Comparative Clinical Pathology (2018) 27:493–497
https://doi.org/10.1007/s00580-017-2618-9

ORIGINAL ARTICLE

Serum protein electrophoresis reference values in the gyrfalcon (*Falco rusticolus*)

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Received: 25 February 2017 / Accepted: 30 November 2017 / Published online: 7 December 2017

Abstract

Blood samples were collected as part of routine clinical examination procedures from 120 clinically normal gyrfalcons (*Falco rusticolus*) during a 4-year period between the months of September and February. Serum protein electrophoresis (SPE) was carried out to establish reference values for the species and to characterize species-specific electrophoretic patterns. The SPE analyses included measurements of pre-albumin, albumin, alpha-1, alpha-2, beta-1, beta-2, gamma globulins, and the albumin/globulin ratio. Reference intervals were determined using a quantile approach with 90% confidence intervals. Female gyrfalcons were found to have significantly lower median albumin and gamma concentrations than males. Juvenile gyrfalcons were found to have significantly lower median total protein, alpha-1, alpha-2, and beta-globulins than adults. Results of this study provide SPE values for gyrfalcons that may contribute to the medical management of this species commonly used in the sport of falconry in the Middle East.

Keywords Gyrfalcon · *Falco rusticolus* · Serum protein electrophoresis · Reference intervals

Introduction

Serum protein electrophoresis (SPE) is used in routine health assessment and management of diseases in avian patients. First applied in human diagnostics more than three decades ago (Cray 1998), SPE is now commonly used in veterinary medicine in the diagnosis of certain diseases and for early detection of humoral and inflammatory responses. In avian medicine, common conditions in which SPE may provide useful information include infectious, toxic, nutritional, and behavioral disorders (Tatum et al. 2000). Moreover, changes in SPE protein fractions may be observed prior to changes in the complete blood count, providing early recognition of altered states of health (Delk et al. 2015). As considerable differences exist between avian species in SPE protein fractions (Cray et al. 2007; Kostka and Janeczek 2013), species-specific references intervals (RIs) are necessary for the interpretation of results. Although some studies have been carried out on a number of avian species, the lack of RIs for numerous other species limits the use of SPE as a routine diagnostic procedure in avian medicine. Moreover, differences in sample processing, methodology, and sample hemolysis may affect results of SPE (Rosenthal et al. 2005; Roman et al. 2009; Cray et al. 2011). Indeed, both plasma and serum have been used for SPE in birds, but fibrinogen in plasma samples may lead to imprecise measurements of the beta- and gamma fractions (Thomas 2000; Gelli et al. 2005) and total protein concentrations approximately 1.7 g/dL higher than in serum (Lumeij and DeBruije 1985; Lumeij et al. 1990). Lastly, the validity of results of some previous studies is questionable due to small sample sizes. Guidelines of the Quality Assurance and Laboratory Standards Committee of the American Society for Veterinary Clinical Pathology and the Clinical Laboratory Standards Institute (CLSI) recommend ideally a minimum of 120 reference individuals to establish non-parametric RIs with 90% confidence intervals (CI) in veterinary species (Horowiz et al. 2008; ASVCP 2011).

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This paper describes the results of SPE analyses carried out in 120 clinically healthy gyrfalcons at the Wrsan Wildlife Division, Abu Dhabi, United Arab Emirates.

Materials and methods

Blood samples were obtained from 120 clinically healthy gyrfalcons (28 males and 92 females) during routine clinical examinations over a 4-year period between the months of September and February. The mean (± SD) body weight of the birds was 972 ± 17.24 g for males and 1382 ± 24.38 g for females. Their ages ranged from 4 months to 3 years old. All birds were accustomed to manual restraint and no medications were given for a minimum of 2 weeks prior to blood sampling. The falcons were fasted for 12 h prior to blood collection. The falcons were manually restrained and anesthetized with a mixture of 5% isoflurane (Aerrane, Baxter, Guayama, Puerto Rico, USA) in 2 L of oxygen/min, delivered via a facemask. An average of 2.5 mL blood was obtained from the right basilic vein (*Vena cutanea ulnaris superficialis*) using a 3-mL disposable syringe fitted with a 23 gauge, 1-in. disposable needle. After collection, 0.5 mL of whole blood was immediately placed into commercial serum gel separation tubes (MiniCollect, Greiner Bio-One, Kremsmunster, Austria) and allowed to clot at room temperature in a tube rack. Serum was harvested after centrifugation for 5 min at 3000 rpm using a benchtop centrifuge (IEC CL30, Thermo Electron Corp., Chateau-Gontier, France) and analyzed within 30 to 60 min. No evidence of gross hemolysis was apparent in any of the serum samples.

