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Functional significance of ultraviolet feeding cues in wild turkeys

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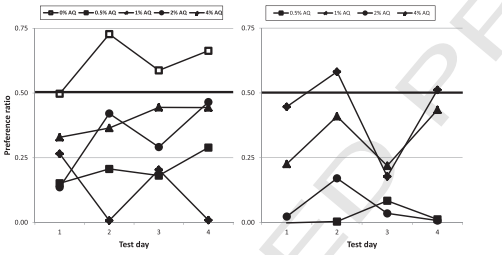
HIGHLIGHTS

- Wild turkeys do not prefer UV feeding cues regardless of feeding experience.
- UV feeding cues are used functionally for avian foraging behavior.
- Postingestive consequences are necessary for conditioned avoidance of UV feeding cues.
- Intestinal parasite infection influences the process of food selection in wild turkeys.

GRAPHICAL ABSTRACT



Preference ratios for female wild turkeys offered untreated food and food treated with an UV-absorbent cue during a 4-day preference test. Preference ratios >0.5 indicate preference for treated food; ratios <0.5 indicate avoidance of treated food. The control group (unfilled squares) was exposed to unadulterated oats and test groups (filled shapes) were exposed to 0.5%, 1%, 2%, or 4% anthraquinone prior to the test of conditioned avoidance.

Preference ratios for male wild turkeys offered untreated food and food treated with an UV-absorbent cue during a 4-day preference test. Preference ratios >0.5 indicate preference for treated food; ratios <0.5 indicate avoidance of treated food. Test groups were exposed to 0.5%, 1%, 2%, or 4% anthraquinone prior to the test of conditioned avoidance.

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ABSTRACT

Most birds are able to sense ultraviolet (UV) visual signals. Ultraviolet wavelengths are used for plumage signaling and sexual selection among birds. The aim of our study was to determine if UV cues are also used for the process of food selection in wild turkeys (*Meleagris gallopavo*). We used avoidance conditioning to test the hypothesis that UV feeding cues can be used functionally for foraging behavior in wild turkeys. Female turkeys exhibited no avoidance of untreated food and 75–98% avoidance of food treated with an UV-absorbent, postingestive repellent (0.5–4% anthraquinone; wt./wt.) during repellent exposure. Male turkeys exhibited 78–99% avoidance of food treated with 0.5–4% anthraquinone. Female and male turkeys that consumed more than 200 mg and 100 mg of anthraquinone, respectively, subsequently avoided food treated only with an UV-absorbent cue. In contrast, unconditioned females consumed 58% more food treated with the UV-absorbent cue than untreated food. Thus, wild turkeys do not prefer foods associated with UV wavelengths regardless of feeding experience. We also observed 1) a weak negative correlation between body condition and intestinal parasite infection and 2) moderate, positive correlations between consumption of food treated with the conditioned UV cue and intestinal parasite infection among male turkeys. The UV feeding cue was used to maintain food avoidance during the four days subsequent to postingestive conditioning. Moreover, the consequences of consuming food treated with the postingestive, UV-absorbent repellent were necessary for conditioned avoidance of the UV-absorbent cue. These findings suggest functional significance of UV feeding cues for avian foraging behavior, the implications of which will enable subsequent investigations regarding the sensory physiology and behavioral ecology of wild birds.

1. Introduction

Most birds appear to be capable of sensing UV visual signals [1], but little is known about how they functionally use this information,

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particularly in the context of foraging. Ultraviolet cues could be used for foraging in two ways: 1) to detect foraging patches and recognize individual food items, and 2) to assess the relative quality of food items [2]. Comparative studies have found that not all bird species that could benefit from the use of UV feeding cues have evolved the retinal color receptors to do so (e.g. plunge-diving seabirds; [3]). Intraspecific studies have demonstrated that some bird species do indeed use UV cues to detect their food. Diurnal, predatory birds such as the Eurasian kestrel (*Falco tinnunculus*), rough-legged buzzard (*Buteo lagopus*) [4] and the great gray shrike (*Lanius excubitor*; [5]) use the UV reflectance of rodent urine to choose foraging patches where they are more likely to find these prey. Similarly, blue tits (*Parus caeruleus*) are able to find the first of a set of experimentally hidden cabbage moth (*Mamestra brassicae*) caterpillars more quickly with UV illumination than without it [6].

