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1 **TITLE: Plasma carotenoids and immune response in a despotic avian scavenger**

2 **RUNNING TITLE: Carotenoids and immune response**

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21 **ABSTRACT**

22 Carotenoids are pigmented compounds acquired through diet that have important functions
23 as antioxidants and immune modulators. We studied the association between immune
24 response and circulating carotenoids in Andean condors (*Vultur gryphus*). We evaluated the
25 relationship between α , β and γ -globulin blood concentrations and different circulating
26 carotenoids in healthy wild individuals and sick individuals received for rehabilitation. In
27 rehabilitating individuals, α , β and γ globulin concentrations were higher than in wild
28 individuals. In addition, circulating carotenoids were lower in rehabilitating than in wild
29 individuals. We found negative correlations between α , β and γ -globulins and the different
30 circulating carotenoids in rehabilitating individuals, but not in wild condors. Xanthophylls
31 were strongly related to α , β and γ -globulin blood concentrations in rehabilitating, but not
32 in wild condors. Our results suggest that a potential relationship between circulating
33 carotenoids and immune response is present in the Andean condor. Given that this species
34 may display a carotenoid-based pigmentation, our results could suggest that a trade-off
35 between the immune system and external coloration could operate in this species, which
36 may have implications in access to food resources and mate selection and conservation.

37 **KEY WORDS:** Carotenoids, Globulins, Health studies, Immune response, Vultures,
38 Xanthophylls.

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3 41 **1-INTRODUCTION**
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6 42 How animals allocate resources to various physiological functions is a key question in the
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8 43 study of life history evolution. When resources are limited, any investment in a particular
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10 44 trait is likely to reduce resources available for other vital functions (Roff, 1992; Roff,
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12 45 Heibo, & Vøllestad, 2006). The underlying costs of these processes can result in negative
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14 46 associations or trade-offs (Schluter, Price, & Rowe, 1991). Immunity is considered a major
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16 47 physiological mechanism regulating survival, and it is shaped by such negative associations
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18 48 (Brace et al., 2017; Schmid-Hempel, 2003; Sheldon & Verhulst, 1996). In this sense,
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20 49 several trade-offs between developing an immune defense and other functions or activities
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22 50 that share common resources, and which contribute to animal fitness, such as energy
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24 51 expenditure, reproductive success or diet requirements, have been described (Cotter,
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26 52 Simpson, Raubenheimer, & Wilson, 2011; Martin, Scheuerlein, & Wikelski, 2003; Schmid-
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28 53 Hempel, 2003). In particular, a trade-off based on the allocation of limited resources to
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30 54 health from external coloration seems to be mediated by carotenoids in several animal
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32 55 species, including birds and fishes (Svensson & Wong, 2011).
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39 56 Vertebrates are unable to synthesize carotenoid pigments, so they must acquire them
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41 57 through diet (Hill & Johnson, 2012). Therefore, these compounds are limited by both
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43 58 environment and physiological uptake (Hill & Johnson, 2012; Olson & Owens, 1998).
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45 59 Besides their signaling role as pigments, carotenoids have important functions in different
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47 60 physiological processes (Hill & Johnson, 2012; Olson & Owens, 1998). For instance,
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49 61 carotenoids have been described as part of the antioxidant system, and are thus involved in
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51 62 the resistance against free radicals and cell protection by quenching reactive oxygen species
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53 63 (Hill & Johnson, 2012; Sies & Stahl, 1995; Von Schantz, Bensch, Grahn, Hasselquist, &
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Wittzell, 1999). Carotenoids also play an important role in relation to immune system functioning in different species (Aguilera & Amat, 2007; Chew & Park, 2004; Kim et al., 2000a, 2000c; Saino, Stradi, Ninni, Pini, & Møller, 1999).

A costly trade-off between the immune system and external coloration mediated by carotenoids was reported in diverse bird species (Faivre, Grégoire, Préault, Cézilly, & Sorci, 2003; Simons, Cohen, & Verhulst, 2012). Supplementation with carotenoids is positively related to feather pigmentation and also improves resistance against parasite infection and stimulates antibody production and T-cell mediated immune response (Aguilera & Amat, 2007; McGraw & Ardia, 2003). For instance, European greenfinch (*Carduelis chloris*) males supplemented with carotenoids in their diets show stronger antibody (γ -globulins) response after an immune challenge or stress, and, in turn, the activation of the immune system decreases the circulating carotenoids and feather pigmentation (Aguilera & Amat, 2007). This suggests that carotenoids enhance the immune system response against pathogens or an immune stress, and, in turn, the immune response diminishes circulating carotenoids and the expression of sexual traits. In this sense, an immune challenge with antigens such as PHA (phytohemagglutinin) subcutaneously injected in the wing web (patagium) decreases circulating carotenoids in different bird species such as the red-legged partridge (*Alectoris rufa*) and the Eurasian blackbird (*Turdus merula*) (Biard, Hardy, Motreuil, & Moreau, 2009; Perez-Rodriguez et al., 2008). In addition, a negative correlation between γ -globulins and carotenoid blood levels in barn swallows (*Hirundo rustica*) suggests that, to exert immune function, carotenoids are diverted from plasma (Saino et al., 1999).