Total protein concentrations were measured using the biuret method on a commercial wet-chemistry analyzer (Miura 500, ISE Srl., Guidonia, Italy), following the manufacturer's recommendations. The SPE was performed using automated electrophoresis systems (GENIO S, Interlab Srl., Rome, Italy, for 63 samples and G26 electrophoresis analyzer, Interlab, Srl., Rome, Italy, for 55 samples) with agarose gels (running conditions: fuse current and type—F1 F2 3,15 AT; power: 80 ÷ 260 V − 50 ÷ 60 Hz; running time: approx. 1 h). The gels were analyzed using the integrated software (Elfolab, Interlab Srl., Rome, Italy). Quantification of SPE fractions was determined by multiplying the total serum protein concentration by the area under the curves for prealbumin, alpha-1, alpha-2, beta-1, beta-2, and gamma globulins. The albumin/globulin ratio was calculated, whereby prealbumin was included in the albumin concentration.

Statistical analysis

All SPE parameters were assessed for normality using Shapiro-Wilk tests and quantile plots, and for homoscedasticity across ages and gender using Levene's test. The reference limits were calculated using the 2.5 and 97.5% quantiles, based on CLSI guidelines and recommendations of the American Society of Veterinary Clinical Pathology (Horowiz et al. 2008; ASVCP 2011). Outliers were detected and removed using both Tukey and Dixon methods. A bootstrap approach was used to determine 90% confidence intervals for the RIs. The influence of age and sex was assessed using Wilcoxon rank-sum tests. For parameters that varied with age, stratified reference intervals were reported (Table 2). All statistical analyses were performed using R (R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org/) (R Development Core Team 2012) and RIs were determined using Reference Value Advisor (Geffré et al. 2011).

Table 1 Reference intervals for serum protein electrophoresis (g/L) determined in 120 clinically healthy gyrfalcons (*Falco rusticolus*) using a non-parametric method

Analyte	*N*	Mean (g/L)	Median (g/L)	SD (g/L)	Range (g/L)	RI (g/L)	Lower limit 90% CI (g/L)	Upper limit 90% CI (g/L)	Method
Total protein	120	27.5	27.5	3.5	18.6–36.4	21.1–35.2	18.6–22.2	33.3–36.4	Non-parametric
Pre-albumin	119	5.3	6.2	3.4	0.0–12.6	0.1–10.9	0.0–0.10	9.9–12.6	Non-parametric
Albumin	119	12.1	12.1	2.7	6.0–18.3	7.5–17.5	6.0–7.9	16.8–18.3	Non-parametric
Alpha-1	119	2.3	1.3	2.7	0.2–12.6	0.4–10.5	0.2–0.5	8.7–12.6	Non-parametric
Alpha-2	117	1.9	1.3	1.7	0.4–8.9	0.4–8.1	0.4–0.6	6.0–8.9	Non-parametric
Beta (total)	118	2.5	2.0	1.6	0.4–8.3	0.4–7.4	0.4–0.8	6.1–8.3	Non-parametric
Beta-1	55	1.7	1.5	1.1	0.2–5.4	0.3–5.1	0.2–0.4	4.0–5.4	Non-parametric
Beta-2	55	1.7	1.1	1.5	0.1–6.8	0.1–6.7	0.1–0.3	4.5–6.8	Non-parametric
Gamma	116	3.8	3.5	2.6	0.1–12.7	0.3–10.7	0.1–0.8	8.8–12.7	Non-parametric
A:G ratio	120	0.3	0.3	1.9	0.0–0.6	0.0–0.6	0.0–0.0	0.5–0.6	Non-parametric

A:*G*, albumin:globulin; *CI*, confidence interval; *RI*, reference interval; *SD* standard deviation

Table 2 Gender-specific differences in albumin and gamma globulin concentrations measures by serum protein electrophoresis in clinically healthy gyrfalcons (*Falco rusticolus*) ($n = 120$)

