

VARIABLES ON THE BLOOD INORGANIC SULPHATE CONCENTRATION IN CATTLE¹

VARIÁVEIS SOBRE A CONCENTRAÇÃO DE SULFATO INORGÂNICO SANGÜÍNEO DE BOVINOS¹

Enrico Lippi Ortolani² Raimundo Alves Barreto Júnior³ Maria Cláudia Araripe Sucupira³
Celso Akio Maruta³

SUMMARY

Fourteen healthy yearling Holstein steers were used to study the influence of the levels of hemolysis and serum storage at different temperatures (4° C, -10°C and at room temperature) on serum inorganic sulphate concentrations. The correlation of inorganic sulphate concentration between plasma and serum was also compared. Three different levels of hemolysis were obtained by adding distilled water to the blood. Precision studies were undertaken to determine the viability of the turbidimetric technique used to measure the inorganic sulphate concentration. No difference in the inorganic sulphate concentration was found between serum and plasma ($p>0.91$), between serum kept at different temperatures ($p>0.87$) and between hemolysed and normal serum ($p>0.85$). Increasing levels of hemolysis did not influence the serum inorganic sulphate concentration. The turbidimetric technique was suitable and accurate for routine analysis of serum inorganic sulphate. It was concluded that serum kept at room temperature can be used for practical routine surveys to assess the sulphur status in cattle.

Key words: inorganic sulphate, serum, blood tests, sulphur, cattle.

RESUMO

Quatorze garrotes sadios holandeses, de um ano de idade, foram utilizados num experimento para verificar a influência do grau de hemólise e de diferentes temperaturas de estoque do soro (4°C, -10°C e temperatura ambiente) sobre a concentração de sulfato inorgânico sérico. Também, foi comparada essa concentração em plasma e soro. Três diferentes graus de hemólise foram obtidos em amostras de soro, através de crescentes adições de água destilada no sangue total. Foram

realizadas provas de precisão para avaliar a viabilidade de um teste turbidimétrico para a determinação de sulfato inorgânico. Não ocorreram diferenças nos teores de sulfato inorgânico no plasma e no soro ($p>0,91$), no soro mantido a diferentes temperaturas de estocagem ($p>0,87$) e em amostras hemolisadas e normais ($p>0,85$). A crescente presença de hemólise no soro não interferiu sobre os teores de sulfato inorgânico nas amostras. O teste turbidimétrico provou ser adequado e preciso para análises rotineiras de sulfato inorgânico sérico. Sugere-se que nos levantamentos rotineiros de avaliação dos teores de enxofre em bovinos seja utilizado soro, mantido em temperatura ambiente.

Palavras-chave: sulfato inorgânico, soro, determinação, variáveis, enxofre, bovinos.

INTRODUCTION

Sulphur is an important macroelement to cattle and other domestic animals. It takes part in the structure of amino acids, such as methionine and cystine, found in most proteins. Sulphur is also important to cellulose and organic matter fermentation by rumen microorganisms. Cattle with sulphur deficiency can exhibit slow growth, loss of body weight, drop in milk production, unthriftiness and death (McDOWEEL, 1999). Recently, a study carried out by BARRETO JÚNIOR (1996) showed that at least 30% of the 480 *Brachiararia sp* samples, collected from different regions in the state of São

¹Este experimento foi parcialmente financiado pelo CNPq.

²Médico Veterinário, MSc., PhD., Professor, Departamento de Clínica Médica da Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo. Av. Prof. Dr. Orlando Marques n. 87, "Cidade Universitária", 05508-000, São Paulo, SP, Brazil. E-mail: ortolani@usp.br. Autor p/ correspondência.

³Médico Veterinário, MSD, Pós-graduandos.

Paulo, contained very low levels of sulphur. This same study revealed that 21% of cattle presented in poor sulphur status, as determined by blood inorganic sulphate concentration.

