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RESEARCH ARTICLE

# Self-supplementation and effects of dietary antioxidants during acute thermal stress

**Michaël Beaulieu*, Anabel Haas and H. Martin Schaefer**

## ABSTRACT

Thermal stress leads to increased production of reactive oxygen species. If an organism is not able to simultaneously mount an efficient antioxidant defense system, this may lead to increased oxidative damage, which is potentially deleterious in terms of health and fitness. Exposure to cold or heat is therefore expected to be associated with a high demand for antioxidants. In agreement, several studies have shown that supplementing the diet of thermally stressed organisms with antioxidants leads to a reduction of oxidative damage. However, whether organisms can actively supplement their diet with antioxidants to alleviate temperature-induced oxidative damage is unknown. Here, we show that captive Gouldian finches (*Erythrura gouldiae*) supplement their diet more with seeds rich in antioxidants below than within their thermoneutral zone. Moreover, having access to seeds rich in antioxidants at temperatures below thermoneutrality decreases their oxidative damage. These results indicate that, when facing a thermal challenge, animals are able to take advantage of the antioxidant properties of their food to improve their oxidative balance. Having access to food resources rich in antioxidants may therefore be of primary importance for organisms in their natural habitat, as it may help them to cope with oxidative constraints due to challenging temperature regimes.

**KEY WORDS: Antioxidants, Birds, Diet, Feeding behavior, Temperature**

## INTRODUCTION

Oxidative stress, i.e. the imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses, can alter the structure and the function of mitochondrial and cellular components. This so-called oxidative damage can have a pronounced impact on fitness by reducing fertility and accelerating ageing. Because of these effects, it has recently received much attention in ecological studies as a potential key actor in the resolution of life-history trade-offs (Catoni et al., 2008; Costantini, 2008; Monaghan et al., 2009; Costantini et al., 2010; Metcalfe and Alonso-Alvarez, 2010).

In order to avoid excessive levels of oxidative damage following an increase in ROS production, organisms can increase their synthesis of endogenous antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) to counteract the action of ROS, or replace molecular constituents susceptible to the action of ROS (e.g. polyunsaturated fatty acids) with less sensitive ones (e.g. monounsaturated fatty acids) (Pamplona and Costantini, 2011). However, these strategies necessarily imply the mobilization of endogenous resources to synthesize new molecules, and therefore appear to be limited to organisms in good condition, able to allocate resources towards antioxidant defenses (Garratt and Brooks, 2012; Fletcher et al., 2013). Moreover, the synthesis of enzymes and, more importantly, the replacement of ROS-sensitive cellular components require time. For instance, mice injected with a pro-oxidant molecule (diquat) experience increased oxidative damage in plasma or liver that disappears after 12–15 h, presumably once endogenous antioxidant defenses are activated (Han et al., 2008). This delay between increased ROS production and the production of endogenous antioxidant defenses may be problematic for organisms frequently and unexpectedly exposed to increased ROS production.

Exploiting antioxidants present in food (e.g. polyphenols, vitamin C, vitamin E, carotenoids) may represent a less costly and more rapid line of defense than the use of endogenous antioxidant defenses (Catoni et al., 2008). First, dietary antioxidants can alleviate the cost related to the use of endogenous antioxidant defenses by allowing organisms to reduce their expression of antioxidant enzymes without experiencing higher oxidative damage (Selman et al., 2006). Second, dietary antioxidants can act rapidly, as their consumption can result in a plasma peak of antioxidants as soon as 30 min post-absorption (Manach et al., 2004).

