Anti-parasite treatment and blood biochemistry in raptor nestlings

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Abstract

We investigated the effects of parasite-removal on various blood clinical-chemical variables (BCCVs). BCCVs are indicators of health, reflecting e.g. homeostasis of liver, kidney function and bone metabolism. The study was conducted in Norway on chicks of two predatory birds: white-tailed eagle *Haliaeetus albicilla* L., 1758 and northern goshawk *Accipiter gentilis* L., 1758. Chicks were treated against both endoparasites (internal parasites) and ectoparasites (external parasites). We treated against ectoparasites by spraying nests with pyrethrins. Within nests, chicks were randomly treated with either an anti-helminthic medication (fenbendazole), or sterile water (controls). Treatment against either ectoparasites or endoparasites led to higher levels of the bone and liver enzyme *alkaline phosphatase*. Bilirubin levels were lower when treated against ectoparasites, while bile acids were higher. Anti-endoparasite treatment led to higher creatinine levels. In northern goshawks, treating against endoparasites led to higher urea levels and lower potassium levels. Treatment against ectoparasites increased uric acid and urea levels and reduced bilirubin levels and protein:creatinine ratios. In conclusion, anti-parasite treatments led to changes in several BCCVs, suggesting differences in nutrient absorption and physiological state of chicks possibly related to costs of parasitism but maybe also the parasite treatment itself.

Introduction

An important aspect of current ecology is to investigate the effects of various stressors on wildlife. By stressor we mean physical, chemical, and biological factors that disturbs or interferes with the normal physiological equilibrium of an organism. Parasites are significant natural stressors in wild organisms, as they use their hosts' resources for own survival and reproduction, and because the hosts' immune defenses against these parasites may be resource demanding (de Lope et al. 1998). Immature individuals experience high growth and increased metabolism and this, in addition to a developing immune system, leads to a high nutrient and energy demand and parasites may therefore be more detrimental to wildlife during their early life stage (Janeway et al. 1999). Parasites induce perturbations in blood biochemistry and in the homeostasis of vertebrate species in general (Schulz et al. 2000; Harr 2002; Braun 2003; Richards and Proszkowiec-Weglarz 2007). Physiological homeostasis is critical for survival and growth of vertebrate species as it maintains the proper functioning of organ systems. Blood clinical-chemical variables (BCCVs) can for example reflect health and homeostasis of liver, kidney function and bone metabolism (de le Court et al. 1995; van Wyk et al. 1998; Thrall et al. 2006), and can indicate the status of energy metabolism, digestion, pancreatic diseases, electrolytic homeostasis and dehydration (Thrall et al. 2006). Measuring levels of (BCCVs) is therefore a valuable tool when assessing health and homeostasis.

Parasites may be classified as either endoparasites (internal parasites) or ectoparasites (external parasites). Many of the larger endoparasites are located in the digestive tract of their host where they absorb nutrients, often attaching to their hosts' intestinal mucosa by various hooks or spikes also leading to local lesions and inflammation (Schmid-Hempel 2011). Ectoparasites, on the other hand, are mostly arthropods that live on their hosts' integument, feeding on their blood, hair or feathers (Price 1980; Schmid-Hempel 2011). Endo- and ectoparasites may have different effects on their host as they may activate different parts of the immune system and drain the host of nutrients and energy (Schmid-Hempel 2011). Experimentally manipulating either ecto- or endoparasite levels in wildlife has been

77 shown to affect reproductive success (Hudson 1986; Møller 1990, 1993; de Lope et al. 1998; Stien et
78 al. 2002), chick survival (Newborn and Foster 2002; Amundson and Arnold 2010), territorial aggression
79 levels (Fox and Hudson 2001), and adult survival (Slattery and Alisauskas 2002; Hanssen et al. 2003;
80 Bustnes et al. 2006). While several of the abovementioned experimental studies have measured
81 reproductive and other fitness related variables in wildlife, an assessment of the effects of
82 experimental manipulation of parasite levels on physiological health indices, such as BCCVs seems to
83 be relatively infrequent (but see Reiner et al. (2009) for an example on domesticated animals).
84 Nonetheless, such health variables are a promising tool to study individual health and fitness since
85 they reflect the proximate mechanisms underlying growth, reproduction, survival and fitness of an
86 individual (Stearns 1992).

