Sources of variation for nutritional condition indices of the plasma of migratory lesser kestrels in the breeding grounds

Airam Rodríguez [a,*], Juan J. Negro [a], Jordi Figuerola [b]

[a] Department of Evolutionary Ecology, Estación Biológica de Doñana (CSIC), Avda. Américo Vespucio S/N, 41092 Seville, Spain
[b] Department of Wetland Ecology, Estación Biológica de Doñana (CSIC), Avda. Américo Vespucio S/N, 41092 Seville, Spain

Keywords:
Cholesterol
Falco naumanni
Free-living birds
Nutritional condition
Plasma
Triglycerides
Urea
Uric acid

abstract

Although published information on reference values for biochemical parameters in birds of prey has increased during the last years, little is known on their sources of variation. We used an insectivorous and small migratory raptor species, the lesser kestrel Falco naumanni, as a model. We looked for sources of variation of nutritional biochemical parameters (i.e. triglycerides, cholesterol, uric acid and urea) of both nestlings and adults. Reference values indicated that, as a rule, lesser kestrel showed more elevated triglycerides, urea and uric acid levels than other raptors. All analyzed factors except gender (i.e. year, colony, sampling time, presence/absence of a geolocator, body mass, laying date and capture date) reached significance for at least one biochemical parameter. In the morning, we found an important postprandial increase in the concentration of all biochemical parameters in nestlings, and uric acid and urea levels in adults. A positive relationship was detected between triglycerides and body mass in nestlings and adults. Although we did not find differences between blood biochemical parameters of the oldest and youngest chick of each brood, we found that cholesterol levels were lower in nestlings from larger broods. Coloration of tarsi (measured as brightness) was related to triglycerides and urea levels of nestlings and adults, respectively. The feeding habits of lesser kestrel probably explain the different levels and patterns of variation of metabolites in comparison to more carnivorous raptors eating mammals or birds.

1. Introduction

During long-term fasting in endotherm vertebrates, three different physiological phases based on changes in body mass and plasma biochemistry can be distinguished (see review in McCue, 2010). The first phase of fasting is characterized by a severe body-mass loss as well as a decrease of the levels of uric acid and urea in plasma. During the second phase, stored lipids are used as an energy source, but the protein catabolism residues (urea and uric acid) maintain a low concentration. In the third phase (prolonged fasting), levels of glucose in blood drop dramatically and uric acid and urea reach high values due to the use of structural proteins as an energy resource (Bauchinger and Biebach, 2001). Finally, animals suffer a severe body mass loss approaching death. During the three fasting phases, triglycerides steadily decrease (Jenni-Eiermann and Jenni, 1998; Alonso-Alvarez and Ferrer, 2001).

Despite an increase of studies publishing reference values of free-living raptors during the last years (e.g. Stein et al., 1998; Casado et al., 2002; Hanauska-Brown et al., 2003; Mealey et al., 2004; Sarasola et al., 2004; Limiñana et al., 2009; Hernández and Margalida, 2010), most of the studies focusing on blood biochemistry have been conducted on captive birds. However, these have non-natural diets, regular access to food, and are forced to live in relative small enclosures in comparison with the natural home ranges, and thus, blood parameters might vary with respect to free-ranging animals (Dobado-Berrios et al., 1998; Balbontín and Ferrer, 2002; Villegas et al., 2002). Therefore, reference values for biochemical parameters of wild individuals are invaluable for ecophysiologists studying free-ranging birds (e.g. nutritional status, presence of diseases or exposure to pollutants; e.g. Artacho et al., 2007), and may also help the managers of rehabilitation centers or captive programs to keep birds in conditions as similar as possible to the wild ones. Furthermore, the majority of studies focusing on blood biochemistry have been conducted in large raptors (e.g. eagles or vultures) which ingest relatively large amounts of food at one time and endure longer fasting periods than smaller species. Therefore, feeding habits could influence the interpretation of some biochemistry values, especially those related to prolonged fasting periods, such as urea and uric acid. In large raptors, high values of these parameters have invariably been interpreted as indicators of low nutritional condition (Ferrer, 1994; Ferrer and Dobado-Berrios, 1998; Balbontín and Ferrer, 2005).

The lesser kestrel Falco naumanni is one of the smallest European raptor species (along with the Merlin Falco columbarius and the Red-Footed Falcon Falco vespertinus). Its diet is basically composed of

* Corresponding author. Tel.: +34 954 23 23 40.
E-mail address: airamrguez@ebd.csic.es (A. Rodríguez).

insects (i.e., grasshoppers, beetles, crickets), but it also feeds on small mammals (Rodríguez et al., 2010; Pérez-Granados, 2010; and references therein). In contrast to larger raptors, the lesser kestrel typically eats several times per day due to the small size of its prey. Starvation is the main cause of nestling mortality (Negro, 1997), suggesting they are not well adapted to long fasting periods. This migratory falcon's breeding range spans from China to the Iberian peninsula and its wintering grounds are located in sub-Saharian Africa (Rodríguez et al., 2009a, 2011). Since the 1950s, its populations have suffered a severe decline (estimated at more than 30% of the world population), leading to its current vulnerable status (BirdLife International, 2011). As a consequence, numerous breeding programs have been put in place for reintroduction purposes (Pomarol, 1993; Alcaide et al., 2010). Therefore, information presented here on sources of variation of nutritional biochemical parameters is valuable to assess condition of the captive stock, which in Spain only numbers several hundred pairs in at least five different breeding centers.

We have analyzed four biochemical parameters related to fat (triglycerides and cholesterol) and protein metabolism (uric acid and urea; see McCue, 2010), in adult and nestling lesser kestrels. These parameters have shown to be related to the physiological and nutritional state of birds (Jenni-Eiermann and Jenni, 1998; Alonso-Alvarez and Ferrer, 2001; Alonso-Alvarez et al., 2002a, 2002b; Sarasola et al., 2004). Our aim is, first, to report reference values of selected plasma biochemical parameters that have been previously reported as nutritional condition indices in larger and mainly vertebrate-eating birds of prey. Second, to examine the influence of different factors, such as sex, colony, year, sampling time, body mass, brood size, capture date –for adults– and laying date –for nestlings–, on nutritional markers. Third, to evaluate differences of biochemical parameters between the eldest and the younger nestlings from the same brood, as in the lesser kestrel death due to starvation typically affects sequentially the youngest and smallest individuals in the brood. Thus, we hypothesize that youngest nestlings will show lower triglyceride and cholesterol levels and higher nitrogenous waste levels (urea and uric acid) as a consequence of protein catabolism. Last, we aim to determine the relationships between certain phenotypic traits (i.e. characteristics of plumage –tail band width– and bare parts coloration –brightness and chroma of tarsi) and blood biochemical parameters. We expect that these traits will be negatively correlated with nutritional condition.

