

Serum levels of proteins and acute-phase proteins in captive emus (*Dromaius novaehollandiae*) of different ages

[Teores séricos de proteínas, inclusive proteínas de fase aguda, em emus (*Dromaius novaehollandiae*) de diferentes faixas etárias e criados em cativeiro]

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ABSTRACT

Protein electrophoresis is a relatively simple technique that allows separating serum protein fractions, and provides important information in the investigation and diagnosis of several diseases. This study determined the levels of acute-phase proteins in the serum of healthy, captive emus (*Dromaius novaehollandiae*). Animals were divided into two groups (n=11 in each) based on age, with 1-year-old and 4-year-old emus. Acute-phase proteins were separated by SDS-PAGE. Ceruloplasmin, transferrin, albumin, haptoglobin, acidic glycoprotein, IgA, and IgG were detected in the serum of all animals. Protein profiles varied significantly with age (P<0.05). Individuals in the 4-year-old emus group had higher values of ceruloplasmin, transferrin, albumin, haptoglobin, and acidic glycoprotein, compared with the group with 1-year-old animals, showing the role of age in the protein profile of this species. Reference values for acute-phase proteins in healthy emus may be useful in the evaluation of health status and in the diagnosis of diseases affecting the species.

Keywords: Acute-phase response, polyacrylamide gel electrophoresis, infection, emus

RESUMO

A eletroforese de proteínas é um método relativamente simples, que permite a separação das proteínas do plasma em frações. Sua interpretação fornece informações importantes para a investigação e o diagnóstico de inúmeras doenças. O objetivo deste estudo foi o de determinar a concentração das proteínas de fase aguda no soro de emus (*Dromaius novaehollandiae*) hígidos e criados em cativeiro. As aves foram separadas em dois grupos: grupo 1: (n=11), aves com um ano de idade; grupo 2: (n=11), aves com quatro anos de idade. As proteínas de fase aguda foram separadas por eletroforese em gel de poliacrilamida (SDS-PAGE). Identificaram-se as proteínas ceruloplasmina, transferrina, albumina, IgG, haptoglobina, glicoproteína ácida, IgA e IgG no soro de todos os emus. Houve diferença (P<0.05) entre os traçados eletroforéticos em função da faixa etária. As aves do grupo 2 apresentaram valores superiores de ceruloplasmina, transferrina, albumina, haptoglobina e glicoproteína ácida quando comparadas às aves do grupo 1. Conclui-se que o perfil eletroforético de emus sofre alterações conforme a idade analisada. O estabelecimento de valores de referência para as proteínas de fase aguda de emus hígidos poderá auxiliar estudos futuros na avaliação da saúde assim como no diagnóstico de doenças em emus.

Palavras-chave: resposta de fase aguda, eletroforese em gel de poliacrilamida, infecção, emus

INTRODUCTION

Electrophoresis of proteins is a common technique for laboratory diagnosis in veterinary

medicine. Though electrophoresis enables to obtain important information about the protein fractions, even acute-phase proteins (APPs) that increase after inflammation and infection so often diagnosed in poultry, the technique remains

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largely unexplored in studies about the emu, *Dromaius novaehollandiae*.

Acute-phase response is a dynamic process that comprises systemic and metabolic changes, which act as unspecific defense mechanism against insult before the specific acquired immunity emerges (Murata, 2004). Such response is triggered by cytokines that act as messengers between the insult site and hepatocytes that produce APP (Petersen *et al.*, 2004). As a rule, APPs are defined as proteins whose serum concentration increases considerably as early as a few hours after a primary stimulus (Gabay and Kushner, 1999). A drop in APP serum levels indicates insult remission (Ballou and Kushner, 1992). Some APPs play a role in the regulation of inflammatory response, promoting the clearance of numerous substances produced by damaged cells or acting directly on the inflammatory response. In addition, APPs are able to modulate the immune response (Thomas, 2000).

In poultry, the measurement of APP serum levels may be useful when monitoring health conditions (Tohjo *et al.*, 1995) and characterizing muscle lesions present in areas where inspection is comparatively complex after slaughter (Saini e Webert, 1991). However, it should be noted that establishing severity and chronic nature of any lesion diagnosed in poultry meat prior to slaughter is essential as a measure to prevent health problems to consumers (Chamanza *et al.*, 1999).

Therefore, the present study describes a polyacrylamide gel electrophoresis protocol (SDS-PAGE) to detect APP serum levels in sera of healthy *D. novaehollandiae* individuals of different age groups.

MATERIALS AND METHODS

Twenty-one healthy emus reared in the nursery managed by Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), Rio de Janeiro (RJ), Brazil were used. The Emus were sorted into two age groups: G1 (n=11), formed by 1-year-old birds, and G2 (n=11), which included 4-year-old birds. The experimental protocol was approved by the Ethics Commission of UENF (n^o 268).

Emus were reared free in the paddocks. G1 and G2 emus were fed commercial feed containing minimum protein levels of 18% and 12% protein, respectively, depending on the nutritional needs of each group. All animals were given water *ad libitum* throughout the experiment.

Approximately 2mL blood samples were collected by venipuncture of the right jugular vein. Collections were carried out in the morning, and samples were immediately centrifuged. The sera obtained was frozen at -20°C upon use in the laboratory tests. Total protein levels in serum (g/L) were measured according to the biuret protein assay using a set of diagnostic reagents (Labtest, Sistema de Diagnósticos Ltda., Lagoa Santa, Brazil) and spectrophotometric readings (E-225-D, Labquest, CELM, Lagoa Santa, Brazil). Protein fractions were determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Molecular weights and concentrations of protein fractions were determined by computed videodensitometry (CS 9000, Shimadzu Corp., Kyoto, Japan). Reference markers (Sigma Chemical Co., St Louis, USA) were used to characterize proteins, with molecular weights of 29, 45, 66, 97.4, 116, and 205kDa. Also, electrophoretic migration of proteins was compared with that of pure proteins including albumin, transferrin, haptoglobin, ceruloplasmin, IgA, IgG, and acidic glycoprotein.

