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RESEARCH ARTICLE

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Resveratrol supplementation reduces oxidative stress and modulates the immune response in free-living animals during a viral infection

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Abstract

1. Diet quality may have an important effect on the regulation of oxidative status and the immune system during an infectious disease. However, the relationship among intake of specific dietary molecules, an individual's oxidative status and the occurrence and progress of a viral disease remains almost unexplored in free-living organisms.
2. Here, we study a wild, long-lived animal, the Magnificent frigatebird *Fregata magnificens* to investigate: (a) the differences in a number of physiological traits (biomarkers of blood oxidative status, corticosterone (CORT), immunity and inflammation) between sick and healthy nestlings; and (b) whether experimentally increased intake of resveratrol (a polyphenol with antioxidant and antiviral properties) affects these physiological markers during the progress of a severe viral disease.
3. Birds with visible clinical signs showed higher oxidative damage, haemolysis and haemagglutination scores and lower antioxidant defences in comparison with birds without clinical signs. At the end of the experiment, supplemented birds showed the following: (a) increased plasma haptoglobin levels and circulating antioxidant defences; (b) reduced generation of lipid oxidative damage; and (c) negligible to no influence on immune markers, baseline CORT levels and activity of antioxidant enzymes.
4. Our work illustrates how the availability of specific organic molecules in the diet may constrain the individuals' capacity to cope with viral infections in free-living animals.

KEYWORDS

antioxidant defences of birds, avian glucocorticoid, avian infectious diseases, Frigatebird, immune response, oxidative stress, stress hormones, wild animals

1 | INTRODUCTION

Infectious agents are an important selective force, potentially reducing survival and reproduction of the host and, eventually, resulting in population declines (Preece et al., 2017; Smith, Sax, & Lafferty, 2006). A well-documented infectious disease is represented by the West Nile virus (WNV), a virus that is mostly found in birds because they are suspected to be the most important amplifying hosts. WNV has been found in 63 bird species 1 year after its first appearance in 1999 (Kramer & Bernard, 2001) and has caused massive mortality in many corvid species (McLean, 2006). Similarly, avian influenza virus (AIV) has caused world-wide severe outbreaks in poultry, wild birds and humans (Chatziprodromidou et al., 2018).

The association between diseases and immune defences (Savage et al., 2016) or the selection of immune traits during outbreaks (Legagneux et al., 2014) has been of great interest in evolutionary ecology and physiology, yet the pathophysiological mechanisms underlying the impact of infectious diseases on wildlife remain poorly documented. An approach that simultaneously quantifies physiological stress and immune status would be ideal to determine how an organism is affected by a given pathogen (Hawley & Altizer, 2011). For instance, estimating stress hormone corticosterone (CORT) levels during infection may be relevant because exposure to stressors stimulates CORT release (Sapolsky, Romero, & Munck, 2000). Although CORT coordinates the stress–response (Sapolsky et al., 2000), high levels and/or chronic release of CORT are known to suppress the immune response (Bourgeon & Raclot, 2006; Gao, Sanchez, & Deviche, 2017) and to increase the impact of virus infection in birds (Owen, Nakamura, Coon, & Martin, 2012).

Similarly, oxidative stress is known to limit immune function in birds (Catoni, Schaefer, & Peters, 2008). Thus, oxidative stress may contribute to the spread of infectious diseases (de Crommenacker, Richardson, Koltz, Hutchings, & Komdeur, 2012; Keles et al., 2010). For instance, Marek's disease increases damage to DNA, lipids and proteins in chickens (Keles et al., 2010), suggesting that oxidative stress might partially explain an organism's vulnerability to viral diseases (Li, Feng, & Sun, 2011) and viral replication (Costantini, Seeber, et al., 2018; Li et al., 2011). Measuring both antioxidant defences and oxidative damage, which respectively reflect the ability to mount a protective response to an adverse condition and the deleterious effects the animals undergo (Beaulieu & Costantini, 2014), might therefore prove valuable to infer the individual's capacity to cope with a pathogen.

Importantly, dietary antioxidants might impact the host's capacity to cope with an infectious disease because antioxidants reduce immunopathology associated with the immune/inflammatory response (Dhinaut, Balourdet, Teixeira, Chogne, & Moret, 2017); they occur in limited supply for free-ranging animals, potentially constraining their capacity to cope with oxidative stress (Catoni et al., 2008; Costantini, Angeletti, et al., 2018) and may limit steroid synthesis (Ozdemir, Ozudogru, Imik, Can, & Sunar, 2011). Accordingly, antioxidants can inhibit the replication of several viruses (i.e., feline immunodeficiency virus Mortola et al., 1998; influenza virus Han et al., 2000; duck enteritis virus Xu et al., 2013; herpes simplex virus Civitelli et al., 2014). Moreover, dietary antioxidants exhibit antiviral effects that are apparently not directly connected to their antioxidant properties (e.g., Abba, Hassim, Hamzah, & Noordin, 2015). However, to the best of our knowledge, experimental supplementation of molecules with antioxidant and antiviral properties to study the impact on oxidative status, CORT levels, and immunity during a viral infection has never been carried out in free-living animals.

Here, we studied nestlings of Magnificent frigatebird (*Fregata magnificens*) coping with a severe viral disease. We investigated whether birds with visible clinical signs (i.e., skin crusts) showed an alteration of their physiological traits (blood oxidative balance, immune status, CORT levels, and inflammation) in comparison with birds without clinical signs. We then tested whether supplementation of resveratrol, a polyphenol with both antioxidant and antiviral activity (Abba et al., 2015), improves the physiological traits, short-term progress of the disease and survival perspectives of supplemented birds. Our experiment was carried out on a protected island in French Guiana, where outbreaks of viral infections occur yearly, causing 85% to 95% nestling mortality (field observations). Bacterial cultures, viral screening and microscopic evaluation of skin samples excluded the presence of ectoparasites, avian poxvirus (de Thoisy et al., 2009) and avian influenza (unpublished results), but detected herpesvirus DNA in body crusts (de Thoisy et al., 2009). Recent work found up to 10 million copies of herpesviral DNA in nestlings with clinical signs of the disease, suggesting that herpesvirus replication is involved in the appearance of clinical signs (Sebastiano, Eens, Abd Elgawad, et al., 2017). This population offers an unprecedented opportunity to investigate the potential connection between viral disease progression and diet quality employing an experimental approach, because clinical signs are associated with several physiological biomarkers of oxidative stress and inflammation (Sebastiano, Eens, Abd Elgawad, et al., 2017; Sebastiano, Eens, Angelier, et al., 2017), and we can experimentally feed frigatebirds in the wild.

2 | MATERIAL AND METHODS

2.1 | Sample collection

The fieldwork was carried out in 2016 on Grand Connétable Island, a protected area off the Northern Atlantic coast of South America (French Guiana, 4°49'30N; 51°56'00W), which hosts approximately 1,300 reproductive pairs of frigatebirds (GEPOG field observations). Most frigatebird pairs in this colony start breeding between the end of November and the beginning of December. Consequently, all nestlings were approximately of the same age (~4 months old) when captured (see also Statistical Analysis section). A total of 26 nestlings without visible clinical signs and 34 sick nestlings showing visible clinical signs were randomly chosen at different sites of the island (Figure 1). Visible clinical signs of the disease include crusts on the head and the

FIGURE 1 Nestlings' classification based on visible clinical signs of the disease: (a) "no signs," (b) "mild" and (c) "severe"

body, hyperkeratosis on eyes and the consequent thickening of the cornea (de Thoisy et al., 2009). All 60 nestlings were captured at the nest by hand on 7 June (D1). Within 3 min after capture, 2 ml of blood was collected from the brachial vein using a heparinized syringe and a 25G needle. Immediately afterwards, each bird was ringed with an aluminium ring for individual recognition and the beak-head distance was measured to control for the age of nestlings. This first sample of blood was used to test any pretreatment difference among groups.