2. Materials and methods

2.1. Study area and trapping procedures

Adult and nestling lesser kestrels (Falco naumanni) were captured during three consecutive breeding seasons (2007–2009) in two neighboring urban colonies distant 10 km, the cereal silo of La Palma del Condado (37º23′N, 6º33′W) and the Purificación church of Manzanilla (37º23′N, 6º25′W), Huelva province, southern Spain (for a detailed description of surrounding habitat see Rodríguez et al., 2006). Adults were captured from March to July at their nest cavities. Nestlings were sampled at the nest at the age of 24.0 ± 3.4 days (± SD). All birds were captured by hand and several body measurements were recorded (body mass, eighth primary, tail and wing length -the latter two only for adults). Body mass was measured with an electronic balance to the nearest 0.1 g and length measurements were done with a ruler to the nearest 1 mm. The width of the black terminal tail band was measured with a caliper at the raquis of the top central tail feather.

2.2. Color measurements

Coloration of the tarsi was measured using a Minolta 2600 spectrometer (Minolta, Osaka, Japan), which uses a high-energy xenon flash illumination and a dual-40-element silicon photodiode array (e.g. Negro et al., 2006). Automatic white calibration was performed before all measurements. Color was evaluated as the averaged reflectance over the wave length interval (360–740 nm) for brightness and as the difference between the maximum and minimum reflectance divided by the averaged reflectance for chroma (Anderson and Prager, 2006).

2.3. Blood sampling and plasma biochemistry

All birds were bled from the brachial vein (approximately 0.5 ml) between 9:00 and 15:00 h to minimize expected variations caused by circadian rhythms of the biochemical parameters (García-Rodriguez et al., 1987; Pérez-Rodríguez et al., 2008). The blood was collected in a heparinized tube and kept in a cooler until centrifugation. Blood was centrifuged (10 min; 4500 g; 4 °C), and the plasma and the cellular fraction were stored at −20 °C until analyses were conducted. Plasma was analyzed for triglycerides, cholesterol, urea, and uric acid using a Screen Point autoanaliser (Hospitex Diagnostics, Sesto Fiorentino, Italy), and commercial kits (Biolabo Labs, Maizy, France). Plasma biochemical analyses were performed by Wildvets S.L.P. (Seville, Spain; Rodríguez et al., 2009b). To assess repeatability (Lessells and Boag, 1987), two measurements of each biochemical parameter were taken from 99 randomly selected samples (30, 44 and 25 samples from 2007, 2008 and 2009, respectively; eleven adults –six females– and 88 nestlings −44 females). Coefficients of repeatability varied between 0.95 and 0.99. For analyses, we employed the mean values of the two readings of these repeated samples.

2.4. Sex determination

As the Lesser Kestrel shows a strong sexual dichromatism, adults were visually sexed according to plumage characteristics. However, gender determination of nestlings is sometimes difficult (Rodríguez et al., 2005). In this case, the cellular fraction was used as a source of DNA. We determined sex by PCR amplification of CHD genes using primers 2550 F and 2718R (Fridolfsson and Ellegren, 1999).

2.5. Statistical analyses

First, an ANOVA was employed to test differences in the plasma metabolite concentrations of nestlings and adults. As data coming from siblings in a same nest are not independent and some adults were sampled twice or more times, we used mean values for each brood and each adult individual, respectively. Second, we used general linear mixed models to explain the variations in blood biochemical parameters. Nestlings' models included sex, year and colony as factors, and sampling time, body mass and laying date as covariates. To avoid the non-independence of data from siblings, brood identity was included as a random effect. As some adult birds were fitted with geolocators during the 2007–2008 winter and some biochemical parameters of nestlings were affected by this procedure (see Rodríguez et al., 2009b), we included an additional fixed factor to control for this source of variation (0 = non geolocator-tagged bird or nestling without tagged parents, 1 = geolocator-tagged bird or nestling with at least one tagged parent). Adults' models included sex, year and age (yearlings or older than 1 year) as factors, and sampling time, body mass and date of capture as covariates. To avoid pseudoreplication, identity was included as a random effect given that some adult individuals were captured in different breeding seasons. Third, to assess the effect of brood size on biochemical parameters of nestlings, we ran models including the significant terms of our previous analyses and an ordinal variable (number of nestlings). In addition, we built general linear mixed models including the sibling order (the youngest and the eldest), brood size and their interaction as fixed factors and brood identity as a random factor. We introduced the interaction because differences between the youngest and the

Table 1
Blood chemistry values and results of ANOVA tests for nestling and adult Lesser Kestrels. All biochemical parameters are expressed as mg/L. Mean, SD and range are absolute values, i.e. they have not been transformed. ANOVA tests were conducted using the mean values of nestlings from the same nest and mean values of adults sampled twice or more times (see text).

Parameter	Nestlings			Adults			F	d.f.	P
	n	Mean ± SD	Range	n	Mean ± SD	Range			
Triglycerides[a]	332	3014 ± 2038	[346, 10380]	135	2572 ± 2553	[532, 13887]	15.23	1, 211	b0.001
Cholesterol[a]	326	2161 ± 651	[871, 4766]	132	2757 ± 917	[879, 7896]	30.17	1, 209	b0.001
Uric acid[b]	320	168 ± 64	[36, 385]	121	151 ± 76	[39, 340]	4.98	1, 203	0.027
Urea[b]	317	172 ± 91	[51, 595]	124	160 ± 94	[7, 475]	3.95	1, 204	0.052

[a] Variable log transformed.
[b] Variable square-root transformed.

eldest nestlings may be more apparent in larger brood sizes. The eighth primary feather of the wing has been used to estimate age of nestlings (Negro, 1997), thus, we assumed the eldest and youngest siblings in a brood showed the longest and the shortest eighth primary, respectively. To test the relationship between brood size and the nutritional condition of adults, we selected those adults captured during chick rearing activities (after June 10th). Given that this dataset contained a smaller number of individuals (n = 39), we used Pearson correlation analyses including breeding success (number of fledglings) and each biochemical parameter. Fourth, to evaluate the relationships between coloration phenotypic traits and biochemical parameters, we built models including the significant terms in the previous analyses. Each phenotypic trait (tail band width, chroma, and brightness) was included individually.

Because variable responses were not normally distributed, they were log or square-root transformed. F-ratio was used to test the significance of fixed effects in our full models (no backward or stepwise procedures were employed). Statistical analyses were conducted using JMP v.9 package. In some cases, the amount of plasma obtained was insufficient to assay all the parameters. For this reason, sample sizes are not uniform.