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86 Because carotenoid availability may influence the immune response, their functional role in
87 different bird species could provide important evidence for processes underlying the
88 relationship between carotenoids and the immune system. For example, vulture species
89 have a diet based on carrion (meat in different states of decomposition, and bones), which
90 is particularly limited in carotenoids (Tella et al., 2004). In addition, this kind of diet
91 contains multiple pathogens, and thus vultures have evolved different mechanisms to cope
92 with them (Blumstein, Rangchi, Briggs, De Andrade, & Natterson-Horowitz, 2017). For
93 instance, low stomach pH (Blumstein et al., 2017), a specialized microbiome (Roggenbuck
94 et al., 2014), and particularities in immune proteins and immune receptors seem to be the
95 adaptive mechanisms that vultures display to resist the harmful effects produced by these
96 pathogens (Blumstein et al., 2017). However, little information is available on the role of
97 carotenoid compounds in immune system functioning for the diversity of vulture species
98 (but see, López-Rull et al. 2015), especially the threatened ones. This information can be
99 important to better understand the overall immune strategy in this avian group.

100 Some vultures may obtain carotenoids from living plants, from the guts of their food animal
101 carcasses, or by consuming vegetal material from herbivore feces (Blanco et al., 2013;
102 Negro et al., 2002). Wild Andean condors (*Vultur gryphus*) feeding on carcasses of wild
103 and domestic ungulates show higher blood concentrations of different carotenoid fractions
104 than condors in a rehabilitation center, which consume a diet restricted to flesh (Blanco et
105 al., 2013). On the other hand, Andean condors in rehabilitation also show higher levels of
106 α , β and γ -globulins in comparison to wild individuals, as a consequence of the immune
107 response associated with different pathologies such as infections, parasitic diseases,
108 poisoning or trauma (Plaza et al., 2019). Therefore, evaluating the relationship between

109 circulating carotenoids and different parameters of immunity, such as globulins, in wild and
110 rehabilitating condors can help to understand how these limiting nutrients are used by the
111 immune system when survival is threatened.

112 Here, we analyze the relationship between circulating carotenoids and α , β and γ -globulin
113 plasma concentrations in two groups of Andean condors with different degrees of immune
114 activation: (1) wild healthy condors from north-western Patagonia not mounting an immune
115 response, and (2) rehabilitating condors mounting an immune response due to the different
116 pathologies that they were undergoing at the time of sampling, upon their arrival at the
117 rehabilitation center. To test the immune system response of the Andean condors studied,
118 we measured their concentrations of plasma globulins (α , β and γ). Rehabilitating condors
119 were expected to show more plasma globulins concentrations than wild healthy individuals.
120 In addition, if individuals invest their resources in essential functions for survival when
121 they are facing unfavorable conditions such as pathologic states, we expect that
122 rehabilitating condors will divert plasma carotenoids to immunity. Therefore, they will
123 show a negative correlation between α , β and γ -globulin concentrations and circulating
124 carotenoids. This association is not expected in healthy wild individuals given that these
125 individuals are not mounting an immune response.

126 **2-MATERIAL AND METHODS**

127 **2.1 Study species**

128 The Andean condor is the largest New World vulture (weighing up to 16 kg, wingspan 3 m)
129 (Alarcón et al., 2017; del Hoyo, Elliott, & Sargatal, 1994) and it is classified worldwide by
130 the IUCN Red List as Near Threatened (IUCN, 2017). Currently, Andean condors are