The experiment started on D2. We administered pills of *trans*-Resveratrol (see Supporting Information Appendix S1) to the experimental groups (12 of 26 healthy nestlings and 18 of 34 sick nestlings, respectively), while the remaining individuals (14 healthy and 16 sick nestlings, respectively; i.e., control groups) were administered an empty pill as a placebo to account for the effect of handling. We chose resveratrol because of its strong antiviral activity against herpesvirus (Abba et al., 2015; Sebastiano, Chastel, de Thoisy, Eens, & Costantini, 2016), which actively replicates in sick nestling frigatebirds (Sebastiano, Eens, Angelier, et al., 2017). Pills were dipped in fish oil (Crafty catcher, Ipswich, UK) to facilitate swallowing. The administration of pills was carried out nine times (D2–4, 8th–10th of June; D12–14, 18th–20th of June; D21–23, 27th–29th of June). On D24, the experiment ended and a second sample of 2 ml of blood was taken from the same individuals. Blood samples were kept cold while in the field and centrifuged within less than 2 hr to separate plasma (used for CORT, oxidative stress biomarkers, inflammatory and immune markers) and red blood cells. Both samples of plasma and red blood cells were then kept in dry ice until the end of the fieldwork and, upon arrival in the laboratory, were kept in a −80°C freezer. Two pictures of each bird were taken from the same distance and same position at the start and the end of the experiment (pre- and post-treatment), to score clinical signs of the disease and assess if visible clinical signs increased or decreased during the experiment (see the specific section below).

2.2 | Molecular analyses

All analyses were performed using established protocols for vertebrates. The determination of nonenzymatic antioxidants was performed using reduced (GSH) and oxidized (GSSG) glutathione in red blood cells. High-performance liquid chromatography (HPLC) with electrochemical detection (Reversed-Phase HPLC of Shimadzu, Hai Zhong Lu, Shanghai) was applied following Sinha et al. (2014), and concentrations were expressed as μmol/g of fresh weight. We also calculated the GSH/GSSG ratio as a metric of oxidative balance. Furthermore, the nonenzymatic antioxidant power of erythrocytes (TAC, an index of circulating nonenzymatic antioxidants) was estimated following Benzie and Strain (1996) and expressed as μmol Trolox/g of fresh weight. The enzymatic antioxidant capacity was measured using three different biomarkers in red blood cells. Superoxide dismutase (SOD) activity was estimated by measuring the inhibition of nitroblue tetrazolium reduction at 560 nm and was expressed as U/mg protein per minute. Catalase activity (CAT) was measured by monitoring the rate of decomposition of hydrogen peroxide (H_2O_2) at 240 nm and was expressed as μmol H_2O_2/mg protein per minute. Glutathione peroxidase (GPX) activity was determined by measuring the decrease in NADPH absorbance at 340 nm and was expressed as μmol NADPH/mg protein per minute. Damage to biomolecules was assessed by quantifying the plasma level of thiobarbituric acid reactive substances (TBARS), which reflect lipid peroxidation, and the level of protein carbonyls in red blood cells as a measure of oxidative damage to proteins. Results are expressed, respectively, as nmol of malondialdeyde (MDA) equivalents/ml (Hodges, DeLong, Forney, & Prange, 1999) and nmol/mg protein (Levine, Williams, Stadtman, & Shacter, 1994).

Plasma haptoglobin concentration was quantified using a commercially available assay (PHASE Haptoglobin assay; Tridelta Development Ltd), and concentrations were expressed as mg/ml. The plasma concentration of nitric oxide (NO) was estimated from the concentration of the stable end products of nitric oxide oxidation (i.e., nitrate and nitrite) and expressed in μmol/L. Innate humoral immunity was determined by the haemolysis-haemagglutination assay as described in Matson, Ricklefs, and Klasing (2005). Finally, the plasma concentration of corticosterone was measured by radioimmunoassay following Lormée, Jouventin, Trouve, and Chastel (2003) and expressed as ng/ml.

Detailed protocols are provided in the Supporting Information Appendix S1.

2.3 | Bird classification based on clinical signs

To classify the sampled birds based on the severity of visible clinical signs, pictures were analysed and blindly scored twice by the same person (1 week apart). Scores ranged from 0 (absolute absence of clinical signs) to 10 (bird fully covered by crusts), including half scores. Reproducibility between the two scores (calculated from variance components derived from a one-way analysis of variance, accordingly to Lessells & Boag, 1987) of each individual was significantly high both before ($r = 0.96$, $F = 49.67$, $p < 0.0001$) and after ($r = 0.93$, $F = 31.64$, $p < 0.0001$) the treatment. An average score was, therefore, calculated and used to further divide nestlings into three study groups to be used for statistical comparisons: healthy group (hereafter "no signs," average score <1), nestlings with few crusts on the neck and around the eyes (hereafter "mild," average score ≥1 or <4) and very sick nestlings showing more widespread and thicker crusts (hereafter "severe," average score ≥4; for reference, see Figure 1). This classification enabled us to detect birds that changed group over the progress of the disease (which matches a change in the visible clinical signs), thus to identify: (a) birds that never showed the appearance of clinical signs, hereafter "always healthy"; (b) birds that showed the appearance of clinical signs, hereafter "new sick"; (c) birds that had an improvement of visible clinical signs, hereafter "better condition"; and (d) birds that did not change their status, hereafter "same severity." Finally, none of the sick birds showed an increased severity of clinical signs, probably because the worsening of an already critical condition coincided with the death of the bird (hereafter called "did not survive").

2.4 | Statistical analyses

Two general linear models were used to analyse pretreatment group differences: (a) among birds classified on the severity of clinical signs (no signs, mild and severe); and (b) among groups based on the progress of the disease (always healthy, new sick, better condition, same severity and did not survive). A third linear model (which included all 34 sick nestling as independent observations, thus not divided into groups) was used to investigate whether the scores assigned to the severity of clinical signs of each nestling (included as a continuous variable) were associated with the analysed biomarkers.

Two linear mixed models with a repeated measures design were used to assess the effects of the treatment: (a) between the two groups based on the presence or absence of visible clinical signs of the disease at the first sampling period (MODEL 1); and (b) among groups based on the progress of the disease (four groups: "always healthy," "new sick," "better condition" and "same severity"; MODEL 2). Mixed models only included nestlings for which we had two measurements (pre- and post-treatment), implying that nestlings that did not survive or that were not found at the second sampling period were not included. As preliminary analysis showed that the head-beak distance (used as a proxy of the age) was similar between healthy and sick (*t* test; $t = -1.85$; $p = 0.07$) and between supplemented and unsupplemented nestlings (*t* test; $t = 1.85$; $p = 0.07$), this variable was not further included in statistical analyses. The reduced model was obtained by sequentially removing nonsignificant interactions from the full model starting from the three-way interaction. Post hoc Tukey tests were used to explore further significant interactions.

Finally, a generalized linear model using a binomial error variance and a logit link function was used to assess whether the treatment influenced the probability of the appearance of clinical signs, of a decrease in visible clinical signs of the disease, or death. Detailed information on data transformation and setup of the linear mixed models can be found in the Supporting Information Appendix S1.