Many of the fruits eaten by birds exhibit high UV contrast with their backgrounds [7,8]. In a field study where UV filters were placed over *Psychotria emetica*, a tropical understory shrub, fewer fruits were taken when UV irradiance onto fruits was blocked compared to when UV transmitting filters were used [9]. Of course birds are not the only taxa to rely upon UV cues to detect their food. Predatory jumping spiders (*Portia labiata*) are preferentially attracted to the webs of their prey spider (*Argiope versicolor*), but only when the web reflects UV wavelengths [10]. Thus, birds and other animals can detect food more easily using UV cues. It is not clear, however, if birds use UV cues to assess the quality of their food.

Although both the strength of UV reflectance and predator preferences are often positively associated with specific prey, it is not known if preferences associated with UV reflectance increase the lifetime fitness of the forager. Are UV-reflecting prey more nutritious (sensu lato)? For example, are the prey biases observed among kestrels, for male rodents and for certain rodent species (see review; [2]), simply due to differences in signal detectability (i.e. greater UV reflectance) or have these predators learned that prey that exhibit greater UV reflectance provide greater benefits (e.g. more fat resources or fewer parasites)? Unfortunately very little is known about how birds utilize UV feeding cues; are there innate preferences for UV-reflecting or UV-absorbing food, or do birds learn to associate UV cues with food quality?

Ecologically-relevant, newborn color preferences and ontogenetic changes in color preferences have been studied experimentally in birds using only human-perceived colors (400–700 nm). Because of their experimental tractability, most of these studies have used domestic fowl (*Gallus gallus domesticus*) chicks as study subjects. Newborn domestic chicks prefer food items that are red or green in color if they are fruit-shaped, but avoid red items that are insect-shaped [11]. Chicks learn more easily to avoid distasteful food items that are red or yellow [12], or that contrast with their background [13], but some combinations of color and palatability are difficult for them to learn. For example, chicks require exposure to high quinine concentrations in their prey to learn that purple is unpalatable, but low quinine levels are sufficient for them to learn to avoid distasteful green prey [13].

Ontogenetic differences have been observed in UV foraging preference in redwings (*Turdus iliacus*; [14]). They discovered that wild-caught adult redwings preferred UV-reflecting bilberry (*Vaccinium myrtillus*) fruits over bilberries whose UV-reflecting waxy coat had been removed, but only when UV illumination was provided. Naïve, captive-reared redwing juveniles, however, showed no preference for the UV-reflecting fruits in either lighting regime, suggesting that redwings must learn to prefer UV wavelengths (or that their UV perception develops later in life). Ripe fruits often reflect more UV wavelengths [9], possibly explaining why many birds are attracted positively to UV wavelengths. Alternatively, plants may have co-opted existing avian preferences for UV-reflecting mates through sensory exploitation [15] in order to achieve greater seed dispersal by avian frugivores. Others posit that UV wavelengths have no special "meaning" via sensory bias [16], but are simply another color for which birds must learn context dependency (just as birds must learn that some red fruits are unpalatable; [17]). To better understand how birds can use UV feeding cues, we experimentally investigated the foraging behavior of avian subjects with UV vision.

We used the wild turkey (*Meleagris gallopavo*) to investigate the functional significance of UV feeding cues. Wild turkeys are omnivores who consume a wide variety of vegetation, fruits, seeds, insects and other invertebrates [18]. Several lines of evidence support our contention that UV vision is important to turkey natural history. First, domestic turkeys (*M. gallopavo*) are attracted to housing with UV lighting [19]. Second, although they lack UV-sensitive opsin photopigments, ocular oil droplets associated with their short-wavelength sensitive cones apparently permit UV vision [20]. Domestic turkeys have considerable sensitivity to wavelengths in the UV-A spectral range (315–400 nm; [20]). Increment threshold psychophysiological tests have shown that domestic turkey poults are maximally sensitive to the UV spectrum at 380 nm [16]. Other studies have demonstrated that UV vision is probably of relevance to the social and sexual interactions of turkeys as well. The intensity of the UV reflectance of iridescent feathers from male wild turkeys is condition-dependent [21] and the plumage of domestic turkey poults exhibits UV-reflective patterning that is associated with body sites of harmful pecking in commercial poultry houses [22]. Moreover, another wild species in the order Galliformes, the black grouse (*Tetrao tetrix*), prefers UV-reflecting morphs of a fruit that is a seasonally important component of their diet [23].

