



Variation in Hematocrit and Total Plasma Proteins of Nestling American Kestrels (*Falco sparverius*) in the Wild

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ABSTRACT. Hematocrits and total plasma proteins were determined at 24 days old for 86 female and 85 male nestling American kestrels (*Falco sparverius*) from the wild in northern Saskatchewan. No sex differences were detected in either hematocrit or plasma protein. For females, hematocrit and plasma protein were not related to time of sampling, temperature at sampling, mass of nestlings or length of the tenth primary flight feather. In males, hematocrit correlated only with the length of tenth primary. For both sexes, concentrations of plasma protein declined as the season progressed. Differences in brood size, natural and experimental, did not affect hematocrit or plasma protein levels. Of six nestlings that died before leaving the nest, three showed depressed hematocrit values compared with those that successfully left the nest, whereas none showed significant deviations in plasma protein levels. For 42 birds, we took a second blood sample 2–5 days after the first. Changes in blood parameters of males between these sampling periods were not related to changes in weight, growth of tenth primary or changes in temperature. Among females, there were trends for changes in weight to be correlated with changes in both hematocrit and plasma protein. Our results suggest that hematocrits and plasma proteins of nestling kestrels are not robust indicators of nutritional status and condition. COMP BIOCHEM PHYSIOL 117A; 3:383–390, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. American kestrel, blood, condition, *Falco sparverius*, hematocrit, mortality, nestlings, total plasma proteins

INTRODUCTION

Studies of hematology and blood chemistry provide measures that are frequently used as indicators of the health and nutritional status of an animal (2,16). Before blood parameters can provide useful information, their relationship with the physical condition of the animal must be validated. Hematocrits and total plasma proteins are relatively easily obtained measures that have been used with some success in mammals to detect differences in nutritional status among populations or years of differing food quality (21,27,47). In contrast to studies of mammals, most studies of avian hematocrits and total plasma proteins have been concerned with simply establishing baseline values. Moreover, many of these investigations have used captive individuals that may not provide results representative of wild birds [e.g., (4,23,33,40)]. Therefore, with a few exceptions [e.g., (6, 17,22)], little is known about how blood parameters relate to condition and nutritional status in wild birds.

This investigation sought to establish baseline informa-

tion on the hematocrits and total plasma proteins of wild American kestrels (*Falco sparverius*), a small falcon, just before their leaving the nest. By investigating sources of variation in these blood parameters with respect to several environmental factors, we hoped to test their utility in evaluating the health and condition of birds. We examined the potential effect of nestling age, sex, time of day, ambient temperature and nesting chronology. These factors to some degree have been shown to influence blood parameters in birds [see (20) and references therein] but are rarely all considered in any one study. We also investigated how blood parameters were associated with food abundance and variation in nestling development and condition. Six nestlings died after sampling but before leaving the nest, allowing a comparison of blood parameters between these birds and those that successfully left the nest.

We also manipulated the number of chicks in nests to determine how brood size affected blood parameters. The fact that in our area at least one nestling typically starved in up to 90% of broods with five chicks (modal brood size) suggests that parent kestrels have limited resources with which to raise offspring [(53), R. Dawson and G. Bortolotti, unpublished data]. Additional evidence for limited resources is that kestrels fine tune their reproductive effort by

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manipulating both the sex ratio and hatching asynchrony of their broods (53,54). Therefore, we expected that the removal of a single nestling from a brood would have significant consequences for offspring condition.

MATERIALS AND METHODS

We studied the American kestrel during 1994 in the boreal forest of northcentral Saskatchewan, Canada, in the vicinity of Besnard Lake (55°N, 106°W). All birds used in this study were reared in nest boxes that contained a few centimeters of wood shavings and were situated along gravel roads and logging trails (7).

Nest boxes were visited every 3–5 days during May and early June until females commenced egg laying and then again after laying was complete so we could ascertain clutch size. We visited nests daily near the time of hatching to determine the day on which the first nestling of each brood emerged. Kestrels within a clutch generally hatch over a period of 1.5–3.5 days and therefore siblings may differ in age by up to several days (54). When the first-hatched nestling was 24 days old (just before leaving the nest), all members of the brood ($n = 52$ nests) were weighed to the nearest gram, and the length of their tenth primary feather was measured to the nearest millimeter. In addition, we noted whether each bird had food in its crop.

