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Functional significance of ultraviolet feeding cues in wild turkeys

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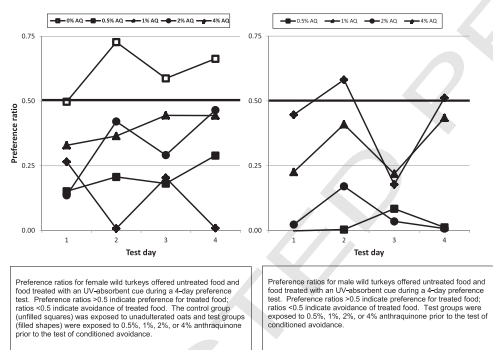
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HIGHLIGHTS

- 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



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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.

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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. 137
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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]. 141
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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. 162
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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. 174
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2. Feeding experiments 186

2.1. Subjects and testing facilities 187

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. 188
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199 2.2. Experimental procedures

200 Experimental investigation of foraging behavior requires that test
 201 subjects be exposed to ecologically relevant feeding conditions, but
 202 scientific ethics require that we minimize and mitigate the pain and
 203 distress of test subjects. In concert with the university veterinarian, we
 204 developed a protocol to meet both of these scientific needs. In the
 205 weeks prior to our study, all test subjects were offered a balanced poul-
 206 try ration ad libitum to ensure that they were in the best condition for
 207 our study. We delayed our study until all test subjects had completed
 208 their molt. We paired hens within test cages to alleviate distress of indi-
 209 viduals and disruption of flock dominance. We selected concentrations
 210 of test materials that had been previously approved for and tested
 211 with wild birds [31–33] to effectively condition and test avoidance
 212 whilst minimizing exposure among test subjects. The health of all sub-
 213 jects was monitored daily by study personnel and university animal
 214 care staff. Veterinary intervention due to our experimental procedures
 215 was never necessary. In accordance with U.S. federal law, all procedures
 216 were conducted only after review by and approval from the University
 217 of Mississippi's Institutional Animal Care and Use Committee (protocol
 218 #12-001; R. Buchholz – Study Director).

219 2.2.1. Exposure to an UV-absorbent, postingestive repellent

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

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

234 Following acclimation, one bowl (1 kg untreated oats for hens,
 235 0.5 kg untreated oats for gobblers) was offered in each cage at approx-
 236 imately 0800 h, daily for three days. Daily oat consumption was mea-
 237 sured within each cage, including spillage, throughout the three-day
 238 pre-test. Paired hens and individual gobblers were ranked based upon
 239 average pre-test consumption and assigned to test groups (five groups
 240 of hens, four groups of gobblers) such that each group was similarly
 241 populated with turkeys that exhibited high–low daily consumption
 242 [31–33]. Test treatments were randomly assigned among groups.

243 On the day subsequent to the pre-test, one bowl (1 kg oats
 244 for hens, 0.5 kg oats for gobblers) was offered in each cage at approx-
 245 imately 0800 h. Turkeys in treatment groups one–four ($n =$
 246 four cages of paired hens per group; $n =$ four individually-caged
 247 gobblers per group) received one bowl of oats treated with 0.5%,
 248 1%, 2%, or 4% anthraquinone (wt./wt.) during the one-day test, res-
 249 pectively. We formulated oat treatments by applying aqueous sus-
 250 pensions (100 ml suspension/kg) to whole oats using a rotating
 251 mixer and household spray equipment [24,25,31,32]. Tested anthra-
 252 quinone concentrations were based upon those previously used to
 253 develop anthraquinone concentration–response relationships for
 254 ring-necked pheasants (*Phasianus colchicus*), Canada geese (*Branta*
 255 *canadensis*), red-winged blackbirds (*Agelaius phoeniceus*) [31] and
 256 common grackles (*Quiscalus quiscula*) [32]. The availability of test
 257 subjects limited the control group (0% anthraquinone) to female
 258 turkeys; thus, the fifth group of hens ($n =$ four control cages)
 259 again received 1 kg of untreated oats during the test. Daily oat con-
 260 sumption was measured within each cage, including spillage, at approx-
 261 imately 0800 h on the day subsequent to repellent exposure.