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3 131 suffering important conservation problems such as lead contamination, intentional and
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5 132 unintentional poisoning, human persecution, and collision with power lines, causing health
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7 133 alterations that require their rehabilitation (Alarcón & Lambertucci, 2018; Pavez & Estades,
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10 134 2016; Wiemeyer et al., 2017). The population of Andean condors studied is the largest
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12 135 along their distribution range, with approximately 300 individuals (Lambertucci, 2010). In
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14 136 the study area, Andean condors feed on large carcasses of sheep, red deer, and cows, but
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16 137 they also feed on carcasses of lagomorphs (Ballejo, Lambertucci, Trejo, & De Santis, 2017;
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18 138 Lambertucci et al., 2009). These food sources provide minimal levels of carotenoids
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20 139 (Blanco et al., 2013). Therefore, condors may obtain a comparatively high concentration of
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22 140 carotenoid compounds by ingesting fresh vegetation and semi-digested vegetal matter
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24 141 contained in carcass viscera (Blanco et al., 2013).
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29 142 Andean condors show a strong sexual dimorphism, with males being more than 30% larger
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31 143 than females (Alarcón et al., 2017); age categories show clear dimorphism, allowing us to
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33 144 separate them by different feather colors (Ferguson-Lees & Christie, 2001; Hoyo et al.,
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35 145 1994). In addition, Andean condors exhibit dominance in their access to food according to
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37 146 sex and age (Donázar et al., 1999; Wallace & Temple, 1987), which may influence their
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39 147 health status and carotenoid levels. Moreover, the intensity of color in the bare parts (e.g.
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41 148 neck) suggests a role as dominance indicators, facilitating the establishment of feeding
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43 149 hierarchies (Blanco et al., 2013; Marinero, Cailly-Arnulphi, Lambertucci, & Borghi, 2018).
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49 **150 2.2 Sample collection**

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51 151 Wild Andean condors were trapped using cannon nets in October–December 2010–2013
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53 152 near San Carlos de Bariloche, Argentine Patagonia (ca. 41°09' S 71°09' W). All samplings
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3 153 were done at the end of the reproductive season, after chicks hatched (Lambertucci, 2007;
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5 154 Lambertucci & Mastrantuoni, 2008). Each trapped individual was physically evaluated by
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7 155 veterinarians (complete physical examination, heart and lung auscultation,
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9 156 electrocardiogram, evaluation of body condition, and evaluation of hydration status) and
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11 157 sampled only if they were apparently healthy. Rehabilitating condors were sampled at the
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13 158 Buenos Aires Zoo, Argentina, during the same period. This zoo admits condors suffering
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15 159 from health problems for rehabilitation, especially from Patagonia. Samples from
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17 160 rehabilitating birds were always taken upon admission at the zoo, reflecting the health
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19 161 status of the birds when they arrived. Previous studies in both wild and rehabilitating
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21 162 condors evaluated different blood parameters (hematocrit, hemoglobin, white blood cell
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23 163 counts, hepatic enzymes, etc.), and found that wild condors do not show alterations in any
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25 164 of the different blood parameters measured (Plaza et al., 2019; Wyemeyer, 2019) compared
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27 165 to available reference values for this species (Doussang et al., 2018). Therefore, the wild
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29 166 condors we sampled can be considered a healthy population not developing relevant
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31 167 immune responses. In the case of rehabilitating condors, they showed different alterations
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33 168 in blood parameters (e.g. white cell counts and globulins over the reference values)
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35 169 according to the combination of pathologies that they were undergoing at the time of
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37 170 admission to the rehabilitation center (Plaza et al., 2019; Wyemeyer, 2019). Therefore,
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39 171 although these condors were admitted based on different health problems, they can be
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41 172 pooled as sick individuals with developing immune responses.
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50 173 In both groups of individuals a sample of blood (10 ml) was extracted from the tarsal or
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52 174 brachial vein, transferred to vials containing dry heparin, and transported to the laboratory
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54 175 in a portable cooler at 4 °C. On the day of collection, blood samples were centrifuged at
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1,300 g for 10 min to obtain plasma, which was frozen at -20 °C until analysis. Sampled individuals included immature (1-6 years old) and adults (>6 years old) of both sexes. We sampled and measured α , β and γ -globulins in a total of 81 Andean condors: 43 wild ones and 38 rehabilitating individuals (Table A1). In addition, we measured circulating carotenoids in a total of 78 Andean condors: 42 wild ones and 36 rehabilitating individuals (Table S1). All the capture and handling methods were approved by Secretaría de Medio Ambiente de la Provincia de Río Negro (Expediente 132730-DF-2010 and Disposición N° 018/13 and 018/16).

2.3 Protein electrophoresis

To measure α , β and γ -globulin concentrations, we performed protein electrophoresis in agar gel (Cray & Tatum, 1998) following the methodology used in Blanco et al. (2006) and Plaza et al. (2019). Briefly, we measured total protein plasma concentrations with a Hitachi 912 Chemistry Analyzer (Roche Diagnostic Corporation, Indianapolis, USA). We performed agarose gel electrophoresis on plasma with the TITAN GEL Serum Protein System (Helena Laboratories, Beaumont, TX, USA) to determine the concentrations of the following protein fractions: α -globulins, β -globulins (acute phase proteins) and γ -globulins (immunoglobulins).