Also at this time, the brachial vein of each chick was lanced with a 27-gauge hypodermic needle and blood was collected in a microcapillary tube coated in sodium heparin as an anticoagulant. Microcapillary tubes were sealed with plasticine and stored in a cooler until they were centrifuged for 5 min in an International Micro-capillary Centrifuge (Model MB, International Equipment Company, Needham Heights, MA) some hours later. Hematocrit was determined directly on a microhematocrit reader. Plasma was obtained by breaking the centrifuged tubes above the column of packed cells, and total plasma protein was estimated using a refractometer (Model 10400A, American Optical Corporation, Keene, NH). Although it has been suggested (35) that the refractometric method may be unreliable for determining plasma protein concentrations in pigeons (*Columba livia domestica*), refractometers are commonly used in clinical practice and have been shown to be reliable for mammalian plasma (46). For a subsample of chicks, a second blood sample was obtained 2–5 days after the first. All blood samples were collected between 4 and 29 July.

To determine whether brood size had significant consequences for blood parameters, we removed one nestling from broods of five chicks ($n = 9$ nests) immediately after hatching, allowing us to compare blood parameters at fledging between natural and manipulated four-chick broods, as well as between natural five-chick broods and five-chick broods reduced to four chicks.

We assessed food abundance by snap-trapping small

mammals, the main food of kestrels (8,31), on most territories during the brood-rearing period (early July). Each trap-line consisted of 10 stations spaced 30 m apart and situated parallel to and 10 m from a road. At each station, two Museum Special snap traps were baited with peanut butter. Each line operated for 3 days, and traps were reset each morning.

A weather station located on the study area recorded ambient temperature at hourly intervals throughout the field season. Ambient temperatures during the study period averaged 17.1°C (range 1.3–30.8°C). Mean ambient temperatures at sampling was 22.4°C (range 13.4–30.8°C). We used these data to test for effects of temperature on blood parameters. Because Rehder *et al.* (43) found significant correlations between hematocrit and mean temperature during the period preceding sampling in captive adult kestrels, we also calculated the mean ambient temperature during the 24 hours before sampling and included this variable in our analyses.

American kestrels are typical Falconiformes in that they exhibit reverse sexual size dimorphism (5). Female nestlings can weigh more than males as young as 6 days old (3). We therefore analyzed data for each sex separately or included sex as a factor in analyses. Condition and growth of individuals within a nest can be highly variable (R. Dawson and G. Bortolotti, unpublished data), so for most analyses we treated each nestling as being independent. Means are presented ± 1 SE. All statistical analyses were performed using the SAS statistical package (45). Tests are two-tailed unless stated otherwise, and we considered results significant at the 0.05 level.

RESULTS

Blood parameters did not appear to be influenced by recent feedings. Two-way analysis of variance (ANOVA) with sex and presence or absence of food in the crop as factors suggested that hematocrits were not different between birds with and without food in their crop ($F = 0.28$, $df = 1, 160$, $P = 0.60$) nor was there an interaction between presence or absence of food in the crop and sex ($F = 0.23$, $df = 1, 160$, $P = 0.63$). Similarly, total plasma proteins were not different between nestlings with and without food in their crops ($F = 2.10$, $df = 1, 160$, $P = 0.15$) nor was the sex-by-food interaction significant ($F = 0.00$, $df = 1, 160$, $P = 0.96$). We therefore did not exclude birds from subsequent analyses because of food in their crops.

Hematocrit

We could not detect a difference between hematocrit values of females ($40.2 \pm 0.46\%$, range 27–52%, $n = 84$) and males ($40.0 \pm 0.50\%$, range 24–53%, $n = 85$; $t = 0.32$, $df = 167$, $P = 0.75$). Similarly, the mean hematocrit level of

TABLE 1. Correlation coefficients and *P* values for correlations between hematocrit and sampling time, ambient temperature at sampling, mean temperature during the 24 hr preceding blood sampling, Julian date weight and length of tenth primary of American kestrel nestlings (age 24 days) in northern Saskatchewan during 1994