All analyses were performed using R (v. 3.3.1, R Core Team, 2013).

3 | RESULTS

3.1 | Bird classification based on clinical signs

Before the experiment, we had a total of 26 birds without clinical signs and 34 sick birds (13 mild and 21 severely affected). Over the course of the experiment, of the 26 birds classified as healthy before the experiment, we had a total of 11 birds (four supplemented) that never showed the appearance of clinical signs, hereafter "always healthy"; 11 birds (four supplemented) that showed the appearance of clinical signs, hereafter "new sick." Of the 34 birds classified as sick before the experiment, eight birds (four supplemented) had an improvement of visible clinical signs, hereafter "better condition"; 18 birds (nine supplemented) did not change their status, hereafter "same severity"; seven birds (four supplemented) died, hereafter called "did not survive." Four healthy and one sick bird (all supplemented) were not found at the end of the experiment.

3.2 | Pretreatment basal differences among groups

Birds with *no clinical signs* showed (a) significantly lower reduced glutathione, oxidized glutathione and oxidative damage to lipids than birds with *severe* ($t > 2.76$, $p < 0.02$; Figure 2a–c) clinical signs; and (b) significantly lower reduced glutathione and oxidative damage to lipids than birds with *mild* ($t > 2.59$, $p < 0.03$, Figure 2a,c) clinical signs. Oxidative damage to lipids was also significantly higher in birds with *severe* clinical signs than in birds with *mild* clinical signs ($t = 2.58$, $p = 0.03$, Figure 2c). *New sick* birds had a tendency to have pretreatment lower oxidized glutathione than birds that were in a *better condition* at the end of the experiment ($t = 2.80$, $p = 0.052$; Figure 3a). Finally, pretreatment oxidative damage to lipids was significantly lower both in birds that were *always healthy* and in *new sick* birds than in birds that (a) that were in a *better condition* at the end of the experiment; (b) showed the *same severity* of clinical signs at the end of the experiment; or (c) *did not survive* ($t > 2.90$, $p < 0.04$; Figure 3b).

Among markers of immunity, the inflammation protein haptoglobin was significantly higher in birds with *severe* clinical signs than birds with *mild* clinical signs ($t = 3.75$, $p < 0.01$, Figure 2d) or with *no clinical signs* ($t = -3.93$, $p < 0.01$, Figure 2d). Furthermore, higher haptoglobin levels

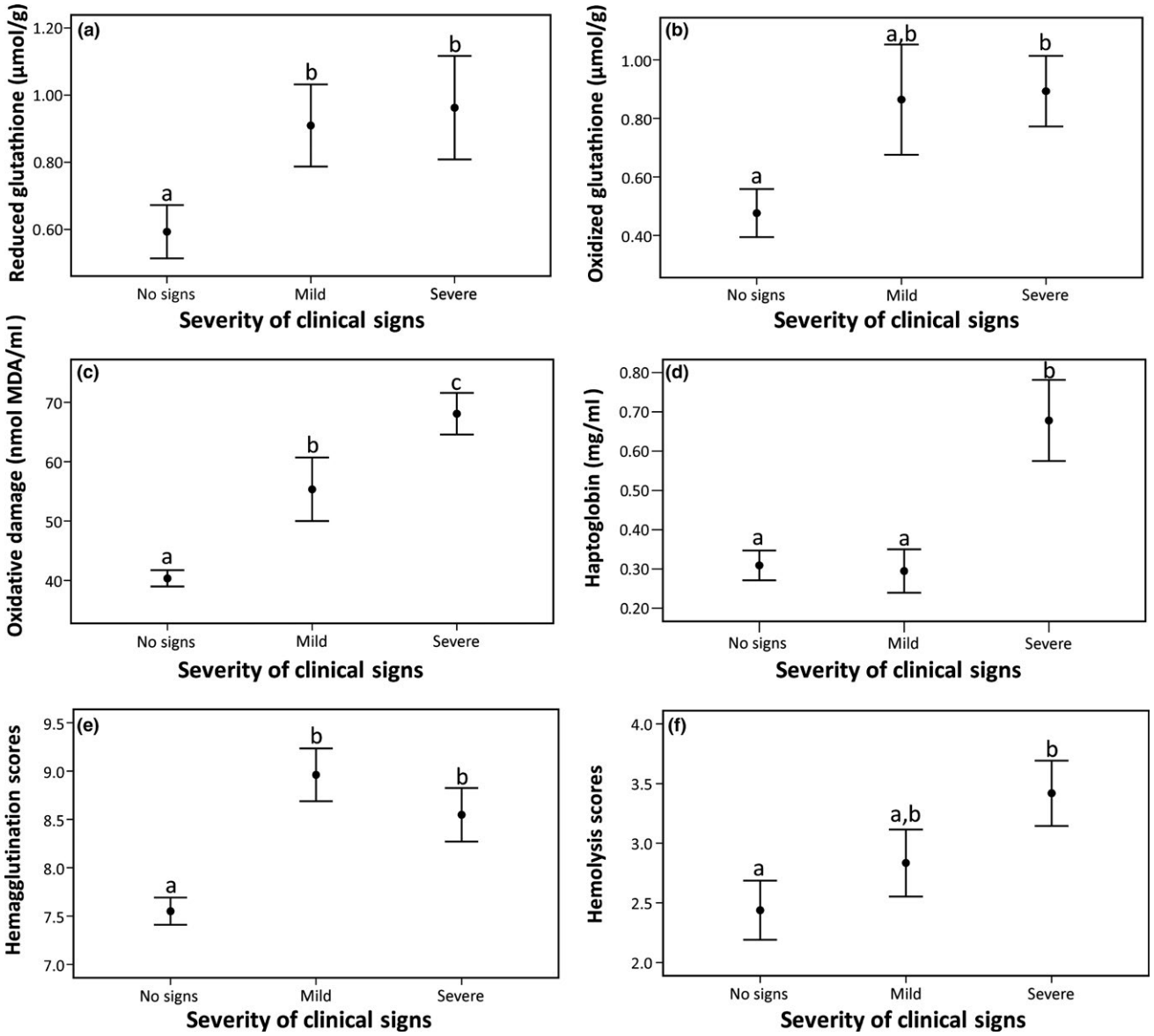

FIGURE 2 Pretreatment differences among birds classified on the severity of visible clinical signs (no signs, $n = 26$; mild, $n = 13$; severe, $n = 21$) of: (a) reduced glutathione (µmol/g of fresh weight); (b) oxidized glutathione (µmol/g of fresh weight); (c) oxidative damage (nmol MDA equivalents/ml); (d) haptoglobin (mg/ml); (e) haemagglutination score; and (f) haemolysis score. Data are shown as mean ± standard error. Values that do not share the same letter are significantly different from each other

were found in birds that *did not survive* ($t > 2.95$, $p < 0.04$, Figure 3c). Birds with *no clinical signs* showed (a) significantly lower haemagglutination than birds with *mild* ($z = -3.88$, $p < 0.01$, Figure 2e) and *severe* ($z = -3.19$, $p < 0.01$; Figure 2e) clinical signs; and (b) lower haemolysis than birds with *severe* clinical signs ($z = -2.72$, $p = 0.02$; Figure 2f). Haemagglutination was also higher in birds that *did not survive* and in birds with the *same severity* of clinical signs than *new sick* birds ($z > 3.49$, $p < 0.01$; Figure 3d), or birds that were *always healthy* ($z > 2.98$, $p < 0.02$; Figure 3d) over the course of the experiment.