87 In the present study, we investigated the cost of parasitism by treating chicks and nests of two raptor
88 species, northern goshawk (*Accipiter gentilis* L., 1758) and white-tailed eagle (*Haliaeetus albicilla* L.,
89 1758), from endoparasites (chicks treated) and ectoparasites (nests treated). The effects of
90 antiparasite treatments on antioxidant defense, oxidant status and humoral immune function of these
91 raptors were already previously addressed (Hanssen et al. 2013). In the previous study by Hanssen et
92 al. (2013) we found that treating raptor chicks against ectoparasites relaxed their investment in
93 humoral immune defence, and also that the total antioxidant capacity was strengthened in all anti-
94 parasite treated groups. Raptors were chosen because parasites often use these as definitive hosts
95 (Crompton and Nickol 1985). Raptors are commonly infected with a variety of endoparasites, including
96 nematodes, trematodes, cestodes, acanthocephalans and coccidiae (Rausch 1983; Upton et al. 1990;
97 Cawthorn 1993; Smith 1993). In addition, raptors often build large nests that they use for several
98 consecutive years, enabling ectoparasites, such as fleas and lice, to winter in the nests and be ready to
99 infest birds when breeding commences in spring (for a review see Philips and Dindal 1977). We chose
100 these two study species in order (i) to examine the inter-species generality of associations between
101 parasites and BCCVs, and (ii) to evaluate how differences in sexual size dimorphism may affect the

costs of parasitism. Female northern goshawks are substantially larger than males, whereas this difference is not as pronounced in white-tailed eagles (Cramp and Simmons 1980). Conducting the same experiment in the two species may enable us to answer questions regarding the inter-species generality of how parasite load and health indices relate to each other, and how differences in sexual size dimorphism may affect the health of juveniles. We investigated the parasite-removal effects on various BCCVs. BCCVs are mostly used in veterinary medicine to assess health and to diagnose disease, thus both higher and lower levels of BCCVs than “normal” may indicate changes in physiological state or disease, including wildlife studies (e.g. Sonne et al. 2012). The challenge in wildlife studies is that different species have different “normal” levels of the different BCCVs, it may therefore be difficult to conclude on the basis of a random measurement of BCCVs if “normal” levels have not been measured for this species. We could not find other studies measuring “normal» levels of BCCVs in chicks of the two species studied here. However, we have a random group of chicks that has not been subjected to any antiparasitic treatment; these are a random subset of chicks from different nests in both species. We assume that these chicks represent a “normal” random sample from the population and thus that the levels of BCCVs in this group should be considered the reference level, and differences in levels from this group should thus be considered an effect of the experimental treatment. BCCVs reflect e.g., energy metabolism by the total concentrations of proteins, uric acid, urea, glucose, fructosamine and creatinine, and digestion and pancreatic diseases can be evaluated by amylase levels (Thrall et al., 2006). Furthermore, magnesium, potassium, sodium, urea, uric acid and proteins are important parameters to reflect electrolytic homeostasis and dehydration (Thrall et al. 2006). In addition, BCCVs reflect health and homeostasis of bone and liver (alkaline phosphatase; alanine aminotransferase; bile acid; total bilirubin; albumin; total protein and cholesterol) while other reflect kidney function (urea, protein, uric acid, creatinine, uric acid:creatinine, protein:creatinine) and bone metabolism (alkaline phosphatase, total protein, inorganic phosphate and calcium) (Viñuela et al. 1991; de le Court et al. 1995; van Wyk et al. 1998; Tilgar et al. 2004, 2008; Thrall et al. 2006). Endoparasites may be more

energetically costly as they absorb food in the intestines. We therefore expected levels of BCCVs that reflect nutritional status to indicate this in birds not treated against endoparasites (e.g. higher uric acid and urea levels, lower plasma creatinine levels). Ectoparasites lead to skin irritation and also drain blood from the host, we therefore predicted that BCCVs related to wound healing should be different in the ectoparasite treated chicks (e.g. lower levels of bilirubin). Furthermore, we expected birds treated against both endo- and ectoparasites to have BCCV levels indicating better overall health and reduced infection than the other treatment/control groups.

METHODS

Study design and sampling

The study was conducted in Troms County, Northern Norway on chicks of two raptor species: white-tailed eagle and northern goshawk. During the winters (February-March) prior to the breeding seasons of 2008 and 2009 all accessible known territories and nests of both species were visited. During this visit in 2008 and 2009 some nests were randomly (every other nest visited) treated with a commercially available ectoparasite removing spray SprayMax (Borregaard Industries Limited, active ingredient pyrethrin and piperonyl butoxide). Each of these nests was treated for one minute, while control nests received a visit of similar length but without any treatment. The sample sizes of the treatments during the different years were as follows: northern goshawk: 2008 (2 sprayed nests, 5 control nests), 2009 (5 sprayed nests, 5 control nests) white-tailed eagle: 2008 (3 sprayed nests, 2 control nests), 2009 (5 sprayed nests, 7 control nests). The nests were visited again shortly after hatching in June (3-4 months after anti-ectoparasite treatment). Northern goshawk clutches contained 2-4 chicks and those of white-tailed eagle 1-2 chicks. During this visit, half of the chicks of the same nest were randomly treated orally with an antihelminthic (Panacur®, active ingredient fenbendazole (25mg/mL)) to reduce levels of endoparasites (1 mL for northern goshawk chicks and 2 mL for white-tailed-eagle chicks), the