Variable	Females (<i>n</i> = 84)		Males (<i>n</i> = 85)	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Sampling time	0.10	0.36	-0.03	0.82
Temperature at sampling	0.001	0.99	0.04	0.72
Temperature during previous 24 hr	0.03	0.77	0.02	0.82
Julian date	-0.15	0.18	-0.02	0.89
Weight	0.11	0.32	0.18	0.09
Length of tenth primary	0.12	0.28	0.34	0.0014

females within a nest did not differ from males from the same nest (paired *t*-test, $t = -0.42$, $n = 38$, $P = 0.68$). For females, there were no significant correlations between hematocrit and sampling time, temperature, date, weight or length of tenth primary (Table 1). There was a significant correlation between the length of tenth primary feather and hematocrit in males, as well as a slight trend between male weight and hematocrit; no other variables were significant (Table 1). Because weight and length of tenth primary were correlated in males ($r = 0.62$, $n = 85$, $P < 0.0001$), we performed a partial correlation analysis. Hematocrit in males was related to length of tenth primary when the effect of weight was controlled for (partial $r = 0.29$, $n = 85$, $P < 0.01$), but the trend between male weight and hematocrit disappeared when we controlled for length of tenth primary (partial $r = -0.04$, $n = 85$, $P = 0.75$).

Snap-trapping small mammals provides an index of food abundance rather than being a measure of absolute quantity. For this reason, we used nonparametric correlation techniques [see (49)] to test for an effect of food supply on blood parameters. As food supply was measured for an entire nest and not individual nestlings, we used mean hematocrit and mean total plasma protein level of each sex in a nest to achieve independence in our observations. We could not detect a relationship between food quantity and hematocrit level for either female ($r_s = 0.24$, $n = 41$, $P = 0.14$) or male chicks ($r_s = -0.11$, $n = 41$, $P = 0.50$).

To test for effects of brood size, ANOVA with sex and brood-size treatment as factors was used. Our treatments were unmanipulated nests with four chicks, unmanipulated nests with five chicks and nests manipulated from five to four chicks. We were unable to detect any differences due to sex ($F = 0.06$, $df = 1$, 124 , $P = 0.81$) or experimental treatment ($F = 0.19$, $df = 2$, 124 , $P = 0.82$) nor was the sex-by-treatment interaction significant ($F = 0.34$, $df = 2$, 124 , $P = 0.71$).

TABLE 2. Correlation coefficients and *P* values for correlations between estimated total plasma proteins and sampling time, ambient temperature at sampling, temperature during the preceding 24 hr, Julian date, weight and length of tenth primary of American kestrel nestlings (age 24 days) in northern Saskatchewan during 1994

Variable	Females (<i>n</i> = 86)		Males (<i>n</i> = 83)	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Sampling time	0.35	0.0008	0.33	0.0024
Temperature at sampling	-0.01	0.92	-0.01	0.90
Temperature during previous 24 hr	-0.14	0.19	-0.15	0.16
Julian date	-0.35	0.001	-0.42	0.0001
Weight	0.08	0.44	0.13	0.25
Length of tenth primary	0.08	0.47	0.13	0.23

Total Plasma Proteins

Total plasma proteins of females (4.03 ± 0.059 g/dl, range 2.8–5.4 g/dl, $n = 86$) were significantly different from those of males (3.81 ± 0.054 g/dl, range 2.7–5.1 g/dl, $n = 83$; $t = 2.65$, $df = 167$, $P < 0.01$, but see below). Total protein correlated positively with hematocrit in both females ($r = 0.48$, $n = 84$, $P < 0.0001$) and males ($r = 0.34$, $n = 83$, $P = 0.002$). Date and time of sampling correlated with total protein for both sexes (Table 2). A partial correlation analysis was performed for both sexes due to a significant correlation between date and time (females, $r = -0.59$, $n = 86$, $P < 0.0001$; males, $r = -0.51$, $n = 83$, $P < 0.0001$). When sampling time was controlled for, plasma protein level declined with date for males (partial $r = -0.31$, $n = 83$, $P < 0.01$); however, sampling time had no effect on male plasma protein when the effect of date was removed (partial $r = 0.15$, $n = 83$, $P = 0.19$). The significant correlation between time and protein was the result of logistics that dictated that samples taken early in the season (when total protein is higher) be taken later in the day. A similar analysis for females indicated that there was a trend for total protein to increase during the day (partial $r = 0.19$, $n = 86$, $P = 0.07$) and to decrease with date (partial $r = -0.18$, $n = 86$, $P = 0.09$). There was no apparent relationship between weight or feather length of nestlings or temperature at sampling and total protein level (Table 2). Food abundance on territories was not related to total plasma proteins for either sex (females, $r_s = -0.003$, $n = 42$, $P = 0.98$; males, $r_s = -0.05$, $n = 41$, $P = 0.76$).