The use of dietary antioxidants by animals to reduce oxidative stress echoes the notion of self-medication. Even though many cases of self-medication against intoxication or infection have been observed (Hart, 2011), clear evidence showing that organisms can use dietary antioxidants to alleviate oxidative damage is currently lacking (Beaulieu and Schaefer, 2013). A central condition necessary for self-medication against oxidative stress to occur is the frequent exposure of organisms to specific situations associated with oxidative stress (Beaulieu and Schaefer, 2013). Thermal stress represents such a situation, as most organisms face daily and/or seasonal temperature changes in their natural habitat. In endotherms, being exposed to temperatures outside their thermoneutral zone results in increased ROS production (Lin et al., 2008). This originates from a metabolism that is elevated to maintain the inner central temperature constant, by producing or eliminating heat when experiencing temperatures below or above thermoneutrality, respectively (Blagojević, 2007). If animals are not able to mount concomitantly an appropriate antioxidant defense system, being exposed to such temperatures results in increased oxidative damage (Al-Azraqi, 2008; Costantini et al., 2012). Interestingly, temperature-induced oxidative damage can be reduced by supplementing the diet of animals with diverse antioxidants (Sahin et al., 2003; Sahin et al., 2006; Ates et al., 2006; Cao et al., 2011; Sahin et al., 2012a; Sahin et al., 2012b; Sahin et al., 2013). However, whether animals are able to actively supplement their diet with antioxidants to alleviate temperature-induced oxidative stress remains entirely unknown.

While the concept of self-medication against oxidative stress has not yet been methodically studied (Beaulieu and Schaefer, 2013), there is indirect evidence showing that it is plausible in the context

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of thermal stress. Woodrats (*Neotoma albigula*) consume more juniper (*Juniperus monosperma*) below than within their thermoneutral zone (Dearing et al., 2008). As juniper exhibits high antioxidant potency (Elmastaş et al., 2006), the higher consumption of juniper by woodrats at low temperature is likely to reinforce their antioxidant defense system, and reduce oxidative damage due to cold temperatures. However, as the demand for antioxidants as well as the effects of juniper consumption on the oxidative balance of woodrats were not determined in that study, it is impossible to clearly conclude that higher consumption of juniper by woodrats in the cold is related to temperature-induced changes in their oxidative balance. This absence of examination of antioxidant demand constitutes the main limitation of most studies examining food selection by animals in relation with its antioxidant content (Beaulieu and Schaefer, 2013).

Here, we examined whether organisms are able to actively increase their antioxidant intake to match their higher demand for antioxidants when exposed to temperatures within (35°C) and below (20°C) their thermoneutral zone. We tested this hypothesis by exposing a tropical granivorous bird, the Gouldian finch [*Erythrura gouldiae* (Gould 1844)], to temperatures within and below its thermoneutral zone, and by considering three key aspects: (1) variation of birds' demand for antioxidants between both temperature conditions, (2) variation of birds' feeding behaviour towards seeds differing in their antioxidant contents between both temperature conditions, and (3) the effects of this diet supplementation on their oxidative balance below thermoneutrality.

## RESULTS

### Seed polyphenol content

Seeds differed significantly in their polyphenol content ($F_{2,13}$=261.816, $P$<0.001; Fig. 1). Red sorghum constituted the main polyphenol source for birds, as it contained six times more polyphenols than white sorghum (Bonferroni test, $P$<0.001) or staple seeds (Bonferroni test, $P$<0.001).

### Effect of temperature on oxidative markers

Temperature affected the antioxidant capacity of Gouldian finches without access to red sorghum ($F_{1,24}$=5.450, $P$=0.011), with 20% higher antioxidant capacity at 20°C than at 35°C (Bonferroni test, $P$=0.012; Fig. 2A). This higher antioxidant capacity was almost reached after 6 h of exposure to 20°C (Bonferroni test between 35°C during 48 h and 20°C during 6 h, $P$=0.088). In contrast, temperature had no effect on oxidative damage [reactive oxygen metabolite (ROM)] levels ($F_{1,12}$=0.001, $P$=0.999; Fig. 2B). This suggests that Gouldian finches were able to maintain oxidative damage stable by rapidly mobilizing endogenous antioxidant defenses after being exposed to temperatures below their thermoneutral zone.

Sex and the temperature × sex interaction had no effects on the antioxidant capacity of birds ($F_{1,12}$=0.799, $P$=0.389 and $F_{1,24}$=0.170, $P$=0.845, respectively) or on their oxidative damage ($F_{1,12}$=1.250, $P$=0.285 and $F_{1,12}$=0.137, $P$=0.872, respectively).