151 other half of the chicks were treated with a corresponding amount of sterile water. Hanssen et al.
152 (2003, 2013) and Bustnes et al. (2006) present more details on this treatment in wild birds. In this way
153 we tried to achieve a balanced split plot design with two factors: ectoparasite treatment (at the nest
154 level), and endoparasite treatment (at the chick level). This design was not possible for white-tailed
155 eagle nests with only one chick and we therefore randomly treated the single chick with either Panacur
156 (treated group) or sterile water (control). The sample sizes at the chick level in the different years were
157 as follows: northern goshawk: 2008 (5 treated chicks, 8 control chicks), 2009 (11 treated chicks, 13
158 control chicks), white-tailed eagle: 2008 (3 treated chicks, 2 control chicks), 2009 (7 treated chicks, 9
159 control chicks). Nests were then visited a third time (white-tailed eagle: 19 ± 2 days later; northern
160 goshawk: 13 ± 0.6 days later) in order to obtain a blood sample, for the analysis for BCCVs, and body
161 feathers, for DNA-based sexing. The blood was sampled from the brachial vein (0.1 - 4.0 mL; heparin-
162 coated syringe) and centrifuged the same day at 1500 G for 10 min and up to 1 mL supernatant plasma
163 was transferred to a sterile 1.5 mL Eppendorf® tube and frozen at -20 °C until BCCV analysis. To
164 minimize the time spent at the nest, and thus the invasiveness of the study, we did not attempt to
165 quantify the reduction in parasite levels in relation to treatment. Nonetheless, several studies have
166 shown that fenbendazole is effective against various intestinal parasites in birds, e.g. nematodes,
167 lungworms and cestodes (Norton et al. 1991; Yazwinsky et al. 1992, 1993), and a study showed that
168 one treatment with fenbendazole eliminated all nematode parasites in 221 out of 230 birds from 38
169 species of six orders (Lawrence 1983). Treatment of nests with pyrethrin has been shown to reduce
170 levels of ticks and fleas on chicks (Szep and Møller 1999; Fessl et al. 2006) and in nests (Dufva and
171 Allander 1996; Christe et al. 2000, 2002). To reduce disturbance of the breeding birds and possible side
172 effects of the pyrethrin-based anti-ectoparasite treatment, this was performed about three months
173 before egglaying. We assumed that the treatment reduced or eliminated active and dormant stages of
174 ectoparasites wintering in the nest material to such a degree that levels of ectoparasites in the treated
175 nests were lower during the chick period even if some reinfection from adults may have occurred.

176

177 *Analyses of BCCVs*

178 All BCCV analyses were conducted at the Central Laboratory at the Department of Veterinary Clinical
179 and Animal Sciences (University of Copenhagen) and included 19 components. These were composed
180 of three liver enzymes and function test compound, i.e. alkaline phosphatase (U L^{-1}), alanine
181 aminotransferase (U L^{-1}), gamma glutamyltransferase (U L^{-1}) and bile acid (µmol L^{-1}), one specific bone
182 enzyme i.e. alkaline phosphatase (U L^{-1}), one digestive enzyme, i.e. amylase (U L^{-1}), two protein groups,
183 i.e. albumin (g L^{-1}) and total protein (g L^{-1}), two erythrocyte metabolism waste products, i.e. total
184 bilirubin (µmol L^{-1}) and bile acids (µmol L^{-1}), cholesterol (mmol L^{-1}), two carbohydrates, i.e. glucose
185 (mmol L^{-1}), fructosamine (µmol L^{-1}), one muscle break-down product, i.e. creatinine (µmol L^{-1}), five
186 electrolytes/minerals, i.e. inorganic phosphate (mmol L^{-1}), calcium (mmol L^{-1}), magnesium (mmol L^{-1}),
187 sodium (mmol L^{-1}) and potassium (mmol L^{-1}), and two protein waste products i.e. urea (mmol L^{-1}) and
188 uric acid (U L^{-1}). The latter one is also used to evaluate renal functioning. In addition, protein:creatinine
189 was included to represent creatinine clearance reflecting filtration rates as a marker of glomerular
190 lesions. The analyses were routinely conducted at the laboratory using an automated
191 spectrophotometrical analyser also containing ion-selective electrodes (ADVIA 1800, Siemens). All
192 assays were subjected to daily internal and quarterly external quality control. Only results from
193 accepted analytical runs are reported here. Information on methods can be found at the Department
194 of Small Animal Clinical Sciences (http://www.life.ku.dk). Further details on BCCV analysis in these
195 raptor chicks can be found in Sonne et al. (2010, 2012).