Because total plasma proteins correlated significantly with date in males and approached significance in females, we re-examined sexual differences in total protein using an analysis of covariance (ANCOVA), with sex as a main effect and date as a covariate. Total protein varied significantly ($F = 12.00$, $df = 3$, 165 , $P < 0.001$) with date of

sampling (covariate, $F = 27.92$, $df = 1$, $P < 0.001$) but not with sex (main effect, $F = 0.05$, $df = 1$, $P = 0.82$) nor was there an interaction between these two factors ($F = 0.10$, $df = 1$, $P = 0.76$).

ANCOVA was also used to investigate whether total plasma proteins varied with brood size. Again, total protein varied significantly ($F = 4.91$, $df = 11,82$, $P = 0.0001$) with date of sampling ($F = 18.65$, $df = 1,82$, $P < 0.0001$) but not among natural broods of four or five or broods of five chicks manipulated to four ($F = 1.61$, $df = 2,82$, $P = 0.21$) or between the sexes ($F = 1.21$, $df = 1,82$, $P = 0.27$). No interactions were significant ($P > 0.24$ in all cases).

Serial Sampling

In addition to taking blood samples at 24 days of age, we also took blood a second time from 42 chicks ranging in age from 26 to 29 days old. We hypothesized that if blood parameters were related to condition indices, then changes in blood parameters between the two sampling periods would be correlated with changes in condition. In addition to correlating changes in blood parameters to absolute changes in weight, we also calculated relative weight changes. If blood parameters are condition dependent, then changes in body weight should have a greater effect on chicks that were in poor condition when first sampled as compared with those that were in good condition. Regardless, results were similar whether we used absolute or relative changes in weight; we therefore present here only results using relative changes. We also tested for the effects of changes in temperature, the amount of growth of the tenth primary and the date of first sampling.

For both sexes, changes in hematocrit levels between successive samples were correlated with changes in plasma protein level (females, $r = 0.48$, $n = 20$, $P = 0.03$; males, $r = 0.59$, $n = 22$, $P = 0.0042$). ANOVA suggested that both the number of days between samples ($F = 3.84$, $df = 3, 34$, $P = 0.02$) and sex ($F = 11.76$, $df = 1, 34$, $P < 0.01$) significantly affected changes in hematocrit. The sex-by-number of days interaction was also significant ($F = 5.48$, $df = 1, 34$, $P < 0.01$). This entire relationship was driven by a single female whose hematocrit fell by 15% between samples, because when we removed this data point and repeated the analysis, all terms of the model became nonsignificant. Hematocrits of males increased between samples (mean difference $3.18 \pm 1.12\%$, paired $t = 3.18$, $n = 22$, $P = 0.01$) and females (excluding the single female) remained the same (mean difference $0.74 \pm 0.75\%$, paired $t = 0.98$, $n = 19$, $P = 0.34$). Change in plasma proteins between samples was not influenced by the number of days between samples ($F = 0.71$, $df = 1, 34$, $P = 0.55$), but there was a trend for female protein to decrease slightly, whereas male levels increased slightly between samples ($F = 3.85$, $df = 1, 34$, $P = 0.06$). No interaction was detected between the num-

ber of days between samples and sex ($F = 1.70$, $df = 1, 34$, $P = 0.18$). Overall, there were no differences in total proteins between the two sampling periods (females, mean difference -0.17 ± 0.173 g/dl, paired $t = -0.98$, $n = 20$, $P = 0.34$; males, mean difference 0.08 ± 0.119 g/dl, paired $t = 0.65$, $n = 22$, $P = 0.52$).