2.4 Determination and quantification of plasma carotenoids

We analyzed carotenoids as described by Blanco et al. (2013). We lyophilized 100 μ L of plasma, and the carotenoid pigments were removed from the dry remainder with 200 μ L of N,N-dimethyl formamide for sixty minutes, including sonication for five minutes every thirty minutes. Then we centrifuged the resulting extract at 12,000 g for five minutes and

the top layer was kept at -30°C until analysis through high-performance liquid chromatography (HPLC). We performed the quantitative analysis of the different carotenoid fractions by HPLC according to the methodology presented in (Minguez-Mosquera & Hornero-Mendez (1993) with modifications. To perform the HPLC, we used a Waters e2695 Alliance chromatograph fitted with a Waters 2998 photodiode array detector, controlled with Empower2 software (Waters Cromatografia, S.A., Barcelona, Spain). We used a C18 reversed phase analytical column (Mediterranea SEA18, 200 mm ×4.6 mm i.d., 3 µm; Teknokroma, Barcelona, Spain). The separation was reached by a binary-gradient elution using an initial composition of 75% acetone and 25% deionized water, which was increased linearly to 95% acetone in ten minutes, then raised to 100% in two minutes, and maintained constant for ten minutes. The initial conditions were achieved in five minutes. A volume of 20 µL was injected and we used a flow rate of 1 mL/min. We performed the detection at 450 nm, and we acquired the online spectra in the 350-700 nm wavelength range. We performed the quantification using external standard calibration curves prepared with β-cryptoxanthin, β-carotene, lutein and zeaxanthin standards formerly isolated. We prepared the curves of calibration with a range of 0.5-45.0 µg/mL, and constructed them by plotting the peak area at 450 nm versus the concentration of the pigments. We used the calibration curve of all-*trans*-lutein to calculate the concentration of the *cis* isomers of lutein. We performed the chromatographic analysis on the same day as the preparation of the extracts. We performed all the operations under dimmed light to avoid isomerization and photo-degradation of carotenoid pigments.

2.5 Statistical methods

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3 220 We first performed a Principal Component Analysis (PCA) (Dunteman, 1989) to show the
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5 221 relationship between the status of condors (wild or rehabilitating birds) and concentrations
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7 222 of circulating carotenoids and α , β and γ globulins. We considered two different groups
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10 223 (fractions) of circulating carotenoids: 1) xanthophylls (all-*trans*-zeaxanthin, all-*trans*-lutein,
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12 224 *cis* isomers of lutein/zeaxanthin, all-*trans*- α -cryptoxanthin, all-*trans*- β -cryptoxanthin and
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14 225 echinenone); and 2) all-*trans*- β -carotene, the only representative of the carotene fraction in
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17 226 Andean condors and the main precursor of vitamin A. We performed this differentiation
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19 227 because xanthophylls and β -carotene have different functions and metabolic pathways in
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21 228 birds (Hill & Johnson, 2012). The mean values from each extracted major axis of the PCA
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23 229 were analyzed through ANOVA. To do this, we used the factor scores of each principal
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25 230 component retained as a response variable and the status of condors (wild and
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28 231 rehabilitating) as predictors. We used the Kolmogorov-Smirnov test to evaluate the
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30 232 normality of each principal component.
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34 233 We used Spearman's rank correlation coefficient to describe how different fractions of
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36 234 circulating carotenoids and α , β and γ -globulin concentrations vary in relation to each other
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38 235 in wild and rehabilitating individuals (Mukaka, 2012). Finally, we evaluated the influence
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40 236 of the age, sex, status (wild or rehabilitating), xanthophyll concentrations, β -carotene
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42 237 concentrations and the interactions between the status of condors and both carotenoid
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44 238 fractions in globulin concentrations. For this purpose, we applied three different linear
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46 239 regression models including either α , β or γ -globulins as response variables and status, age,
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48 240 sex, xanthophyll concentrations, β -carotene concentrations and the interactions between
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50 241 status and carotenoids as main effects (full model). Response variables were first log-
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53 242 transformed. We performed a stepwise selection (backward/forward direction) (Derksen &
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243 Keselman, 1992) starting with the full model and eliminating predictors one at a time, at
244 each step considering whether the criterion will be improved by adding back in a variable
245 removed at a previous step (Derksen & Keselman, 1992). All statistical analyses were
246 performed with R core team (2015) (R Core Team, 2015) and we considered p-values <
247 0.05 as significant.

248 **3-RESULTS**

249 The principal component analysis resulted in a first principal component (PC1) explaining
250 55% of the variance, and the second principal component (PC2) explaining 26% of the
251 variance (Table 1). The PCA showed a first group corresponding to rehabilitating
252 individuals with higher values of α , β and γ -globulins and lower values of xanthophylls and
253 β -carotene (Fig. 1 and Table S1). The second group corresponded to wild individuals with
254 lower levels of α , β and γ -globulins and higher values of xanthophylls and β -carotene (Fig.
255 1 and Table S1). There was a statistical difference in the PC1 and PC2 values between wild
256 and rehabilitating condors, with rehabilitating birds having higher levels of PC1 and PC2
257 than wild individuals (F-value= 24.18, $P < 0.001$, F-value= 4.357, $P = 0.040$, respectively).

