Total plasma protein level as an indicator of condition in wild American kestrels (*Falco sparverius*)

Russell D. Dawson and Gary R. Bortolotti

Abstract: Total plasma protein levels were determined for 292 female and 228 male American kestrels (*Falco sparverius*) in the wild. Plasma protein levels were significantly higher in females than in males, and higher during prelaying than during incubation. For both sexes, plasma protein levels did not vary significantly with the number of days before or after egg laying on which the sample was taken, time of sampling, prey abundance, age, molt, or infection by the blood parasite *Haemoproteus* sp. Protein levels in females increased with date of sampling and body condition during prelaying, while the same pattern was seen in males during incubation. With the exception of those of prelaying females, plasma protein levels increased with ambient temperature. The results of this study suggest that at least some of the variation observed in total protein levels is attributable to physical condition. However, further investigation is required before the reliability of using total plasma protein level as a tool to assess the health and condition of kestrels is known.

Résumé : Les concentrations totales des protéines plasmatiques ont été évaluées chez 292 femelles et 228 mâles de la Crècerelle d’Amérique (*Falco sparverius*) en nature. Les concentrations étaient significativement plus élevées chez les femelles que chez les mâles et plus élevées au cours de la période avant la ponte que pendant l’incubation. Il n’y avait pas de corrélation significative entre les concentrations plasmatiques de protéines et le nombre de jours avant ou après la ponte, le moment de l’échantillonnage, l’abondance des proies, l’âge, la mue ou les infections par les hémoparasites *Haemoproteus* sp., ni chez les mâles ni chez les femelles. Chez les femelles, les concentrations de protéines augmentaient en fonction de la date d’échantillonnage et de la condition physique avant la ponte et ce même pattern a été observé chez les mâles durant l’incubation. Chez tous les oiseaux sauf les femelles avant la ponte, les protéines plasmatiques augmentaient avec la température ambiante. Les résultats de cette étude indiquent qu’au moins une partie de la variation observée dans les concentrations de protéines totales est attribuable à la condition physique. Cependant, il est encore prématuro d’affirmer que les concentrations totales de protéines plasmatiques peuvent constituer des indicateurs valables de la santé et de la condition physique des crêcerelles.

[Traduit par la Rédaction]

**Introduction**

Documenting the physical condition and general health of a wild animal is invaluable in studies of its ecology and behavior. Ferrer (1992a, 1992b, 1993a) has used concentrations of urea and uric acid in blood as a measure of condition to gain insight into such ecological issues as dispersal in Spanish imperial eagles (*Aquila adalberti*). However, evaluating urea concentration requires specialised laboratory equipment and a relatively large quantity of blood, precluding its use on a practical basis by field ecologists and behaviorists.

In contrast, total protein level in the blood is easily measured in the field with a minimal amount of blood and equipment. Circulating levels of protein in the blood are thought to be an index of total protein reserves in an animal (Allison 1955). Total plasma protein levels may become elevated when dietary protein intake increases (Leveille and Sauberlich 1961). Depressed total protein levels may result from gluconeogenesis, and therefore may have the potential for use to assess dietary inadequacies (LeResche et al. 1974).

The purpose of our study was to establish base-line levels of total plasma proteins in the American kestrel (*Falco sparverius*), a small falcon. Despite the potential for using this blood parameter to assess condition, few blood studies have been done so. We also tested whether normal plasma protein levels vary with the characteristics of a bird, such as sex, age, reproductive status, molt, and parasite load, as well as the abundance of prey where it nested and the time and temperature at sampling. These factors all have the potential to influence blood parameters (see Ferrer 1993b and references therein) and need to be taken into account before inferences can be made about the relationship between condition and blood parameters.

**Materials and methods**

A wild population of American kestrels was studied during 1994 and 1995 in the boreal forest near Besnard Lake, north-central Saskatchewan, Canada (55°N, 106°W). Nest boxes were made available in a variety of habitats ranging from densely forested road sides to nearly treeless clearcuts (see Bortolotti 1994). Kestrels arrived on our study area in mid to late April after migration, and began laying eggs in mid-May. We visited nest boxes every 3–5 days during May and early June to determine laying date, and again to capture adults once laying was completed.