3. Results

3.1. Reference values

A total of 334 nestling, belonging to 112 broods, and 104 adult lesser kestrels were studied. Twenty eight adult birds were sampled two or three times, increasing the sample size for adults to 135. Excepting for urea levels, mean values for all biochemical parameters were significantly different between nestlings and adults, although ranges overlapped (Table 1).

3.2. Factors affecting blood parameters

The main factor affecting the blood biochemical parameters of nestlings was sampling time (Table 2), which reached significance for all parameters analyzed. A positive relationship was detected through the morning for all biochemical parameters (Fig. 1). Cholesterol, uric acid and urea levels were affected by year of sampling, indicating that it is a crucial factor in the nutritional status of lesser kestrel nestlings. Triglyceride levels were also affected by colony, and they showed a

Table 2
Summary of fixed effects and the overall variability explained (R^2) by the general linear mixed models on biochemical parameters of nestling and adult lesser kestrels. All response variables were log transformed, excepting uric acid (nestlings' model) and urea (adults' model) which were square-root transformed.

	Triglycerides		Cholesterol		Uric acid		Urea	
	Estimate ± SE	P	Estimate ± SE	P	Estimate ± SE	P	Estimate ± SE	P
Nestlings								
Intercept	1.009 ± 0.290	b0.001	2.035 ± 0.167	b0.001	1.011 ± 0.966	0.297	0.378 ± 0.241	0.119
Sex	–	0.102	–	0.087	–	0.398	–	0.187
Female	0.023 ± 0.001	–	0.011 ± 0.006	–	0.036 ± 0.042		0.013 ± 0.010	
Year	–	0.611	–	b0.001	–	b0.001	–	b0.001
2007	0.022 ± 0.025	–	−0.050 ± 0.015	–	−0.294 ± 0.082	–	−0.113 ± 0.020	–
2008	0.010 ± 0.022	–	0.032 ± 0.013	–	0.282 ± 0.074	–	0.061 ± 0.018	–
Colony	–	0.005	–	0.515	–	0.912	–	0.534
Silo	−0.053 ± 0.019	–	−0.008 ± 0.012	–	−0.007 ± 0.062	–	−0.010 ± 0.016	–
Geolocator	–	0.111	–	0.825	–	0.195	–	0.287
Untagged	−0.035 ± 0.022	–	−0.003 ± 0.014	–	−0.096 ± 0.074	–	−0.020 ± 0.018	–
Time of day	0.056 ± 0.011	b0.001	0.017 ± 0.007	0.011	0.142 ± 0.037	b0.001	0.029 ± 0.009	0.002
Body mass	0.002 ± 0.001	0.012	0.000 ± 0.001	0.822	0.003 ± 0.003	0.269	−0.000 ± 0.001	0.826
Laying date	0.004 ± 0.002	0.024	0.001 ± 0.001	0.522	0.008 ± 0.005	0.148	0.004 ± 0.001	0.002
Brood identity (%)	6.1	–	38.4	–	17.3	–	22.2	–
R^2 (%)	26.9	–	55.7	–	36.1	–	45.8	–
Adults								
Intercept	0.678 ± 0.323	0.038	2.527 ± 0.183	b0.001	0.094 ± 0.265	0.778	0.564 ± 1.483	0.705
Sex	–	0.478	–	0.264	–	0.750	–	0.751
Female	−0.023 ± 0.032	–	−0.020 ± 0.018	–	0.008 ± 0.026	–	0.046 ± 0.143	–
Year	–	b 0.001	–	0.157	–	0.506	–	0.031
2007	0.185 ± 0.042	–	−0.031 ± 0.024	–	−0.035 ± 0.035	–	−0.344 ± 0.198	–
2008	−0.056 ± 0.043	–	0.046 ± 0.024	–	0.040 ± 0.036	–	0.511 ± 0.191	–
Age	–	0.168	–	0.262	–	0.405	–	0.514
	0.048 ± 0.034	–	0.022 ± 0.019	–	0.023 ± 0.028	–	0.105 ± 0.161	–
Time of day	0.028 ± 0.017	0.103	−0.003 ± 0.010	0.797	0.049 ± 0.014	b0.001	0.173 ± 0.078	0.028
Body mass	0.009 ± 0.002	b0.001	−0.000 ± 0.001	0.947	0.001 ± 0.002	0.357	0.003 ± 0.008	0.722
Capture date	0.000 ± 0.001	0.796	−0.001 ± 0.001	0.199	0.002 ± 0.001	0.006	0.006 ± 0.004	0.162
Identity (%)	10.0	–	7.0	–	8.1	–	2.7	–
R^2 (%)	46.8	–	20.5	–	38.4	–	26.1	–