None of the other biomarkers of oxidative status, immunity and basal plasma CORT showed a significant association with the disease status (Table 1). Finally, none of the biomarkers showed a significant relationship when clinical signs were used as a continuous variable, with the exception of haptoglobin (i.e., haptoglobin increases with increasing clinical signs; $F = 30.47$, $p < 0.01$; Supporting Information Figure S1).

3.3 | Effect of resveratrol administration

We then examined whether administration of resveratrol would affect the oxidative status of the birds. Circulating nonenzymatic antioxidants increased in supplemented birds with *no clinical signs* ($t = -3.94$, $p < 0.01$; Figure 4, Supporting Information Table S1). Specifically, this increase in nonenzymatic antioxidants only occurred in supplemented birds that were *always healthy* during the experiment ($t = -3.70$, $p = 0.03$; Figure 5). Oxidative damage to lipids did not change in supplemented birds (Figure 4), while it strongly increased in unsupplemented sick birds ($t = -6.19$,

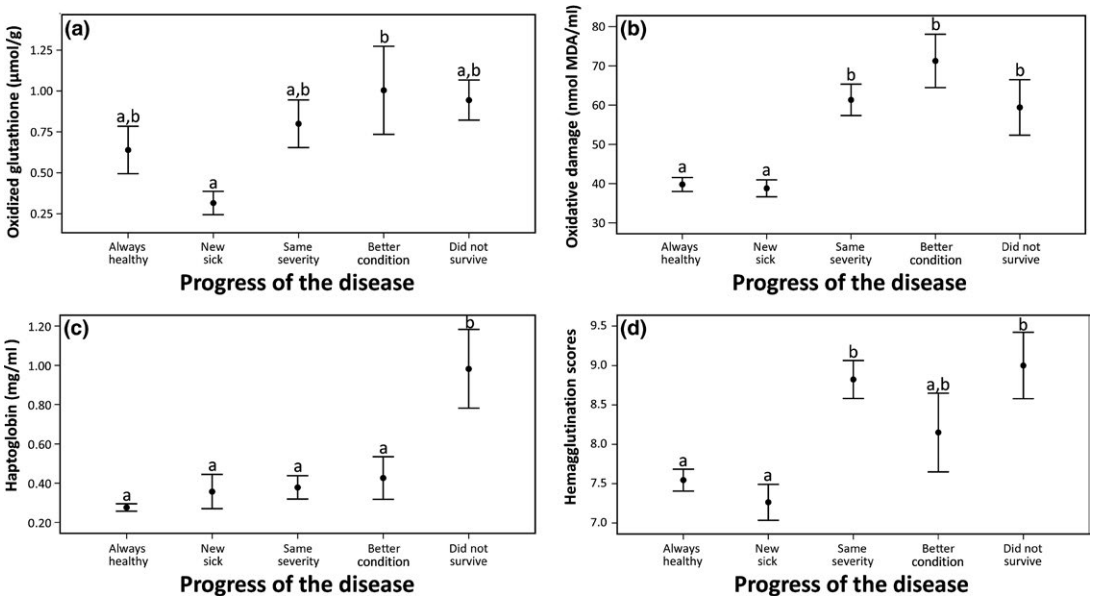

FIGURE 3 Pretreatment differences among birds classified based on the progress of the disease (always healthy, *n* = 11; new sick, *n* = 11; better condition, *n* = 8; same severity, *n* = 18; did not survive, *n* = 7) of: (a) oxidized glutathione (µmol/g of fresh weight); (b) oxidative damage (nmol MDA equivalents/ml); (c) haptoglobin (mg/ml); and (d) haemagglutination score. Data are shown as mean ± standard error. Values that do not share the same letter are significantly different from each other

TABLE 1 Linear models explaining whether the pre-treatment level of a specific biomarker differed among groups classified on the severity of clinical signs (no signs, mild and severe) or the progress of the disease (always healthy, new sick, same severity, better condition, and did not survive)

	Grouping factor			
	Severity of clinical signs		Progress of the disease	
Biomarker	*F*-value	*p*-value	*F*-value	*p*-value
Carbonyls	2.07	0.14	0.74	0.57
Catalase	0.48	0.62	0.80	0.53
Corticosterone	0.32	0.73	0.28	0.89
Glutathione peroxidase (GPX)	1.84	0.17	0.88	0.48
GSH/GSSG ratio	0.30	0.74	0.78	0.54
Haptoglobin	10.1	**<0.01**	5.16	**<0.01**
Hemagglutination	9.78	**<0.01**	6.18	**<0.01**
Hemolysis	3.05	**<0.06**	1.84	0.14
Nitric oxide (NOX)	0.20	0.82	1.55	0.20
Non-enzymatic antioxidants (TAC)	1.07	0.35	0.93	0.45
Oxidative damage (TBARS)	22.2	**<0.01**	8.83	**<0.01**
Oxidized glutathione (GSSG)	4.33	**0.02**	2.71	**0.04**
Reduced glutathione (GSH)	5.18	**<0.01**	2.45	0.07
Superoxide dismutase (SOD)	0.89	0.41	0.72	0.58

Note. The linear model on the progress of the disease included all nestlings excluding the five individuals that were not found at the second sampling period. *p*-values are in bold when post-hoc comparisons were significant.

$p < 0.01$; Figure 4, Supporting Information Table S1), indicating that resveratrol prevented increased production of oxidative damage. This increase in oxidative damage to lipids occurred mostly in birds that showed the *same severity* of clinical signs during the experiment ($t = -4.91$, $p < 0.01$; Figure 5, Supporting Information Table S2).