We trapped kestrels throughout the breeding season using balchatri traps (Berger and Mueller 1959), and in the nest box with traps or by hand. Birds were generally captured between 07:00 and 20:00 CST. Each bird was examined for molt and weighed to the

---

Received September 18, 1996. Accepted December 18, 1996.

R.D. Dawson and G.R. Bortolotti. Department of Biology, University of Saskatchewan, 112 Science Place, Saskatoon, SK S7N 5E2, Canada.

Author to whom all correspondence should be addressed (e-mail: dawson@sask.usask.ca).

nearest gram, and 6 measures of size were taken: lengths of the unflattened wing chord, tenth primary, outer rectrix, central rectrix, and culmen and width of the tarsus. These 6 linear measures were used as input variables in a principal-component analysis (PCA). The first component (PC1) was used as a measure of size of each bird, as multivariate measures of structural size are preferred over univariate measures (Rising and Summers 1989; Freeman and Jackson 1990). PCAs were performed separately for females ($n = 454$) and males ($n = 336$) (for further details see Bortolotti and Iko 1992). A condition index was then calculated for each bird by dividing its mass by its PC1.

The brachial vein of each bird was lanced with a hypodermic needle and blood was collected in a heparinized microcapillary tube. A blood smear was made, air-dried, and fixed immediately in 100% ethanol. Prevalence and intensity of haematozoa were determined at the International Reference Centre for Avian Haematozoa at Memorial University, St. John’s, Newfoundland, Canada, by G.F. Bennett. Haematozoa were quantified by counting the number of parasites in 100 microscope fields under oil using a 12X ocular and a 100X objective. We restricted our analyses to birds parasitised with *Haemoproteus* sp., which were the parasites most commonly found in kestrels on our study area.

The blood remaining in the microcapillary tube was stored in a cooler until it was centrifuged for 5 min in an International Microcapillary Centrifuge some hours later. Total plasma protein level was estimated using a refractometer (Model 10400A, American Optical Corporation, Keene, N.H.). Our blood-sampling protocol was approved by the University of Saskatchewan Animal Care Committee on behalf of the Canadian Council on Animal Care.

Minimum age was known for birds banded in previous years and which returned to the study area. We also attempted to identify kestrels captured for the first time as adults according to whether they were in their second year (SY) of life, based on plumage characteristics and feather fault bars (Smallwood 1989). As aging techniques are not always precise, we restricted our analyses of age effects on plasma protein levels to birds classified unequivocally as SY, or those known from banding to be SY or older (after second year, ASY).

Food abundance was assessed by determining the relative abundance of small mammals, the main prey of kestrels, on territories (Bortolotti et al. 1991). During early July 1994, trap lines consisting of 10 stations spaced 30 m apart and situated parallel to and 10 m from a road were set on most territories. At each station two Museum Special snap traps were baited with peanut butter. Each line operated for 3 days. The same protocol was used in July 1995, except that some lines had only 5 stations spaced 60 m apart. We examined the effect of food abundance on blood parameters for incubating birds only. During prelaying, both sexes can be fairly transient and often switch between one or more territories before egg laying begins (see Bortolotti and Iko 1992). By testing for preabundance effects only during incubation, we were more confident that birds had been present on territories long enough for food-mediated alterations of blood parameters, if they existed, to become apparent.

Ambient temperatures were recorded at hourly intervals throughout both years by a weather station located on the study area. Temperatures at sampling averaged 12.1 ± 0.39°C (range -0.3 to 24.3°C) during prelaying and 21.5 ± 0.30°C (range = 6.9–33°C) during incubation.

We obtained blood samples from adult kestrels during the prelaying and incubation stages of the breeding season. Both of these periods were treated separately in analyses, owing to differences in behavior and physiology. We included in our analyses only one estimate of total plasma protein level per bird at each stage of the breeding season: however, birds sampled during both prelaying and incubation were treated as independent because of the separation in time, differences in physiological processes, and in some cases, changes in physical condition.

We analysed data for each sex separately, or included sex as a factor in analyses, because kestrels show reverse sexual size dimorphism (Bird 1988), and because the sex roles differ during reproduction. Males undertake nearly all of the hunting duties for the pair from the time of pair formation to about 10 days after eggs hatch (Balgooyen 1976). Additionally, physiological processes within each sex differ during reproduction, owing to females laying eggs.

