the vessel and blood was aspirated without access of air. A place where the needle was inserted was pressed for approx. 2 minutes after taking a blood sample in order to prevent haematoma formation. A set that consisted of a holder equipped with a syringe was, right after taking a blood sample, put in the IDEXX VetStat Electrolyte and Blood Gas Analyzer OPTI4 apparatus and the blood pH was determined as well as partial pressure of  $\text{CO}_2$  ( $\text{pCO}_2$ ), a total content of  $\text{CO}_2$  in blood serum ( $\text{tCO}_2$ ), concentration of bicarbonates ( $\text{HCO}_3^-$ ), and ions  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$ . The

data obtained were used to calculate a value of the anion gap for the arterial and venous blood of each horse according to parameters used by other authors for AG calculation before (Gossett and French 1983, Constable et al. 1998)  $AG = ([Na^+ \text{ mmol/l}] + [K^+ \text{ mmol/l}]) - ([Cl^- \text{ mmol/l}] + [HCO_3^- \text{ mmol/l}])$ . The data were subjected to a statistical analysis. A mean value and standard deviation were calculated and, in order to find differences among the samples, the Wilcoxon's pair sequence test for linked variables was applied. The test was carried out at a level of statistical significance  $p < 0.05$ .

## Results

The obtained values of pH, pCO<sub>2</sub>, tCO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup>, and the concentrations of Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup> determined in the arterial and venous blood are presented in Tables 1 and 2. The examined ABB parameters: pCO<sub>2</sub>, tCO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup> differed from each other significantly in respect of statistics for the two samples examined, whereas the pH of the arterial and venous blood did not differ from each other. The mean values of AG

Table 1. Mean values (and standard deviations: ±) of the acid-base balance parameters of the arterial and venous blood determined at a temperature of 37°C (n = 20).

	Arterial blood	Venous blood
pH	7.44 ± 0.02	7.42 ± 0.01
pCO <sub>2</sub> mmHg	41.00 ± 3.74	48.16* ± 4.91
tCO <sub>2</sub> mmol/l	27.4 ± 1.23	30.25* ± 2.09
HCO <sub>3</sub> <sup>-</sup> mmol/l	26.16 ± 1.14	28.20* ± 1.94

\* – statistically significant differences at  $p < 0.05$

Table 2. Mean serum concentrations (and standard deviations: ±) of ions in the arterial and venous blood determined at a temperature of 37°C (n = 20).

	Arterial blood	Venous blood
Na <sup>+</sup> mmol/l	144.33 ± 3.88	146 ± 3.33
K <sup>+</sup> mmol/l	4.45 ± 0.32	4.38 ± 0.15
Cl <sup>-</sup> mmol/l	100.83 ± 3.37	100.33 ± 3.01

Table 3. Serum values of the anion gap – AG (mean and standard deviations: ±) calculated for the arterial and venous blood (n = 20).

	Arterial blood	Venous blood
AG mmol/l	20.90 ± 1.39	19.93 ± 2.7
Range of the values obtained	19.90 – 23.60	16.50 – 23.90

in the arterial and venous blood did not differ from each other. The values obtained and their range are presented in Table 3.

## Discussion

The mean values of pH, pCO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup> of the arterial and venous blood were consistent with the results obtained by Nolte et al. (1982). The values of pH and pCO<sub>2</sub> of the arterial blood are also within the range provided by Matthews et al. (1992) as these authors did not give any mean values. Looney et al. (1998) studied the influence of the methodology for carrying out examinations of the arterial and venous blood on the obtained results of pH, pCO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup>, tCO<sub>2</sub> and Na<sup>+</sup>, and K<sup>+</sup>. However, their presentation of the data only includes the method by which the determination was performed and not the division into arterial and venous blood, therefore comparison of the results obtained in this study with the former findings is impossible. So far, Fenger et al. (2000) have performed the most detailed comparison in their gasometrical analysis of arterial and venous blood. They examined the pH and pCO<sub>2</sub>, and concentration of the ions: Na<sup>+</sup>, K<sup>+</sup>, and Cl<sup>-</sup> before and after exercise in both types of blood. The authors found that the pH and pCO<sub>2</sub>, and Cl<sup>-</sup> of the arterial blood and venous blood differ from each other significantly in horses without exercise. The values of pH and pCO<sub>2</sub>, and concentration of the ions: K<sup>+</sup> and Cl<sup>-</sup> provided by them are different from those obtained in this study, only the concentration of Na<sup>+</sup> is similar with the one we obtained.

The acid-base balance of the organism is described by Henderson-Hasselbach equation where the blood pH is the resultant of the metabolic component – HCO<sub>3</sub><sup>-</sup> and the respiratory component – pCO<sub>2</sub>.

$$pH = 6.11 + \log \frac{[HCO_3^-]}{pCO_2 \times 0.226}$$

What results from the mutual dependencies of pCO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup> is that respiratory acidosis is characterized by a drop in the pH of arterial blood and an increase in pCO<sub>2</sub>, and, in the case of the acidosis compensation, or equalization – an increase in the concentration of HCO<sub>3</sub><sup>-</sup> and normalization of the pH (Haskins 1977ab, Di Bartola 2006). The comparative study performed here, in which a significantly higher concentration of pCO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup> and lower pH (yet not significant statistically) were found in the venous compared to arterial blood, proves that an analysis of the ABB parameters determined for the venous blood of a healthy horse is not sufficient as it may lead to a wrong diagnosis of the compensated respiratory acidosis.

The obtained values of AG were close to the ones obtained by Shull (1978). The change in the anion gap size is very useful for diagnostics of the colic (Bristol 1982) and metabolic diseases in horses (Gossett et al. 1987), and in the case of foals, the AG value is a good prognostic of their survival rate (Bedenice et al. 2003, Saulez 2007). When making use of AG in diagnostics, one should always take into account the age of the horses examined, in accordance with the suggestion by Gossett and French's (1983). The above-mentioned authors divided the clinically healthy foals into 5 age groups from newborn foals to two-year-old foals and described very clear changes in AG related to the age of horses. Its fluctuations could be explained by a changing concentration of phosphates, calcium, and globulins in the blood serum. The results obtained by Gossett and French (1983) can, therefore, be taken as reference results for age group examinations. The available literature does not mention such an accurate study performed in horses of the different ages, therefore the authors of this paper consider it to be important to calculate the anion gap in mature horses of one breed that have not been subject to exercise and at the age at which they are most frequently used. As, in this study, a significantly different value of  $\text{HCO}_3^-$  between arterial and venous blood was obtained, the value of anion gap for both types of blood samples was calculated.

## Conclusions

The results obtained in this study allow reaching the following conclusions:

1) ABB parameters such as  $\text{pCO}_2$ ,  $\text{HCO}_3^-$  and  $\text{tCO}_2$  determined both in the arterial and venous blood of the Małopolski horses differ from each other significantly;

2) In spite of the lack of statistically significant differences between pH of the arterial and venous blood, the ABB parameters in horses should be determined in the arterial blood, because the performed comparative study proves that the analysis of the ABB parameters determined for the venous blood of a healthy horse may lead to a wrong diagnosis of the compensated respiratory acidosis.

3) The mean value of anion gap in horses aged 8-12 years amounts to 20.9 mmol/l for the arterial blood and 19.93 for the venous blood; the difference between the two values is not statistically significant.

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