Because the implications of UV cues are poorly understood for avian foraging behavior, we compared the feeding response of conditioned and unconditioned wild turkeys offered food treated with an UV-absorbent cue subsequent to conditioning with an UV-absorbent, postingestive repellent. If wild birds prefer foods associated with UV wavelengths regardless of feeding experience (hypothesis 1), then conditioned and unconditioned wild turkeys will prefer foods treated with an UV cue. If UV feeding cues, like other visual and gustatory cues [24,25], are used functionally for avian foraging behavior (hypothesis 2), then wild turkeys conditioned with an UV-absorbent, postingestive repellent will subsequently avoid food treated with an UV-absorbent cue, even in the absence of the aversive consequence.

Although intestinal parasite infection (e.g. *Eimeria* spp.) decreases food consumption in domestic turkeys [26–29], the effects of body condition and parasite load are poorly understood for the process of food selection. Coccidia infection influences sexual selection among female wild turkeys [30] and UV plumage signaling among male wild turkeys [21]. Body condition or parasite infection of wild turkeys may also influence an individual's selection of food treated with an UV cue previously paired with negative postingestive consequences. If body condition or parasite infection influences the process of avian food selection (hypothesis 3), then consumption of food treated with an aversively-conditioned UV cue will be least among wild turkeys with poor body condition or high parasite infection.

2. Feeding experiments

2.1. Subjects and testing facilities

Wild turkeys (4–6 years of age) were maintained at the Department of Biology's Avian Research Facility at the University of Mississippi Field Station in Lafayette County, Mississippi, USA. The wild turkey flock of game farm origin was raised in captivity from hatching. Twenty netted enclosures (4.0 × 3.7 × 1.8 m) were established within a 0.04-ha flight pen for the study of hens (i.e. female wild turkeys; body mass average = 4.07 kg, range = 3.02–5.75 kg). We used 16 individual cages (2.4 × 1.5 × 1.8 m) within an open-sided research aviary for the study of gobblers (male wild turkeys; body mass average = 9.87 kg, range = 7.45–11.50 kg). Clean water was provided ad libitum to all test subjects throughout the study.

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2.2. Experimental procedures

Experimental investigation of foraging behavior requires that test subjects be exposed to ecologically relevant feeding conditions, but scientific ethics require that we minimize and mitigate the pain and distress of test subjects. In concert with the university veterinarian, we developed a protocol to meet both of these scientific needs. In the weeks prior to our study, all test subjects were offered a balanced poultry ration ad libitum to ensure that they were in the best condition for our study. We delayed our study until all test subjects had completed their molt. We paired hens within test cages to alleviate distress of individuals and disruption of flock dominance. We selected concentrations of test materials that had been previously approved for and tested with wild birds [31–33] to effectively condition and test avoidance whilst minimizing exposure among test subjects. The health of all subjects was monitored daily by study personnel and university animal care staff. Veterinary intervention due to our experimental procedures was never necessary. In accordance with U.S. federal law, all procedures were conducted only after review by and approval from the University of Mississippi's Institutional Animal Care and Use Committee (protocol #12-001; R. Buchholz — Study Director).

2.2.1. Exposure to an UV-absorbent, postingestive repellent

An anthraquinone-based repellent (Avipel®; Arkion Life Sciences, New Castle, DE, U.S.A.) was used to condition food avoidance among wild turkeys in captivity. Anthraquinone is a cathartic purgative [34] and is the active ingredient of avian repellents developed for the protection of rice [35–38], turf [39,40], corn [41] and sunflower crops [31,32]. We previously used spectrophotometry to determine that Avipel repellent absorbs UV wavelengths [33] throughout the spectrum visible to *M. gallopavo* (i.e. 315–400 nm; [20]).

Female wild turkeys ($N = 40$, experimentally naïve) acclimated within group cages (two hens per cage) and male turkeys ($N = 16$, experimentally naïve) acclimated within individual cages for five days prior to the study. During the acclimation period, one food bowl (1 kg untreated oats for hens, 0.5 kg untreated oats for gobblers) was presented in each cage at approximately 0800 h, daily.