The values obtained were compiled and presented as means \pm SD. The data were analyzed using an ANOVA with repeated measures. Significant means were compared using the Tukey test with significance at $P < 0.05$.

RESULTS AND DISCUSSION

Twenty-seven proteins with molecular weights between 23 and 231kDa were identified in sera of all G1 and G2 emus. Serum levels of proteins of 105kDa (celuloplasmin), 175kD (IgA), 81kDa (IgG), 81kDa (transferrin), 61kDa (albumin), 41kDa (haptoglobin), and 38kDa (acidic glycoprotein) were nominally identified (Tab. 1).

Serum levels...

Table 1. Serum protein concentrations (means \pm SD) in healthy 1-year-old (G1) and 4-year-old (G2) emus determined using sodium dodecyl sulphate-polyacrylamide gel electrophoresis

Protein	Groups	
	G1 (one year old)	Group 2 (four year old)
Total serum protein (g/dL)	4.05 \pm 0.46	4.24 \pm 0.35
Albumin (mg/dL)	2.72 \pm 3.38b	3.06 \pm 2.28a
Ceruloplasmin (mg/dL)	16.68 \pm 8.04b	18.21 \pm 6.33a
Transferrin (mg/dL)	297.19 \pm 59.74b	323.91 \pm 44.69a
Haptoglobin (mg/dL)	13.11 \pm 5.83b	17.54 \pm 4.21a
Acid glycoprotein (mg/dL)	9.51 \pm 4.31b	14.48 \pm 15.77a
Immunoglobulin A (mg/dL)	102.90 \pm 25.15	98.45 \pm 17.92
Immunoglobulin G (mg/dL)	405.12 \pm 84.33	397.91 \pm 121.85

* Significantly different ($P < 0.05$) between groups.

Total protein levels did not vary between G1 and G2. However, a significant difference ($P < 0.05$) was observed between the protein fraction levels determined by electrophoresis for both age groups. G2 emus had higher albumin, ceruloplasmin, transferrin, haptoglobin, and acidic glycoprotein values.

A study about the ostrich *Rhea Americana* of different ages also revealed the differences in electrophoretic run due to age. Older birds had higher APPs, compared with young animals. These results are due to the more intense exposure of animals of each age group to pathogens (Conrado *et al.*, 2007).

In an immune stress situation, an organism reacts first with an innate response that precedes specific responses. The acute-phase response (APR) is described as an intense systemic reaction to local or systemic changes. Infection, tissue lesion, trauma, surgery, neoplastic growths, and immune disorders trigger APR. For Chamanza *et al.* (1999), increased transferrin levels were observed in broiler chickens after a *Staphylococcus aureus* infection and administration of turpentine. Yet, transferrin levels did not vary with lesion severity or evolution. In other words, mild, severe, acute, and chronic lesions triggered similar responses. Therefore, the authors claim that transferrin should not be measured as a tool to determine the severe or chronic characteristic or lesions in broiler chickens.

Chickens carrying the avian infectious bronchitis virus (IBV) or the infectious laryngotracheitis virus present significantly high levels of

haptoglobin (Nazifi *et al.*, 2011) and acidic glycoprotein (Nakamura *et al.*, 1996). Tracheitis, bronchitis, and edema in the trachea, bronchi, and aerial sacs point to a generalized secondary inflammatory reaction to IBV, producing, releasing and therefore rising serum levels of haptoglobin. Holt and Gast (2002) observed a significant increase in acidic glycoprotein levels in sera of chicken infected with *Salmonella enteritidis*. Even higher acidic glycoprotein levels were detected in sera of chicken infected with *S. enteritidis* under stress conditions. The authors maintained that acidic glycoprotein was a fast and reliable indicator of infection of chickens with the bacterium.

Birds and broiler chickens inoculated with *E. coli* lipopolysaccharides had significantly high serum levels of acidic glycoproteins and haptoglobin (Nakamura *et al.*, 1998; Miller *et al.*, 2007). These changes were detected as early as 6h after inoculation of lipopolysaccharides, and persisted for 4 days. The highest acidic glycoprotein and haptoglobin levels were recorded 2 days after inoculation. However, no changes suggestive of infection with lipopolysaccharides were noticed in gross inspection and histologic tests of samples of liver, spleen, kidneys, heart, lungs, eyes, gastrointestinal tract, trachea, and bursa of Fabricius. Lipopolysaccharides present in the cell wall of Gram-negative bacteria trigger an innate immune response by eliciting the production of pro-inflammatory cytokines that, in turn, stimulate APP production (Sijben *et al.*, 2013). The measurement of APP levels after inoculation with lipopolysaccharides is a useful tool in the evaluation of induced innate immunity (Martinely and Reichhart, 2005).

The results obtained show that, while the response pattern mediated by APPs may differ across the various kinds of stimuli and organisms, changes in APP serum levels are sensitive, though unspecific, indicators of the presence of inflammation. However, previous studies have shown that a variety of factors unrelated to diseases may rise serum APP levels in birds, such as stress, for instance. For this reason, reference values may be useful in the interpretation and comparison of protein levels of healthy and sick emus, in addition to determining whether APP levels are influenced by management practices.

CONCLUSIONS

The protein profile of emus varies with age of animals. The reference values for APP levels in healthy emus established in the present study may be used in research on pathologies, diagnosis, and as indicators of these birds' overall health status.

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