As change in female hematocrit between samples was affected by the number of days between samples, we used a series of ANCOVAs to examine relationships between changes in female hematocrits and other variables. When the number of days between samples was held constant, change in female hematocrit level correlated weakly with relative weight change ($F = 4.03$, $df = 1, 13$, $P = 0.07$) but not with growth of the tenth primary ($F = 0.88$, $df = 1, 13$, $P = 0.37$), change in temperature ($F = 2.59$, $df = 1, 13$, $P = 0.13$) or date of first sampling ($F = 0.22$, $df = 1, 13$, $P = 0.65$). Among 22 males, change in hematocrit did not correlate with relative weight change ($r = 0.12$, $P = 0.59$), growth of the tenth primary ($r = 0.11$, $P = 0.61$), change in temperature ($r = 0.36$, $P = 0.10$) or date of first sampling ($r = 0.12$, $P = 0.61$).

Changes in plasma proteins of 20 females bled twice were related to both relative weight change ($r = 0.65$, $P = 0.0018$) and date of sampling in females ($r = 0.64$, $P = 0.0025$). Because sampling date and relative weight changes were correlated ($r = 0.65$, $P = 0.0019$), a partial correlation analysis was performed. When relative weight changes were controlled for, sampling date was no longer significantly related to changes in female plasma protein levels (partial $r = 0.37$, $n = 20$, $P = 0.12$). With the effect of sampling date removed, the relationship between relative weight changes and changes in plasma protein concentrations of females approached significance (partial $r = 0.41$, $n = 20$, $P = 0.08$). Growth of the tenth primary ($r = 0.13$, $P = 0.58$) and change in temperature ($r = 0.24$, $P = 0.31$) were not related to change in plasma protein levels. Of the 22 males bled twice, changes in plasma protein levels were not related to relative weight changes ($r = 0.36$, $P = 0.10$), growth of the tenth primary ($r = 0.20$, $P = 0.38$), change in temperature ($r = 0.22$, $P = 0.33$) or sampling date ($r = -0.07$, $P = 0.75$).

Nestling Mortality

Six nestlings died, presumably from starvation, after they were sampled at 24 days old but before they left the nest box (one female, five males). Hematocrits of males that died ($35.0 \pm 3.05\%$) were significantly different than those that fledged ($40.3 \pm 0.46\%$; $t = -2.63$, $df = 86$, $P = 0.01$), although two of the male chicks that died had hematocrits higher than the mean value of those that survived. We could not detect a difference between plasma protein levels of males that died (3.60 ± 0.13 g/dl) and those that survived (3.81 ± 0.06 g/dl; $t = -0.94$, $df = 84$, $P = 0.35$),

although this test lacks statistical power. The hematocrit (43%) and plasma protein level (3.9 g/dl) of the single female that died was similar to our sample of fledged females (hematocrit $40.2 \pm 0.45\%$, plasma protein level 4.02 ± 0.06 g/dl).

DISCUSSION

Hematocrit

Hematocrit values of approximately 40% in wild American kestrel chicks found in this study are similar to those reported previously for healthy captive adult raptors (4,23, 40,43). Hematocrits of 10-day-old captive kestrel chicks have been reported to be 34.1% (29). Hematocrit values in our study were higher and this is likely attributed to the fact that the number of red blood cells increases with age in juvenile birds (13,41), causing an increase in hematocrit levels as they grow (28,29). Our data from successive samples of males, but not females, indicate that hematocrit does increase with age. Daily increases in hematocrits of growing birds are small (e.g., 0.16% per day in glaucous-winged gulls [*Larus glaucescens*] (29)). The length of time between our sampling periods was relatively short and so we may not have easily been able to detect ontogenetic changes in females.

We found no sex differences in hematocrit. Although male birds in general are thought to have higher hematocrits than females (50), it has been suggested that there is little influence of gender on hematocrit before sexual maturity (19). Indeed, most studies have found little variation between the sexes even in adult birds (23,30,33,39,48). Where sex differences have been noted, they are often inconsistent and have been attributed to different effects of circulating sex steroids in the blood (37,42,43). Androgens have been shown to increase erythropoiesis, whereas estrogen is thought to have little effect on erythropoiesis (50).