258 Rehabilitating individuals presented multiple negative correlations between α , β and γ -
259 globulins and the different fractions of circulating carotenoids (Table 2). However, in wild
260 condors, we did not find any negative relationship between α , β and γ -globulins and the
261 different fractions of circulating carotenoids (Table 2).

262 Finally, our models indicated that the status of individuals (wild or rehabilitating),
263 xanthophyll concentrations, β -carotene concentrations, and the interaction between condor
264 status and xanthophylls explained α , β and γ -globulin blood concentrations (Table 3). Age,

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3 265 sex and the interaction between status- β -carotene were consistently excluded from the
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5 266 models (Table 3). The models in every globulin fraction showed that the interaction
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7 267 between xanthophyll-status explained α , β and γ -globulin blood concentrations (Table 3).
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10 268 Rehabilitating Andean condors with higher concentrations of α , β and γ -globulins had
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12 269 lower levels of circulating xanthophylls compared to wild individuals that did not show this
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14 270 pattern (Fig. 2). In addition, β and γ -globulin concentrations were also explained by β -
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16 271 carotene concentrations, showing that individuals with higher levels of this carotenoid had
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18 272 higher β and γ -globulin concentrations, but this variable was statistically significant only
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20 273 for γ -globulins (Table 3).
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24 274 **4-DISCUSSION**

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27 275 Our results suggest a potential association between the immune system (α , β and γ -globulin
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29 276 concentrations) and circulating carotenoids in Andean condors. We found that
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31 277 rehabilitating and wild condors differed in their concentrations of α , β , γ -globulins and in
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33 278 their β -carotene and xanthophylls concentrations. Andean condors that arrived for
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35 279 rehabilitation and were sampled upon admission showed higher blood concentrations of α ,
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37 280 β and γ -globulins than wild individuals. This suggests that, upon arrival at the rehabilitation
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39 281 center, this group of condors were mounting an immune response associated with the
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41 282 different combinations of pathologies including both infectious and non-infectious
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43 283 conditions. In addition, rehabilitating condors showed lower concentrations of all
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45 284 circulating carotenoids, mostly β -carotene and xanthophylls, than wild individuals.
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47 285 Moreover, the multiple negative correlations between circulating carotenoids and α , β and
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49 286 γ -globulins in condors developing an immune response (rehabilitating), but not in healthy
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51 287 condors (wild), suggest that carotenoids could be diverted from the plasma to supply the
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immune system, as was reported for other bird species (Aguilera & Amat, 2007; Saino et al., 1999). In summary, our results suggest that the immune system is probably using circulating carotenoids to deal with immune stress, by increasing acute phase proteins and immunoglobulins (α , β and γ -globulins), which are an important component of the immune system (Cray & Tatum, 1998; Gruys, Toussaint, Niewold, & Koopmans, 2005; Werner & Reavill, 1999).

Our models excluded the interaction between β -carotene and status and included the interaction between xanthophylls and status of condors for each globulin fraction. This last interaction was present in each of the selected models for α , β and γ -globulins. Condors developing an immune response (rehabilitating condors) but not healthy individuals (wild) showed a strong relationship between xanthophyll concentrations and α , β and γ -globulins. Therefore, this carotenoid fraction could be the main one involved in the association with acute phase proteins and immunoglobulins. This may be expected because xanthophylls are considered potent immune enhancers both in cell-mediated and humoral responses in humans (Chew, 1993; Chew & Park, 2004), other mammal species (Kim et al., 2000a, 2000b), and birds (Leclaire et al., 2015; McGraw & Ardia, 2003; Rajput, Naeem, Ali, Rui, & Tian, 2012). In addition, given that Andean condors may display carotenoid-based pigmentation (Blanco et al., 2013; Marinero et al., 2018), our results could suggest that a trade-off between external coloration and immune system may occur in this species, but this merits further research. The positive association between β -carotenes and γ -globulins we found is not easy to explain. However, it may be associated with the fact that some individuals, especially individuals developing an immune response, could have alterations

in the transformation rates between β -carotene (pro-vitamin A) and vitamin A (Hill & Johnson, 2012), causing β -carotene to accumulate in blood.

