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COMPARATIVE HEMATOLOGY AND PLASMA BIOCHEMISTRY OF RED-TAILED HAWKS AND AMERICAN KESTRELS WINTERING IN CALIFORNIA

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ABSTRACT.—In December and January of the winters of 1990–91, 1991–92, and 1992–93, blood samples were collected from 52 Red-tailed Hawks (*Buteo jamaicensis*) and 91 American Kestrels (*Falco sparverius*). Twenty-five blood parameters were measured, including white blood cell components, plasma enzyme activities, and plasma chemistry concentrations. Interspecific comparisons were made to identify species specific differences pertinent to health assessment. American Kestrels had a higher incidence of hematozoa infection, higher alkaline phosphatase and acetylcholinesterase activities, and higher plasma cholesterol, blood urea nitrogen, uric acid and sodium concentrations. Red-tailed Hawks had higher white blood cell concentration and eosinophil count estimates, aspartate aminotransferase and butrylcholinesterase activities, and a higher plasma albumin concentration.

KEY WORDS: *Red-tailed Hawk*; Buteo jamaicensis; *American Kestrel*; Falco sparverius; *hematology*; *plasma biochemistry*; *hematozoa.*

Hematología comparativa y bioquímica de plasma de *Buteo jamaicensis* y *Falco sparverius* durante el invierno en California

RESUMEN.—En Diciembre y Enero de los inviernos de 1990–91, 1991–92, y 1992–93, se tomaron muestras de sangre de 52 *Buteo jamaicensis* y de 91 *Falco sparverius.* Veinticinco parametros de sangre fueron medidos incluyendo componentes de células de globulos blancos, actividad de enzimas del plasma y concentraciones químicas del plasma. Se efectuaron comparaciones inter-específicas con el fin de identificar diferencias relacionadas con la salud de cada especie. *Falco sparverius* tuvo una mayor incidencia de infecciones por hematozoarios, mayor fosfatasa alkalina y actividad de acetilcolinesterasa, mayor colesterol en el plasma, nitrógeno de urea en la sangre, acido úrico y concentraciones de sodio. *Buteo jamaicensis* tuvo mayor concentracion de células blancas y estimativos de conteo de eosinófilos, aspartato aminotransferosa y actividad de butrycolinesterosa y altas concentraciones de albúmina en plasma.
[Traducción de César Márquez]

Hematological and plasma biochemistry measures are routinely used in monitoring bird health, and potentially provide information regarding nutritional and immunological status, toxicant exposure, and other aspects of physiological function. Critical to meaningful interpretation of these values are reference data from wild birds sampled under specific criteria. Blood samples from Red-tailed Hawks (*Buteo jamaicensis*) and American Kestrels (*Falco sparverius*) wintering in the Central Valley of California were analyzed for hematozoa infection and 25 hematological and biochemical parameters. These data were used to assess the general health of all birds captured, to establish reference ranges for these species in California, and to determine interspecific differences pertinent to health assessment.

METHODS

During December and January of the winters of 1990–91, 1991–92, and 1992–93, Red-tailed Hawks and American Kestrels were captured in orchard areas of Stanislaus County (37°35′N, 120°50′W) and nonorchard areas of Yolo County (38°35′N, 121°41′W), California using balchatri traps. Within 2 hr of capture, 1–1.5 ml of blood was collected from the metatarsal or brachial vein using a heparinized syringe and a 26-gauge needle. Immediately after collection, whole blood was transferred to a microcentrifuge tube and spun for 10 min at 1000 × g to separate cells from plasma. Plasma was divided into three

aliquots: 250 μl were stored at 0–4°C for biochemical analyses and two 100 μl fractions were stored initially on dry ice, then at 70°C, for cholinesterase assay. When sufficient blood was available, three blood smears were prepared and air-dried. A qualitative physical examination was conducted on each bird to check for evidence of burns associated with surviving electrocution, a pronounced or "sharp" keel, excessive ectoparasite load, and discharge at the eyes or beak. Each bird was banded with a USGS band and released at its capture site.