196

197 *Sexing*

198 DNA was extracted from body feathers (approx. 2 mm root tip) or blood (5-10 µl) using Nexttec™
199 Genomic DNA Isolation Kit for Tissue and Cells. We used primers 2550F and 2718R to amplify an intron

200 of the CHD1 genes on the Z and W chromosomes (Fridolfsson and Ellegren 1999). For details of these
201 methods, see Hanssen et al. (2013).

202

203 *Experimental design and statistical methods*

204 Sample sizes may differ slightly between analyses because not all laboratory tests could be run on all
205 samples. Furthermore, the number of sprayed nests versus control nests were not equal because not
206 all nests selected at the first visit would eventually produce nestlings. We therefore include the sample
207 size used for each analysis in Table 1. The dependent variables creatinine and bile acid were $\log_{10}$-
208 transformed to conform to the normality assumptions of parametric statistics. Each response variable
209 was analyzed in a mixed analysis design (proc mixed in SAS 9.3). Nest identity was always included as
210 a random variable to avoid pseudo-replication of chicks within nests. Selecting the models used for
211 inference was performed within a model selection framework using Akaike's Information Criterion
212 (AIC) (e.g. Buckland et al. 1997; Anderson et al. 2000; Burnham and Anderson 2002) as follows: We
213 formed a set of candidate models where models were rescaled and ranked relative to the model with
214 the lowest AIC value (Δi denotes this difference for model i). We selected the simplest model, i.e. the
215 model with the fewest degrees of freedom, with a $\Delta i \leq 2$ (Table S2). In all the analyses we kept at least
216 one of the key predictors (anti-endoparasite or anti-ectopararasite experimental treatment) in the
217 models based on our a priori expectations, whereas covariates (sex and species) and the first order
218 interactions was excluded and included in the model used for inference based on how they affected
219 the AIC (and the Δi). (See supplement S2 for details) (Table S2). Chick body mass at the last capture
220 was tested as covariate in the full models, however it did not significantly contribute to any of the
221 models and was therefore not included. Mean values are presented as mean ± standard error. All
222 analyses were performed with the statistical software SAS version 9.3.

223

RESULTS

Sex ratio and body mass

The sexing analyses showed that 15 northern goshawk chicks were females and 16 were males. The corresponding numbers for white-tailed eagles were 8 females and 12 males. As expected, there was marked size dimorphism between the sexes in goshawks and no significant size difference in white-tailed sea eagles. Female goshawk chicks were heavier than males (body mass females 1101 ± 44g, males 783 ± 41g, ANOVA $F = 37.40$, $p < 0.0001$) from Hanssen et al. (2013). Body mass was not significantly different between the sexes in white-tailed sea-eagles even though female chicks tended to be heavier (body mass females 4408 ± 269g, males 4100 ± 199g, ANOVA $F = 0.85$, $p = 0.37$) from Hanssen et al. (2013). In a previous analysis of this experiment in relation to oxidative stress we showed that there was no significant differences in body mass or structural size related to the treatment groups (Hanssen et al. 2013).

Combined experimental effects

BCCVs: Of the 19 BCCVs measured, the analysis for effects of the experimental anti-parasite treatments did not lead to a significant final model for gamma glutamyl transferase, inorganic phosphate, albumin, alanine aminotransferase, glucose, cholesterol, fructosamine, calcium, magnesium and sodium (all $P>0.05$). The mean values for these BCCVs in relation to experiments and sex are presented in Table S1 for reference. Table 1 presents the results of the final models, with main effects, covariates and interactions, for the remaining BCCVs.

Liver and bone enzymes: Removing ectoparasites or endoparasites led to significantly higher levels of alkaline phosphatase, in contrast to control chicks and chicks receiving both endoparasite and ectoparasite treatments (Table 1, Figure 1a). Furthermore, alkaline phosphatase levels were significantly higher in females (Table 1). In males, removing ectoparasites led to higher alkaline phosphatase levels (Table 1, Figure 1b).

Digestive enzyme: Anti-endoparasite treatment led to higher amylase levels (Table 1). Females had significantly higher levels (Table 1), and northern goshawk chicks also had significantly higher levels (Table 1).

Protein groups: Northern goshawk chicks had lower levels of total protein when compared to white-tailed eagles (Table 1).