### Effect of temperature on sorghum consumption

Gouldian finches supplemented their diet with white sorghum similarly at 35°C and at 20°C ($F_{1,12}$=1.253, $P$=0.285; Fig. 3A). In contrast, they adjusted their consumption of red sorghum to temperature, as they consumed more red sorghum at 20°C than at 35°C ($F_{1,12}$=7.579, $P$=0.018; Fig. 3B).

Males and females consumed white sorghum or red sorghum similarly ($F_{1,12}$=0.245, $P$=0.630 and $F_{1,12}$=0.065, $P$=0.803, respectively), irrespective of the thermal environment they were exposed to (sex × temperature interaction: $F_{1,12}$=0.013, $P$=0.910 and $F_{1,12}$=1.401, $P$=0.259, respectively).

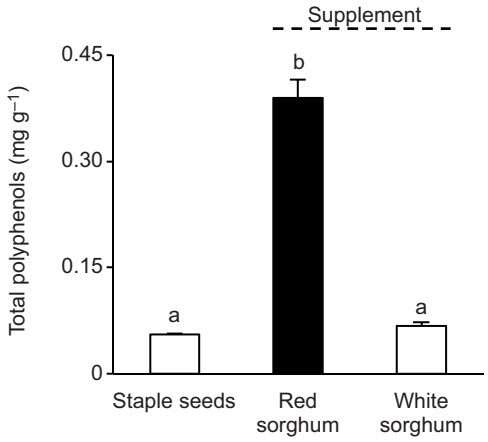


**Fig. 1. Polyphenol content (means ± s.e.m.) of the staple food given to Gouldian finches and of the two varieties of sorghum given as a supplement to their staple food.** Different letters above data points indicate a statistical difference between seed varieties.

### Effects of sorghum consumption on oxidative balance

Gouldian finches had similar antioxidant capacities when they had access to white sorghum or red sorghum at 20°C ($F_{1,12}$=0.224, $P$=0.645; Fig. 4A). In contrast, oxidative damage was 25% lower when they could supplement their diet with red sorghum versus with white sorghum ($F_{1,12}$=11.489, $P$=0.005; Fig. 4B).

Sex and the sorghum variety × sex interaction had no effect on the antioxidant capacity of birds ($F_{1,12}$=0.174, $P$=0.684 and $F_{1,12}$=2.022, $P$=0.180, respectively) or on their oxidative damage ($F_{1,12}$=0.339, $P$=0.571 and $F_{1,12}$=0.181, $P$=0.678, respectively).

## DISCUSSION

We found that Gouldian finches responded to cold conditions by rapidly increasing their antioxidant capacity. Moreover, under these conditions, they increased their consumption of seeds rich in antioxidants, which led to reduced oxidative damage. These results suggest that: (1) antioxidant requirements increase in cold conditions, and (2) organisms are able to exploit the antioxidant properties of their food to respond to this increased demand for antioxidants, and thereby alleviate oxidative damage.

Exposing Gouldian finches to a temperature lower than thermoneutrality rapidly resulted in an endogenous antioxidant response, as reflected by higher antioxidant capacity in birds exposed to 20°C for 6 h without access to food rich in antioxidants. Because oxidative damage remained concomitantly stable, this suggests that Gouldian finches were able to satisfy endogenously the increased demand for antioxidants due to cold conditions. However, we caution that these results may only apply to organisms in good condition, which can invest easily in endogenous antioxidant defenses. The antioxidant response of animals with more limited resources than our captive birds is likely to be less pronounced and may be insufficient to avoid an increase in oxidative damage in the cold.