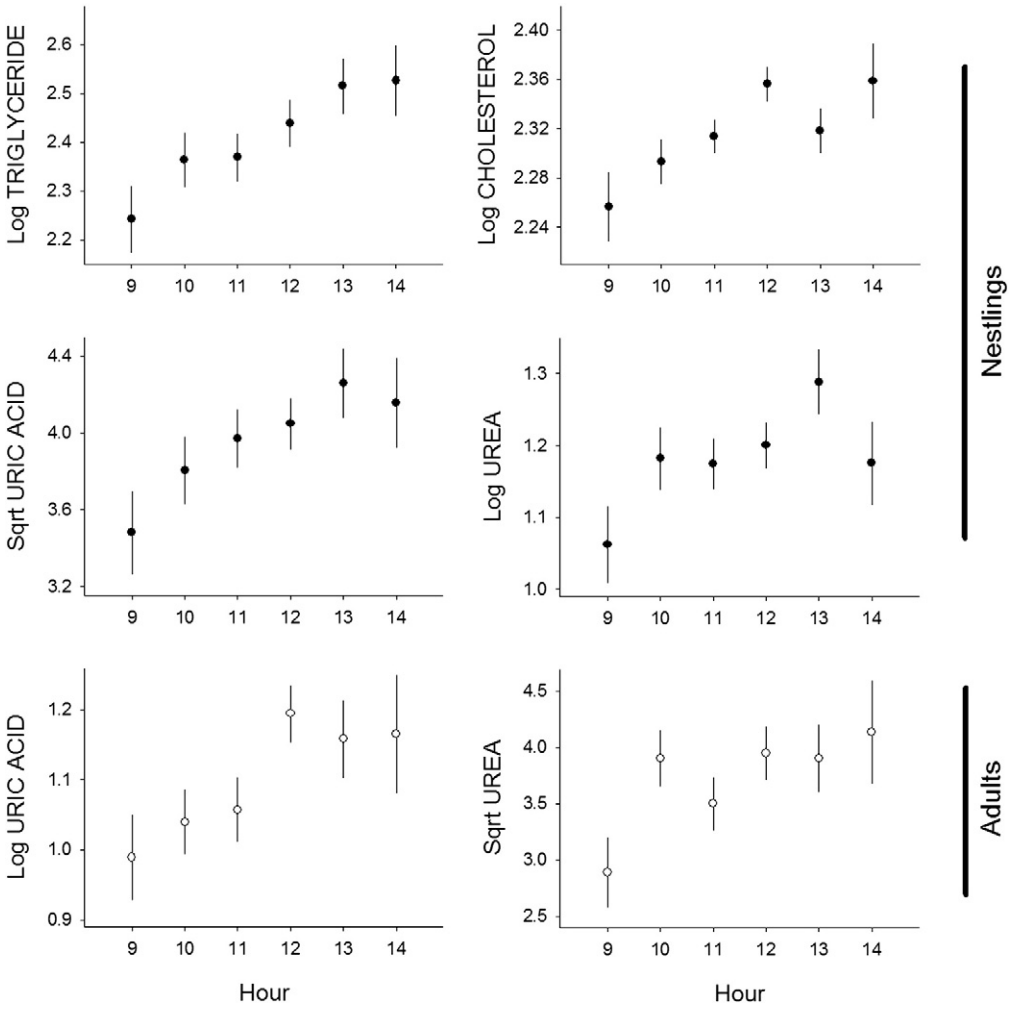

Fig. 1. Relationship between biochemical parameters of nestling (filled circles) and adult (open circles) lesser kestrels and sampling time. Least square means (± SE) from saturated models from Table 2 are shown.

positive relationship with body mass (Fig. 2) and laying date. Last, levels of urea increased with laying date. The geolocator tagging effect was not significant for any parameter. Models explained from 26.9 to 55.7% of the total variation in the blood parameters (Table 2). Brood identity (random factor) explained from 6.1 to 38.5% of the total variation (Table 2).

For adults, levels of triglycerides varied between years and showed a positive relationship with body mass (Fig. 2). Cholesterol concentration was not explained by any independent variable measured in this study. Uric acid and Urea were affected by sampling time, and additionally by date of capture (positive relationship) and by year of sampling, respectively (Table 2). Models explained from 20.5 to 46.8% of the total variation in the blood parameters (Table 2). The random factor (individual identity) explained low percentages of the total variation (from 2.7 to 10%).

3.3. Effect of brood size on biochemical parameters

Models including the significant terms listed in Table 2 for each biochemical parameters and the number of nestlings (as an ordinal variable) indicated that cholesterol was negatively affected by brood size in nestlings ($F = 3.88$; d.f. = 4, 120.1; $P = 0.005$; Fig. 3A). Number of nestlings tended to be positively related to triglycerides ($F = 2.07$; d.f. = 4, 126.8; $P = 0.087$), but in a second analysis excluding the single nestlings, the relationship reached significance ($F = 2.47$; d.f. = 3, 94.5; $P = 0.047$; see Fig. 3B). Number of siblings was not related to uric acid nor urea ($F = 1.18$; d.f. = 4, 113.3; $P = 0.322$ and $F = 0.16$; d.f. = 4, 103.5; $P = 0.956$, respectively. No significant differences were found between the youngest and eldest nestlings for any biochemical parameter (all P-values > 0.125) or the interaction between hatching order and brood size (all P-values > 0.163). In adults, only urea levels were correlated with number of fledglings ($r = 0.325$; $P = 0.049$, Fig. 4).

3.4. Correlates of biochemical parameters and phenotypic traits

Tarsi brightness was the single phenotypic trait which correlated with some blood biochemical parameter. Negative relationships were observed between brightness and triglyceride levels, and brightness and urea levels for nestlings and adults, respectively (see Table 3).

4. Discussion

4.1. Reference values

We provide for the first time reference values for four plasma biochemical parameters indicative of nutritional condition for free-living nestling and adult lesser kestrels. Nearly all ranges of plasma metabolites for nestlings and adults overlapped with those described for free-living larger raptorial birds (Dobado-Berrios et al., 1998; Ferrer and Dobado-Berrios, 1998; Stein et al., 1998; van Wyk et al., 1998; Bowerman et al., 2000; Balbontín and Ferrer, 2002; Casado et al.,

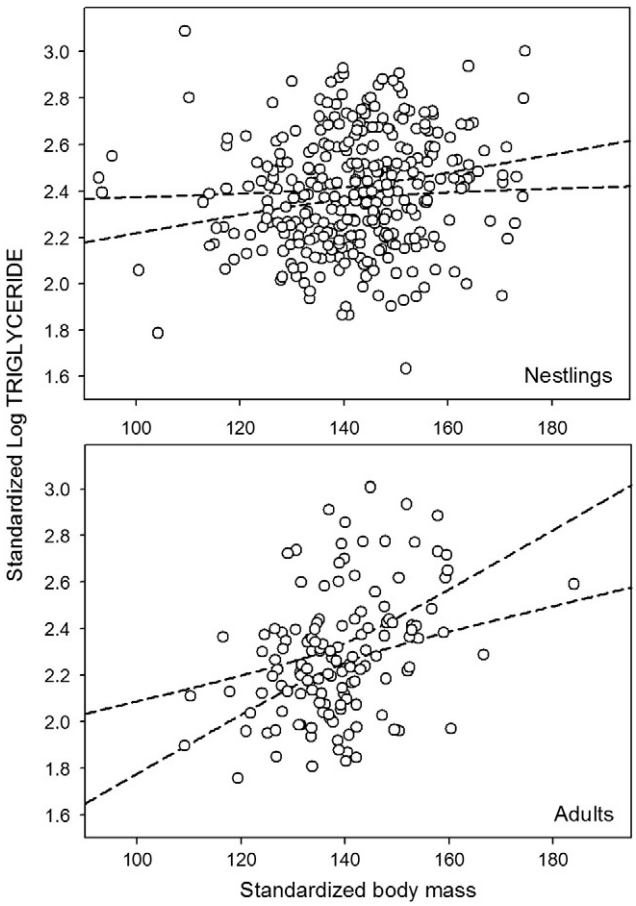

Fig. 2. Relationship between triglyceride plasma levels and body mass (both variables standardized by regression of the other variables in the models in Table 2, i.e. leverage plots) for nestlings and adults. Dashed lines indicate 95% confidence bands of simple linear regressions.

Fig. 3. Relationship between cholesterol (A) and triglycerides (B) levels of nestlings and number of nestlings. Least square means (± SE), from models including the significant terms from Table 2 and number of nestlings as an ordinal variable, are shown.