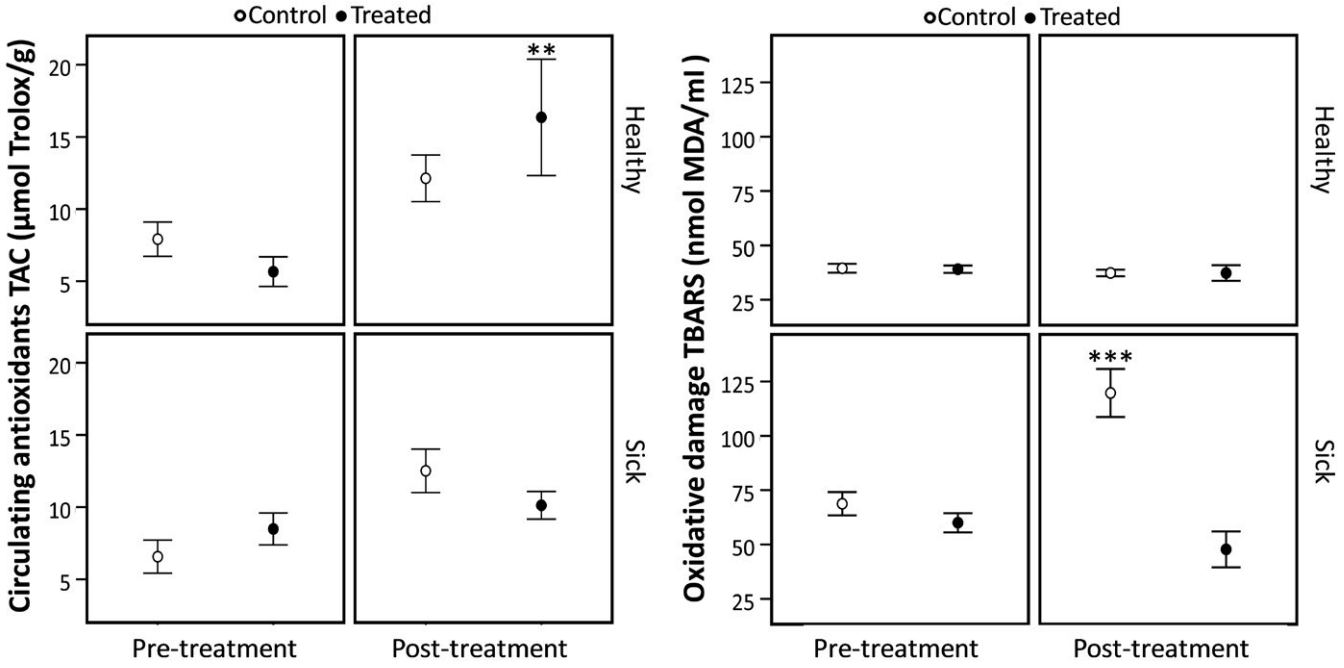

FIGURE 4 Effect of resveratrol administration on the levels of (*left)* circulating nonenzymatic antioxidants (µmol Trolox/g of fresh weight), and (*right)* oxidative damage to lipids (nmol of MDA equivalents/ml) levels. Asterisks indicate significant differences: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. Data are shown as mean ± standard error

Among immune markers, the plasma concentration of haptoglobin increased in supplemented *new sick* birds (birds that showed the appearance of clinical signs at the end of the experiment, $t = -3.86$, $p = 0.03$; Figure 5, Supporting Information Table S2). The concentration of nitric oxide did not change in supplemented birds, while it significantly decreased in unsupplemented birds ($t = 2.96$, $p < 0.01$, Supporting Information Tables S1 and S2). Other biomarkers of immunity and stress were not significantly influenced by the treatment considering both birds divided based on the presence/absence of clinical signs (MODEL 1) and birds divided on the progress of the disease (MODEL 2; Supporting Information Tables S1 and S2).

3.4 | Effects of resveratrol administration on the progress of the disease

When we compared supplemented and unsupplemented birds, no significant difference in the progress of the disease was detected. Resveratrol supplementation did not influence the probability of developing clinical signs ($z = 0$, $p = 1$; exact same number of individuals in each group), nor did it influence the probability of mortality and/or reduction in visible clinical signs ($z = 0.19$, $p = 0.85$).

4 | DISCUSSION

Our study is the first to measure the effect of resveratrol administration on oxidative status, inflammation, immunity and CORT levels in a wild vertebrate facing a severe virus outbreak. Before the start of the experiment, there were significant differences in the oxidative and immune statuses between sick and healthy birds in our study population. There was also a strong increase in lipid oxidative damage during the progress of the disease. The experimental part of our study demonstrated that resveratrol supplementation increased antioxidant defences and limited the generation of lipid oxidative damage during the progress of the disease.

The analysis of several biomarkers prior to the antioxidant treatment enabled us to discover that a viral disease can affect diverse functional pathways. Viral diseases are known to affect the oxidative status (Durgut, Ataseven, Sagkan-Ozturk, & Ozturk, 2013; Keles et al., 2010) and immunity (Staley & Bonneaud, 2015) of exposed animals. Accordingly, we found that nestlings with visible clinical signs showed a pronounced alteration of their immune status and cellular oxidative balance in comparison with birds without clinical signs. Contrary to our expectation, baseline CORT levels did not differ among the different groups. CORT also did not increase during the progress of the disease, which is in agreement with our previous results (Sebastiano, Eens, Angelier, et al., 2017). By dividing birds according to the severity of clinical signs and the progress of the disease over the course of the experiment, instead, we found novel findings. Birds without clinical signs showed lower haemolysis and haemagglutination scores, results that had not previously emerged (Sebastiano, Eens, Angelier, et al., 2017).

Supplementation with resveratrol did not affect haemolysis and haemagglutination scores, which might suggest that these particular aspects of the immune system play a minor role in coping with a viral infection in frigatebirds (Sebastiano, Eens, Angelier, et al., 2017). In contrast, nestlings supplemented with resveratrol showed

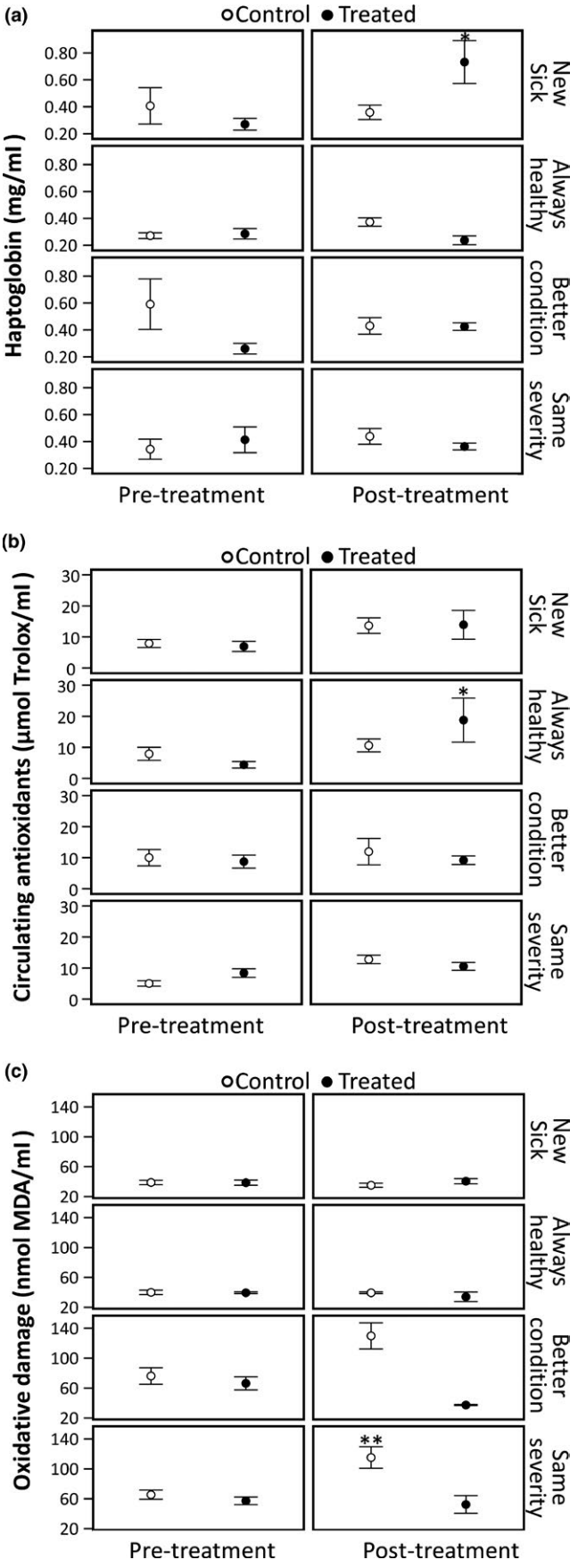

FIGURE 5 Effect of resveratrol administration on the levels of (a) haptoglobin (mg/ml), (b) circulating nonenzymatic antioxidants (µmol Trolox/g of fresh weight) and (c) oxidative damage to lipids (nmol of MDA equivalents/ml). Asterisks indicate significant differences: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. Data are shown as mean ± standard error

