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Serum protein concentrations, including acute phase proteins, in calves with hepatogenous photosensitization

[*Teores séricos de proteínas, inclusive proteínas de fase aguda, em bovinos com fotossensibilização hepatógena*]

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ABSTRACT

One hundred 6- to 12-month-old Nelore calves were allotted into control group (G1; 50 healthy calves) and photosensitization group (G2; n= 50). Blood samples were collected 12 to 24 hours after the onset of dermatitis (M1), and 15 to 30 days after that (M2), at time of resolution of clinical signs. Serum protein electrophoresis was performed by means of sodium dodecyl sulphate–polyacrylamide gel electrophoresis. Eighteen serum proteins with molecular weights ranging from 16,000 to 189,000 daltons (Da) were identified in all calves. In M1 and M2 serum concentrations of proteins with molecular weights of 115,000Da (ceruloplasmin), 61,000Da (α_1-antitrypsin), 45,000Da (haptoglobin), and 40,000Da (acid glycoprotein) were significantly increased in calves. In conclusion, measurement of serum acute phase protein concentrations may be useful in monitoring the progression of bovine hepatogenous photosensitization, including guide probable alteration on therapeutic procedures.

Keywords: calf, hepatogenous photosensitization, acute phase protein

RESUMO

Foram examinados 100 bezerros da raça Nelore com 6 a 12 meses de idade, distribuídos em: grupo controle (G1; 50 bezerros sadios) e grupo fotossensibilização (G2; n= 50). As amostras de sangue foram coletadas 12 a 24 horas após o início da dermatite (M1) e 15 a 30 dias após (M2), época da cura das lesões cutâneas. O proteinograma sérico foi obtido por eletroforese em gel de acrilamida. Em todos os bezerros foram identificadas 18 proteínas com pesos moleculares (PM) entre 16.000 a 189.000 dáltons (Da). Em M1 e M2, as concentrações séricas das proteínas de PM 115.000Da (ceruloplasmina), 61.000Da (α_1-antitripsina), 45.000Da (haptoglobina) e 40.000Da (glicoproteína ácida) foram significativamente maiores em bezerros com fotossensibilização hepatógena em comparação com aquelas dos animais do grupo-controle. A determinação dos teores séricos de proteínas de fase aguda pode ser útil no monitoramento da progressão da fotossensibilização hepatógena em bovinos, inclusive orientando possíveis alterações em procedimentos terapêuticos.

Palavras-chave: bezerro, fotossensibilização hepatógena, proteína de fase aguda

INTRODUCTION

Bovine hepatogenous photosensitization has been reported in Brazil since 1975 (Dobereiner et al., 1976). Hepatotoxic saponins (Meagher et al., 1996; Cruz et al., 2000) and/or sporidesmin, mycotoxin from *Pithomyces chartarum* spores (Dobereiner et al., 1976; Fagliari et al., 1993ab; Fioravanti, 1999), have been incriminated as underlying cause of the disease in cattle grazing *Brachiaria decumbens* pastures. The disease causes substantial losses in animal production in several countries, including Brazil, where *B. decumbens* is a major forage provided to cattle.

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As bovine hepatogenous photosensitization is an inflammatory condition, it is likely to induce changes in the serum concentration of acute phase proteins that could be identified by means of electrophoresis. If so, measuring changes in the acute phase proteins might be useful to detect and monitor progression of bovine photosensitization. Cellulose acetate (Fagliari et al., 1991) and agarose gel electrophoresis (Keay and Doxey, 1982) have been used to analyze plasma protein concentrations, but these techniques are limited, because they can identify only 5 to 7 groups of proteins. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), on the other hand, can be used to indetify much more proteins, to separate proteins present in very low quantities, and can be performed on small serum or plasma samples (Gordon, 1975). The technique has been used to analyze plasma or serum protein concentrations (Coyne et al., 1990; Fagliari et al., 2003) but has not been previously used to determine changes in serum protein concentrations associated with hepatogenous photosensitization. The purpose of this study was to determine, by SDS-PAGE, the spectrum of serum protein alterations in bovine hepatogenous photosensitization, including acute phase proteins, and the rate at which these changes occur.