Following acclimation, one bowl (1 kg untreated oats for hens, 0.5 kg untreated oats for gobblers) was offered in each cage at approximately 0800 h, daily for three days. Daily oat consumption was measured within each cage, including spillage, throughout the three-day pre-test. Paired hens and individual gobblers were ranked based upon average pre-test consumption and assigned to test groups (five groups of hens, four groups of gobblers) such that each group was similarly populated with turkeys that exhibited high–low daily consumption [31–33]. Test treatments were randomly assigned among groups.

On the day subsequent to the pre-test, one bowl (1 kg oats for hens, 0.5 kg oats for gobblers) was offered in each cage at approximately 0800 h. Turkeys in treatment groups one–four ($n =$ four cages of paired hens per group; $n =$ four individually-caged gobblers per group) received one bowl of oats treated with 0.5%, 1%, 2%, or 4% anthraquinone (wt./wt.) during the one-day test, respectively. We formulated oat treatments by applying aqueous suspensions (100 ml suspension/kg) to whole oats using a rotating mixer and household spray equipment [24,25,31,32]. Tested anthraquinone concentrations were based upon those previously used to develop anthraquinone concentration–response relationships for ring-necked pheasants (*Phasianus colchicus*), Canada geese (*Branta canadensis*), red-winged blackbirds (*Agelaius phoeniceus*) [31] and common grackles (*Quiscalus quiscula*) [32]. The availability of test subjects limited the control group (0% anthraquinone) to female turkeys; thus, the fifth group of hens ($n =$ four control cages) again received 1 kg of untreated oats during the test. Daily oat consumption was measured within each cage, including spillage, at approximately 0800 h on the day subsequent to repellent exposure.

2.2.2. Conditioned avoidance of an UV-absorbent feeding cue

A titanium dioxide cue (Aeroxide® P25; Acros Organics, Fair Lawn, NJ, U.S.A.) was used to test food avoidance previously conditioned with the anthraquinone-based repellent. We previously used spectrophotometry to determine that this titanium dioxide cue absorbs UV wavelengths similarly to Avipel repellent [33] and throughout the spectrum visible to *M. gallopavo*.

Two bowls (1 kg oats per bowl for hens, 0.5 kg oats per bowl for gobblers) were offered in each cage at approximately 0800 h, daily, during the four days subsequent to repellent exposure. One bowl contained untreated oats. The alternate bowl contained oats treated only with the UV-absorbent cue (0.2% titanium dioxide, wt./wt.; [33]). We formulated oat treatments by applying aqueous suspensions (85 ml suspension/kg) to whole oats using a rotating mixer and household spray equipment. The east–west placement of treated and untreated oats was randomized on test day one, and was thereafter alternated daily, throughout the test. Daily consumption of treated and untreated oats was independently measured within each cage, including spillage, at approximately 0800 h throughout the test of conditioned avoidance.

2.3. Analytical chemistry

Reversed-phase, high performance liquid chromatography (HPLC) with UV detection (254 nm) was used to quantify anthraquinone residues for all repellent-treated oats (±100 ppm anthraquinone). We collected a 200 g sample of each treatment used for repellent exposure. Subsequent to formulations, all samples were transferred to a 4 °C refrigerator at the National Wildlife Research Center (Fort Collins, CO, U.S.A.) where they were stored for the duration of the analysis period.

Triplicate subsamples from each repellent treatment were extracted and analyzed. All samples were cryogenically homogenized. Control samples were fortified with 1,500 ppm and 40,000 ppm anthraquinone, and extracted to determine the recovery rate for the assay. We weighed 0.5 (±0.05) g of ground whole oats into 25-ml glass test tubes fitted with Teflon lined caps. We pipetted 8 ml of 25% hexane in chloroform (vol/vol) into each tube. Extraction was accomplished by vortexing each tube for 20 s, placing on a horizontal shaker for 30 min, sonicating for 30 min, and then centrifuging at 2,000 rpm for 10 min. The supernatant was carefully filtered through a 0.45 μm Teflon filter into a 25-ml volumetric flask. The entire extraction procedure was replicated three times and the supernatants were combined. The sample was diluted to volume with the 25% hexane in chloroform solution and an aliquot was placed in a clean 25-ml glass test tube. The aliquot was evaporated to dryness at 50 °C under a gentle stream of nitrogen. The extract was reconstituted using 10 ml of methanol, sonicated for 30 min, and again centrifuged at 2,000 rpm for 10 min. Sample solutions were transferred into autosampler vials and analyzed by HPLC using an Agilent 1200 liquid chromatograph (Agilent Technologies, Inc., Santa Clara, CA, U.S.A.).