Rehabilitating and wild condors differed in their concentrations of globulins (α , β and γ), β -carotene and xanthophylls, but there is some overlap between the two groups (see Fig. 1). The individual variation in the concentrations of the different carotenoid compounds may be associated with different levels of access to these compounds in the diet due to hierarchical rank in carcasses (Donázar et al., 1999; Marinero et al., 2018; Wallace & Temple, 1987). For instance, the hierarchical rank could influence the quantity of food ingested (Donázar et al., 1999) and thus, could influence the carotenoid concentrations reached both in wild and rehabilitating individuals. In addition, the variability in the concentrations of the different globulin fractions in both groups of condors may depend on the health status and physiological state, but may also depend on the individual differences in the immune response (Plaza et al., 2019). Therefore, the overlap that the PCA analysis shows between the two groups of Andean condors (wild and rehabilitating) may be associated with the inherent variability of the studied variables. Moreover, some wild condors with low carotenoid concentrations and high globulin levels could have been suffering from a sub-clinical pathology not detected in our examination or blood analyses. In addition, some rehabilitating condors with high carotenoid concentrations and low globulin levels could have been suffering a mild pathologic process without mounting a strong immune response.

An alternative explanation for the differences in circulating carotenoids in rehabilitating and wild individuals could be associated with other factors not related to a physiological association between carotenoids and the immune system. For instance, in the days previous

to the rescue and transport to the rehabilitation center, condors may have been subject to different clinical alterations such as anorexia, diarrhea, or depression associated with the pathological processes they were undergoing (Pavez & Estades, 2016; Ritchie, Harrison, Zantop, & Harrison, 1997). Therefore, they may have been poorly fed before being rescued and thus, carotenoid concentrations in the blood could have been reduced. Regrettably, there is no information available about carotenoid depletion in vultures. Consequently, it is difficult to distinguish when the reduction of circulating carotenoids is related to an association with the immune system, a lack of food intake due to the concomitant pathologic process, or both. However, we studied a large number of individuals in different rescue situations, undergoing different pathologic problems with diverse levels of intensity, and we sampled them upon admission. Therefore, apart from the depletion of carotenoids due to diet restriction, an association between carotenoids and immune system should be present.

In conclusion, we show that an association between circulating carotenoids and the immune system seems to occur in Andean condors. These compounds are probably not available for other vital functions such as external coloration in individuals developing an immune response. This may be the first piece of evidence that a trade-off between external coloration and the immune system could be present in this species. However, more research is needed to evaluate this. Andean condors are a large, highly despotic species that exerts dominance in carcasses according to sex, age, and also by external coloration (Marinero et al., 2018). Therefore, the association we found between carotenoids and immunity could have important implications in the ecology and conservation of this species, because it could guarantee honest signaling produced by carotenoid pigmentation. This honest

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356 signaling can have important consequences in access to food sources containing
357 carotenoids, better breeding or roosting locations, and mate selection, and therefore merits
358 particular attention.

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For Peer Review

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CONFLICT OF INTEREST: No conflict of interest declared

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Figure 1: Principal Component Analysis ordering individuals by their different fractions of globulins and circulating carotenoids. X (xanthophylls), BCar (β -carotene), AG (α -globulins), BG (β -globulins), GG (γ -globulins).

Figure 2: Regression models for α -globulins, β -globulins and γ -globulins as a function of xanthophyll concentrations. Rehabilitating condors (black dots) and wild condors (red dots).

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Table 1: Variable loadings for the two eigenvectors (PC1 and PC2) from the principal component analysis performed with the different fractions of circulating carotenoids and globulins in wild and rehabilitating Andean condors.

Parameters	PC1	PC2
Xanthophylls	-0.3825341	-0.5692098
β-carotene	-0.3308039	-0.6471098
α-globulins	0.5308006	-0.2560456
β-globulins	0.477477	-0.3401789
γ-globulins	0.4842545	-0.2756230
Proportion of variance	0.5469618	0.2604503
Cumulative proportion	0.5469618	0.8074121

Table 2: Correlations (Spearman’s rank correlation coefficient) between the different carotenoid compounds and the different fraction of globulins according to the status of condors (wild or rehabilitating).