Most studies of avian hematocrit have attempted to control for diurnal variation in blood parameters by limiting sampling to a few hours during the day. Logistical constraints dictated that our samples be collected during most hours of daylight; however, there appeared to be no relationship with time of sampling and hematocrit in kestrels (Table 1), concurring with a previous study of captive adult kestrels (43). Rehder *et al.* (44) found that hematocrit declined throughout the day in red-tailed hawks (*Buteo jamaicensis*), although it is possible that this was caused by hemodilution resulting from repeated sampling [see (51)] rather than any biologically meaningful pattern.

We found no relationship between ambient temperature at sampling nor mean temperature during the preceding 24 hours and hematocrits (Table 1), even though it has been hypothesized that one of the principal factors influencing variation in adult hematocrit is changes in temperature (42). Correlations between temperature and hematocrit

have been noted in previous investigations of kestrels (42,43), and many studies have shown that winter hematocrits are higher than those found in summer [(1,30,52); but see (12)], presumably to increase oxygen-carrying capacity to sustain higher levels of thermogenesis (12). The lack of temperature effects in our birds may be due to samples being collected over a relatively short period during summer when little variation in temperature existed.

Seasonal effects on hematocrit have been found in both birds (1,30,37,42) and mammals (10). Rehder and Bird (42) suggested that seasonal variation in hematocrits of captive adult kestrels resulted from the combined effects of photoperiod, ambient temperature, reproduction and molt. We found no relationship between Julian date and hematocrit. The disparity between our study and Rehder and Bird's is likely the result of our birds being juveniles and not undergoing molt or reproduction. Similarly, the effect of photoperiod would not be pronounced as our samples were collected over a relatively short period of time.

There was no relationship between hematocrit and weight in either sex (Table 1). Although few avian studies have examined weight-hematocrit relationships, experimental starvation experiments in geese suggest that hematocrit declines throughout the period of food deprivation, particularly during the first few days of fasting (6,34). Work on black bears (*Ursus americanus*) showed that declines in hematocrit occurred between spring and early summer, a time when bears are losing body weight and nutritional condition due to the poor quality of food ingested (27). Data on kestrels from other studies are ambiguous, with no relationship between weight and hematocrit during summer months (43), but during nonreproductive and nonmolting periods, hematocrit varied directly with body weight in captive adults after body weight reached a certain threshold (42). We found that relative change in body weight correlated weakly with change in hematocrit level in females but not males. These results may suggest that females that had weight changes between sampling periods had corresponding changes in the rates of erythropoiesis. This relationship may have been weak in females, and nonexistent in males, because birds that lost weight may also have become dehydrated resulting in hemoconcentration [but see (34)].

The length of the tenth primary flight feather was weakly correlated with hematocrit in males but not females (Table 1). Although no studies have specifically examined the relationship between feather growth and hematocrit in nestling birds, it has been suggested that intensity of molt in adult birds is an important factor influencing hematocrit (18); however, previous studies have found that hematocrit declines during molt (15,42,37). Growing feathers are extensively vascularized, which results in declines in hematocrit because the increase in plasma volume is not accompanied by an increase in erythrocyte number (15). It seems likely that the relationship we found is between some other factor

that is also correlated with the growth of this feather rather than the degree of feather growth per se. Other studies have found a positive relationship between hematocrit and age. Both age and feather growth are really correlates of maturity in nestling birds.

The amount of food resources on individual territories did not affect hematocrit. Similarly, the addition or removal of one chick from broods had no discernable effect. We have observed that parent kestrels occupying territories with few small mammals and those that raise five chicks instead of four often have insects making up a larger portion of the prey items fed to nestlings. Despite variance in food supply, kestrel chicks appear to not be affected to such a degree as to cause declines in the rate of erythropoiesis.

Total Plasma Proteins

Levels of total protein in the blood of kestrel chicks was within the range of values previously reported for raptors (4,40). It is perhaps not prudent to make rigorous comparisons of total protein levels that have been reported in the literature. Certainly, variation exists in these data due to different methodology (35) and the fact that workers have used either serum or plasma in their determinations.

Total protein is generally thought to increase as young birds grow (9), and this has been confirmed in canvasbacks (*Aythya valisineria*) (32) and white spoonbills (*Platalea leucorodia*) (17). However, our repeated sampling of some chicks failed to detect a consistent direction in the change of total protein between successive sampling periods. As with hematocrit, it is likely that the differences in age between sampling periods was insufficient for age-related increases in total protein to become apparent.