The HPLC instrument included a Waters X-Bridge Phenyl column (2.5 μm, 2.1 × 50 mm). The mobile phase gradient included 90% Millipore water and 10% methanol at 0 and 2 min, 20% Millipore water and 80% methanol at 4 and 7 min, and 100% methanol at 10 min. The HPLC flow rate, injection volume, and temperature were 0.3 ml/min, 5 μl and 40 °C, respectively. A four-point external calibration curve was used to calibrate our HPLC instrument. Samples were run in triplicate each day and we checked single calibration points upon each ten injections. The average response was plotted against anthraquinone concentrations. Linear regression was used to calculate anthraquinone concentrations among samples.

2.4. Statistical analyses

The dependent measure for the repellent exposure phase of our study was calculated as test consumption of anthraquinone-treated oats relative to average pre-test consumption of untreated oats (i.e. percent

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repellency = (1 − (test consumption × pre-test consumption^{-1})) × 100; [31,32]). Logarithmic regression procedures (SAS v9.2) were used to analyze repellency as a function of actual anthraquinone concentration (±100 ppm) and predict a threshold anthraquinone concentration (i.e. 80% repellency; [31,32]) for hens and gobblers. Descriptive statistics ($\bar{x} \pm$ SE) were used to summarize oat and anthraquinone consumption during repellent exposure.

The dependent measure for the test of conditioned avoidance was average daily consumption of untreated oats and oats treated with the UV-absorbent cue throughout the test. Test consumption data for hens and gobblers were subjected to a repeated measures ANOVA. The random effect of our model was cages (i.e. paired hens, individual gobblers), the between-subjects effects were oat treatments (treated, untreated) and test groups (i.e. previous exposure to 0%, 0.5%, 1%, 2%, or 4% anthraquinone-treated oats), and the within-subject effect was test day. The group-by-treatment interaction was analyzed using the mixed procedure (SAS v9.2). Tukey's tests were used to separate the means of significant interactions ($\alpha = 0.05$), and descriptive statistics ($\bar{x} \pm$ SE) and preference ratios [daily average TiO_2 consumption × (daily average TiO_2 consumption + untreated consumption)$^{-1}$] were used to summarize and illustrate test consumption, respectively.

To test our prediction regarding the influence of subject body condition, we measured the condition (body mass × tarsus length^{-1}) and enumerated intestinal parasites (*Eimeria* spp., *Capillaria* spp., other nematodes) from collected fecal samples [30] for each tested gobbler (i.e. independent of test groups; $n = 16$). Body condition and parasite data were not available for individual hens that were paired for our feeding experiments. These indices of body condition were correlated with 4-day average test consumption and relative test consumption [4-day average TiO_2 consumption × (4-day average TiO_2 consumption + untreated consumption)$^{-1}$] of food treated with the UV cue during the test of conditioned avoidance.

3. Results

3.1. Exposure to an UV-absorbent, postingestive repellent

Hens in the control group consumed 216.1 ± 21.0 g of untreated oats during the exposure phase of our study; their average, pre-test consumption of untreated oats was 193.3 ± 22.5 g. In contrast, hens exposed to oats treated with 0.5–4% anthraquinone exhibited 75–98% repellency during repellent exposure (Table 1). Hen repellency (y) was a function of anthraquinone concentration (x): $y = 10.746 \ln(x) - 12.029$ ($r^2 = 0.94$, $P = 0.030$). We therefore predicted a threshold concentration of 5,300 ppm anthraquinone (i.e. 80% repellency), or 47.0 ± 18.3 mg anthraquinone × kg body mass^{-1}, for hens offered treated oats.

Gobblers exposed to oats treated with 0.5–4% anthraquinone exhibited 78–99% repellency during repellent exposure (Table 1). Gobbler repellency (y) was a function of anthraquinone concentration (x): $y = 9.921 \ln(x) - 2.260$ ($r^2 = 0.93$, $P = 0.034$). We therefore predicted a threshold concentration of 4000 ppm anthraquinone, or 13.7 ± 8.3 mg anthraquinone × kg body mass^{-1}, for gobblers offered treated oats.