We used Pearson’s product moment correlation analysis to test for effects of date, time, and ambient temperature when samples were taken, the number of days before or after egg laying on which the sample was taken (“breeding chronology”), and physical condition on plasma protein level. Spearman’s rank correlation coefficient was used to examine the relationship between plasma protein level and parasitism by *Haemoproteus* sp., as intensities of infection must be considered indices rather than absolute values (G.F. Bennett, personal communication). We feel that prey abundance, measured as the number of small mammals per 100 trap-nights, is better considered an index than an absolute value. We therefore also used nonparametric correlation to examine the relationship between prey abundance and blood parameters.

We also compared blood parameters between parasitised and unparasitised birds, molting and non-molting birds, different intensities of molt, and different age-classes. Where significant correlations were detected between plasma protein level and other variables (Table 1), we entered these variables as covariates in an analysis of covariance (ANCOVA). However, as sample sizes in ANCOVAs were often smaller than in the original correlations, and the original relationships were not strong, these variables did not always contribute significantly to the ANCOVA model. We therefore removed nonsignificant covariates from the models and repeated the analyses. When none of the variables that were originally correlated with plasma protein level contributed significantly to the ANCOVA model, we tested for differences between groups using analysis of variance (ANOVA).

Means are presented ± 1 standard error. All statistical analyses were performed using the SAS statistical package (SAS Institute Inc. 1990) and results were considered significant at the 0.05 level.

**Results**

Total plasma protein levels were estimated for 292 females and 228 males. Three-way ANOVA suggested that plasma protein levels did not differ between years ($F_{[1,512]} = 2.06$, $P = 0.15$). Females had higher protein levels than males ($F_{[1,512]} = 67.41$, $P < 0.0001$), and levels were higher during the prelaying stage than the incubation stage of the breeding season ($F_{[1,512]} = 17.5$, $P < 0.001$). A significant sex × stage of the breeding season interaction ($F_{[1,512]} = 36.67$, $P < 0.0001$) resulted from protein levels in females declining from prelaying to incubation, while those in males increased slightly (Fig. 1). No other interactions were significant. As no differences in protein levels could be attributed to year effects, we pooled data from both years to increase statistical power.

During prelaying, female plasma protein level increased with the date on which the sample was taken and with physical condition (Table 1, Fig. 2). There was a weak trend for plasma protein levels of females to increase as the commencement of egg laying approached (breeding chronology). Date of sampling, physical condition, and breeding chronology were all intercorrelated for prelaying females, so we performed a series of partial correlations. Plasma protein levels correlated significantly with both sampling date (partial $r = 0.35$, $n = 50$, $P = 0.01$) and physical condition.
Fig. 1. Mean total protein levels in the plasma of adult American kestrels during prelaying and incubation. Open symbols represent prelaying birds and solid symbols incubating birds. Error bars represent ± 1 standard error. Numbers below the x axis are sample sizes for each group.

Table 1. Correlation coefficients and P values for correlations between estimates of total plasma protein level and Julian date of sampling, breeding chronology, time of sampling, ambient temperature at sampling, prey abundance as measured by an index of small-mammal numbers, and condition of American kestrels during prelaying and incubation in northern Saskatchewan in 1994–1995.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sex</th>
<th>Prelaying</th>
<th></th>
<th>Incubation</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>r</td>
<td>P</td>
<td>n</td>
<td>r</td>
</tr>
<tr>
<td>Sampling date</td>
<td>F</td>
<td>0.37</td>
<td>0.0002</td>
<td>97</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>-0.15</td>
<td>0.17</td>
<td>81</td>
<td>0.16</td>
</tr>
<tr>
<td>Breeding chronology</td>
<td>F</td>
<td>0.26</td>
<td>0.06</td>
<td>53</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>-0.23</td>
<td>0.17</td>
<td>39</td>
<td>0.13</td>
</tr>
<tr>
<td>Time of sampling</td>
<td>F</td>
<td>-0.11</td>
<td>0.27</td>
<td>97</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>0.18</td>
<td>0.11</td>
<td>81</td>
<td>0.02</td>
</tr>
<tr>
<td>Temperature at sampling</td>
<td>F</td>
<td>0.12</td>
<td>0.23</td>
<td>97</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>0.21</td>
<td>0.06</td>
<td>81</td>
<td>0.19</td>
</tr>
<tr>
<td>Prey abundance</td>
<td>F</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>-0.04</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>-0.09</td>
</tr>
<tr>
<td>Physical condition b</td>
<td>F</td>
<td>0.51</td>
<td>0.0001</td>
<td>93</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>0.09</td>
<td>0.42</td>
<td>79</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Note: Correlation coefficients are Pearson's for all variables except prey abundance, which is Spearman's (see Methods for the rationale).