an elevated production of nitric oxide during the progress of the disease, while nitric oxide production drastically decreased in unsupplemented birds. Previous studies have found that cells from knockout mice lacking nitric oxide production showed a lower antimicrobial activity during *Salmonella* infection (Vazquez-Torres, Jones-Carson, Mastroeni, Ischiropoulos, & Fang, 2000), indicating that nitric oxide is important to defeat pathogens. Previous studies have shown that dietary antioxidants can act as immunostimulants in birds (Catoni et al., 2008; Hooda, Tyagi, Tyagi, & Sharma, 2005). Thus, the stimulating effect of resveratrol on the production of nitric oxide might underlie its antiviral activity because nitric oxide controls the function of natural killer cells and cytokines that are essential to combat pathogens (Bogdan, Rollinghoff, & Diefenbach, 2000). Although our results support a potential role of resveratrol in promoting nitric oxide production in a wild bird (Gülçin, 2010), it is unclear why there was a decrease in nitric oxide in unsupplemented birds that never showed the appearance of clinical signs. Similarly to nitric oxide, synthesis of haptoglobin increases during infections to protect from oxidative damage (MacKellar & Vigerust, 2016). In nestlings supplemented with resveratrol, haptoglobin was upregulated during the early stage of the infection. In contrast, in those nestlings that were not given resveratrol, haptoglobin was higher in birds at an advanced stage of the disease. This is in agreement with previous results (Asasi, Mohammadi, Boroomand, Hosseinian, & Nazifi, 2013; Sebastiano, Eens, Angelier, et al., 2017) and suggests that haptoglobin production is upregulated at an advanced stage of the disease, but resveratrol stimulated the production of haptoglobin at an earlier phase of the disease. The consequences of this change in haptoglobin production for individual survival are unclear and need further investigation.

Supplementation of organic molecules with antioxidant properties (e.g., vitamins, polyphenols) may reduce oxidative stress (Costantini, 2014). However, an increased intake of these compounds may also interfere with the endogenous antioxidant systems, leading to a decrease in enzymatic antioxidant activity (Wang, Chien, & Pan, 2006) and expression of antioxidant genes (Selman et al., 2006). We found an increase in nonenzymatic antioxidant capacity in erythrocytes, which occurred in birds that never showed the appearance of clinical signs, suggesting that resveratrol had antioxidant effects. To date, the high antioxidant activity of resveratrol has been linked with its capacity to induce glutathione synthesis (Bellaver, Souza, Souza, & Quincozes-Santos, 2014; Kode et al., 2008), but our results do not show such relationship, implying that this mechanism needs further assessment.

Resveratrol is also known to have a very strong inhibition power against lipid peroxidation (Gülçin, 2010), and its administration

prevented an increase in oxidative damage to lipids. This result might have fundamental implications for the progress of the disease because (a) high levels of oxidative damage are associated with reduced short-term survival probability (Sebastiano, Eens, Abd Elgawad, et al., 2017); (b) birds that were naturally recovering from clinical signs did not show an increase in oxidative damage; and (c) a cell condition of oxidative stress appears to facilitate virus replication, while cells that are able to upregulate antioxidant defences and limit damage can survive viral infections (Qiang et al., 2006). This result, however, does not enable us to assess whether the protection from oxidative damage was due to the antioxidant or the antiviral activity of resveratrol, and further studies are warranted to clarify this mechanism.

Increased production of CORT is another mechanism activated in animals facing an infection because it induces a number of physiological changes (e.g., mobilization of stored energy, stimulation of immune function) that sustain the body function and maintain cellular homoeostasis (Sapolsky et al., 2000). However, our results provide no support for this relationship between virus-induced stress and CORT release, but the high variation in CORT levels among individuals could arise from exposure to strong environmental stressors (Martinet & Blanchard, 2009; Sebastiano, Bustamante, et al., 2016, 2017), and clearly deserves further investigations.

The antiviral and antioxidant effects of resveratrol might also result in life extension as previously suggested by a meta-analytic study (Hector, Lagisz, & Nakagawa, 2012). However, we could not detect the effect of resveratrol supplementation on the survival probability of birds. This might have been due to the relatively short treatment period compared with the rapid progress of the disease. A longer-term treatment would prove useful to understand whether resveratrol increases survival probabilities of sick birds and whether it can prevent the appearance of clinical signs. This might have fundamental implications for the long-term viability of this population, which is now at risk due to the massive mortality events of nestlings. In long-lived species with low fecundity, as the Magnificent frigatebird, even a small rate of nestling mortality can indeed have important negative demographic effects (Finkelstein, Doak, Nakagawa, Sievert, & Klavitter, 2010).

5 | CONCLUSIONS

We have provided experimental support to the hypothesis that dietary compounds may constrain the capacity of organisms to cope with a viral disease. Our work shows that the effects of these dietary organic molecules may come through both antioxidant protection and antiviral properties. It will be important to expand our study to other species and environmental conditions to further assess the conditions under which the quality of diet may affect the capability of animals to cope with a viral disease. Although our work focused on the effects of a polyphenol (i.e., resveratrol), naturally occurring diets may be rich in many other compounds that show similar properties (e.g., carotenoids, vitamins) to those of polyphenols, but whose effects on viral diseases in free-ranging animals have not been explored so far.

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CONFLICT OF INTEREST

All authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

D.C., O.C. and M.S. designed the study; M.E., D.C., O.C., G.B. and K.P. coordinated different phases of the study; M.S., S.M. and K.P. collected the samples; M.S., S.M. and H.A. performed laboratory analyses; M.S. analysed the data and wrote the manuscript with the contribution of all authors.

DATA ACCESSIBILITY

Data are deposited in the Dryad Digital Repository: https://doi.org/10.5061/dryad.r75251m (Sebastiano et al., 2018).

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REFERENCES

Abba, Y., Hassim, H., Hamzah, H., & Noordin, M. M. (2015). Antiviral activity of resveratrol against human and animal viruses. *Advances in Virology*, *2015*, 1–7. https://doi.org/10.1155/2015/184241

Asasi, K., Mohammadi, A., Boroomand, Z., Hosseinian, S. A., & Nazifi, S. (2013). Changes of several acute phase factors in broiler chickens in response to infectious bronchitis virus infection. *Poultry Science*, *92*, 1989–1996. https://doi.org/10.3382/ps.2012-02902

Beaulieu, M., & Costantini, D. (2014). Biomarkers of oxidative status: Missing tools in conservation physiology. *Conservation Physiology*, *2*, cou014. https://doi.org/10.1093/conphys/cou014

Bellaver, B., Souza, D. G., Souza, D. O., & Quincozes-Santos, A. (2014). Resveratrol increases antioxidant defenses and decreases proinflammatory cytokines in hippocampal astrocyte cultures from newborn,

adult and aged Wistar rats. *Toxicology in Vitro*, *28*, 479–484. https://doi.org/10.1016/j.tiv.2014.01.006

Benzie, I. F. F., & Strain, J. J. (1996). The Ferric Reducing Ability of Plasma (FRAP) as a measure of "Antioxidant Power": The FRAP Assay. *Analytical Biochemistry*, *239*, 70–76. https://doi.org/10.1006/abio.1996.0292

Bogdan, C., Rollinghoff, M., & Diefenbach, A. (2000). The role of nitric oxide in innate immunity. *Immunological Reviews*, *173*, 17–26. https://doi.org/10.1034/j.1600-065X.2000.917307.x

Bourgeon, S., & Raclot, T. (2006). Corticosterone selectively decreases humoral immunity in female eiders during incubation. *Journal of Experimental Biology*, *209*, 4957–4965. https://doi.org/10.1242/jeb.02610