2002; Villegas et al., 2002; Hanauska-Brown et al., 2003; Mealey et al., 2004; Sarasola et al., 2004; Limiñana et al., 2009; Hernández and Margalida, 2010). However, we consistently detected higher mean values of triglycerides in lesser kestrel than in the above cited studies on larger raptors. Given that plasma triglyceride levels decline after short periods of fasting (Jenni-Eiermann and Jenni, 1997), these differences might be explained by the higher feeding frequency of the lesser kestrel in comparison with larger raptors, which endure longer fasting periods, sometimes more than a week, due to unpredictability of their food. In this sense, adult lesser kestrels need 3.1–9 min to acquire a prey item during hunting activities (Donázar et al., 1993; Tella et al., 1998) and the prey delivery rates to nestlings vary between 2.04–3.98 items per hour (Tella et al., 1996), although under food shortage conditions, prey delivery rates can notably decrease (Negro et al., 2000; Rodríguez et al., 2006).

While reported in fewer studies, urea and uric acid also were lower in some raptor species (Lavin et al., 1992; Stein et al., 1998; Bowerman et al., 2000; Balbontín and Ferrer, 2002; Villegas et al., 2002; Mealey et al., 2004; Sarasola et al., 2004; Limiñana et al., 2009; Hernández and Margalida, 2010), or in non-raptorial species such as the greater flamingo Phoenicopterus roseus or the white stork Ciconia ciconia (Amat et al., 2007; Jerzak et al., 2010). Plasma urea and uric acid are protein catabolism residues and good indicators of low nutritional condition because protein catabolism is active during the last phase of fasting (McCue, 2010). However, high levels of these parameters can be induced by protein-rich diets (Jenni-Eiermann and Jenni, 1998). Although a non negligible part is sequestered in indigestible exoskeleton, grasshoppers contain higher levels of proteins than mammals or birds (Bird et al., 1982). Thus, we think differences reported here are a consequence of the lesser kestrel mainly insectivorous diet (based on Ortopthera; e.g. Rodríguez et al., 2010) more than to the effect of

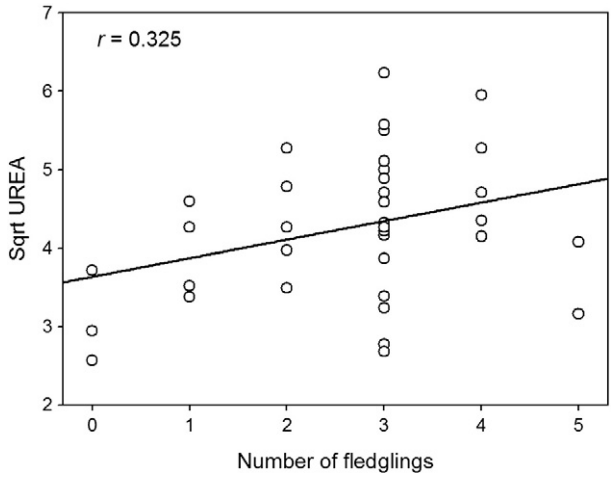

Fig. 4. Relationship between urea levels of adult lesser kestrels captured after June 10th and breeding success (measured as number of fledglings).

Table 3
Results of general linear mixed models for phenotypic traits introducing significant terms of Table 2 and brood identity (for nestlings) and individual identity (for adults) as random factors.

Variable response	Variable independent	Estimate ± SE	F	d.f.	P
Nestlings					
Triglycerides[a]	Tail Band Width	0.00038 ± 0.00385	0.01	1275.9	0.921
	Chroma	0.10854 ± 0.13807	0.91	1278.3	0.341
	Brightness	−0.01157 ± 0.00487	5.65	1269.2	0.018
Cholesterol [a]	Tail Band Width	0.00055 ± 0.00169	0.10	1285.8	0.745
	Chroma	−0.08467 ± 0.05869	2.08	1301.3	0.150
	Brightness	0.00041 ± 0.00216	0.03	1302.8	0.851
Uric acid[b]	Tail Band Width	−0.00758 ± 0.01195	0.40	1273.1	0.527
	Chroma	0.01123 ± 0.39688	0.00	1276.4	0.977
	Brightness	−0.01057 ± 0.01484	0.50	1281.1	0.477
Urea [a]	Tail Band Width	0.00114 ± 0.00295	0.14	1272.4	0.699
	Chroma	−0.16672 ± 0.09967	2.79	1281.3	0.096
	Brightness	0.00339 ± 0.00371	0.83	1290.8	0.361
Adults					
Triglycerides[a]	Tail band width	0.00665 ± 0.00415	2.56	1104.3	0.112
	Chroma	0.04139 ± 0.14824	0.07	1120.6	0.781
	Brightness	−0.01163 ± 0.00731	2.52	1123.7	0.115
Cholesterol [a]	Tail band width	0.00217 ± 0.00206	1.11	1100.7	0.294
	Chroma	−0.03074 ± 0.07727	0.15	1126.4	0.691
	Brightness	0.00216 ± 0.00400	0.29	1126.7	0.591
Uric acid [a]	Tail band width	0.00221 ± 0.00301	0.53	182.12	0.466
	Chroma	0.06414 ± 0.12143	0.25	1104.9	0.599
	Brightness	−0.00511 ± 0.00604	0.71	1113.3	0.399
Urea[b]	Tail band width	−0.00183 ± 0.01705	0.01	166.86	0.915
	Chroma	0.15205 ± 0.65867	0.05	1112.6	0.818
	Brightness	−0.06485 ± 0.03199	4.11	1112.2	0.045

[a] Variable log transformed.
[b] Variable square-root transformed.

starvation (i.e. protein catabolism; Jenni-Eiermann and Jenni, 1998; McCue, 2010) given the high levels of triglycerides.

4.2. Factors affecting blood parameters

The important effect of sampling time has been reported in several studies using captive birds (García-Rodriguez et al., 1987; Ferrer et al., 1994; Pérez-Rodríguez et al., 2008), but, as far as we know, it has been only described in a published field study for triglycerides and uric acid levels in adult passerines (Jenni-Eiermann and Jenni, 1997). We found an increase in the concentration of all four biochemical parameters with sampling time as daytime advanced (triglycerides, cholesterol, uric acid and urea in nestlings, and uric acid and urea in adults; Fig. 1). The increase in the values of blood biochemical parameters detected in nestlings seems to be related to the increase of feeding rates in the morning. Thus, during the first daytime hours we sampled nestlings that had fasted overnight (at least for the nine nighttime hours), but that were subsequently fed by parents during the morning. In adults, the same pattern was found for uric acid and urea levels (Fig. 1), and it may be due to energy cost incurred in food provisioning given that an increase for triglyceride and cholesterol levels was not observed. The positive relationship between plasma uric acid concentration and date of capture, which suggests an important energy cost of breeding duties, also supports this idea. However, we cannot discard that the same hypothesis suggested for nestlings can be acting on adults, i.e. a postprandial increase in the morning.