Exposing Gouldian finches to cold conditions led them to increase their consumption of seeds rich in antioxidants, which had direct effects on their oxidative balance. Indeed, birds having access to seeds rich in antioxidants in the cold had 25% lower oxidative damage than the same birds having only access to seeds poor in

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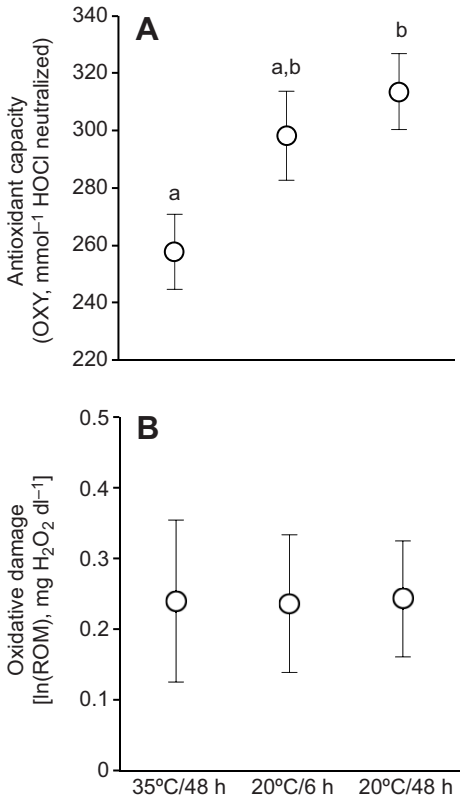


**Fig. 2. Oxidative markers at different temperatures.** Plasma antioxidant capacity (A) and oxidative damage (B) of Gouldian finches (*N*=14) exposed to 35°C for 48 h (BS1 in Fig. 5), to 20°C for 6 h (BS3 in Fig. 5) and to 20°C for 48 h (BS2 in Fig. 5). Different letters above data points indicate a statistical difference between temperature treatments. Results are presented as means ± s.e.m.

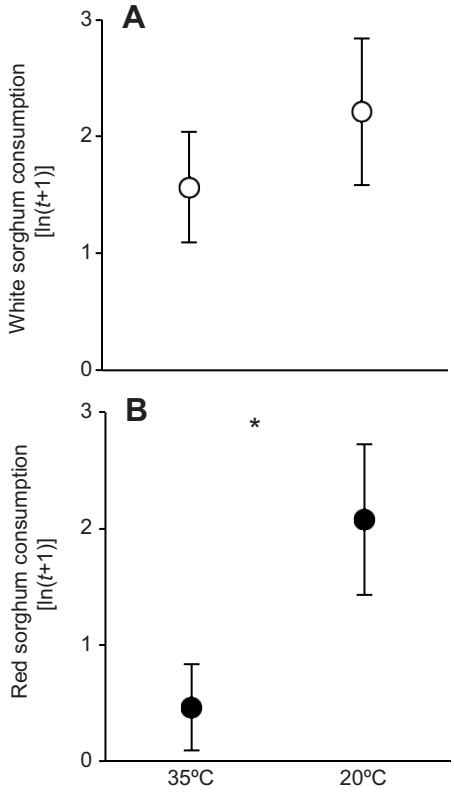


**Fig. 3. Consumption of sorghum seeds at different temperatures.** Time (*t*; in seconds) spent by Gouldian finches (*N*=14) eating (A) white sorghum and (B) red sorghum for 6 h at 35°C and 20°C. An asterisk indicates a statistical difference (*$P$<0.05) between temperature treatments. Results are presented as means ± s.e.m.

antioxidants. This result is in agreement with studies showing that artificially supplementing the diet of thermally stressed animals with various antioxidants improves their oxidative balance (Sahin et al., 2003; Sahin et al., 2006; Ates et al., 2006; Cao et al., 2011; Sahin et al., 2012a; Sahin et al., 2012b; Sahin et al., 2013). However, a fundamental difference between these studies and ours is that from our study it appears that animals can actively supplement their diet with antioxidants when exposed to temperatures outside their thermoneutral zone.