Catoni, C., Schaefer, H. M., & Peters, A. (2008). Fruit for health: The effect of flavonoids on humoral immune response and food selection in a frugivorous bird. *Functional Ecology*, *22*, 649–654. https://doi.org/10.1111/j.1365-2435.2008.01400.x

Chatziprodromidou, I. P., Arvanitidou, M., Guitian, J., Apostolou, T., Vantarakis, G., & Vantarakis, A. (2018). Global avian influenza outbreaks 2010–2016: A systematic review of their distribution, avian species and virus subtype. *Systematic Reviews*, *7*, 17. https://doi.org/10.1186/s13643-018-0691-z

Civitelli, L., Panella, S., Marcocci, M. E., De Petris, A., Garzoli, S., Pepi, F., … Angiolella, L. (2014). In vitro inhibition of herpes simplex virus type 1 replication by *Mentha suaveolens* essential oil and its main component piperitenone oxide. *Phytomedicine*, *21*, 857–865. https://doi.org/10.1016/j.phymed.2014.01.013

Costantini, D. (2014). *Oxidative stress and hormesis in evolutionary ecology and physiology: A marriage between mechanistic and evolutionary approaches*. Berlin: Springer.

Costantini, D., Angeletti, D., Strinati, C., Trisolino, P., Carlini, A., Nascetti, G., & Carere, C. (2018). Dietary antioxidants, food deprivation and growth affect differently oxidative status of blood and brain in juvenile European seabass (*Dicentrarchus labrax*). *Comparative Biochemistry and Physiology Part A Molecular Integrative Physiology*, *216*, 1–7.

Costantini, D., Seeber, P. A., Soilemetzidou, S.-E., Azab, W., Bohner, J., Buuveibaatar, B., … Greenwood, A. D. (2018). Physiological costs of infection: Herpesvirus replication is linked to blood oxidative stress in equids. *Scientific Reports*, *8*, 10347.

de Thoisy, B., Lavergne, A., Semelin, J., Pouliquen, J.-F., Blanchard, F., Hansen, E., & Lacoste, V. (2009). Outbreaks of disease possibly due to a natural avian herpesvirus infection in a colony of young magnificent Frigatebirds (*Fregata magnificens*) in French Guiana. *Journal of Wildlife Diseases*, *45*, 802–807. https://doi.org/10.7589/0090-3558-45.3.802

Dhinaut, J., Balourdet, A., Teixeira, M., Chogne, M., & Moret, Y. (2017). A dietary carotenoid reduces immunopathology and enhances longevity through an immune depressive effect in an insect model. *Scientific Reports*, *7*, 12429. https://doi.org/10.1038/s41598-017-12769-7

Durgut, R., Ataseven, V. S., Sagkan-Ozturk, A., & Ozturk, O. H. (2013). Evaluation of total oxidative stress and total antioxidant status in cows with natural bovine herpesvirus-1 infection. *Journal of Animal Science*, *91*, 3408–3412. https://doi.org/10.2527/jas.2012-5516

Finkelstein, M. E., Doak, D. F., Nakagawa, M., Sievert, P. R., & Klavitter, J. (2010). Assessment of demographic risk factors and management priorities: Impacts on juveniles substantially affect population viability of a long-lived seabird. *Animal Conservation*, *13*, 148–156. https://doi.org/10.1111/j.1469-1795.2009.00311.x

Gao, S., Sanchez, C., & Deviche, P. J. (2017). Corticosterone rapidly suppresses innate immune activity in the house sparrow (*Passer domesticus*). *Journal of Experimental Biology*, *220*, 322–327.

Gülçin, İ. (2010). Antioxidant properties of resveratrol: A structure–activity insight. *Innovative Food Science & Emerging Technologies*, *11*, 210–218. https://doi.org/10.1016/j.ifset.2009.07.002

Han, S. N., Meydani, M., Wu, D., Bender, B. S., Smith, D. E., Vina, J., … Meydani, S. N. (2000). Effect of long-term dietary antioxidant supplementation on influenza virus infection. *Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, *55*, B496–B503. https://doi.org/10.1093/gerona/55.10.B496

Hawley, D. M., & Altizer, S. M. (2011). Disease ecology meets ecological immunology: Understanding the links between organismal immunity and infection dynamics in natural populations. *Functional Ecology*, *25*, 48–60. https://doi.org/10.1111/j.1365-2435.2010.01753.x

Hector, K. L., Lagisz, M., & Nakagawa, S. (2012). The effect of resveratrol on longevity across species: A meta-analysis. *Biology Letters*, *8*, 790–793. https://doi.org/10.1098/rsbl.2012.0316

Hodges, M. D., DeLong, M. J., Forney, F. C., & Prange, K. R. (1999). Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta*, *207*, 604–611. https://doi.org/10.1007/s004250050524

Hooda, S., Tyagi, P. K., Tyagi, P. K., & Sharma, D. (2005). Effect of higher dietary concentration of alpha tocopherol on growth performance and immune response in growing quails. *Indian Journal of Poultry Science*, *40*, 32–35.

Keles, H., Fidan, A. F., Cigerci, I. H., Kucukkurt, I., Karadas, E., & Dundar, Y. (2010). Increased DNA damage and oxidative stress in chickens with natural Marek's disease. *Veterinary Immunology and Immunopathology*, *133*, 51–58. https://doi.org/10.1016/j.vetimm.2009.07.003

Kode, A., Rajendrasozhan, S., Caito, S., Yang, S. R., Megson, I. L., & Rahman, I. (2008). Resveratrol induces glutathione synthesis by activation of Nrf2 and protects against cigarette smoke-mediated oxidative stress in human lung epithelial cells. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *294*, L478–L488. https://doi.org/10.1152/ajplung.00361.2007

Kramer, L. D., & Bernard, K. A. (2001). West Nile virus infection in birds and mammals. *Annals of the New York Academy of Sciences*, *951*, 84–93. https://doi.org/10.1111/j.1749-6632.2001.tb02687.x

Legagneux, P., Berzins, L. L., Forbes, M., Harms, N. J., Hennin, H. L., Bourgeon, S., … Burness, G. (2014). No selection on immunological markers in response to a highly virulent pathogen in an Arctic breeding bird. *Evolutionary Applications*, *7*, 765–773. https://doi.org/10.1111/eva.12180

Lessells, C. M., & Boag, P. T. (1987). Unrepeatable repeatabilities: A common mistake. *The Auk*, *104*, 116–121. https://doi.org/10.2307/4087240

Levine, R. L., Williams, J. A., Stadtman, E. R., & Shacter, E. (1994). Carbonyl assays for determination of oxidatively modified proteins. *Methods in Enzymology*, *233*, 346–357.