The higher the dietary sulphur intake, the greater the blood inorganic sulphate concentration. Thus, the blood sulphate concentration is considered a good marker of the sulphur status of cattle, especially when they are fed a sulphur deficient diet (QI *et al.*, 1992; BARRETO JÚNIOR, 1996). Nevertheless, the determination of blood inorganic sulphate has been frequently carried out in controlled experiments, but scarcely under field condition. Blood sulphate concentration is mostly determined by a turbidimetric technique described by KRIJGSHELD *et al.* (1979), though no precision studies were carried out by the authors. Many times blood samples collected under these conditions are submitted to varying temperatures and delayed processing and hemolysis can supervene. Blood samples kept at room temperature or that have mild levels of hemolysis can result in a gradual and significant increase in serum inorganic phosphorus concentrations (DAYRELL *et al.*, 1973). During storage, the erythrocytes can convert ATP to ADP, liberating in the process a molecule of inorganic phosphorus. As most phosphorus is located within erythrocytes rather than serum, the higher the level of hemolysis, the greater the serum inorganic phosphorus concentration. Sulphur can also be found in erythrocytes and serum. However, unlike phosphorus, no studies have been carried out to determine if storage at different temperatures and hemolysis increase inorganic sulphate concentrations.

Thus, the main objective of this experiment was to study the influence of storage temperature and hemolysis on serum inorganic sulphate concentrations in cattle. The correlation of inorganic sulphate concentration between plasma and serum was also compared. Precision studies of a turbidimetric technique to measure inorganic sulphate were carried out.

MATERIALS AND METHODS

Fourteen healthy yearling Holstein steers were used in this experiment. The animals were fed coast-cross (*Cynodon dactylon*) hay and common salt *ad libitum*, for two months before the beginning of the experiment. The chemical composition of the

coast-cross hay was: dry matter 86.4%; crude protein 7.6%; ether extract 1%, crude fibre (CF) 34%, non-nitrogen extractives 51.4%, mineral content 6%, sulphur 0.17%. Standard methods were used for determination of chemical composition in the samples of coast-cross hay (AOAC, 1985).

The experiment was divided into three different periods of one week each. Firstly, the concentration of inorganic sulphate in plasma and serum was compared, followed by the evaluation of the influence of the degree of hemolysis on the inorganic sulphate concentration, and finally by the effect of storage temperature on serum inorganic sulphate levels.

The steers were always bled at the 4thh after feeding. Blood was drawn from the jugular vein into vacutainer tubes with no anticoagulant to obtain serum and with sodium heparinate for plasma. The blood samples were centrifuged within two hours of collection and plasma and serum stored at 5°C until analysed. The blood inorganic sulphate levels were determined by a turbidimetric technique described by KRIJGSHELD *et al.* (1979). To 0.5mℓ of serum 2mℓ of trichloroacetic acid (5%) was added. The mixture was allowed to stand for 10min at room temperature. After centrifugation, 1mℓ of the clear supernatant was added to 0.25mℓ BaCl₂ reagent (20g BaCl₂ and 10g dextran in 1ℓ water) and the absorbance was read after 35min at 350nm.

Precision studies were undertaken to determine the viability of the turbidimetric technique. For linearity studies the turbidimetric technique was tested through a series of aqueous standards by serially diluting a 1.8mMol/ℓ (NH₄)₂SO₄ stock solution. Within-run precision was assessed by analysis of aqueous standards with inorganic sulphate concentrations ranging from 0.20 to 1.8mMol/ℓ. For within-run study, 15 samples were analysed in the same run. Between-run precision was assessed in the three samples with triplicates determined in three different days. Analytical recovery trial was performed in which 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2mMol/ℓ of inorganic sulphate were added to a serum pool containing 0.60mMol/ℓ of inorganic sulphate.

Hemolysis was induced by adding three different amounts of distilled water (0.65mℓ; 0.85mℓ and 1.0mℓ) to 10mℓ of blood immediately after its collection, as described elsewhere (DAYRELL *et al.*, 1973). To quantify the presence of hemolysis,

serum free hemoglobin concentrations were determined by a classical technique (VAN KAMPEN & ZIJLSTRA, 1961). Only hemoglobin concentrations between 2.9 and 14.1g/l were used in the study.

The influence of temperature on the inorganic sulphate concentration was analysed using serum. Serum samples were divided in three aliquots and kept for 24h at 4°C, -10°C or room temperature. The inorganic sulphate collection was determined afterwards.