Contrary to other studies focused on larger raptors (Casado et al., 2002; Sarasola et al., 2004) or other bird species (e.g. Artacho et al., 2007; Jerzak et al., 2010), no differences between sexes were found for plasma concentration of the four analyzed parameters, despite the sexual size dimorphism of lesser kestrel and the mate-feeding by males to their female mates during the breeding period (Donázar et al., 1992). However, no sexual differences have been detected in free-living nestlings of other sexual size dimorphic raptors such as the Spanish imperial eagle Aquila adalberti, the Bonelli's eagle Aquila fasciata or the Montagu's harrier Circus pygargus (Ferrer and Dobado-Berrios, 1998; Balbontín and Ferrer, 2002; Limiñana et al., 2009) or in free-living adults of the American kestrel Falco sparverius or the northern goshawk Accipiter gentilis (Stein et al., 1998; Hanauska-Brown et al., 2003).

Inter-annual variation seems to be another important factor explaining differences in the biochemical parameters of lesser kestrel nestlings, as it reached significance for cholesterol, uric acid and urea. For these three parameters, lowest values were detected in 2007 and the highest ones in 2008 (data not shown). Also for adults, year of sampling reached significance for triglyceride and urea levels, although the pattern for triglycerides was opposite to nestlings (data not shown). These annual differences could be related to quantitative and qualitative differences in prey abundance as a consequence of annual weather conditions, which have a strong effect on breeding success (Rodríguez and Bustamante, 2003). However, we detected a decrease in the breeding success during the studied years (2007, 2008 and 2009 reaching productivity values of 3.3, 3.2 and 2.5 fledglings per successful nest, respectively), that suggests the involvement of multiple factors, apart from diet.

Triglycerides level seems to be one of the best indicators of fasting in field studies given its interspecific consistency (Jenni-Eiermann and Jenni, 1997). Positive relationships between triglycerides concentration and body mass (or body condition) have been described in passerines, raptors and pelecaniformes (Jenni-Eiermann and Jenni, 1998; Sarasola et al., 2004; Villegas et al., 2004). Our data also indicate a relationship between triglyceride and condition, both in nestlings and adults (Fig. 2).

We found a positive relationship between urea levels and laying date of nestlings (Table 2). The same pattern was found in Spanish imperial eagle nestlings, but not in Bonelli's eagle nestlings (Balbontín and Ferrer, 2005), and the former was interpreted as earlier broods having better-nourished nestlings (Ferrer, 1994). However, triglyceride levels of nestlings also increased with the laying date in our

study (Table 2), suggesting that a poorer diet is not the actual explanation, but more probably a change in the diet (Rodríguez et al., 2010).

4.3. Effect of brood size on biochemical parameters

For nestlings, we found a negative relationship between cholesterol and brood size (Fig. 3). The differences were pronounced between extreme values of brood size, thus nestlings from nests with brood size equal to 1 showed the highest values and nestlings in broods with 5 nestlings showed the lowest levels. In the case of triglycerides and brood size, a high variability was observed in the single nestlings, but a clear positive relationship was found for nests with 2–5 nestlings. Although we do not have data to prove it, different type of prey delivered to nestlings by parents according to brood size may explain the observed trends.

Spanish imperial eagle nestlings from nests with larger brood sizes were better nourished (Ferrer, 1994) and Bonelli's eagle nestlings from nests with two siblings showed a poorer condition than single nestlings during a year of adverse environmental circumstances (heavy rainfall; Balbontín and Ferrer, 2005). However, these conclusions were obtained based on urea and uric acid plasma levels. Interestingly, we did not find any relationship with these two parameters, and there were not clear differences between the oldest and youngest siblings. This suggests that nestlings were equally fed by parents independently of their age, probably as a consequence of the high habitat quality and productivity of our colonies. The two colonies studied here (Silo and Purificación) showed the best habitat quality in comparison with four other colonies, and their habitat quality is high enough for population stability, according to a modeling approach based on prey abundance and prey size (see Rodríguez et al., 2006).

The relationship between urea levels and breeding success of adults may be explained by the work load of reproduction duties (Fig. 4). It has been experimentally demonstrated that kestrels adjust the provisioning rates according to brood size (Dawson and Bortolotti, 2003). Thus, the parents rearing a high number of nestlings have high feeding provisioning rates, reducing time for themselves. This would fit with the negative correlation between brightness and urea levels (see below).

4.4. Correlates of biochemical parameters and phenotypic traits

Melanin-based traits are static signals and may provide information on nutritional status at the time when feathers were growing. This may explain the lack of correlation between tail band width and biochemical parameters, which are indicative of short-term nutritional status (Jenni-Eiermann and Jenni, 1998). However, colored patches of bare skin are a reflection of recent physiological events, indicating the current physiological condition of the individual (e.g. Negro et al., 2006). High carotenoid plasma concentrations and brilliant coloration of fleshy parts have been reported as indicators of quality in kestrels (Bostrom and Ritchison, 2006; Casagrande et al., 2006; Dawson and Bortolotti, 2006; Vergara and Fargallo, 2011). Carotenoid pigments are responsible of yellow-orange coloration of kestrel tarsi (Bortolotti et al., 1996; Casagrande et al., 2009), and important physiological modulators, but these pigments can not be synthesized de novo by birds. Thus, a trade-off between the show and health functions have been suggested (Negro et al., 1998).

Brightness is inversely proportional to pigment concentration (Anderson and Prager, 2006). The negative relationships between brightness and triglyceride levels in nestlings suggest that the nestlings better nourished (higher triglyceride levels) had the higher carotenoid concentration in tarsi (lower brightness). In contrast, the negative relationship between brightness and urea levels in adults may be a consequence of higher work loads of high quality individuals (low brightness) rearing higher number of nestlings (see above).

5. Conclusions

The small size (atypical for the majority of raptor species studied so far for biochemical parameters) and its insectivorous diet suggest lesser kestrel may be more alike, ecologically speaking, to a medium sized passerine, such a shrike, than to a typical raptor. The high feeding frequency seems to be responsible for the general higher values of triglycerides, while the insectivorous diet may explain the higher uric acid and urea levels observed here. Also the increase of feeding rates during the morning may be the explanation for the increase of metabolite concentration, which is a key factor for future studies sampling at different times.

Acknowledgments

We thank Luis Cadahía, Álvaro Ramos and Miguel Alcaide for helping us with the molecular sex determination of nestlings. Javier Balbontín and an anonymous reviewer offered useful comments on early drafts. A.R. was supported by a pre-doctoral grant (I3P-BPG2006) from the Spanish National Research Council (CSIC). Projects CGL2006-07481/BOS, CGL2009-10652/BOS and HORUS Project P06-RNM-01712 funded this research. This study has been carried out in accordance with EC Directive 86/609/EEC for animal experiments and the current laws from Spain and Andalusia.