Analyte	*N*	Mean (g/L)	Median (g/L)	SD (g/L)	Range (g/L)	RI (g/L)	Lower limit 90% CI (g/L)	Upper limit 90% CI (g/L)	Method
Albumin									
Males	28	13.8	13.6	2.1	8.6–17.5	9.5–18.4	8.3–10.7	17.0–19.6	Robust
Females	91	11.5	11.5	2.6	6.0–18.3	5.6–17.1	6.0–7.7	16.1–18.3	Non-parametric
Gamma									
Males	28	4.5	4.3	1.8	1.7–8.4	0.6–8.3	0.0–1.5	7.2–9.2	Robust
Females	92	3.4	2.8	2.8	0.1–12.7	0.2–10.8	0.1–0.4	8.9–12.7	Non-parametric

CI, confidence interval; *RI*, reference interval; *SD*, standard deviation

Results

Results of the SPE fractions measured are presented in Tables 1, 2, and 3. There were significant differences between females and males for albumin (median, 11.5 and 13.6 g/L for females and males, respectively, Wilcoxon rank-sum test, $p < 0.001$) and gamma globulins (median, 2.8 and 4.3 g/L for females and males, respectively, Wilcoxon rank-sum test, $p < 0.001$). There were significant differences between juveniles and adults for total proteins (median, 25.3 and 28.4 g/L for juveniles and adults, respectively, Wilcoxon rank-sum test, $p < 0.001$), alpha-1 globulins (0.9 and 1.4 g/L for juveniles and adults, respectively, Wilcoxon rank-sum test, $p < 0.001$), alpha-2 globulins (1.1 and 1.4 g/L for juveniles and adults, respectively, Wilcoxon rank-sum test, $p < 0.001$), and beta-globulins (1.6 and 2.2 g/L for juveniles and adults, respectively, Wilcoxon rank-sum test, $p < 0.001$).

Discussion

Although SPE has become a commonly used diagnostic tool for evaluation of diseases and health status in psittacine birds, only little information is available on its application in falcon medicine. Previous studies have evaluated both plasma and serum protein electrophoresis for some species, including bald eagles (*Haliaeetus leucocephalus*), Spanish imperial eagles (*Aquila adalberti*), Steller's sea eagles (*Haliaeetus pelagicus*), gyrfalcons (*Falco rusticolus*), peregrine falcons (*Falco peregrinus*), saker falcons (*Falco cherrug*), red-naped shaheens (*Falco pelegrinoides babylonicus*), gyr-hybrid falcons (gyr-peregrine, gyr-saker, gyr-red-naped shaheen), red-tailed hawks (*Buteo jamaicensis*), Harris' hawks (*Parabuteo unicinctus*), common buzzards (*Buteo buteo*), black kites (*Milvus migrans*), barn owls (*Tyto alba*), great horned owls (*Bubo virginianus*), barred owls (*Strix varia*), screech owls

Table 3 Age-specific differences in serum protein electrophoretic fractions in clinically healthy gyrfalcons (*Falco rusticolus*) ($n = 120$)

Analyte	*N*	Mean (g/L)	Median (g/L)	SD (g/L)	Range (g/L)	RI (g/L)	Lower limit 90% CI (g/L)	Upper limit 90% CI (g/L)	Method
Total protein									
Juvenile	35	25.2	25.3	2.5	18.6–29.7	20.1–30.5	18.9–21.3	29.2–31.6	Robust
Adult	85	28.5	28.4	3.3	21.1–36.4	21.7–36.1	21.1–23.1	33.5–36.4	Non-parametric
Alpha-1									
Juvenile	35	1.6	0.9	2.6	0.3–12.6	0.3–8.8	0.3–0.4	3.5–9.8	Robust
Adult	85	2.5	1.4	2.7	0.2–10.5	0.4–10.3	0.2–0.4	8.7–10.5	Non-parametric
Alpha-2									
Juvenile	34	1.1	1.1	0.5	0.4–2.9	0.0–2.1	0.0–0.3	1.7–2.4	Robust
Adult	84	2.1	1.4	1.8	0.4–8.8	0.4–7.9	0.4–0.6	6.1–8.8	Non-parametric
Beta									
Juvenile	35	2.0	1.6	1.5	0.4–7.4	0.4–6.2	0.3–0.6	4.1–9.1	Robust
Adult	85	2.7	2.2	1.7	0.4–8.3	0.4–7.8	0.4–0.7	5.9–8.3	Non-parametric