For the linearity and recovery studies of the turbidimetric technique the correlation coefficient was assessed; the relationship between expected sulphate and observed sulphate was evaluated by linear regression and analysis of variance. For the within-run, between-run assays the overall mean, standard deviation and coefficient of variation were calculated. For other comparisons, differences between means were determined using a paired t test. A pooled sample of hemolysed serum was compared to normal serum by the same test. The effect of the level of hemolysis on the inorganic sulphate concentration was evaluated by linear regression and analysis of variance (SNEDCOR & COCKRAM, 1967).

RESULTS

The precision studies are presented in figure 1 and 2, and table 1. There was a high linearity ($r=0.926$; $p<0.0001$) between the expected sulphate level and the observed sulphate level (Figure 1). The between-run standard deviation (SD) was only marginally greater than the within-run SD (Table 1). Nevertheless, the coefficient of variation in both assays was small (not more than 7.7%) revealing the low variability of the between-run and within-run data. The higher the level of inorganic

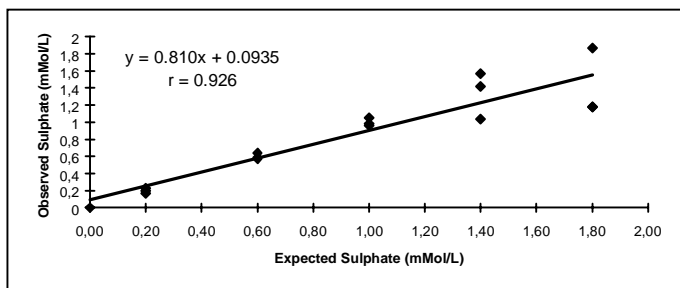


Figure 1 – Relationship between expected sulphate level and observed sulphate level of the standard curve.

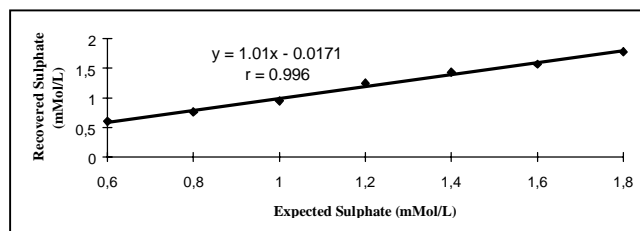


Figure 2 – Relationship between expected sulphate level and recovered sulphate level in a serum sample added of different standard solution.

sulphate added to the serum, the greater the inorganic sulphate recovered ($r=0.996$; $p<0.0001$; Figure 2).

There were no differences in the inorganic sulphate concentration between plasma and serum ($p>0.91$), between normal and hemolysed serum ($p>0.85$) and between serum kept at different temperatures ($p>0.87$; Table 2). There was no influence of the level of hemolysis on the serum inorganic sulphate concentration ($p>0.65$; $r=-0.072$). The level of free hemoglobin in the serum varied from 2.9 to 17.3g/l with a mean of 8.2 ± 4.7 g/l.

DISCUSSION

Adequate linearity, reproducibility and recovery were obtained in the precision studies of the turbidimetric technique, to measure inorganic sulphate, described by KRIJGSHELD *et al.* (1979); (Table 1; Figures 1 and 2). This technique is easy to be performed, very economical (none of the reagents are expensive), and is suitable and accurate for rapid routine analysis of serum inorganic sulphate.

The concentration of inorganic sulphate was similar between serum and plasma (Table 1). The clotting process removes from plasma basically some proteins (thrombin, fibrin etc) and some calcium, but not sulphate (KANEKO *et al.*, 1997). The sulphur present in these proteins is in the form of sulphur-containing amino acids which are not measured by the turbidimetric method. Serum is easier to obtain than plasma under routine conditions, and should therefore be used to assess the sulphur status of cattle.

The level of hemolysis produced in the serum samples varied from mild to intense. Nevertheless, hemolysis did not interfere with the serum inorganic sulphate

Table 1 – Precision studies of a turbidimetric technique to measure inorganic sulphate in serum. Overall mean values, standard variation (SD) and coefficient of variation (CV) of the within-run and between-run assays.