References

Alcaide, M., Negro, J.J., Serrano, D., Antolin, J.L., Casado, S., Pomarol, M., 2010. Captive breeding and reintroduction of the lesser kestrel Falco naumanni: a genetic analysis using microsatellites. Conserv. Genet. 11, 331–338.

Alonso-Alvarez, C., Ferrer, M., 2001. A biochemical study of fasting, subfeeding, and recovery processes in Yellow-Legged Gulls. Physiol. Biochem. Zool. 74, 703–713.

Alonso-Alvarez, C., Ferrer, M., Velando, A., 2002a. The plasmatic index of body condition in Yellow-legged Gulls Larus cachinnans: a food-controlled experiment. Ibis 144, 147–149.

Alonso-Alvarez, C., Velando, A., Ferrer, M., Veira, J.A.R., 2002b. Changes in plasma biochemistry and body mass during incubation in the Yellow-legged Gull. Waterbirds 25, 253–258.

Amat, J.A., Hortas, F., Arroyo, G.M., Rendón, M.A., Ramírez, J.M., Rendón-Martos, M., Pérez-Hurtado, A., Garrido, A., 2007. Interannual variations in feeding frequencies and food quality of greater flamingo chicks (Phoenicopterus roseus): evidence from plasma biochemistry and effects on body condition. Comp. Biochem. Physiol. A 147, 569–576.

Anderson, S., Prager, M., 2006. Quantifying colors. In: Hill, G.E., McGraw, K.J. (Eds.), Bird coloration. Mechanisms and measurements, vol. 1. Harvard University Press, Boston, pp. 41–89.

Artacho, P., Soto-Gamboa, M., Verdugo, C., Nespolo, R.F., 2007. Blood biochemistry reveals malnutrition in black-necked swans (Cygnus melanocoryphus) living in a conservation priority area. Comp. Biochem. Physiol. A 146, 283–290.

Balbontín, J., Ferrer, M., 2002. Plasma chemistry reference values in free-living Bonelli's Eagle (Hieraaetus fasciatus) nestlings. J. Raptor Res. 36, 231–235.

Balbontín, J., Ferrer, M., 2005. Condition of large brood in Bonelli's Eagle Hieraaetus fasciatus. Bird Study 52, 37–41.

Bauchinger, U., Biebach, H., 2001. Differential catabolism of muscle protein in Garden Warblers: flight and leg muscle act as a protein source during long-distance migration. J. Comp. Physiol. B 171, 293–301.

Bird, D.M., Shio-Kuen, H., Paré, D., 1982. Nutritive values of three common prey items of the American kestrel. Comp. Biochem. Physiol. A 73, 513–515.

BirdLife International, 2011. Species factsheet: Falco naumanniDownloaded from http://www.birdlife.org on 19 April 2011.

Bortolotti, G.R., Negro, J.J., Tella, J.L., Marchant, T.A., Bird, D.M., 1996. Sexual dichromatism in birds independent of diet, parasites and androgens. Proc. R. Soc. Lond. B 263, 1171–1176.

Bostrom, M.R., Ritchison, G., 2006. Possible relationships between morphology, territory quality, and skin color of American Kestrels. J. Field Ornithol. 77, 392–398.

Bowerman, W.W., Stickle, J.E., Sikarskie, J.G., Giesy, J.P., 2000. Hematology and serum chemistries of nestling bald eagles (Haliaeetus leucocephalus) in the lower peninsula of MI, USA. Chemosphere 41, 1575–1579.

Casado, E., Balbontín, J., Ferrer, M., 2002. Plasma chemistry in booted eagle (Hieraaetus fasciatus) during breeding season. Comp. Biochem. Physiol. A 131, 233–241.

Casagrande, S., Csermely, D., Pini, E., Bertacche, V., Tagliavini, J., 2006. Skin carotenoid concentration correlates with male hunting skill and territory quality in the kestrel Falco tinnunculus. J. Avian Biol. 37, 190–196.

Casagrande, S., Costantini, D., Tagliavini, J., Dell'Omo, G., 2009. Phenotypic, genetic, and environmental causes of variation in yellow skin pigmentation and serum carotenoids in Eurasian kestrel nestlings. Ecol. Res. 24, 273–279.

Dawson, R.D., Bortolotti, G.R., 2003. Parental effort of American kestrels: the role of variation in brood size. Can. J. Zool. 81, 852–860.

Dawson, R.D., Bortolotti, G.R., 2006. Carotenoid-dependent coloration of male American kestrels predicts ability to reduce parasitic infections. Naturwissenschaften 93, 597–602.

Dobado-Berrios, P.M., Tella, J.L., Ceballos, O., Donázar, J.A., 1998. Effects of age and captivity on plasma chemistry values of the Egyptian Vulture. Condor 100, 719–725.

Donázar, J.A., Negro, J.J., Hiraldo, F., 1992. Functional analysis of mate-feeding in the Lesser Kestrel Falco naumanni. Ornis Scand. 23, 190–194.

Donázar, J.A., Negro, J.J., Hiraldo, F., 1993. Foraging habitat selection, land-use changes and population decline in the lesser kestrel Falco naumanni. J. Appl. Ecol. 30, 515–522.

Ferrer, M., 1994. Nutritional condition of Spanish Imperial Eagle nestlings Aquila adalberti. Bird Study 41, 120–123.

Ferrer, M., Dobado-Berrios, P., 1998. Factors affecting plasma chemistry values of the Spanish imperial Eagle, Aquila adalberti. Comp. Biochem. Physiol. A 120, 209–217.

Ferrer, M., Amat, J.A., Viñuela, J., 1994. Daily variations of blood chemistry values in the chinstrp penguin (Pygoscelis antarctica) during the Antarctic summer. Comp. Biochem. Physiol. A 107, 81–84.

Fridolfsson, A.K., Ellegren, H., 1999. A simple and universal method for molecular sexing of non-ratite birds. J. Avian Biol. 30, 116–121.

García-Rodriguez, T., Ferrer, M., Recio, F., Castroviejo, J., 1987. Circadian rhythms of determined blood chemistry values in Buzzards and Eagle owls. Comp. Biochem. Physiol. A 88, 663–669.

Hanauska-Brown, L.A., Dufty, A.M., Roloff, G.J., 2003. Blood chemistry, cytology, and body condition in adult Northern Goshawk (Accipiter gentiles). J. Raptor Res. 37, 299–306.

Hernández, M., Margalida, A., 2010. Hematology and blood chemistry reference values and age-related changes in wild bearded vultures (Gypaetus barbatus). J. Wildl. Dis. 46, 390–400.

Jenni-Eiermann, S., Jenni, L., 1997. Diurnal variation of metabolic responses to short-term fasting in passerine birds during the postbreeding, molting and migratory period. Condor 99, 113–122.