Erythrocyte metabolism waste products: Treatment against ectoparasites led to significantly reduced total bilirubin and increased bile acid levels (Table 1). Bile acid levels were also significantly higher in northern goshawk chicks (Table 1).

Muscle break down product: Creatinine levels were significantly higher in chicks treated against endoparasites, and also higher in female chicks of both species (Table 1).

Electrolytes/minerals: In northern goshawk chicks, potassium levels were lower in chicks treated against endoparasites (Table 1, Figure 2). In white-tailed eagle chicks, potassium levels were significantly higher than in northern goshawk chicks (Table 1).

Protein waste materials: Treatment against ectoparasites significantly increased both uric acid and urea levels (Table 1). Uric acid levels tended to be higher in treated male chicks (Table 1, Figure 3). For urea, this difference was larger in northern goshawk chicks (Table 1, Figure 4). Urea levels were also significantly higher in northern goshawk chicks when compared to white-tailed eagle chicks (Table 1, Figure 4).

Renal functioning: Treatment against ectoparasites led to significantly reduced protein:creatinine ratios (Table 1).

DISCUSSION

Anti-parasite treatments led to changes in several BCCVs, suggesting differences in nutrient absorption and physiological and homeostatic state of chicks that may be related to the cost of parasitism.

Ectoparasites

Anti-ectoparasite treatment led to higher uric acid levels in chicks of both species, and tended to be higher in treated male chicks. Also urea levels where higher in chicks treated against ectoparasites, with differences larger in northern goshawk chicks than in white-tailed eagle chicks. There are differing opinions among authors on the interpretation of uric acid and urea levels in wildlife studies. High uric acid and urea levels may indicate poor nutritional condition since it reflects increased muscle degradation from energy consumption during periods of starvation (Cherel and Le Maho 1985; Robin et al. 1998; Casado et al. 2002). Alternatively, higher levels of urea and uric acid may suggest higher protein intake (Okumura and Tasaki 1969; Voss and Siems 2006). In this respect, low concentrations of urea and uric acid in herring gulls (*Larus argentatus*) were interpreted as signs of low diet quality (Fox et al. 2007). Also, blood urea concentration has been reported to vary greatly within short periods of time in raptors and other birds in response to fasting and dehydration (Lumeij 1987; Lumeij and Remple 1991; Liminana et al. 2009). We found that presumably having reduced levels of ectoparasites as a consequence of treatment of the nest with pyrethrin led to higher levels of uric acid and urea in raptor chicks. It is unlikely that reduced levels of external parasites should lead to increased feeding by the parents. On the other hand, perhaps better health in the treated chicks led to improved appetite and digestion of food. However, as the treated chicks did not show signs of improved growth (Hanssen et al. 2013), further and more detailed studies are necessary to explain this effect. Treatment against ectoparasites led to reduced protein:creatinine. A lowered protein:creatinine ratio indicates renal disorders with urine loss of protein and a reduced creatinine clearance due to glomerular lesions

294 (Maxie 1993; Hochleithner 1994; Confer and Panciera 1995; Ettinger and Feldman 1995). Thus, it may
295 seem that reducing ectoparasite levels led to an increased strain on the raptor chicks' kidney function
296 possibly caused by the SprayMax treatment. However, other factors like increased immune functioning
297 (antibody production) and dehydration from e.g. parasite burdens may also cause such changes
298 (Harrison and Lightfoot 2005). Total bilirubin levels were lower in raptor chicks treated against
299 ectoparasites. Bilirubin is a powerful endogenous antioxidant and is one of the catabolites of heme
300 oxygenases that is active during the healing process of for instance bruises and the sequestration of
301 old erythrocytes (Kikuchi et al. 2005). Lower bilirubin levels in treated chicks may indicate a reduced
302 wound-healing activity as a consequence of reduced levels of skin biting ectoparasites. However,
303 during hepatic disease, infection and reduced kidney function; bilirubin increases in birds which could
304 be a likely explanation in the present study (Harrison and Lightfoot 2005). In domestic pigs,
305 experimental infection with the endoparasitic protozoan *Sarcocystis miescheriana* led to increased
306 bilirubin levels (Reiner et al. 2009). Regarding bile acid that increased in the treatment groups; it is
307 usually associated with liver function and disease such as hepatitis (Harrison and Lightfoot 2005).
308 Whether it could also be caused by an increased production as a result of parasite removal and
309 coherent increased nutrient uptake is uncertain (Harrison and Lightfoot 2005). The treatments against
310 ectoparasites were performed 2-4 months before hatching, so any toxic side-effects of pyrethrin are
311 highly unlikely. Moreover, this substance has been used in numerous studies to remove ectoparasites
312 in birds' nests during breeding without any reported side effects (Møller 1990; Dufva and Allander
313 1996; Szep and Møller 1999; Christe et al. 2000, 2002).
314