MATERIALS AND METHODS

One hundred 6- to 12-mounth-old Nelore calves were allotted into control group (G1; 50 healthy calves) and photosensitization group (G2; n= 50). Affect calves were kept or grazed on *Brachiaria decumbens* pastures. Diagnosis of hepatogenous photosensitization was based on history, clinical signs, and laboratorial findings (Towers and Stratton, 1978; Fagliari et al., 1993b). Blood samples were collected 12 to 24 hours after the onset of dermatitis (M1), and 15 to 30 days after that (M2), at time of resolution of skin lesions. Serum protein concentration was determined by SDS-PAGE (Weber and Osborn, 1969). Gels were stained for 5 minutes in 200ml of coomassie brilliant blue and destained in 7% acetic acid solution until the gel background was completely clear. Concentration of protein fractions was determined by use of computer-assisted videodensitometry[1] Proteins were identified by use of reference markers[2] with molecular weights of 29,000, 45,000, 66,000, 97,400, 116,000, and 205,000 daltons (Da) and by comparison with electrophoretic mobility of purified albumin[1], transferrin[1], haptoglobin[1], ceruloplasmin[1], IgG[1], and α_1-antitrypsin[1].

Results of determinations were compiled, and results are presented as mean±SD. Data were analyzed by use of repeated measure ANOVA. Means of interest were compared by use of Tukey test. A P value <0.05 was considered significant.

RESULTS AND DISCUSSION

Eighteen serum proteins with molecular weights ranging from 16,000 to 189,000Da were identified in all calves. Serum concentrations of proteins with molecular weights of 115,000Da (ceruloplasmin), 61,000Da (α_1-antitrypsin), 45,000Da (haptoglobin), and 40,000Da (acid glycoprotein) were significantly increased in calves with hepatogenous photosensitization compared with concentrations in control calves (Table 1). These proteins have previously been identified as acute phase proteins (Gruys et al., 1994). Acute phase proteins are synthesized by the liver in response to inflammatory cytokines, particularly interleukin-6 (Heindrich et al., 1990).

Twelve to 24 hours after onset of dermatitis (M1), the protein with the highest percentage increase (377.3±43.6%) in concentration was haptoglobin, compared with control group values (Table 2). At this time, percentage increase for ceruloplasmin, antitrypsin, and acid glycoprotein were 309.1±40.7%, 251.7±23.8%, and 146.2±22.5%, respectively. Fifteen to 30 days after the onset of dermatitis (M2), at time of resolution of skin lesions, the protein with the highest percentage increase (122.7±9.8%) in concentration was again haptoglobin, compared with control group values. At this time, percentage increase for ceruloplasmin, α_1-antitrypsin, and acid glycoprotein were 51.4±8.9%, 42.9±7.5%, and 89.2±7.4%, respectively, significantly lower than M1 values. Although acute phase protein concentrations in calves with photosensitization obtained in M1 have been lower than M2 (Table 1), these values were significantly higher than those verified in control calves, suggesting some persistent active lesion, probably in the liver, as showed in literature (Alessi et al., 1994; Fioravanti, 1999; Cruz et al., 2000). Even though acute phase proteins were produced in affected liver by stimulation of underlying cause of photosensitization, percentages increase were similar to those showed in calves with experimentally induced pneumonic pasteurellosis (Fagliari et al., 2003).

[1]Shimadzu CS 9000, Shimadzu Corp. - Kyoto, Japan.
[2]Sigma Chemical Co. - St Louis, USA.

Table 1. Serum protein concentrations (means±SD), determined by means of sodium dodecyl sulphate-polyacrylamide gel electrophoresis, in healthy calves (group 1) and in calves with hepatogenous photosensitization (group 2) obtained 12-24 hs after the onset of dermatitis (M1) and, 15-30 days after that (M2), at time of resolution of clinical signs