* Number of days before or after egg laying commenced on which the sample was taken.

b Mass/PC1.

(partial $r = 0.54$, $n = 50$, $P < 0.0001$) but not breeding chronology (partial $r = -0.18$, $n = 50$, $P = 0.22$). We also examined whether plasma protein levels of females became elevated as laying approached by examining the relationship between chronology and protein level only during the 10 days prior to egg laying rather than for the entire prelaying period; however, we could not detect a significant correlation between protein level and number of days before egg laying during this time in females ($r = 0.26$, $n = 18$, $P = 0.30$). Time of sampling and ambient temperature at sampling were not related to plasma protein levels of females during prelaying (Table 1).

Among prelaying males, no variables correlated significantly with plasma protein level, although there was a trend for protein level to increase with ambient temperature (Table 1).
During incubation, plasma protein levels of both sexes increased with ambient temperature (Table 1). Male plasma protein levels during incubation varied marginally with sampling date, and were positively related to physical condition (Fig. 2). Although numbers of small mammals ranged from 0 to 71.1/100 trap-nights (median = 15.4/100 trap-nights, \( n = 168 \) territories), prey abundance was not related to protein level in either sex (Table 1).

ANOVA with age, sex, and stage of the breeding season as factors could not detect differences in plasma protein levels between birds classified as SY and those classed as ASY (\( F_{1,205} = 1.04, P = 0.31 \)). Similarly, no interactions
among age, sex, and (or) stage of the breeding season were significant \((P > 0.70\text{ in all cases})\).

Molt of kestrel flight feathers generally occurs during incubation, and approximately 50\% of birds we caught during this time had missing or growing feathers. ANCOVA showed that plasma protein levels of incubating kestrels increased with ambient temperature (covariate, \(F_{1,130} = 11.64, P < 0.001\)), but no differences could be attributed to the presence (least square mean = 4.37 ± 0.05 g/dL, \(n = 173\)) or absence of molt (least square mean = 4.31 ± 0.05 g/dL, \(n = 165\); \(F_{1,130} = 1.43, P = 0.23\)), nor were there significant interactions between temperature, sex, and (or) molt status \((P > 0.15\text{ in all cases})\).

We also tested whether the intensity of molt affected plasma protein levels. The intensity of molt in birds with 1 or 2 remiges molting was classified as low, whereas birds with 4 or more remiges molting were classified as having a high intensity of molt. Again, ANCOVA suggested that plasma protein levels of kestrels undergoing molt increased with ambient temperature (covariate, \(F_{1,139} = 5.54, P = 0.02\)), but there were no differences in protein levels between birds classified as having high (least square mean = 4.29 ± 0.09 g/dL, \(n = 78\)) or low intensities of molt (least square mean = 4.37 ± 0.04 g/dL, \(n = 249\); \(F_{1,139} = 0.25, P = 0.62\)). Interactions between temperature, sex, and (or) molt intensities were not significant \((P > 0.33\text{ in all cases})\).

Using ANOVA with parasite infection status, sex, and stage of the breeding season as factors, we could not detect any difference in plasma protein levels between birds parasitized by *Haemoproteus* sp. and those in which no infection was detected \(F_{1,292} = 0.94, P = 0.33\). No interactions were detected among the three factors \((P > 0.43\text{ in all cases})\). There was considerable variation among birds harboring *Haemoproteus* sp. (median = 53.5 parasites/100 fields, range = 1–2000 parasites/100 fields, \(n = 262\)); however, the level of infection was not related to plasma protein levels during prelaying (females: \(r_s = -0.07, n = 68, P = 0.58\); males: \(r_s = 0.18, n = 45, P = 0.23\)) or incubation (females: \(r_s = -0.16, n = 80, P = 0.14\); males: \(r_s = 0.02, n = 56, P = 0.87\)).