315 *Endoparasites*

316 Internal parasites may be more energetically costly as they absorb food in the intestines, and we
317 therefore expected that levels of BCCVs that reflect nutritional status should be lower in birds not
318 treated against endoparasites. Creatinine levels were lower in chicks not treated against endoparasites

(control chicks). Creatinine is a breakdown product of creatinine phosphate in muscle and is usually produced at a fairly constant rate by the liver (depending on muscle mass) (You et al. 2008). Lower plasma creatinine levels may indicate worse nutritional condition as creatinine levels have been suggested to decline with food supply which in turn is reflected in poor-growing chicks (Rosskopf et al. 1982; Alonso-Alvarez and Ferrer 2001; Casado et al. 2002). However, a higher plasma creatinine level could reflect malnutrition leading to elevated muscle catabolism (Hotchleithner 1994; Casado et al. 2002) or due to renal dysfunction caused by prolonged starvation (Alonso et al. 2001). The increase of amylase may indicate an increase in pancrase activity due to elevated nutrient uptake (Harrison and Lightfoot 2005).

BCCVs affected by both treatments

In theory, increasing plasma concentrations of liver enzymes may be a result of e.g. hypoxia, inflammation, diet, infection, neoplasia, trauma, metabolic abnormalities (storage diseases), endocrine diseases or hepatocyte regeneration (Hochleithner 1994; Ettinger and Feldman 1995; Thrall et al. 2006). In the present study, we observed that the levels of bone and liver enzymes (alkaline phosphatase) as well as amylase originating from the pancreas were affected by the anti-parasite treatments. Alkaline phosphatase levels increased in chicks treated against either endoparasites or ectoparasites, but not in the chicks receiving both treatments. Alkaline phosphatase is also associated with growth and has been found to be higher in chicks during the growth/bone formation period (Viñuela et al. 1991; Dobado-Berrios and Ferrer 1997; Tilgar et al. 2004, 2008). However, no measurable growth differences were found between the treatment groups (Hanssen et al. 2013). Low levels of alkaline phosphatase have been found to be related to parasitic infections in pigs (*Sus scrofa*) (Reiner et al. 2009), and as such the increased levels in treated birds are consistent with the reduced parasite levels. Such comparisons should, however, be done with great cautions as BCCVs vary greatly even between raptorial species (Sonne et al. 2010, 2012).

Interestingly, alkaline phosphatase levels were not reduced in the double-treated nestlings. If reduced alkaline phosphatase levels are an indication of reduced parasite levels, then one might speculate that being treated against only one of the parasite groups reduced parasite levels but that being treated against both parasite groups did not reduce levels of parasitic infection. This may be because the experimental removal of a wide range of parasites might have led to increased infections with other types of macroparasites or microparasites such as bacteria and fungi (Van Oers et al. 2002; Pedersen and Antonovics 2013).

Sex, size and species

As the sexual size dimorphism was more pronounced in northern goshawks (females are larger) compared to white-tailed eagles, we expected more pronounced differences between males and females in the former. It could also be that parasite removal is more important for female northern goshawk chicks as these grow faster than their male siblings and could thus be more sensitive to negative energetic effects of parasitic infections. The results showed that there were marked sex differences in levels of several of the measured BCCVs. Alkaline phosphatase, amylase and creatinine levels were higher in females of both species (total protein levels tended to be a lower P=0.06). There thus seems to be physiological differences between males and females that may be related to higher growth or hormonal differences. Regarding species differences, we found that amylase, bile acid, and urea levels were higher in northern goshawk chicks, while total protein and potassium levels were higher in white-tailed eagles. Higher protein levels may indicate dehydration, faster growth or a combination (Ettinger and Feldman 1995; Ferrer and Dobado-Berrios 1998; Thrall et al. 2006; Waikar and Bonventre 2008). One might therefore speculate that higher levels of total protein in white-tailed eagles may be related to faster growth in these large birds. It cannot be excluded, either, that the protein concentrations simply reflect protein dietary intake meanwhile proteins also maintain osmotic

pressure and PhD regulation (Sturkie 1976; Harrison and Lightfoot 2005). One should be cautious when interpreting these species differences as natural levels of BCCVs vary greatly between raptorial species (Sonne et al. 2010, 2012).

