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The proper time for antioxidant consumption

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HIGHLIGHTS

- How animals prioritise antioxidant resources in their diet is unknown.
- Gouldian finches consume most seeds rich in antioxidants in the middle of the day.
- This feeding pattern is out of phase with the consumption of energy resources.
- This indicates that antioxidant intake is constrained by energy requirements.
- Antioxidant intake can therefore occur only when energy requirements are relaxed.

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ABSTRACT

Consuming food rich in antioxidants may help organisms to increase their antioxidant defences and avoid oxidative damage. Under the hypothesis that organisms actively consume food for its antioxidant properties, they would need to do so in view of other physiological requirements, such as energy requirements. Here, we observed that Gouldian finches (*Erythrura gouldiae*) consumed most seeds rich in antioxidants in the middle of the day, while their consumption of staple seeds more profitable in energy intake (and poor in antioxidants) was maximal in the morning and the evening. This consumption of seeds rich in antioxidants in the middle of the day may be explicable (1) because birds took advantage of a time window associated with relaxed energy requirements to ingest antioxidant resources, or (2) because birds consumed antioxidant resources as a response to the highest antioxidant requirements in the middle of the day. If the latter hypothesis holds true, having the possibility to ingest antioxidants should be most beneficial in terms of oxidative balance in the middle of the day. Even though feeding on seeds rich in antioxidants improved Gouldian finches' overall antioxidant capacity, we did not detect any diurnal effect of antioxidant intake on plasma oxidative markers (as measured by the d-ROM and the OXY-adsorbent tests). This indicates that the diurnal pattern of antioxidant intake that we observed was most likely constrained by the high consumption of staple food to replenish or build up body reserves in the morning and in the evening, and not primarily determined by elevated antioxidant requirements in the middle of the day. Consequently, animals appear to have the possibility to increase antioxidant defences by selecting food rich in antioxidants, only when energetic constraints are relaxed.

1. Introduction

The imbalance between the production of reactive oxygen species (ROS) and antioxidant defences can strongly affect health and fitness, through the generation of elevated levels of oxidative damage [1]. As such, this so-called oxidative stress is a potential key mechanism shaping life history trade-offs [2–4]. Several studies have shown that artificially supplementing antioxidants to the diet of animals can decrease abnormally high levels of oxidative damage (*e.g.*, [5–7], but see [8]). This suggests that animals might alleviate oxidative stress by consuming food resources rich in antioxidants in their natural habitat to preserve health and fitness [9]. However, whether organisms are able to actively consume antioxidants to avoid oxidative stress, and how they prioritise antioxidant requirements relative to other physiological requirements remain unknown [10].

The consumption of dietary antioxidants to reduce oxidative stress echoes the notion of self-medication in animals. When resources used for self-medication differ physically from resources consumed for energetic requirements, animals are expected to consume resources used for self-medication only when the benefits of ingesting them exceed the benefits of ingesting energy-rich resources [10]. The examination of feeding patterns across the day represents an interesting situation to assess this hypothesis. Indeed, because of fasting at night, energy requirements are high in the morning (to replenish body reserves) and in the evening (to build up body reserves) in diurnal animals. This translates into a U-shaped consumption of food across the day, with high feeding activity early in the morning and in the evening, as demonstrated in a variety of free-living and captive passerine birds (*e.g.*, [11,12]). Accordingly, mass gain is maximal in the morning and

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in the evening, but minimal in the middle of the day [12,13]. Importantly, increasing energy requirements (through exercise or cold exposure) reduces or even eliminates feeding intermission in the middle of the day [11,14], which indicates that it occurs once energetic conditions are relaxed. The benefits of ingesting antioxidant resources relative to energy resources should therefore be lowest in the morning and in the evening (when energy requirements are high), and highest in the middle of the day (if energy requirements are relaxed). A temporal dichotomy should then be observed across the day between food resources consumed for their energy profitability and those consumed for their antioxidant content. Accordingly, we expect high intake of energetic resources in the morning and in the evening, and high intake of antioxidant resources in the middle of the day (hypothesis 1).

The benefits of ingesting antioxidant resources relative to energy resources may not only depend on varying energetic requirements, but also on varying antioxidant requirements across the day. First, because most organisms fast at night, they have to be active and feed early in the morning. This sudden increase in physical activity coupled with high food intake results in increased metabolism and ROS production [15,16]. Moreover, hormonal changes occurring in the morning, such as an increase in glucocorticoids, are likely to increase oxidative stress in diurnal animals [17,18]. This may explain the observed increase in ROS production in the morning with a consecutive peak in the middle of the day [19–21]. Second, this increase in ROS production is coupled with low endogenous antioxidant defences, as the expression of antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) is tightly contingent upon nocturnal secretion of melatonin (itself a powerful antioxidant [22]). The overall antioxidant status of the organism is therefore low during the day [23–26]. This situation entails that the highest risk of oxidative stress occurs in the middle of the day, when ROS production is high while endogenous antioxidant defences are low. The consumption of antioxidants could normalise this situation through their direct antioxidant effects, or by upregulating the expression of antioxidant enzymes [27–29]. In line with the concept of self-medication against oxidative stress [10], animals may be able to actively consume antioxidant resources as a response to their vulnerability to oxidative stress in the middle of the day. This should translate into high antioxidant intake in the middle of the day coupled with higher benefits of antioxidant consumption on oxidative markers in the middle of the day than in the morning and in the evening (hypothesis 2).