On average, hens and gobblers consumed 114 ± 88 mg and 48 ± 15 mg of anthraquinone when exposed to oats treated with 4% anthraquinone, respectively. In comparison, average consumption among hens and gobblers was 204 ± 34 mg and 129 ± 38 mg anthraquinone, respectively, when exposed to oats treated with 0.5%, 1%, or 2% anthraquinone. Thus, conditioned food avoidance was positively related to the amount of the postingestive repellent consumed during the one-day exposure.

3.2. Conditioned avoidance of an UV-absorbent feeding cue

The five test groups of hens consumed different amounts of oats treated with the UV-absorbent cue and untreated oats during the four-day test of conditioned avoidance ($F_{9,27} = 11.66$, $P < 0.0001$; Fig. 1a). Unconditioned (control) hens consumed similar amounts of untreated

Table 1
Feeding repellency of oats treated with an anthraquinone-based repellent (Avipel®; Arkion Life Sciences, New Castle, DE, U.S.A.) among wild turkeys, *Meleagris gallopavo*. Actual anthraquinone concentrations among oat seed treatments were quantified using high performance liquid chromatography. The method detection limit (MDL) of our analyses was 0.50 μg anthraquinone/g. Percent repellency represents daily consumption of repellent-treated oats relative to average pre-treatment consumption of untreated oats among five groups of females (n = four cages of paired hens per group) and four groups of males (n = four individually-caged gobblers per group).

Targeted anthraquinone concentration (%)	Actual anthraquinone concentration (ppm)	Hen repellency (%)	Gobbler repellency (%)
0	<MDL	−12	
0.5	4100	75	78
1	8800	89	91
2	19,100	95	97
4	34,400	98	99

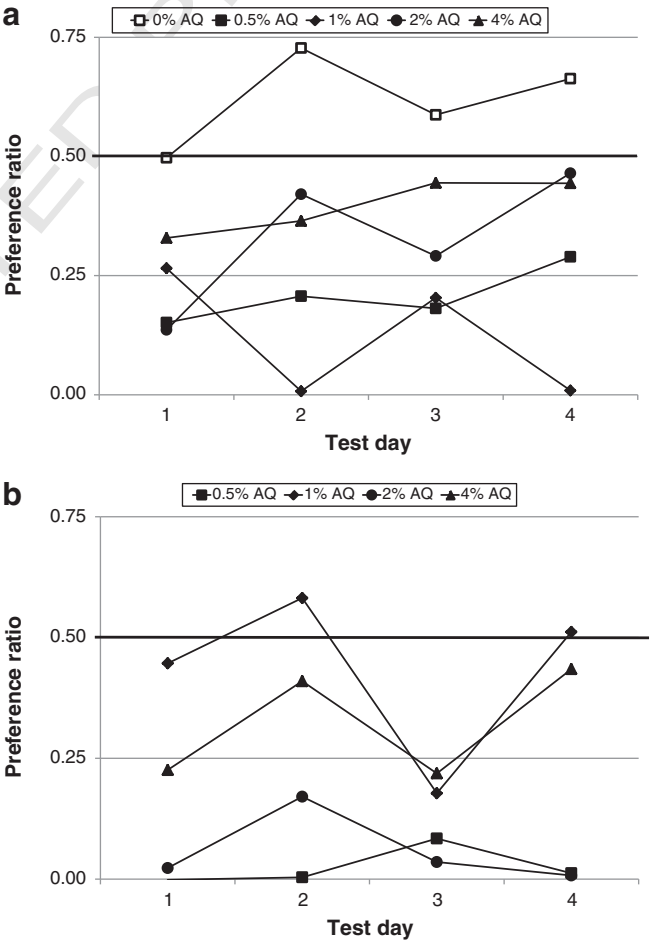

Fig. 1. Preference ratios for (a) five test groups of female wild turkeys (*Meleagris gallopavo*; n = four cages of paired hens per group) and (b) four test groups of male wild turkeys (n = four individually-caged gobblers per group) offered untreated food and food treated with an UV-absorbent cue (a.i. titanium dioxide; Acros Organics, Fair Lawn, NJ, U.S.A.) subsequent to one-day exposure to an UV-absorbent, postingestive repellent (a.i. 9,10-anthraquinone; Arkion Life Sciences, New Castle, DE, U.S.A.). Preference ratios >0.5 indicate preference for treated food; ratios <0.5 indicate avoidance of treated food. The control group of females (unfilled squares) was exposed to untreated oats and test groups (filled shapes) were exposed to 0.5%, 1%, 2%, or 4% anthraquinone (AQ, wt./wt.) prior to the test of conditioned avoidance.