Considerations

The therapeutic use of fenbendazole is rarely associated with side effects. The primary mechanism is binding to parasite tubulin and interfering with microtubule assembly, which is necessary for cell division (Zajac 1993). Fenbendazole is poorly absorbed by the host animal and selectively absorbed by the parasite due to its strong specificity for invertebrate tubulin (Weiss and Adams 1987). However, some studies have indicated adverse effects of fenbendazole in birds (e.g. Howard et al. 2002; Gozalo et al. 2006). These reported effects seem to be related to food intake and lead to weight loss and even reduced survival (Gozalo et al. 2006). Pigeons and doves (family *Columbidae*) are more frequently affected (Howard et al. 2002; Gozalo et al. 2006), while studies on other bird orders report no adverse effects (Lawrence 1983; Kirsh 1984; Yazwinski et al. 1986). The therapeutic treatment with fenbendazole reported in the studies above also requires the dose to be repeated 2-6 times, whereas in this study we only administered one dose. We do however suggest that more studies are done regarding possible negative effects of fenbendazole in birds.

CONCLUSIONS

The results showed that treating against the different types of parasites (fenbendazole against endoparasites and pyrethrin against ectoparasites) had effects on different BCCVs. Treatment against ectoparasites affected biomarkers related to energy metabolism (uric acid), bone metabolism (alkaline phosphatase, uric acid), fat metabolism (bile acid), diet or protein consumption (urea) in addition to the antioxidant bilirubin. In contrast, treatment against endoparasites affected biomarkers related to

392 energy metabolism and kidney function (creatinine), and digestion/liver function (potassium,
393 amylase). The only group of BCCVs that was affected by both experimental treatments was liver and
394 bone enzyme alkaline phosphatase levels. A decreased protein:creatinine ratio may indicate an effect
395 on the glomerular function from the parasite treatment. In conclusion, anti-parasite treatments led to
396 changes in several BCCVs, suggesting differences in nutrient absorption and physiological state of
397 chicks including growth that may be related to costs of parasitism. Thus, parasites but maybe also the
398 treatment seem to have multifaceted effects on the homeostasis and physiological condition in chicks
399 of the two raptor species. Future studies should examine further the effects of infectious organisms
400 via physiological homeostasis on fitness (survival and reproduction) in wildlife, and aim at quantifying
401 the parasite load.

402

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593

Table 1 Effects of reducing ectoparasitic (ecto) and endoparasitic (endo) burdens on different blood clinical-chemical variables (BCCVs) in chicks of northern goshawk *Accipiter gentilis* L., 1758 and white-tailed eagle *Haliaeetus albicilla* L., 1758 in Northern Norway in the breeding seasons 2008 and 2009. All variables presented are from the final mixed models, analysed with restricted maximum likelihood estimation method. Estimates (±SE) are presented for variables with *P*-values less than 0.10 and are least square means from the presented final models. C=control group, T=treated group, NG=northern goshawk, WTE=white-tailed eagle.