Carotenoids fractions	α -globulins		β -globulins		γ -globulins	
	Rehabilitating	Wild	Rehabilitating	Wild	Rehabilitating	Wild
all-trans-Zeaxanthin	-0.34 (<i>P</i>=0.019)	-0.01 (<i>P</i> =0.453)	-0.16 (<i>P</i> =0.172)	0.16 (<i>P</i> =0.849)	-0.29 (<i>P</i>=0.041)	0.23 (<i>P</i> =0.931)
all-trans-Lutein	-0.33 (<i>P</i>=0.023)	-0.07 (<i>P</i> =0.319)	-0.18 (<i>P</i> =0.145)	0.15 (<i>P</i> =0.836)	-0.28 (<i>P</i>=0.044)	0.13 (<i>P</i> =0.805)
cis-isomers of Lutein/Zeaxanthin	-0.27 (<i>P</i> =0.052)	-0.09 (<i>P</i> =0.277)	-0.11 (<i>P</i> =0.245)	0.12 (<i>P</i> =0.789)	-0.24 (<i>P</i> =0.073)	0.14 (<i>P</i> =0.818)
all-trans-α-Cryptoxanthin	-0.31 (<i>P</i>=0.032)	-0.10 (<i>P</i> =0.252)	-0.11 (<i>P</i> =0.256)	0.04 (<i>P</i> =0.617)	-0.20 (<i>P</i> =0.116)	0.07 (<i>P</i> =0.670)
all-trans-β-Cryptoxanthin	-0.27 (<i>P</i> =0.051)	-0.13 (<i>P</i> =0.193)	-0.15 (<i>P</i> =0.179)	0.07 (<i>P</i> =0.686)	-0.15 (<i>P</i> =0.180)	0.11 (<i>P</i> =0.761)
Echinenone	-0.36 (<i>P</i>=0.015)	-0.01 (<i>P</i> =0.470)	-0.24 (<i>P</i> =0.076)	0.20 (<i>P</i> =0.904)	-0.23 (<i>P</i> =0.079)	0.17 (<i>P</i> =0.868)
β-Carotene	-0.42 (<i>P</i>=0.004)	-0.14 (<i>P</i> =0.187)	-0.27 (<i>P</i>=0.049)	0.18 (<i>P</i> =0.875)	-0.25 (<i>P</i> =0.066)	0.14 (<i>P</i> =0.813)
Total Xanthophylls	-0.36 (<i>P</i>=0.014)	-0.04 (<i>P</i> =0.394)	-0.18 (<i>P</i> =0.134)	0.16 (<i>P</i> =0.856)	-0.31 (<i>P</i>=0.029)	0.17 (<i>P</i> =0.870)
Total carotenoids	-0.40 (<i>P</i>=0.007)	-0.05 (<i>P</i> =0.353)	-0.27 (<i>P</i>=0.049)	0.14 (<i>P</i> =0.824)	-0.28 (<i>P</i>=0.044)	0.18 (<i>P</i> =0.873)

Table 3: Selected linear regression models evaluating the effect of carotenoids (β -Carotene and xanthophylls), sex, age, status (wild or rehabilitating) and the interactions between carotenoids and status of condors on α , β and γ -globulin concentrations.

Type of globulins	Variables	Estimate	SD	t- value	P-value
α -globulins	Intercept	0.4680	0.1601	2.924	0.004
	Status [rehabilitating- wild]	-0.7513	0.2640	-2.846	0.005
	Sex [female-male]	0.2637	0.1719	1.534	0.129
	Xanthophylls	-0.5278	0.1174	-4.494	<0.001
	Status: Xanthophylls	0.5691	0.1340	4.247	<0.001
β -globulins	Intercept	0.07767	0.11233	0.691	0.491
	Status [rehabilitating- wild]	-0.77141	0.20391	-3.783	<0.001
	β -carotene	0.25389	0.13801	1.840	0.069
	Xanthophylls	-0.52511	0.11984	-4.382	<0.001
	Status: Xanthophylls	0.52104	0.11319	4.603	<0.001
γ -globulins	Intercept	-0.0962	0.1370	-0.702	0.4847
	Status [rehabilitating- wild]	-1.0625	0.2487	-4.273	<0.001

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β-carotene	0.3976	0.1683	2.363	0.020
Xanthophylls	-0.7801	0.1461	-5.338	<0.001
Status:	0.7416	0.1380	5.374	<0.001
Xanthophylls				

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Principal Component Analysis ordering individuals by their different fractions of globulins and circulating carotenoids. X (xanthophylls), BCar (β -carotene), AG (α - globulins), BG (β -globulins), GG (γ -globulins).

Regression models for α -globulins, β -globulins and γ -globulins as a function of xanthophyll concentrations. Rehabilitating condors (black dots) and wild condors (red dots).