CI, confidence interval; *RI*, reference interval; *SD*, standard deviation

(*Otus asio*), tawny owls (*Strix aluco*), turkey vultures (*Cathartes aura*), and black vultures (*Coragyps atratus*) (Del Pilar Lanzarot et al. 2001; Garcia-Montijano et al. 2002; Kummrow et al. 2012; Ordonneau et al. 2005; Spagnolo et al. 2008; Tatum et al. 2000). Although two studies evaluated SPE in falcons, RIs were only reported for nestling peregrine falcons (15 to 27 days old, $n = 32$) (Del Pilar Lanzarot et al. 2001) and falcon species in general (including gyrfalcons, peregrine, saker, and gyr-hybrid falcons, $n = 73$) (Kummrow et al. 2012). In the latter study, the RI was established based on SPE in 73 falcons, including 14 gyrfalcons, but these were grouped together and values for individual species were not reported. Moreover, RIs for different genders or age groups were not reported. To the knowledge of the authors, gyrfalcon-specific RIs have not been previously established.

The RI for pre-albumin in the present study (0.1–10.9 g/L) was comparable to that previously reported for falcon species (0–12.7 g/L, Kummrow et al. 2012), and no significant differences between genders or ages were found. However, significant differences were found between genders for albumin and gamma globulins. This contrasts with previous findings in which no differences were found between genders for all falcon species (Kummrow et al. 2012). However, the previous study included only 20 males, of which only two were gyrfalcons, and SPE was performed using a different system and different gels, making direct comparison difficult. In addition, significant differences were found in the present study between juvenile and adult gyrfalcons for total proteins and both alpha- and beta-globulins. Previous studies have not evaluated differences between age groups, but this finding may significantly impact the interpretation of results. In particular, the upper RI limit of the alpha-2 fraction in adult gyrfalcons was over threefold that of juveniles in the present study. These findings suggest that not only species-specific but also gender- and age-specific RIs may be necessary, at least in some avian species.

After albumin and pre-albumin, gamma globulins represented the predominant SPE fraction in the present study. This contrasts to findings in psittacines and phoenicopteriformes (Delk et al. 2015), in which beta-globulins are predominant. The median gamma globulin value for gyrfalcons in the present study was lower than that found in free-living nestling peregrine falcons in a former study (Del Pilar Lanzarot et al. 2001). However, the former study used a different SPE method on cellulose acetate gels and RIs were even wider than those found in the present study. Nevertheless, elevated gamma globulins in free-living falcons may indeed be expected to be higher than in captive birds due to a greater potential for antigenic exposure or subclinical inflammation or infection (Hausmann et al. 2015). The median albumin/globulin ratio found in the present study was somewhat lower than previously reported values in other bird species, in which higher values are typically expected in healthy birds (Vergneau-Grosset et al. 2016).

Findings of the present study contribute to the scarce reports on gyrfalcon SPE and the established reference intervals may be helpful in the interpretation of SPE results in this species. Further studies are necessary to establish whether the age and gender differences observed are common to all falcon species.

Acknowledgements We thank HH Sheikh Sultan bin Zayed Al Nahyan for his interest and continuing support of the research program of the Veterinary Science Department, Wildlife Division. We thank Angelito Viola and Philippe Graf for assistance in this study. We also would like to acknowledge the technical staff of the Veterinary Science Department for assistance in handling the birds.

Compliance with ethical standards All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Conflict of interest The authors declare that they have no conflict of interest.

References

Cray C (1998) Applications of protein electrophoresis in avian diagnostics. J Avian Med Surg 12:4–10

Cray C, Rodriguez M, Zaias J (2007) Protein electrophoresis of psittacine plasma. Vet Clin Pathol 36(1):64–72. https://doi.org/10.1111/j.1939-165X.2007.tb00184.x

Cray C, King E, Rodriguez M, Decker LS, Arheart KL (2011) Differences in protein fractions of avian plasma among three commercial electrophoresis systems. J Avian Med Surg 25(2):102–110. https://doi.org/10.1647/2010-019.1

Del Pilar Lanzarot M, Montesinos A, San Andrés MI, Rodriguez C, Barahona MV (2001) Hematological, protein electrophoresis and cholinesterase values of free-living nestling peregrine falcons in Spain. J Wildl Dis 37(1):172–177. https://doi.org/10.7589/0090-3558-37.1.172

Delk KW, Wack RF, Burgdorf-Moisuk A, Kass PH, Cray C (2015) Acute phase protein and electrophoresis protein fraction values for captive American flamingos (*Phoenicopterus ruber*). J Zoo Wildl Med 46(4):929–933. https://doi.org/10.1638/2014-0191.1