Jenni-Eiermann, S., Jenni, L., 1998. What can plasma metabolites tell us about the metabolism, physiological satate and condition of individual birds? An overview. Biol. Cons. Fauna 102, 312–319.

Jerzak, L., Sparks, T.H., Kasprzak, M., Bochenski, M., Kaminski, P., Wisniewska, E., Mroczkowski, S., Tryjanowski, P., 2010. Blood chemistry in white stork Ciconia ciconia chicks varies by sex and age. Comp. Biochem. Physiol. B 156, 144–147.

Lavin, S., Cuenca, R., Marco, I., Velarde, R., Viñas, L., 1992. Hematology and blood chemistry of Marsh Harrier (Circus aeruginosus). Comp. Biochem. Physiol. A 103, 493–495.

Lessells, C.M., Boag, P.T., 1987. Unrepeatable repeatabilities: a common mistake. Auk 104, 116–121.

Limiñana, R., López-Overa, J.R., Gallardo, M., Fordham, M., Urios, V., 2009. Blood chemistry and hematologic values in free-living nestlings of Montagu's Harriers (Circus pygargus) in a natural habitat. J. Zoo Wild. Med. 40, 687–695.

McCue, M.D., 2010. Starvation physiology: Reviewing the different strategies animals use to survive a common challenge. Comp. Biochem. Physiol. A 156, 1–188.

Mealey, B., Parks, G.M., Pages, C.M., Millsap, B.A., Bass, O.L., Bossart, G.D., 2004. Serum chemistry values for nestling Bald Eagles (Haliaeetus leucocephalus) in Florida Bay. J. Raptor Res. 38, 96–100.

Negro, J.J., 1997. Lesser Kestrel Falco naumanni. BWP Update 1, 49–56.

Negro, J.J., Bortolotti, G.R., Tella, J.L., Fernie, K.J., Bird, D.M., 1998. Regulation of integumentary colour and plasma carotenoids in American Kestrels consistent with sexual selection theory. Funct. Ecol. 12, 307–312.

Negro, J.J., Bustamante, J., Melguizo, C., Ruiz, J.L., Grande, J.M., 2000. Nocturnal activity of lesser kestrels under artificial lighting conditions in Seville, Spain. J. Raptor Res. 34, 327–329.

Negro, J.J., Sarasola, J.H., Fariñas, F., Zorrila, I., 2006. Function and occurrence of facial flushing in birds. Comp. Biochem. Physiol. A 143, 78–84.

Pérez-Granados, C., 2010. Diet of adult lesser kestrels Falco naumanni during the breeding season in central Spain. Ardeola 57, 443–448.

Pérez-Rodríguez, L., Alonso-Alvarez, C., Martínez-Haro, M., Viñuela, J., 2008. Variation in plasma biochemical parameters in captive adult red-legged partridges (Alectoris rufa) during daylight hours. Eur. J. Wildl. Res. 54, 21–26.

Pomarol, M., 1993. Lesser Kestrel recovery project in Catalonia. In: Nicholls, M.K., Clarke, R. (Eds.), Biology and Conservation of Small Falcons: Proceedings of the 1991 Hawk and Owl Trust Conference. The Hawk and Owl Trust, London, pp. 24–28.

Rodríguez, C., Bustamante, J., 2003. The effect of weather on lesser kestrel breeding success: can climate change explain historical population declines? J. Anim. Ecol. 72, 793–810.

Rodríguez, C., Bustamante, J., Martínez-Cruz, B., Negro, J.J., 2005. Evaluation of methods for gender determination of Lesser Kestrel nestlings. J. Raptor Res. 39, 127–133.

Rodríguez, C., Johst, K., Bustamante, J., 2006. How do crop types influence breeding success in lesser kestrels through prey quality and availability? A modelling approach. J. Appl. Ecol. 43, 587–597.

Rodríguez, A., Negro, J.J., Bustamante, J., Fox, J.W., Afanasyev, V., 2009a. Geolocators map the wintering grounds of threatened lesser kestrels in Africa. Divers. Distrib. 15, 1010–1016.

Rodríguez, A., Negro, J.J., Fox, J.W., Afanasyev, V., 2009b. Effects of geolocator attachments on breeding parameters of Lesser Kestrels. J. Field Ornithol. 80, 399–407.

Rodríguez, C., Tapia, L., Kieny, F., Bustamante, J., 2010. Temporal changes in lesser kestrel (Falco naumanni) diet during the breeding season in southern Spain. J. Raptor Res. 44, 120–128.

Rodríguez, A., Alcaide, M., Negro, J.J., Pilard, P., 2011. Using major histocompatibility complex markers to assign the geographic origin of migratory birds: examples from the threatened lesser kestrel. Anim. Conserv. 14, 306–313.

Sarasola, J.H., Negro, J.J., Travaini, A., 2004. Nutritional condition and serum biochemistry for free-living Swainson's Hawks wintering in central Argentina. Comp. Biochem. Physiol. A 137, 697–701.

Stein, R.W., Yamamoto, J.Y., Fry, M.F., Wilson, B.W., 1998. Comparative hematology and plasma biochemistry of Red-Tailed Hawks and American Kestrel wintering in California. J. Raptor Res. 32, 163–169.

Tella, J.L., Hiraldo, F., Donázar, J.A., Negro, J.J., 1996. Costs and benefits of urban nesting in the lesser kestrel. In: Bird, D., Varland, D., Negro, J.J. (Eds.), Raptors in Human Landscapes: Adaptations to Built and Cultivated Environments. Academic Press, London, pp. 53–60.

Tella, J.L., Forero, M.G., Hiraldo, F., Donázar, J.A., 1998. Conflicts between lesser kestrel conservation and European agricultural policies as identified by habitat analyses. Conserv. Biol. 12, 593–604.

van Wyk, E., van der Bank, H., Verdoorn, G.H., 1998. Dynamics of haematology and blood biochemistry in free-living African whitebacked vulture (Pseudogyps africanus) nestlings. Comp. Biochem. Physiol. A 120, 495–508.

Vergara, P., Fargallo, J.A., 2011. Multiple coloured ornaments in male common kestrels: different mechanisms to convey quality. Naturwissenschaften 98, 289–298.

Villegas, A., Sánchez, J.M., Costillo, E., Corbacho, C., 2002. Blood chemistry and haematocrit of the black vulture (Aegypius monachus). Comp. Biochem. Physiol. A 132, 489–497.

Villegas, A., Sánchez, J.M., Corbacho, C., Corbacho, P., Vargas, J.M., 2004. Blood values of Bald Ibis (Geronticus eremita) in captivity: comparative ranges and variability with age, sex and physical condition. J. Ornithol. 145, 98–104.