Hematological and plasma biochemical analyses were performed by Consolidated Veterinary Diagnostics, Inc. (CVD), West Sacramento, CA. Whole blood slides were examined at 40× power magnification to estimate white blood cell concentration, perform differential cell counts, and identify hematozoa (Campbell 1988, Hawkey and Dennet 1989). Hematocrit was not measured due to limited sample volume; therefore, the white blood cell concentration estimates and white cell differential count estimates reported here were not corrected for anemia. Five plasma enzyme activities: lactate dehydrogenase (LDH), alkaline phosphatase (AP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatine kinase (CK) (AP, Bessey et al. 1946; LDH, Wolf et al. 1972; CK, Szasz et al. 1976; AST, Bergmeyer et al. 1977; ALT, Bergmeyer and Horder 1980) and 11 plasma constituent concentrations: total protein, albumin, globulin (total protein minus albumin), albumin/globulin ratio, cholesterol, blood urea nitrogen, uric acid, glucose, sodium, potassium, calcium, and phosphorous (total protein, Layne 1957; glucose, Schmidt 1961; blood urea nitrogen, Talke and Schubert 1965; albumin, Doumas and Biggs 1972; calcium, Baginski et al. 1973; cholesterol, Allain et al. 1974; phosphorus, Woo and Cannon 1984; uric acid, Merdes et al. 1985) were determined with a Hitachi 736 autoanalyzer. Three additional plasma enzyme activities, total cholinesterase (CHE), acetylcholinesterase (ACHE), and butrylcholinesterase (BCHE) were measured using the methods of Ellman et al. (1961).

Intraspecific and interspecific comparisons were performed on 23 hematological and biochemical measurements using a *t*-test (*t*-test procedure, SAS). Based on this number of comparisons, a *P*-value < 0.002 was used to determine significant differences. A few Red-tailed Hawks and American Kestrels that were captured and sampled were excluded from the analyses presented here. Individuals were excluded based on findings during a qualitative physical examination; individuals showing evidence of burns associated with surviving electrocution, a pronounced or "sharp" keel, excessive ectoparasite load, or discharge from the eyes or beak, were excluded. Individuals were also excluded based on elevated organophosphate (OP) residue levels. In conjunction with pesticide exposure studies on wintering raptors (Wilson et al. 1993), those Red-tailed Hawks ($N = 37$) and American Kestrels ($N = 30$) captured in orchard areas of Stanislaus County were sampled for OP residues. To limit the analyses to measures from birds with low and nondetectable OP exposure (see Hooper et al. 1989 for description of OP analyses), we excluded three Red-tailed Hawks and four American Kestrels with more than 5.0 μg and 1.0 μg total OP residues on their feet, respectively. Linear regression was used to detect relationships between these allowed OP levels and hematological and biochemical parameters for each species (regression procedure, SAS). Samples from 15 Red-tailed Hawks and 61 American Kestrels that were captured in nonorchard areas of Yolo County and that met the physical examination criteria were included in the analyses. OP residues were not measured on birds captured in Yolo County; however, it is unlikely that they would have encountered OP spraying in these nonorchard areas. Individuals are represented in the analyses only once.

RESULTS

There were no intraspecific differences between Red-tailed Hawk age classes (adult vs. immature) or American Kestrel genders for any of the hematological or biochemical parameters (*t*-test, $P < 0.002$). Therefore, data from adult and immature Red-tailed Hawks were pooled as were data from male and female American Kestrels. Linear regression yielded no significant relationships between OP levels and any of the hematological or biochemical parameters for the 34 Red-tailed Hawks with low (26 of 34) and nondetectable (eight of 34) OP residues, or for the 26 American Kestrels with low (21 of 26) and nondetectable (five of 26) OP residues (regression procedure, $P < 0.05$ for slope). Sample sizes were not consistent due to the variable quality of whole blood smears and insufficient plasma to measure all parameters.