How can cold exposure lead animals to increase their consumption of food rich in antioxidants? Being exposed to cold conditions has been described as altering noradrenergic and serotoninergic systems in the brain, thus relecting psychological stress (Stillman et al., 1998; Lapiz-Bluhm et al., 2009). At the same time, cold exposure also alters cerebral oxidative markers, and can result in increased levels of oxidative damage in the brain (Buzadzić et al., 1997; Şahin and Gümüşlü, 2004). This suggests that psychological stress and oxidative stress due to cold exposure occur simultaneously and are therefore interconnected. This relationship between psychological stress and oxidative stress has also been documented for stressors other than cold. For instance, in mice, whisker removal translates into both high levels of oxidative damage and altered levels of dopamine neurotransmitters in the brain (Rahman et al., 2008). The consumption of food rich in antioxidants therefore offers a double benefit for organisms exposed to stressful conditions such as cold exposure: it restores neurotransmitter levels (thereby alleviating psychological stress) and at the same time it improves oxidative balance (Rahman et al., 2008; Papandreou et al., 2009; Xu et al., 2010; Yu et al., 2013). In our study, Gouldian finches, which may not be able to perceive variation in their oxidative balance directly but can presumably experience psychological stress due to cold conditions, may have opted to consume more food rich in antioxidants not for its antioxidant potency per se but because of the associated alleviation of psychological stress. To confirm this hypothesis, further studies should examine the concomitant effects of cold exposure on brain neurotransmitters and oxidative markers in birds with and without access to food rich in antioxidants.

Gouldian finches increased their consumption of seeds rich in antioxidants in the cold while they were able to increase their endogenous antioxidant defenses. This suggests that the combined effect of antioxidant supplementation and endogenous response must be more beneficial for birds in the cold than relying solely on endogenous antioxidant defenses. Following the evolutionary framework of self-medication, a first advantage may be an alleviation of the cost related to the use of endogenous antioxidant defenses (Beaulieu and Schaefer, 2013). In our study, birds may have indeed reduced the use (and thus the cost) of endogenous antioxidants when feeding on red sorghum, as their antioxidant capacity remained stable despite higher antioxidant intake (the measurement of the activity of antioxidant enzymes when birds have access or not to food rich in antioxidants would nevertheless be necessary to confirm this point). A second advantage that we observed in our study is a reduction of oxidative damage, which may be related to an improvement of fitness

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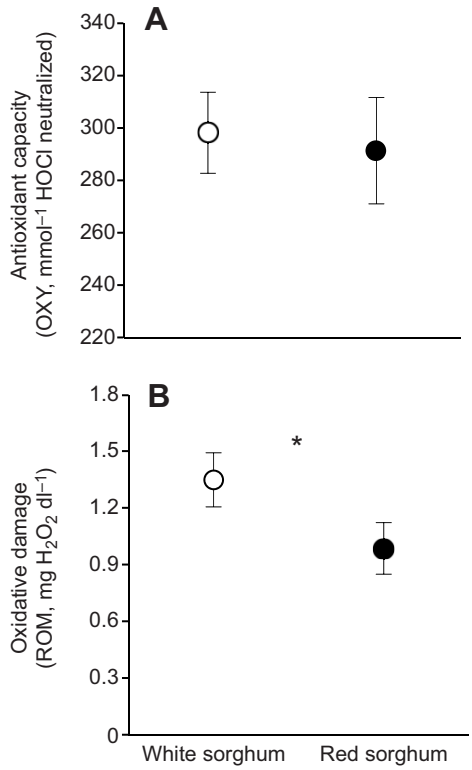


**Fig. 4. Oxidative markers as a function of seed treatment at 20°C.** Plasma antioxidant capacity (A) and oxidative damage (B) of Gouldian finches (*N*=14) with the possibility of supplementing their diet with white sorghum or red sorghum at 20°C. An asterisk indicates a statistical difference (*$P$<0.05) between temperature treatments. Results are presented as means ± s.e.m.

components in birds (Freeman-Gallant et al., 2011; Noguera et al., 2012). This suggests that increasing antioxidant intake in cold conditions may have adaptive advantages, which may explain why this behavior has evolved.