Protein and group (G)	M1	M2
Total serum protein (g/dl)		
G1	6.43±0.52	6.51±0.60
G2	6.71±0.66	6.60±0.57
No identified‡ (MW, 189,000; mg/dl)		
G1	10.7±2.4	11.4±2.9
G2	11.9±3.0	12.6±3.2
Immunoglobulin A (MW, 168,000; mg/dl)		
G1	258.3±15.1	265.4±13.9
G2	249.0±18.7	256.1±16.7
No identified‡ (MW, 145,000; mg/dl)		
G1	192.4±20.6	186.3±21.6
G2	209.6±18.3	198.7±23.1
Ceruloplasmin (MW, 115,000; mg/dl)		
G1	12.0±4.1*	13.4±2.7*
G2	49.1±6.2a	20.3±3.1b
No identified‡ (MW, 103,000; mg/dl)		
G1	36.8±4.1	35.0±3.5
G2	34.3±2.9	36.8±4.0
Phosphorylase A (MW, 91,000; mg/dl)		
G1	45.7±3.8	44.0±3.2
G2	47.0±4.2	48.1±5.0
Transferrin (MW, 80,000; mg/dl)		
G1	264.9±19.5	271.1±21.7
G2	252.7±20.9	264.3±25.3
Albumin (MW, 66.000; g/dl)		
G1	3.31±0.26	3.41±0.29
G2	3.18±0.40	3.27±0.33
IgG high-chain (MW, 62,000; mg/dl)		
G1	564.4±50.6	572.3±46.7
G2	581.7±55.1	570.0±51.0
α_1-antitrypsin (MW, 59,000; mg/dl)		
G1	23.4±3.6*	21.4±2.2*
G2	82.3±14.4a	30.6±4.3b
No identified‡ (MW, 53,000; mg/dl)		
G1	18.6±3.1	17.6±3.4
G2	21.4±4.0	20.4±3.9
Haptoglobin (MW, 45,000; mg/dl)		
G1	32.3±4.0*	36.5±3.7*
G2	154.2±26.2a	81.3±10.4b
Acid glycoprotein (MW, 40,000; mg/dl)		
G1	14.5±2.8*	11.2±1.9*
G2	35.7±5.8a	21.2±2.8b
No identified‡ (MW, 38,000; mg/dl)		
G1	28.6±3.9	26.0±3.5
G2	30.5±3.4	31.1±4.0
No identified‡ (MW, 36,000; mg/dl)		
G1	141.7±12.6	152.1±15.0
G2	150.8±14.1	161.4±18.3
IgG light-chain (MW, 32,000; mg/dl)		
G1	822.7±53.9	836.1±60.3
G2	880.1±76.3	902.0±82.4
No identified‡ (MW, 26,000; mg/dl)		
G1	371.6±28.9	360.8±31.9
G2	392.3±32.6	381.7±40.6
Hemoglobin (MW, 16,000; mg/dl)		
G1	30.6±5.1	28.7±4.5
G2	36.0±6.4	33.2±5.0

*Significantly (P<0.05) different from values for calves with hepatogenous photosensitization.
In each row, values with different letters were significantly (P<0.05) different from each other.
MW= molecular weight.
‡No identified proteins because there were no purified proteins to compare (like ceruloplasmin, haptoglobin, transferring)

Table 2. Serum acute phase protein concentrations (means±SD) and percentage increase in serum acute phase protein concentrations, compared with healthy calves concentrations, in calves with hepatogenous photosensitization obtained 12-24 hs after the onset of dermatitis (M1) and, 15-30 days after that (M2), at time of resolution of clinical signs

Protein	M1	M2
Ceruloplasmin	G1: 12.0mg/dl	G1: 13.4mg/dl
	G2: 49.1mg/dl	G2: 20.3mg/dl
	309.1±40.7%	51.4±8.9%
α_1-antitrypsin	G1: 23.4mg/dl	G1: 21.4mg/dl
	G2: 82.3mg/dl	G2: 30.6mg/dl
	251.7±23.8%	42.9±7.5%
Haptoglobin	G1: 32.3mg/dl	G1: 36.5mg/dl
	G2: 154.2mg/dl	G2: 81.3mg/dl
	377.3±43.6%	122.7±9.8%
Acid glycoprotein	G1: 14.5mg/dl	G1: 11.2mg/dl
	G2: 35.7mg/dl	G2: 21.2mg/dl
	146.2±22.5%	89.2±7.4%

CONCLUSION

Measurement of serum acute phase protein concentrations may be useful in monitoring the progression of bovine hepatogenous photosensitization, including guide probable alteration on therapeutic procedures.

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