American Kestrels had a higher incidence of hematozoa infection than Red-tailed Hawks (45.5% vs. 26.3%). *Haemoproteus* occurred more frequently than *Leukocytozoa* in American Kestrels while the inverse was true for Red-tailed Hawks. Of 90 American Kestrels, 38 were infected with *Haemoproteus* (42.2%) and four were infected with *Leukocytozoa* (4.4%); one bird was infected with both (1.1%). Of 38 Red-tailed Hawks, one was infected with *Haemoproteus* (2.6%) and nine were infected with *Leukocytozoa* (23.7%).

Several interspecific differences in hematological and plasma parameters were identified. American Kestrels had lower white blood cell concentration estimates and eosinophil count estimates than Red-tailed Hawks (Table 1). However, the mean heterophil/lymphocyte ratio estimate was 2.3 for both Red-tailed Hawks and American Kestrels. Plasma enzyme activity differences between Red-tailed hawks and American Kestrels were most notable for AP, AST and ACHE (Table 2). Red-tailed Hawks had higher plasma concentrations of total protein and albumin than American Kestrels, but had lower plasma concentrations of cholesterol, blood urea nitrogen and uric acid (Table 3). The mean

Table 1. White blood cell concentration estimates and differential cell counts of wild Red-tailed Hawks (RTHA) and American Kestrels (AMKE). White blood cell concentration estimates are uncorrected for anemia and differential cell counts are parameter estimates.

SPECIES	WBC EST[a] 10^3 (CELLS/μl)	HETERO 10^3 (CELLS/μl)	LYMPHO 10^3 (CELLS/μl)	MONO (CELLS/μl)	EOSINO (CELLS/μl)	BASO (CELLS/μl)
RTHA						
Mean	16	9.0	5.6	214	1300	258
SEM	2.0	1.1	0.9	82	256	68
Range	4–48	1.4–26.2	1.0–22.6	0–1710	0–5520	0–1440
N	31	31	31	31	31	31
AMKE						
Mean	8.6	5.0	3.4	17	31	102
SEM	0.9	0.7	0.6	9.0	15	28
Range	1.7–17	1.1–13.6	0.4–12	0–150	0–300	0–450
N	21	21	21	21	21	21
P-value	0.001	0.004	0.05	0.02	0.0001	0.04

[a] WBC EST is white blood cell concentration estimate; HETERO is heterophil; LYMPHO is lymphocite; MONO is monocyte; EOSINO is eosinophil; BASO is basophil.

albumin/globulin ratios were 0.56 and 0.43 for Red-tailed Hawks and American Kestrels, respectively. American Kestrels had a higher plasma sodium concentration than Red-tailed Hawks (Table 4).

DISCUSSION

Cooper (1989) emphasized the importance of health monitoring for migrating raptors due to the stresses of competition, contact with diseased or contaminated prey, fatigue, reduced food intake and metabolism of body reserves. Therefore, hematological and plasma parameters from wild raptors are essential tools for assessing the health and understanding the physiology of these birds. However, detailed data are limited for wild raptors (Hunter and Powers 1980, Gessaman et al. 1986, Gonzalez and Hiraldo 1991, Lavin et al. 1992, Powers et al. 1994).

Hematozoa infection rates reported for American Kestrels and Red-tailed Hawks from Colorado are nearly identical to those detected in wintering

Table 2. Plasma enzyme activities of wild Red-tailed Hawks (RTHA) and American Kestrels (AMKE).

SPECIES	LDH[a] (IU/L)	AP (IU/L)	ALT (IU/L)	AST (IU/L)	CK (IU/L)	CHE (μmol/ml/min)	ACHE (μmol/ml/min)	BCHE (μmol/ml/in)
RTHA								
Mean	798	27	38	327	1948	0.66	0.14	0.52
SEM	107	1.6	4.1	17	429	0.03	0.01	0.02
Range	176–3284	12–46	2–138	182-761	332-16 126	0.28–1.14	0.04–0.37	0.21–1.04
N	45	37	45	45	44	44	44	44
AMKE								
Mean	798	242	52	98	891	1.9	1.6	0.26
SEM	28	12	3.4	7.6	37	0.05	0.05	0.01
Range	520–1710	57–522	16–171	44–405	286–2078	0.78–3.41	0.61–3.04	0.10–0.49
N	64	60	60	64	64	86	86	86
P-value	0.9	0.0001	0.01	0.0001	0.02	0.0001	0.0001	0.0001

[a] LDH is lactate dehydrogenase; AP is alkaline phosphatase; ALT is alanine aminotransferase; AST is aspartate aminotransferase; CK is creatine kinase; CHE is total cholinesterase; ACHE is acetylcholinesterase; BCHE is butrylcholinesterase.