In line with the concept of self-medication against oxidative stress (Beaulieu and Schaefer, 2013), the present study is the first to show that organisms can respond to higher demand for antioxidants in the cold by increasing their antioxidant intake. However, increasing antioxidant intake is not likely to be restricted to cold conditions but may also occur in other situations associated with oxidative stress, such as reproduction or migration (Monaghan et al., 2009). Considering thermal conditions, it may also apply to heat exposure, which is also associated with increased ROS production (Lin et al., 2008). This is important to consider in the context of climate change. For instance, declining populations of *Pygoscelis* penguins from Antarctic regions already experiencing climate warming exhibit lower antioxidant capacity than increasing populations from other regions (Beaulieu et al., 2013). This shows that climate changes may appreciably alter the capacity of organisms to satisfy their antioxidant requirements, which may contribute to their decline. This may be even more important to consider in tropical species such as Gouldian finches, which regularly experience temperatures very close to their upper critical temperature in their natural habitat (Marschall and Prinzinger, 1991; Burton and Weathers, 2003; Evans and Fidler, 2005), and for which a moderate increase of 3–4°C [as expected in the next decades in their native range in Northern Australia (Preston and Jones, 2006)] will force them to live in a thermally constraining environment likely to increase ROS production and oxidative damage. Consequently, the ability of these species to protect themselves against oxidative damage through the consumption of antioxidants may help them to better resist climate warming.

In conclusion, our study confirms the fact that the demand for antioxidants increases for organisms exposed to temperatures outside thermoneutrality. Gouldian finches respond to this higher demand for antioxidants by increasing their consumption of seeds rich in antioxidants, which results in lower oxidative damage. Altogether, these results emphasize the necessity for investigators to examine the need for antioxidants before studying the feeding behavior of animals in relation with the antioxidant content of their food as well as the effects of antioxidant intake on their oxidative balance. Because we examined both of these aspects in our study, our results may qualify as the first example of self-medication against oxidative stress through the consumption of food rich in antioxidants (Beaulieu and Schaefer, 2013). However, further studies should be conducted in other biological models exposed to different stressors to assess the generality of this finding.

## MATERIALS AND METHODS

### Biological model

Fourteen black-headed, wild-type Gouldian finches, between 1 and 2 years old, were housed in pairs (one male + one female) in individual cages (70×50×50 cm) at the University of Freiburg, Germany. Each bird stayed in its respective cage, and cages, which were all in the same room, were not moved during the study. Between experiments, the temperature of the room was set at 27.5°C (i.e. between the two experimental temperatures used in our study; see Fig. 5). Birds underwent a 12 h:12 h light:dark cycle (lights on: 07:00 h, lights off: 19:00 h). The fluorescent lamps of the room (Lumilux De Luxe Cool Daylight, Munich, Germany) covered the full light spectrum, as UV reflectance may affect birds' feeding selection (Church et al., 2001). Each cage was equipped with a digital camera (Samsung SEB-1005R, Gyeonggi-do, South Korea) connected to a digital video recorder (Samsung SDE-5001N) and a monitor.

The staple food of birds consisted of a seed mix for tropical finches (Deli Nature 40, Exoten Basis, Beyers, Belgium), mostly composed of millet [60% millet (*Panicum miliaceum*), 22% canary seed (*Phalaris canariensis*) and 18% foxtail millet (*Setaria italica*)], naturally poor in antioxidants (Dykes and Rooney, 2007). Our aim was to examine whether birds were able to supplement this antioxidant-poor diet with seeds rich in antioxidants when their demand for antioxidants varied because of thermal constraints. Because energetic demand also fluctuates with temperature changes and may primarily dictate feeding decisions (Burness et al., 2010; Salvante et al., 2010), energetic constraints due to temperature variation may blur more subtle food selection due to fluctuating oxidative constraints (Beaulieu and Schaefer, 2013). Therefore, to dissociate food resources consumed for their energetic or oxidative properties, we gave birds the possibility to supplement their staple diet with larger seeds, less profitable in terms of energy gain than staple seeds because of their larger size (Díaz, 1996), and differing in their antioxidant contents. Specifically, birds could supplement their staple diet with white sorghum (*Sorghum bicolor*; Rath Futtermittel, Nordkirchen, Germany), which is poor in antioxidants, or red sorghum (*S. bicolor*; Mühle Gladen, Lembeck, Germany), which is rich in antioxidants (Awika and Rooney, 2004; Dykes and Rooney, 2007). To confirm that polyphenol content was higher in red sorghum than in white sorghum (and in the staple food), we ground 5 g of each seed type (*N*=5 for red sorghum, *N*=5 for white sorghum and *N*=4 for the staple food), and measured the polyphenol content of 0.8 g of the resulting flour using the Prussian Blue assay method (Graham, 1992). The results were expressed as tannic acid equivalents.