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oats and oats treated with titanium dioxide throughout the test (Fig. 1a). The control group consumed an average of 138.6 ± 13.1 g of oats treated with titanium dioxide and 87.7 ± 17.5 g of untreated oats per day (Tukey $P = 0.667$). Thus, unconditioned wild turkeys did not significantly prefer foods treated with an UV feeding cue.

In contrast, hens conditioned with the UV-absorbent, postingestive repellent subsequently avoided oats treated with the UV-absorbent cue throughout the test (Fig. 1a). Hens previously exposed to oats treated with 0.5% anthraquinone consumed an average of 51.0 ± 17.1 g of oats treated with titanium dioxide and 197.6 ± 21.5 g of untreated oats per day (Tukey $P < 0.001$). The group of hens exposed to oats treated with 1% anthraquinone subsequently consumed an average of 34.8 ± 17.9 g of oats treated with titanium dioxide and 229.7 ± 26.4 g of untreated oats per day (Tukey $P < 0.0001$). Hens previously exposed to oats treated with 2% anthraquinone consumed an average of 78.3 ± 16.5 g of oats treated with titanium dioxide and 173.7 ± 24.7 g of untreated oats per day (Tukey $P = 0.038$). Thus, the UV-absorbent cue was used to maintain avoidance during the four days subsequent to postingestive conditioning.

The group of hens previously exposed to oats treated with 4% anthraquinone consumed an average of 81.9 ± 13.7 g of oats treated with titanium dioxide and 128.1 ± 14.9 g of untreated oats per day (Tukey $P = 0.774$). Thus, conditioned avoidance of food treated with the UV-absorbent cue was influenced by the amount of repellent-treated oats consumed during exposure (i.e. the negative postingestive consequence).

The four test groups of gobblers also consumed different amounts of oats treated with the UV-absorbent cue and untreated oats during the four-day test of conditioned avoidance ($F_{7,21} = 14.20$, $P < 0.0001$; Fig. 1b). Gobblers previously exposed to oats treated with 0.5% anthraquinone consumed an average of 4.2 ± 4.3 g of oats treated with titanium dioxide and 196.0 ± 18.6 g of untreated oats per day (Tukey $P < 0.0001$). The group of gobblers exposed to oats treated with 1% anthraquinone subsequently consumed an average of 86.6 ± 23.6 g of oats treated with titanium dioxide and 117.4 ± 28.0 g of untreated oats per day (Tukey $P = 0.961$). Two gobblers in the group previously exposed to 1% anthraquinone consumed more oats treated with the UV-absorbent cue than untreated oats on test days one–four, and test days one, two and four, respectively. Of these two gobblers, one had the highest parasite infection measured in the study [i.e. greatest abundance of *Eimeria* spp. (203/fecal g), *Capillaria* spp. (1267/g) and other nematodes (34/g)] and the other gobbler had an intermediate parasite infection among tested gobblers.

Gobblers previously exposed to oats treated with 2% anthraquinone consumed an average of 12.4 ± 7.6 g of oats treated with titanium dioxide and 206.3 ± 24.5 g of untreated oats per day (Tukey $P < 0.0001$). The group of gobblers exposed to oats treated with 4% anthraquinone subsequently consumed an average of 77.4 ± 21.6 g of oats treated with titanium dioxide and 175.3 ± 25.8 g of untreated oats per day (Tukey $P = 0.054$). Two gobblers in the group previously exposed to 4% anthraquinone consumed more oats treated with the UV-absorbent cue than untreated oats on test days two and four, and test days two, three and four, respectively. Of these two gobblers, one consumed the least amount (0.7 g) of 4% anthraquinone-treated oats during repellent exposure. Similar to the hens, conditioned avoidance of UV-absorbent food was influenced by the amount of repellent-treated oats consumed by tested gobblers during exposure.