In this study, we examined whether the consumption of antioxidant resources is constrained by energetic requirements (hypothesis 1) or if it is actively determined by higher demand for antioxidants (hypothesis 2), by considering three key aspects. First, we examined the temporal pattern of food consumption across the day for food profitable in terms of energy gain but poor in antioxidants (called 'staple food' thereafter). Second, to assess whether food consumption depends both on daytime and the antioxidant content of food, we examined how organisms supplement their staple food with food less profitable in terms of energy gain but richer in antioxidants. Finally, we measured the impact of consuming food rich in antioxidants on the oxidative balance throughout the day, to examine if the benefits of consuming food rich in antioxidants vary across the day.

2. Methods

2.1. Biological model

Granivorous passerine birds are good candidates to test for diurnal patterns of food selection and antioxidant intake. First, they feed continuously throughout the day owing to their high metabolism. Second, small seeds are faster to process and hence more profitable in terms of energy gain than large seeds [30]. Thus, they can be given to birds primarily to cover energetic requirements, while larger seeds can be given to cover other requirements. Third, seeds vary strongly in their polyphenol content, which determines their antioxidant potency [31,32]. Consequently, seeds consumed to cover energy and antioxidant requirements can be dissociated. Finally, passerine birds have the capacity to visually evaluate the polyphenol content of their food, which could provide a robust functional mechanism underlying food selection in order to optimise antioxidant intake [33].

For our study, 18 one-year old black-headed, wild-type Gouldian finches (*Erythrura gouldiae*) were bought from a local breeder and housed as pairs (1 male + 1 female) in individual cages (70 × 50 × 50 cm) at the University of Freiburg, Germany. The temperature of the room was set at 26 °C, the humidity at 50% and the airflow at 700 m^3/h, as recommended for captive Gouldian finches [34]. Birds underwent a 12/12 h light/dark cycle (lights on: 07:00, lights off: 19:00), as they would experience in their natural habitat in Northern Australia. Because birds perceive UV reflectance, which may affect their feeding selection [35], the fluorescent lamps of the room (Lumilux deluxe Cool Daylight, Munich, Germany) covered the full light spectrum.

We provided birds with a seed mix for tropical finches (Deli Nature 40 — Exoten Basis, Beyers, Belgium) as staple food. This seed mix was primarily composed of millet (60% millet (*Panicum miliaceum*), 22% canary seed (*Phalaris canariensis*), and 18% foxtail millet (*Setaria italica*) naturally poor in antioxidants [36]. To dissociate resources consumed in relation with energetic and oxidative constraints, we also gave birds the possibility to supplement staple seeds with larger seeds, expected to be less profitable in terms of energy gain than small staple seeds because of their large size [30], and presenting different antioxidant contents. More specifically, we provided birds with (1) dehusked red sorghum (*Sorghum bicolor*, Mühle Gladen, Lembeck, Germany), as these seeds are among the cereal grains with the highest polyphenol content and antioxidant potency [37], and (2) dehusked white sorghum (*S. bicolor*; Rath Futtermittel, Nordkirchen, Germany) poor in polyphenols. White sorghum was used to control that the polyphenol content of red sorghum was responsible for a diurnal pattern of seed consumption, as white sorghum has been described as having digestibility, energy and macronutrient contents very similar to red sorghum [38] but much lower polyphenol content [37].

To confirm that staple seeds were smaller than sorghum seeds, we measured the length and the width of 50 seeds of each variety (accounting for the proportion of each seed for staple seeds) with a digital calliper (Lux-Tools, Wermelskirchen, Germany; ±0.01 mm). We extracted the scores of the first axis of a principal component analysis (PCA) on length and width measurements (explaining 80% of size variance) and used them as size index for seeds. To verify 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 = 4 for red sorghum, and n = 4 for white sorghum, n = 4 for the staple food), and measured the polyphenol content of 0.8 g of the resulting flour by using the Prussian blue assay method [39]. The results were expressed as tannic acid equivalent.

2.2. Behavioural monitoring

Seven cages were equipped with a digital camera (Samsung SEB-1005R, Gyeonggi-do, South Korea), connected to a digital video recorder (Samsung SDE-5001N, Gyeonggi-do, South Korea) and a monitor. Cameras were attached to the wire of the cage to visualise each seed cup, containing staple seeds, white sorghum or red sorghum, on the bottom of the cage. The daily food selection of each bird was examined after filming each cage from 07:00 to 19:00 during six days. To measure how birds consumed staple and sorghum seeds across the day, we summed the duration of all feeding events (in seconds) for each seed variety for each individual and for every hour of video. A feeding event was defined as a series of pecks, starting with a first peck and finishing when birds stopped eating for at least 5 s. In total, 17,035 feeding events were observed in 504 h of videos.