### Effect of temperature on oxidative markers

During the first part of our study, birds had access to one cup with staple seeds and one cup with white sorghum (i.e. a diet poor in antioxidants) placed on the floor of the cage, so that they could not alter their oxidative balance

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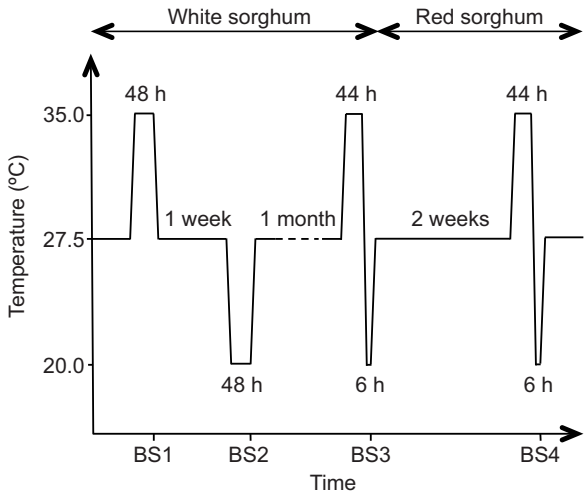


**Fig. 5. Timeline summarizing the order and the duration of temperature treatments to which Gouldian finches were exposed in our study.** The time when blood sampling (BS) occurred is represented on the time axis. The duration of each temperature treatment or of periods between treatments is written next to the temperature line. Access to white or red sorghum is represented above the graphic.

through their food intake. To confirm that the demand for antioxidants was higher below than within the thermoneutral zone, birds were first exposed to a room temperature of 35°C [i.e. in the middle of their thermoneutral zone: 31.7–38°C (Marschall and Prinzinger, 1991; Burton and Weathers, 2003)], and then 1 week later to 20°C (i.e. 12°C below their lower critical temperature). These two temperatures are in the range of temperatures that Gouldian finches can naturally experience in their native habitat in Northern Australia (Evans and Fidler, 2005). Each temperature treatment lasted 48 h (Fig. 5), because oxidative markers may vary after a few hours to several days of exposure to temperature treatment in birds (Sahin et al., 2003; Sahin et al., 2006; Lin et al., 2008; Sahin et al., 2012a; Sahin et al., 2012b).

This first treatment allowed us to examine in which direction and in which proportion oxidative markers vary in Gouldian finches exposed to different thermal conditions. Towards this end, we took blood (50–100 µl) after 48 h of temperature treatment in the middle of the day from the wing vein with heparinized capillaries (Natelson blood collecting tubes, Fisherbrand, Fisher Scientific, Pittsburgh, PA, USA). After centrifugation (15 min, 6000 ***g***), plasma and red blood cells were separated in Eppendorf tubes and stored at −20°C. We assessed plasma oxidative damage and antioxidant defences using assays that have already been applied in bird studies (Costantini and Dell'Omo, 2006; Rubolini et al., 2006): oxidative damage was analysed within 10 days using the d-ROM test (Diacron International, Grosseto, Italy), which measures the concentration of hydroperoxide, an ROM resulting from the attack of ROS on organic substrates (and therefore reflecting oxidative damage), and antioxidant capacity was examined using the OXY-adsorbent test (Diacron International) [for details on the procedure, see Beaulieu et al. (Beaulieu et al., 2010)]. Spectrophotometric readings were conducted at 510 nm, and intra-assay coefficients of variation for the d-ROM and the OXY-adsorbent tests were 4% and 7%, respectively. A higher demand for antioxidants at low temperature was expected to translate into higher oxidative damage (if birds were not able to mount an appropriate antioxidant defense system) or higher antioxidant capacity (if birds were able to mount an appropriate antioxidant defense system).