With further regard to the relationship between body condition and conditioned avoidance of food treated with an UV cue, we observed moderate, positive correlations [42] between consumption of food treated with the conditioned UV cue and intestinal parasite infection among tested gobblers (Table 2). We also observed a weak negative correlation between body condition and intestinal parasite infection (Table 2). Thus, intestinal parasites moderately decreased conditioned avoidance of food treated with an UV cue previously paired with negative postingestive consequences during the gobbler test.

Table 2
Correlation coefficients for empirical relationships between body condition (body mass × tarsus length^{-1}), intestinal parasite infection (abundance × fecal g^{-1}), and test consumption and relative test consumption of food treated with an UV cue among male wild turkeys, *Meleagris gallopavo*, used to test conditioned avoidance of food treated with an UV cue previously associated with negative postingestive consequences.

	Body condition	*Eimeria* spp.	*Capillaria* spp.	Other nematodes
Consumption of UV-treated food	0.013	0.496	0.479	0.433
Relative consumption of UV-treated food	−0.002	0.519	0.503	0.452
Eimeria spp.	−0.213			
Capillaria spp.	−0.219			
Other nematodes	−0.249			

4. Discussion

Female turkeys exhibited no avoidance of untreated food and 75–98% avoidance of food treated with an UV-absorbent, postingestive repellent (0.5–4% anthraquinone; wt./wt.) during one day of repellent exposure. Male turkeys exhibited 78–99% avoidance of food treated with 0.5–4% anthraquinone. Hens and gobblers that consumed more than 200 mg and 100 mg of the UV-absorbent, postingestive repellent, respectively, subsequently avoided food treated only with an UV-absorbent cue. Ultraviolet feeding cues were therefore specifically related to the postingestive consequences of the subsequent reinforcer [43]. In contrast, unconditioned hens consumed 58% more food treated with the UV-absorbent cue than untreated food. Thus, conditioned food avoidance was positively related to the amount of the postingestive repellent consumed during the one-day exposure, and the consequences of consuming oats treated with the postingestive, UV-absorbent repellent were necessary for conditioned avoidance of the UV-absorbent cue. Wild turkeys do not prefer foods associated with UV wavelengths regardless of feeding experience (hypothesis 1).

In the absence of negative postingestive feedback [44,45], UV feeding cues are therefore unlikely to function as aposematic signals [46] or elicit food avoidance in wild birds. Ultraviolet foraging behavior is therefore a function of its consequences [47] and UV feeding cues are used functionally for foraging behavior in wild turkeys (hypothesis 2). Subsequent investigations should be focused to relate food preference with the chromatic and achromatic characteristics of natural foods [7]. Newborn and ontogenetic color preferences can be better understood by investigating the full spectrum visible to and used by avian subjects.

We predicted that consumption of food treated with an aversively-conditioned UV cue would be least among wild turkeys with poor body condition or high parasite infection (Hypothesis 3). Rather, we observed moderate, positive correlations between consumption of food treated with the conditioned UV cue and intestinal parasite infection (Table 2). In context of food selection, aversive feedback or a lack of positive feedback from the gut to the central nervous system causes animals to reduce food consumption [44,45]. Perhaps poor body condition or high parasite infection can interfere with feedback-mediated consumption of foods, including those previously associated with negative postingestive consequences. Supplemental studies are recommended to further investigate the influence of parasite infection and subject body condition for the process of avian food selection.

In conclusion, we discovered that wild turkeys do not prefer foods associated with UV wavelengths regardless of feeding experience. Rather, we found that wild turkeys can use UV feeding cues to avoid foods previously associated with negative postingestive consequences, and that this cue–consequence association was dependent upon the amount of previously experienced, postingestive consequences. Thus, UV feeding cues, like other visual and gustatory cues, have functional significance for avian foraging behavior. Not all individuals in our study, however, exhibited conditioned avoidance of foods treated with an UV feeding cue, an effect moderately related to intestinal parasite infection. Our study of the functional use of UV feeding cues in wild

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turkeys contributes to a broader avian data set and will enable subsequent investigations regarding the sensory physiology and behavioral ecology of wild birds.

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