Li, X., Feng, J., & Sun, R. (2011). Oxidative stress induces reactivation of Kaposi's Sarcoma-associated herpesvirus and death of primary effusion lymphoma cells. *Journal of Virology*, *85*, 715–724. https://doi.org/10.1128/JVI.01742-10

Lormée, H., Jouventin, P., Trouve, C., & Chastel, O. (2003). Sex-specific patterns in baseline corticosterone and body condition changes in breeding Red-footed Boobies *Sula sula*. *Ibis*, *145*, 212–219. https://doi.org/10.1046/j.1474-919X.2003.00106.x

MacKellar, M., & Vigerust, D. J. (2016). Role of haptoglobin in health and disease: A focus on diabetes. *Clinical Diabetes: A Publication of the American Diabetes Association*, *34*, 148–157. https://doi.org/10.2337/diaclin.34.3.148

Martinet, V., & Blanchard, F. (2009). Fishery externalities and biodiversity: Trade-offs between the viability of shrimp trawling and the conservation of Frigatebirds in French Guiana. *Ecological Economics*, *68*, 2960–2968. https://doi.org/10.1016/j.ecolecon.2009.06.012

Matson, K. D., Ricklefs, R. E., & Klasing, K. C. (2005). A hemolysis-hemagglutination assay for characterizing constitutive innate humoral immunity in wild and domestic birds. *Developmental and Comparative Immunology*, *29*, 275–286. https://doi.org/10.1016/j.dci.2004.07.006

McLean, R. G. (2006) *West Nile virus in North American birds*. Ornithological Monographs No. 60, pp. 44–64. American Ornithologists' Union.

Mortola, E., Okuda, M., Ohno, K., Watari, T., Tsujimoto, H., & Hasegawa, A. (1998). Inhibition of apoptosis and virus replication in feline immunodeficiency virus-infected cells by N-acetylcysteine and ascorbic acid. *Journal of Veterinary Medical Science*, *60*, 1187–1193. https://doi.org/10.1292/jvms.60.1187

Owen, J. C., Nakamura, A., Coon, C. A. C., & Martin, L. B. (2012). The effect of exogenous corticosterone on West Nile virus infection in Northern Cardinals (*Cardinalis cardinalis*). *Veterinary Research*, *43*, *9*. https://doi.org/10.1186/1297-9716-43-34

Ozdemir, D., Ozudogru, Z., Imik, H., Can, M., & Sunar, M. (2011). Effects of dietary antioxidant supplementation on the adrenal glands in quails (*Coturnix coturnix japonica*) reared under heat stress. *Revue De Medecine Veterinaire*, *162*, 8–12.

Preece, N. D., Abell, S. E., Grogan, L., Wayne, A., Skerratt, L. F., van Oosterzee, P., … Epstein, J. H. (2017). A guide for ecologists: Detecting the role of disease in faunal declines and managing population recovery. *Biological Conservation*, *214*, 136–146. https://doi.org/10.1016/j.biocon.2017.08.014

Qiang, W., Kuang, X., Liu, J., Liu, N., Scofield, V. L., Reid, A. J., … Wong, P. K. (2006). Astrocytes survive chronic infection and cytopathic effects of the ts1 mutant of the retrovirus Moloney murine leukemia virus by upregulation of antioxidant defenses. *Journal of Virology*, *80*, 3273–3284. https://doi.org/10.1128/JVI.80.7.3273-3284.2006

R Core Team (2013). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews*, *21*, 55–89. https://doi.org/10.1210/er.21.1.55

Savage, A. E., Terrell, K. A., Gratwicke, B., Mattheus, N. M., Augustine, L., & Fleischer, R. C. (2016). Reduced immune function predicts disease susceptibility in frogs infected with a deadly fungal pathogen. *Conservation Physiology*, *4*, cow011. https://doi.org/10.1093/conphys/cow011

Sebastiano, M., Bustamante, P., Costantini, D., Eulaers, I., Malarvannan, G., Mendez-Fernandez, P., … Chastel, O. (2016). High levels of mercury and low levels of persistent organic pollutants in a tropical seabird in French Guiana, the Magnificent frigatebird, *Fregata magnificens*. *Environmental Pollution*, *214*, 384–393.

Sebastiano, M., Bustamante, P., Eulaers, I., Malarvannan, G., Mendez-Fernandez, P., Churlaud, C., … Chastel, O. (2017). Trophic ecology drives contaminant concentrations within a tropical seabird community. *Environmental Pollution*, *227*, 183–193.

Sebastiano, M., Chastel, O., de Thoisy, B., Eens, M., & Costantini, D. (2016). Oxidative stress favours herpes virus infection in vertebrates: A meta-analysis. *Current Zoology*, *62*, 325–332. https://doi.org/10.1093/cz/zow019

Sebastiano, M., Eens, M., Abd Elgawad, H., de Thoisy, B., Lacoste, V., Pineau, K., … Costantini, D. (2017). Oxidative stress biomarkers are associated with visible clinical signs of a disease in frigatebird nestlings. *Scientific Reports*, *7*, 1599.

Sebastiano, M., Eens, M., Angelier, F., Pineau, K., Chastel, O., & Costantini, D. (2017). Corticosterone, inflammation, immune status and telomere length in frigatebird nestlings facing a severe herpesvirus infection. *Conservation Physiology*, *5*, cow073.

Sebastiano, M., Eens, M., Messina, S., AbdElgawad, H., Pineau, K., Gerrit, B., … Costantini, D. (2018). Data from: Resveratrol supplementation reduces oxidative stress and modulates the immune response in free-living animals during a viral infection. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.r75251m

Selman, C., McLaren, J. S., Meyer, C., Duncan, J. S., Redman, P., Collins, A. R., … Speakman, J. R. (2006). Life-long vitamin C supplementation in combination with cold exposure does not affect oxidative damage or lifespan in mice, but decreases expression of antioxidant protection genes. *Mechanisms of Ageing and Development*, *127*, 897–904. https://doi.org/10.1016/j.mad.2006.09.008

Sinha, A. K., AbdElgawad, H., Giblen, T., Zinta, G., De Rop, M., Asard, H., … De Boeck, G. (2014). Anti-oxidative defences are modulated differentially in three freshwater teleosts in response to ammonia-induced oxidative stress. *PLoS One*, *9*, e95319. https://doi.org/10.1371/journal.pone.0095319

Smith, K. F., Sax, D. F., & Lafferty, K. D. (2006). Evidence for the role of infectious disease in species extinction and endangerment. *Conservation Biology*, *20*, 1349–1357. https://doi.org/10.1111/j.1523-1739.2006.00524.x

Staley, M., & Bonneaud, C. (2015). Immune responses of wild birds to emerging infectious diseases. *Parasite Immunology*, *37*, 242–254. https://doi.org/10.1111/pim.12191

van de Crommenacker, J., Richardson, D. S., Koltz, A. M., Hutchings, K., & Komdeur, J. (2012). Parasitic infection and oxidative status are associated and vary with breeding activity in the Seychelles warbler. *Proceedings of the Royal Society B: Biological Sciences*, *279*, 1466–1476. https://doi.org/10.1098/rspb.2011.1865

Vazquez-Torres, A., Jones-Carson, J., Mastroeni, P., Ischiropoulos, H., & Fang, F. C. (2000). Antimicrobial actions of the NADPH phagocyte oxidase and inducible nitric oxide synthase in experimental salmonellosis. I. Effects on microbial killing by activated peritoneal macrophages in vitro. *Journal of Experimental Medicine*, *192*, 227–236. https://doi.org/10.1084/jem.192.2.227

Wang, Y.-J., Chien, Y.-H., & Pan, C.-H. (2006). Effects of dietary supplementation of carotenoids on survival, growth, pigmentation, and antioxidant capacity of characins, *Hyphessobrycon callistus*. *Aquaculture*, *261*, 641–648. https://doi.org/10.1016/j.aquaculture.2006.08.040

Xu, J., Yin, Z., Li, L., Cheng, A., Jia, R., Song, X., … Shi, F. (2013). Inhibitory effect of resveratrol against duck enteritis virus in vitro. *PLoS One*, *8*, e65213. https://doi.org/10.1371/journal.pone.0065213

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