Oxidative markers can vary within a few hours (3–6 h) following a change in temperature (Davidović et al., 1999; Tan et al., 2010). In order to examine how quickly oxidative markers of Gouldian finches vary during the initial 48 h of temperature treatment, we examined whether they already varied within 6 h following a change in temperature from within to below thermoneutrality. Towards this end, we exposed birds in the second part of our study to 35°C for 44 h and then shortly (6 h) to 20°C (Fig. 1). The temperature of the 23-$m^3$ room where birds were housed decreased from 35°C to 20°C within 3 h (as measured by a temperature logger placed in the room to control temperature; Testo 174T, Lenzkirch, Germany). Because we wanted to measure the oxidative status of the birds in the middle of the day (similarly to the previous part of the experiment) and after 6 h of cold and stable temperatures, we changed the temperature settings at 04:00 h so that temperature became stable (20°C) at 07:00 h, when the lights were turned on. We took blood as previously described at 13:00 h (i.e. after 6 h at 20°C), and the temperature of the room was set back to 27.5°C after blood sampling at 14:00 h (Fig. 1).

### Effect of temperature on antioxidant supplementation

To examine whether temperature affected white sorghum consumption, we filmed birds between 07:00 and 13:00 h 1 day before blood sampling (i.e. at 35°C) and on the day of blood sampling during the 6 h of exposure to 20°C (each bird was therefore its own control). Food consumption was calculated as the sum (in seconds) of all feeding events towards white sorghum for each individual. A feeding event was defined as a series of pecks, starting with the first peck and finishing when birds stopped eating for at least 5 s.

To examine whether temperature affected the birds' intake of antioxidants, we repeated the experiment described above but provided birds with red sorghum (rich in antioxidants) instead of white sorghum. To avoid any effects related to food novelty, birds were given red sorghum for the 2 weeks preceding analyses of feeding behavior (Fig. 5). After these 2 weeks, the birds' feeding behavior towards red sorghum was monitored at 35°C and 20°C as previously described for white sorghum.

### Effect of red sorghum consumption on oxidative markers at 20°C

To examine the effects of the consumption of red sorghum relative to white sorghum on the oxidative balance when birds were exposed to temperature below thermoneutrality, we collected blood at the end of the 6 h of exposure to 20°C, both when birds had fed on white sorghum and 2 weeks later, when they had fed on red sorghum. Oxidative markers were measured as previously described. For measurements following red sorghum consumption, intra-assay coefficients of variation for the d-ROM and the OXY-adsorbent tests were 2% and 8%, respectively, and inter-assay coefficients of variation were 1% and 12%, respectively.

### Statistical analysis

We compared the polyphenol content of seeds using a general linear model with seed type (staple seeds, white sorghum, red sorghum) as the fixed factor and polyphenol content as the dependent variable. We used Bonferroni *post hoc* tests to highlight differences between seed varieties.

To examine the effects of temperature on oxidative markers (independently of antioxidant intake through red sorghum consumption), we performed a general linear mixed model (GLMM) with oxidative damage or antioxidant capacity as dependent variables, individual nested within cage as a random factor, and sex, temperature treatment (48 h at 35°C with white sorghum, 6 h at 20°C with white sorghum, 48 h at 20°C with white sorghum) and their interaction as fixed factors. ROM data were ln transformed for normality of residuals (tested for each model using a Shapiro–Wilk test). We used Bonferroni *post hoc* tests to highlight differences between temperature treatments.

We conducted the same GLMM to examine changes in the consumption of white sorghum or red sorghum between 35°C and 20°C. For both sorghum varieties, feeding duration ($t$) was ln transformed [ln($t$+1) because of null values] for normality of residuals.

Finally, to examine the effects of the consumption of red sorghum relative to white sorghum on oxidative balance at 20°C, we conducted a GLMM with oxidative damage or antioxidant capacity as dependent variables, individual nested within cage as a random factor, and sex, sorghum variety (red, white) and their interaction as fixed factors.

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### Competing interests
The authors declare no competing financial interests.

### Author contributions
M.B. and H.M.S. designed the study; M.B. and A.H. conducted the experiment and performed analyses; and M.B. and H.M.S. wrote the article.

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