Dependent variable	*n*	Main effects	*F*-value/ *P*-value	Estimates (± standard error)	Covariates	*F*-Value/ *P*-value	Estimates (± standard error)	Interaction effects	*F*-Value *P*-value
Alkaline phosphatase	51	Anti-ectoparasite	$F_{1,16}$=0.02 *P*=0.88		Sex	$F_{1,16}$=5.60 ***P*=0.03**	♂ 1135±43 U L^{-1}, ♀ 1274±48 U L^{-1}	ecto×endo (Fig 1a)	$F_{1,16}$=5.49 ***P*=0.03**
		Anti-endoparasite	$F_{1,16}$=0.46 *P*=0.51		Species	$F_{1,16}$=1.89 *P*=0.19		ecto×sex (Fig 1b)	$F_{1,16}$=5.86 ***P*=0.03**
Amylase	50	Anti-endoparasite	$F_{1,17}$=5.00 ***P*=0.04**	C: 635.2±24 U L^{-1} T: 707.5±26 U L^{-1}	Sex	$F_{1,17}$=16.65 ***P*=0.0008**	♂ 602±24 U L^{-1}, ♀ 741±26 U L^{-1}	ecto×endo	$F_{1,17}$=0.02 *P*=0.90
		Anti-ectoparasite	$F_{1,17}$=0.74 *P*=0.4		Species	$F_{1,17}$=82.36 ***P*<0.0001**	NG: 848±26 U L^{-1}, WTE: 494±28 U L^{-1}	ecto×species	$F_{1,17}$=2.00 *P*=0.18
Total protein	50	Anti-endoparasite	$F_{1,17}$=1.02 *P*=0.41		Sex	$F_{1,17}$=4.01 *P*=0.06	♂ 26.3±0.4 g L^{-1}, ♀ 27.3±0.4 g L^{-1}	endo×species	$F_{1,17}$=2.09 *P*=0.17
		Anti-ectoparasite	$F_{1,17}$=2.78 *P*=0.11		Species	$F_{1,17}$=21.96 ***P*=0.0002**	NG: 25.3±0.4 g L^{-1} WTE: 28.3±0.5 g L^{-1}		
Total bilirubin	50	Anti-ectoparasite	$F_{1,16}$=7.47 ***P*=0.02**	C: 17.0±0.9 µmol L^{-1} T: 13.4±0.9 µmol L^{-1}	Sex	$F_{1,16}$=0.22 *P*=0.65		ecto×endo	$F_{1,16}$=0.02 *P*=0.88
		Anti-endoparasite	$F_{1,16}$=0.09 *P*=0.76		Species	$F_{1,16}$=0.07 *P*=0.79		endo×sex	$F_{1,16}$=2.01 *P*=0.18
Bile acid	51	Anti-ectoparasite	$F_{1,20}$=4.86 ***P*=0.04**	C: 1.6±0.1 µmol L^{-1} T: 2.0±0.1 µmol L^{-1}	Species	$F_{1,20}$=17.11 ***P*=0.0005**	NG: 2.2±0.1 µmol L^{-1}, WTE: 1.4±0.1 µmol L^{-1}		
Creatinine	51	Anti-endoparasite	$F_{1,18}$=4.47 ***P*=0.05**	C: 0.04±0.01 µmol L^{-1} T: 0.07±0.01 µmol L^{-1}	Sex	$F_{1,18}$=4.35 ***P*=0.05**	♂ 0.03±0.01 µmol L^{-1}, ♀ 0.07±0.01 µmol L^{-1}		
Potassium	45	Anti-endoparasite	$F_{1,13}$=0.75 *P*=0.40		Species	$F_{1,13}$=20.58 ***P*=0.0006**	NG: 1.9±0.1 mmol L^{-1} WTE: 2.7±0.1 mmol L^{-1}	endo×species (Fig 2)	$F_{1,13}$=5.89 ***P*=0.03**
Uric acid	50	Anti-ectoparasite	$F_{1,15}$=5.51 ***P*=0.03**	C: 666±53 U L^{-1} T: 847±56 U L^{-1}	Sex	$F_{1,15}$=0.00 *P*=0.96		ecto×sex (Fig 3)	$F_{1,15}$=4.11 ***P*=0.06**
		Anti-endoparasite	$F_{1,15}$=1.89 *P*=0.19		Species	$F_{1,15}$=2.45 *P*=0.14		ecto×endo	$F_{1,15}$=0.26 *P*=0.61
Urea	50	Anti-ectoparasite	$F_{1,20}$=19.63 ***P*=0.0003**	C: 2.21±0.09 mmol L^{-1} T: 2.83±0.10 mmol L^{-1}	Species	$F_{1,20}$=158.85 ***P*<0.0001**	NG: 3.41±0.09 mmol L^{-1} WTE: 1.64±0.11 mmol L^{-1}	ecto×species (Fig 4)	$F_{1,20}$=3.92 ***P*=0.06**
Protein:creatinine	50	Anti-ectoparasite	$F_{1,18}$=5.05 ***P*=0.04**	C:2.3±0.1 T:1.8±0.1	Species	$F_{1,18}$=2.16 *P*=0.16		ecto×sex	$F_{1,18}$=2.19 *P*=0.16
					Sex	$F_{1,18}$=2.94 *P*=0.10			

Figure legends

Figure 1. a) Combined effects from removing ecto- and endoparasites on plasma concentrations of alkaline phosphatase in northern goshawk *Accipiter gentilis* L., 1758 and white-tailed eagle *Haliaeetus albicilla* L., 1758 chicks. b) Effects of treatment against ectoparasites on plasma concentrations of alkaline phosphatase in female and male northern goshawk *Accipiter gentilis* L., 1758 and white-tailed eagle *Haliaeetus albicilla* L., 1758 chicks. Values are predicted least square means values (with standard error bars) from the models presented in Table 1.

Figure 2. Effects of treatment against endoparasites on plasma concentrations of potassium in northern goshawk *Accipiter gentilis* L., 1758 and white-tailed eagle *Haliaeetus albicilla* L., 1758 chicks. Values are predicted least square means values (with standard error bars) from the model presented in Table 1.

Figure 3. Effects of treatment against ectoparasites on plasma concentrations of uric acid in female and male northern goshawk *Accipiter gentilis* L., 1758 and white-tailed eagle *Haliaeetus albicilla* L., 1758 chicks. Values are predicted least square means values (with standard error bars) from the model presented in Table 1.

Figure 4. Effects of treatment against ectoparasites on plasma concentrations of urea in northern goshawk *Accipiter gentilis* L., 1758 and white-tailed eagle *Haliaeetus albicilla* L., 1758 chicks.