**Discussion**

Plasma protein levels of females were significantly higher during prelaying than during incubation, while protein levels in males remained relatively similar between prelaying and incubation (Fig. 1). During both prelaying and incubation, plasma protein levels were higher in females than males. Studies cited by Grimminger (1976) suggest that oestrogen increases the plasma protein level, which would account for both the seasonal and sex differences observed in kestrels. Further evidence for an oestrogen effect comes from numerous studies that have been unable to demonstrate sex differences outside the breeding season (Gee et al. 1981; Snyder et al. 1981; Gessaman et al. 1986; Lavin et al. 1992; Phalen et al. 1995).

Plasma protein levels have been correlated with condition in both birds (de le Court et al. 1995) and mammals (Brannon 1985; Messier et al. 1987), although the predictive power of this relationship has always been poor. Plasma protein levels of males during incubation, but not prelaying, were positively related to condition indices (Table 1, Fig. 2). Plasma protein levels of prelaying females increased with both the date on which the sample was taken and with condition (Table 1, Fig. 2). After arriving on the study area from migration, female kestrels generally increase in mass, and hence condition, until incubation (Dawson and Bortolotti 1997). It has been suggested that prior to laying, protein levels in the blood of females should increase as yolk proteins are transported to the ovary from the liver; however, using partial correlation analysis, we could not detect any relationship between the number of days before egg laying on which the sample was taken and plasma protein level in females. In fact, after laying was completed, there was a slight trend for female protein levels to increase with the number of days following laying on which the sample was taken (Table 1). Additionally, there was no indication that protein levels of females increased dramatically during the 10 days prior to laying. These results suggest that the increase in plasma protein levels during prelaying is related to increases in the condition of females as they prepare for egg laying, rather than to an amplification of circulating protein levels for egg formation per se. These results suggest that plasma protein levels in kestrels are at least partly a function of nutritional condition.

Reproductive parameters of kestrels in our study area are very sensitive to fluctuations in small-mammal numbers (Wiebe and Bortolotti 1992, 1994, 1995). Despite considerable variation in prey abundance on territories, plasma protein levels were not influenced by small-mammal numbers (Table 1). Abelenda et al. (1993) detected a seasonal decrease in plasma protein levels in common cranes (*Grus grus*) during winter, which they suggested might be the result of differential protein intake throughout the sampling period. In contrast, we detected a marginally significant trend for male plasma protein levels to increase with the date of sampling during incubation (Table 1). The fact that protein levels were not related to the number of days following initiation of laying suggests that this relationship is a seasonal increase rather than being a function of the stage of breeding. However, given the lack of association between prey abundance and plasma protein level, differential protein intake throughout the sampling period is not likely a plausible explanation for the observed relationship.

With the exception of prelaying females, plasma protein levels increased as ambient temperature increased (Table 1). There appears to be a paucity of data in the literature regarding this type of relationship. One possible explanation, at least during incubation, when ambient temperatures are high, is that plasma water is used to help dissipate body heat through evaporation, therefore concentrating proteins as the ambient temperature rises.

Previous studies have been unable to detect temporal changes in plasma protein levels of chickens (Sturkie and Newman 1951), eagle owls (*Bubo bubo*), or common buzzards (*Buteo buteo*) (García-Rodríguez et al. 1987). Similarly, our results suggest that the time of day at which the sample was taken does not significantly influence the plasma protein levels of kestrels of either sex (Table 1).

The effects of age on total protein levels are unclear. Some studies have found juvenile birds to have higher protein levels than adults (Kocan and Pitts 1976; Grimminger
plasma proteins and traditional condition indices such as mass scaled to body size.

Acknowledgments

We thank J. Ball, E. Dzus, C. Fehr, M. Gloutney, M. Hart, H. Heavin, M. Miller, and S. Tomassi for assistance in the field. G.F. Bennett kindly examined blood smears for haematooza, and G. Wobeser generously loaned equipment. Saskatchewan Environment and Resource Management provided permits and weather data from our study area. Funding for this project was provided by the Natural Sciences and Engineering Research Council of Canada through a research grant to G.R.B. and a postgraduate scholarship to R.D.D., and by the Canadian Wildlife Federation and Northern Scientific Training Program in research awards to R.D.D.

References