Garcia-Montijano M, Garcia A, Lemus JA, Montesinos A, Canales R, Luaces I, Pereira P (2002) Blood chemistry, protein electrophoresis, and hematologic values of captive Spanish imperial eagles (*Aquila adalberti*). J Zoo Wildl Med 33(2):112–117. https://doi.org/10.1638/1042-7260(2002)033[0112:BCPEAH]2.0.CO;2

Geffré A, Concordet D, Braun JP, Trumel C (2011) Reference value advisor: a new freeware set of macroinstructions to calculate reference intervals with Microsoft Excel. Vet Clin Pathol 40(1):107–112. https://doi.org/10.1111/j.1939-165X.2011.00287.x

Gelli D, Franceschini F, Bedin M, Ferrari V, Bernardini D, Romagnoli S (2005) Serum biochemical and electrophoretic patterns in the Eurasian buzzard (*Buteo buteo*): reference values. Proc Eur Ass Avian Vet 166–170

Guidelines for the determination of reference intervals and other related topics (2011) American society for veterinary clinical pathology, Quality Assurance and Laboratory Standards Committee, Madison, WI, USA. http://www.asvcp.org/pubs/qas/index.cfm. Accessed July 2012

Hausmann JC, Cray C, Hartup BK (2015) Comparison of serum protein electrophoresis values in wild and captive whooping cranes (*Grus Americana*). J Avian Med Surg 29(3):191–199

Horowiz GL, Altaie S, Boyd JC, Ceriotti F, Garg U, Horn P, Pesce A, Sine HE, Zakowski Z (2008) CLSI. Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline—third edition. CLSI document C28-A3

Kostka V, Janeczek F (2013) Diagnostic value of protein electrophoresis in large psittacines. International Conference on Avian, Herpetological and Exotic Mammal Medicine, Germany, pp 421–422

Kummrow M, Silvanose C, Di Somma A, Bailey TA, Vorbrüggen S (2012) Serum protein electrophoresis by using highresolution agarose gel in clinically healthy and Aspergillus speciesinfected falcons. J Avian Med Surg 26:213–220

Lumeij JT, DeBruije JJ (1985) Evaluation of the refractometric method for the determination of total protein in avian plasma or serum. Avian Pathol 14(3):441–444. https://doi.org/10.1080/03079458508436245

Lumeij JT, DeBruije JJ, Kwant MM (1990) Comparison of different methods of measuring protein and albumin in pigeon sera. Avian Pathol 19(2):255–261. https://doi.org/10.1080/03079459008418678

Ordonneau D, Roman Y, Chaste-Duvernoy D, Bomsel MC (2005) Plasma electrophoresis reference ranges in various bird species. Proc Eur Ass Avian Vet 283–289

Roman Y, Bomsel-Demontoy M, Levrier J, Chaste-Duvernoy D, Saint Jalme M (2009) Effect of hemolysis on plasma protein levels and plasma electrophoresis in birds. J Wildl Dis 45(1):73–80. https://doi.org/10.7589/0090-3558-45.1.73

Rosenthal K, Johnston MS, Shofer FS (2005) Assessment of the reliability of plasma electrophoresis in birds. Amer J Vet Res 66(3):375–378

Spagnolo V, Crippa V, Marzia A, Alberti I, Sartorelli P (2008) Hematologic, biochemical, and protein electrophoretic values in captive tawny owls (*Strix aluco*). Vet Clin Pathol 37(2):225–228. https://doi.org/10.1111/j.1939-165X.2008.00038.x

Tatum LM, Zaias L, Mealey BK, Cray C, Bossart GD (2000) Protein electrophoresis as a diagnostic and prognostic tool in raptor medicine. J Zoo Wildl Med 31(4):497–502. https://doi.org/10.1638/1042-7260(2000)031[0497:PEAADA]2.0.CO;2

Thomas JS (2000) Protein electrophoresis. In: Feldman BF, Zinkl JG and Jain Nc (eds): Schalm's veterinary hematology, 5th edn. Lippincott Williams and Wilkins, Baltimore, pp 899–903

Vergneau-Grosset C, Beaufrère H, Ammersbach M (2016) Clinical biochemistry. In: Speer BL (ed) Current therapy in avian medicine and surgery. Elsevier, St. Louis, pp 486–501