Table 3. Plasma biochemistry concentrations of wild Red-tailed Hawks (RTHA) and American Kestrels (AMKE).

SPECIES	TP[a] (g/dl)	ALB (g/dl)	GLOB (g/dl)	CHOLEST (mg/dl)	BUN (mg/dl)	UA (mg/dl)	GLUC (mg/dl)
RTHA							
Mean	3.6	1.3	2.4	191	5.0	8.3	424
SEM	0.1	0.04	0.1	6.0	0.4	1.4	9.2
Range	2.2–5.3	0.7–2.0	1.0–3.9	95–296	1–12	1.5–38.1	306–574
N	45	39	39	45	45	45	45
AMKE							
Mean	3.1	0.9	2.2	277	7.4	18.5	428
SEM	0.1	0.02	0.1	7.7	0.4	1.1	6.7
Range	2.2–6.2	0.4–1.4	1.5–5.0	181–569	2–12	3–49.2	329–618
N	64	60	60	64	60	64	64
P-value	0.0002	0.0001	0.2	0.0001	0.0001	0.0001	0.8

[a] TP is total protein; ALB is albumin; GLOB is globulin; CHOLEST is cholesterol; BUN is blood urea nitrogen; UA is uric acid; GLUC is glucose.

American Kestrels and Red-tailed Hawks in California (Stabler and Holt 1965). It is not clear whether hematozoa infection is associated with pathogenesis in birds (Sibley and Werner 1984, Olsen and Gaunt 1985). However, there were no outward indications of disease or emaciation observed in the birds reported on here.

Total white blood cell counts or concentration estimates and white cell differential count reference ranges are important to establish, since a shift in the proportions of white cell types, rather than a change in absolute numbers, may be the only consequence of disease (Campbell and Dein 1984). Response to stressors can also induce a shift in the proportions of white cell types in birds, particularly lymphocytes, which decrease, and heterophils, which increase (Gross and Siegel 1983). In our study, the prolonged holding period (1–2 hr) prior to blood sample collection makes it unlikely that the effects of capture stress on heterophil and lymphocyte levels were avoided. This, together with the fact that white blood cell estimates were not corrected for anemia, should be taken into consideration in the interpretation of the white blood cell parameters reported here.

Table 4. Plasma trace element concentrations of wild Red-tailed Hawks (RTHA) and American Kestrels (AMKE).

SPECIES	NA[a] (MEQ/L)	K (MEQ/L)	CA (mg/dl)	P (mg/dl)
RTHA				
Mean	151	4.9	8.0	2.6
SEM	0.5	0.6	0.2	0.2
Range	144–158	2.1-15.3	4–10	0.9–5.7
N	31	35	38	39
AMKE				
Mean	158	6.9	7.9	2.4
SEM	0.6	0.7	0.1	0.1
Range	155–160	2.4–24.6	6.4–11.7	0.4–5.6
N	7	60	64	60
P-value	0.0001	0.03	0.7	0.5

[a] NA is sodium; K is potassium; CA is calcium; P is phosphorous.