Parameters	Within-run					Between-run		
Standard, mMoℓ/ℓ	0.2	0.6	1.0	1.4	1.8	0.5	1.0	1.5
Mean, mMoℓ/ℓ	0.21	0.58	1.05	1.43	1.82	0.52	1.03	1.46
SD, mMoℓ/ℓ	0.01	0.02	0.02	0.03	0.05	0.04	0.04	0.05
CV (%)	4.76	3.45	1.90	2.1	2.74	7.69	3.88	3.42

concentrations compared to normal serum (Table 1). This lack of interference may be related to the form of sulphate measured by the turbidimetric method. According to QI *et al.* (1992) about 93% of the serum and plasma sulphate is in an inorganic form, while the remaining sulphate is bound to protein. Conversely, most of the erythrocyte sulphate is bound to protein. The turbidimetric method precipitates the protein, through the use of trichloroacetic acid (5% v/v), carrying with it the organic sulphate. Consequently, the sulphate in this fraction is not measured by the turbidimetric method. The small amount of inorganic sulphate present in the serum originating from hemolysed erythrocytes was not significant to increase the total concentration of inorganic sulphate measured.

There was no effect of the temperature during storage on the inorganic sulphate levels. Thus, serum kept at room temperature can be used for practical routine surveys.

In conclusion, either serum or plasma can be used to measure the sulphur status in cattle, but for practical routine surveys, serum is suggested to be used; different levels of blood hemolysis and temperatures of storage do not interfere with accurate measurement of the serum inorganic

Table 2 – Mean values of inorganic sulphate in serum and plasma, hemolysed normal serum and serum kept at different temperatures for 24h.

Treatments	Sample		Serum		Temperature (°C)		
	serum	plasma	hemolysis	normal	-10	4	24
Sulphate (mMoℓ/ℓ)	0.71	0.70	0.66	0.69	0.71	0.72	0.71
Standard deviation	0.30	0.30	0.31	0.28	0.27	0.30	0.26

sulphate concentration. The turbidimetric technique described by KRIJGSHELD *et al.* (1979) is suitable and accurate for routine analysis of serum inorganic sulphate.

ACKNOWLEDGMENTS

The authors express their appreciation to Clara Satsuki Mori and Regina Miekeo Sakata Mirandola for technical support in the laboratory analysis.

REFERENCES

- AOAC. **Official and tentative methods of analysis**. 6 ed. Washington : Georg Barita, 1985. 1015p.
- BARRETO, JÚNIOR, R. A **Influência de algumas variáveis sobre a concentração de sulfato inorgânico sanguíneo de bovinos. Estudo da relação das concentrações de enxofre de capins e de sulfato inorgânico sérico de bovinos, criados no Estado de São Paulo**. São Paulo-SP, 1996. 53p. Dissertação (Mestrado em Clínica Veterinária) - Curso de Pós-graduação em Medicina Veterinária, Universidade de São Paulo, 1996.
- DAYRELL, M.S., LOPES, H.S., SAMPAIO, I.V., *et al.* Fatores a serem considerados na interpretação de valores analíticos de fósforo inorgânico no soro sanguíneo de bovinos. **Pesq Agrop Bras**, v.8, p.43-47, 1973
- KANEKO, J.J., HARVEY, J.H., BRUSS, M.L. **Clinical biochemistry of domestic animals**. 5 ed. San Diego : Academic , 1997. 932p.
- KRIJGSHELD, K.R., FRANKENA, H., SCHOLTENS, E., *et al.* Absorption, serum levels and urinary excretion of inorganic sulfate after oral administration of sodium sulfate in the conscious rat. **Bioch Biophys Acta**, v.586, n.3, p.492-500, 1979.
- MCDOWELL, L.R. **Minerais para ruminantes sob pastejo em regiões tropicais, enfatizando o Brasil**. 3 ed. Gainesville : University of Florida, 1999. 92p.
- SNEDCOR, G.W., COCKRAM, W.G. **Statistical methods**. 6 ed. Ames : Iowa State University, 1967. 593p.
- QI, K., LU, C.D., OWENS F.N., *et al.* Sulfate supplementation of Angora goats: Metabolic and mohair responses. **J Ani Sci**, v.70, p.2828-2837, 1992.
- VAN KAMPEN, E.J., ZIJLSTRA, W.G. Standardization of hemoglobimometry. II The hemiglobincyanide method. **Clin Chim Acta**, v.6, p.538- 544, 1961.