Total plasma proteins were not related to the weight of chicks nor the length (Table 2) or the changes in length of their tenth primary feather between samples. Total protein has been correlated with condition indices in both birds (17) and mammals (11,36). Geese subjected to an experimental period of starvation showed declines in total proteins during the first 6 days of fasting (6). However, protein levels then increased to normal levels until starvation became more critical, suggesting that geese were not successful in using protein-sparing strategies during starvation. When we sampled birds 24 days old, the chicks were extremely variable in weight (61–156 g for females, 69–148 g for males), suggesting that some were on the verge of starvation. Indeed, a lack of response of total protein to weight at this time may indicate that kestrels are similar to geese in being relatively unsuccessful at protein sparing during times of food deprivation.

Total proteins tended to decline with date (Table 2). If protein levels in the plasma are an indicator of quality, then it appears that later-hatched chicks have poorer nutritional status than those hatched earlier in the breeding season, although we were unable to detect this relationship using

weight or tenth primary length. There is evidence that selection favors birds that breed early in the season (38). As later-hatched young are often thought to have reduced post-fledging survival and recruitment to the breeding population, parents should not invest as heavily in later nestlings (14,25). Therefore, parents may be feeding late chicks less because they perceive these chicks as being less valuable to their reproductive success. Alternatively, parents that delay breeding may do so because they are of poor quality and are constrained by their ability to acquire the necessary resources to breed earlier in the year. If late nesters are being constrained, this would also account for the observed declines in total protein as the breeding season progressed.

At first, there appeared to be a sex difference in total proteins; however, reanalyzing the data using ANCOVA with date as a covariate showed that in fact none existed. The initially significant results were due to the fact that more males were sampled later in the season at a time when total protein levels were low in all birds, regardless of sex. Most studies of adult birds have found no differences in total protein between the sexes (17,23,24,33,39,48), although adult males tend to have lower levels of proteins in their plasma than reproductively active females, presumably as a result of estrogen (26,51).

Brood size and experimental treatment did not have any effect on total protein levels. Parents may be able to feed nestlings at a sufficient rate that stresses associated with increasing brood size were not manifested in differences in total protein. Given the low weights of some birds at fledging, this seems unlikely. Alternatively, if kestrels are not successful at protein sparing during periods of food shortages, we might expect that their levels of total protein would be similar between broods of four and five chicks, a situation akin to fasting geese (6).

CONCLUSIONS

Overall, there was little evidence to support the notion that hematocrit and total plasma protein levels could be used with any certainty in assessing the nutritional status of nestling American kestrels. Although several condition-related variables correlated significantly with both hematocrit and total plasma protein, these relationships were often sex-specific and had poor predictive power. It may be that even though our sample sizes were large, there was not sufficient variation in condition to manifest itself in alterations of blood parameters. Considering the variation in weight when samples were taken (see above) and the observed mortality, this seems unlikely. Similarly, of the six birds that died, three had hematocrits higher than the mean values, and none showed reduced levels of total protein. Taken together, these results suggest that the use of hematocrit and total plasma proteins as indicators of health or condition in wild kestrels seems tenuous at best.

It has been suggested that birds might operate at some

“optimal” hematocrit level and that increasing the number of erythrocytes to improve oxygen delivery may increase the viscosity of blood which would actually impede flow rates (12). The tradeoff between increased oxygen carrying capacity and optimal flow rates would result in an optimal hematocrit level where oxygen transport is maximized. Similarly, birds that are in good general health need to maintain the colloidal pressure of their plasma as well as the pH level. Therefore, reasonably healthy birds may not show large variations in either their total plasma protein concentration or hematocrit level that coincides with changes in their nutritional status. If this is the case, we concur with Messier *et al.* (36) who state that if “blood studies can only detect advanced stages of malnutrition, their merit in evaluating the nutritional condition of (animals) on a practical basis remains somewhat limited. Indeed, a simple external examination of the animals will reveal severe malnutrition.” At present, we believe that too few rigorous field studies of avian blood profiles have been performed to make this a useful management and prognostic aid.

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