The predominant leukocytes of wild Red-tailed Hawks and American Kestrels were heterophils and lymphocytes, accounting for 95% or more of the white blood cells, on average. The numbers of monocytes, eosinophils and basophils were low, though variable. The degree of variability observed is not unique to these two raptor species (Powers et al. 1994) or to wild raptors (Hernandez et al. 1990). This variability is most likely indicative of individual response to different immunological challenges and different stages of response. Eosinophils play a defensive role against parasites such as worms and protozoa, while monocytes are critical in defense against intracellular parasites such as viruses and certain bacteria. The role of basophils is less well understood; however, they are involved in the early inflammatory response of allergic reaction (Maxwell 1993). The mean total white blood cell count and white cell differential counts reported by Hernandez et al. (1990) for captive

Common Buzzard (*B. buteo*) were consistently lower than those of wild Red-tailed Hawks, except for eosinophils and basophils. White blood cell estimates and heterophil count estimates of wild Red-tailed Hawks were two times higher, while lymphocyte and monocyte count estimates were four times higher than those of captive Common Buzzards. These differences are consistent with heterophil and lymphocyte shifts associated with capture stress. Wild American Kestrels were more similar in these measures to captive Common Buzzard than were wild Red-tailed Hawks.

Enzyme activities of wild Red-tailed Hawks and American Kestrels were similar to values previously reported for captive hawks and falcons. Wild Red-tailed Hawks had similar enzyme activities to captive Red-tailed Hawks for AP, ALT and AST (Kollias and McLeish 1978) and to captive Common Buzzards for LDH, ALT and AST (Hernandez et al. 1990). CK activity was higher and more variable in wild Red-tailed Hawks than in captive Common Buzzards; this may be indicative of capture myopathy exhibited by the wild Red-tailed Hawks. Dabbert and Powell (1993) reported elevated CK activity as evidence of capture myopathy in Mallards (*Anas platyrhynchos*) sampled after a similar time duration as the raptors reported on here. Wild American Kestrels had higher AP activity, but similar LDH and AST activities compared with captive Peregrine Falcons (*F. peregrinus tundrius*) (Gee et al. 1981). For most enzyme activities, wild birds had larger ranges than those reported in the literature for captive raptors. Presumably, this is largely due to natural variability in the condition of wild birds (e.g., due to differences in migratory status, time of capture, activity level, time since last meal and contact with pathogens and other stressors prior to capture).

Plasma concentrations of total protein, albumin, globulin, cholesterol, uric acid, calcium and phosphorus of wild Red-tailed Hawks were similar to means and ranges reported for captive Common Buzzards (Ferrer et al. 1987, Garcia-Rodriguez et al. 1987b, Hernandez et al. 1990). Plasma concentrations of total protein, albumin, blood urea nitrogen, calcium and phosphorus of wild American Kestrels were also similar to means and ranges reported for captive Peregrine Falcons (Gee et al. 1981). Glucose concentration was lower for both captive Common Buzzards and Red-tailed Hawks than wild Red-tailed Hawks; similarly, glucose and uric acid concentrations were lower for captive Peregrine Falcons than wild American Kestrels (Kollias and McLeish 1978, Gee et al. 1981, Hernandez et al. 1990). Garcia-Rodriguez et al. (1987a) reported increases in the concentrations of blood urea nitrogen, uric acid, glucose and cholesterol during long-term fasting of captive Common Buzzards. An analogous situation may exist for wintering raptors, which experience relatively low prey densities and increased metabolic demands, and may account for the higher levels of these components found in our study.

Comparison of Red-tailed Hawk and American Kestrel hematological parameters, enzyme activities and other plasma constituents indicate important interspecific differences in hematological profiles, knowledge of which is critical for use in health assessment. In an attempt to report data from healthy wild raptors, individuals that did not meet qualitative health criteria or were known to carry elevated OP residues were excluded from the analyses. However, since white blood cell estimates were not corrected for anemia and substantial time elapsed between capture and blood sampling, capture stress may have influenced the heterophil and lymphocyte count estimates and CK activity values reported here. Furthermore, determining whether statistically significant differences are biologically important is not straightforward; however, a number of the highly significant differences may reflect differences important for clinical consideration during health assessment, particularly AP, AST, ACHE activities and cholesterol concentration. Clearly, further studies are desirable for expansion and confirmation of values reported here for raptors wintering in California. In particular, investigations on factors contributing to variability in hematological values such as sampling time, body condition, diet, season, geographical location and captive vs. wild status, are warranted.

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