203x101mm (300 x 300 DPI)

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3 16-Jan-2020
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5 Dear Dr. Plaza:
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8 Thank you for submitting your manuscript for publication in Journal of Avian Biology.
9 After carefully reading your manuscript and considering it within the board of editors,
10 we must decline your submission for publication in Journal of Avian Biology without
11 further evaluation. This decision is not subject to appeal or reconsideration.
12

13 Although your study might be interesting from a carotenoid and immunological point of
14 view, I unfortunately rate the probability of publication in J. Avian Biol. as very low. I
15 find it hard to draw conclusions on correlations between two so drastically different
16 groups of birds as wild and sick ones, especially in JAB which has its backbone in
17 testing hypotheses in ecology, evolution and behaviour. Furthermore, JAB does not
18 publish primarily descriptive papers and thereby your paper is out of the scope of our
19 publishing policy. Currently, JAB receives far more manuscripts than can be accepted,
20 resulting in that only papers of the highest quality and conforming to the publication
21 policy can be accepted. Although, I realise that a decision not to send out a paper on
22 review is disappointing for the authors, I hope that the quick decision will be to the
23 advantage in the long run.
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27 While your manuscript certainly has merit, we do not believe that it will attract the level
28 of support required for publication in the Journal of Avian Biology. In such cases we
29 choose to return the manuscript to the author(s) to allow them to submit their work to
30 another journal without further delays. Page space in the Journal of Avian Biology is
31 unfortunately quite limited in relation to pressure from submissions. Currently, 75% of
32 all received manuscripts are sent for external review, but there is room to print only
33 about 30% of these in Journal of Avian Biology. Decisions to send manuscripts out for
34 review are based on several criteria, including novelty, appeal to our general
35 readership and connection to topics listed in our self-declared mission as an outlet for
36 empirical and theoretical research in all areas of ornithology. Many manuscripts that we
37 decline are in fact highly publishable, but given constraints on journal space we are
38 simply unable to consider them all.
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42 We wish you good luck in finding a suitable alternative outlet for your manuscript. While
43 I understand that this message will be disappointing, I sincerely hope that you will
44 consider Journal of Avian Biology for future submissions.
45

46 Thank you for considering Journal of Avian Biology for the publication of your
47 research. I hope the outcome of this specific submission will not discourage you from
48 the submission of future manuscripts.
49

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51 Sincerely,
52 Prof. Jan-Åke Nilsson
53 Editor-in-Chief, Journal of Avian Biology
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16-Jan-2020

Dear Dr. Plaza:

Thank you for submitting your manuscript for publication in Journal of Avian Biology. After carefully reading your manuscript and considering it within the board of editors, we must decline your submission for publication in Journal of Avian Biology without further evaluation. This decision is not subject to appeal or reconsideration.

1) Although your study might be interesting from a carotenoid and immunological point of view, I unfortunately rate the probability of publication in J. Avian Biol. as very low. I find it hard to draw conclusions on correlations between two so drastically different groups of birds as wild and sick ones, especially in JAB which has its backbone in testing hypotheses in ecology, evolution and behaviour. Furthermore, JAB does not publish primarily descriptive papers and thereby your paper is out of the scope of our publishing policy. Currently, JAB receives far more manuscripts than can be accepted, resulting in that only papers of the highest quality and conforming to the publication policy can be accepted. Although, I realise that a decision not to send out a paper on review is disappointing for the authors, I hope that the quick decision will be to the advantage in the long run.

Response: We understand and agree that the groups of birds are drastically different and this is the point of our article; In this sense, we performed an exhaustive clinical examination of each individual trapped. We considered as a healthy individual if any clinical alteration was found by the veterinarians. Unhealthy individuals in this population were very rare (in fact, between 2010 to 2019 we trapped just one unhealthy condor of more almost 100; this bird was not part of this study). As we explained, in our manuscript version of the article, we highlight that in previous studies (Plaza et al. 2019; Wiemeyer 2019) different blood parameters were measured. Wild condors did not show any alterations in the different blood parameters we measured compared with the reference values for this species (Doussang et al., 2018). In the case of rehabilitating condors, they showed alterations in different blood parameters according to the pathology/pathologies that they were undergoing.

2) While your manuscript certainly has merit, we do not believe that it will attract the level of support required for publication in the Journal of Avian Biology. In such cases we choose to return the manuscript to the author(s) to allow them to submit their work to another journal without further delays. Page space in the Journal of Avian Biology is unfortunately quite limited in relation to pressure from submissions. Currently, 75% of all received manuscripts are sent for external review, but there is room to print only about 30% of these in Journal of Avian Biology. Decisions to send manuscripts out for review are based on several criteria, including novelty, appeal to our general readership and connection to topics listed in our self-declared mission as an outlet for empirical and theoretical research in all areas of ornithology. Many manuscripts that we decline are in fact highly publishable, but given constraints on journal space we are simply unable to consider them all.

Response: Thank you for this comment.

We wish you good luck in finding a suitable alternative outlet for your manuscript. While I understand that this message will be disappointing, I sincerely hope that you will consider Journal of Avian Biology for future submissions.

Thank you for considering Journal of Avian Biology for the publication of your research. I hope the outcome of this specific submission will not discourage you from the submission of future manuscripts.

Sincerely,
Prof. Jan-Åke Nilsson
Editor-in-Chief, Journal of Avian Biology

For Peer Review