262 2.2.2. Conditioned avoidance of an UV-absorbent feeding cue

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

269 Two bowls (1 kg oats per bowl for hens, 0.5 kg oats per bowl for
 270 gobblers) were offered in each cage at approximately 0800 h, daily, dur-
 271 ing the four days subsequent to repellent exposure. One bowl contained
 272 untreated oats. The alternate bowl contained oats treated only with the
 273 UV-absorbent cue (0.2% titanium dioxide, wt./wt.; [33]). We formulated
 274 oat treatments by applying aqueous suspensions (85 ml suspension/kg)
 275 to whole oats using a rotating mixer and household spray equipment.
 276 The east–west placement of treated and untreated oats was randomized
 277 on test day one, and was thereafter alternated daily, throughout the
 278 test. Daily consumption of treated and untreated oats was independ-
 279 ently measured within each cage, including spillage, at approximately
 280 0800 h throughout the test of conditioned avoidance.

281 2.3. Analytical chemistry

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

289 Triplicate subsamples from each repellent treatment were extracted
 290 and analyzed. All samples were cryogenically homogenized. Control
 291 samples were fortified with 1,500 ppm and 40,000 ppm anthraqui-
 292 none, and extracted to determine the recovery rate for the assay. We
 293 weighed 0.5 (± 0.05) g of ground whole oats into 25-ml glass test
 294 tubes fitted with Teflon lined caps. We pipetted 8 ml of 25% hexane in
 295 chloroform (vol/vol) into each tube. Extraction was accomplished by
 296 vortexing each tube for 20 s, placing on a horizontal shaker for
 297 30 min, sonicating for 30 min, and then centrifuging at 2,000 rpm for
 298 10 min. The supernatant was carefully filtered through a 0.45 μ m Teflon
 299 filter into a 25-ml volumetric flask. The entire extraction procedure was
 300 replicated three times and the supernatants were combined. The sam-
 301 ple was diluted to volume with the 25% hexane in chloroform solution
 302 and an aliquot was placed in a clean 25-ml glass test tube. The aliquot
 303 was evaporated to dryness at 50 °C under a gentle stream of nitrogen.
 304 The extract was reconstituted using 10 ml of methanol, sonicated for
 305 30 min, and again centrifuged at 2,000 rpm for 10 min. Sample solu-
 306 tions were transferred into autosampler vials and analyzed by HPLC
 307 using an Agilent 1200 liquid chromatograph (Agilent Technologies,
 308 Inc., Santa Clara, CA, U.S.A.).

309 The HPLC instrument included a Waters X-Bridge Phenyl column
 310 (2.5 μ m, 2.1 \times 50 mm). The mobile phase gradient included 90%
 311 Millipore water and 10% methanol at 0 and 2 min, 20% Millipore
 312 water and 80% methanol at 4 and 7 min, and 100% methanol at
 313 10 min. The HPLC flow rate, injection volume, and temperature were
 314 0.3 ml/min, 5 μ l and 40 °C, respectively. A four-point external calibra-
 315 tion curve was used to calibrate our HPLC instrument. Samples were
 316 run in triplicate each day and we checked single calibration points
 317 upon each ten injections. The average response was plotted against an-
 318 traquinone concentrations. Linear regression was used to calculate an-
 319 traquinone concentrations among samples.

320 2.4. Statistical analyses

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

324 repellency = $(1 - (\text{test consumption} \times \text{pre-test consumption}^{-1})) \times$
 325 100; [31,32]). Logarithmic regression procedures (SAS v9.2) were used
 326 to analyze repellency as a function of actual anthraquinone concentration
 327 (± 100 ppm) and predict a threshold anthraquinone concentration
 328 (i.e. 80% repellency; [31,32]) for hens and gobblers. Descriptive statistics
 329 ($\bar{x} \pm \text{SE}$) were used to summarize oat and anthraquinone consumption
 330 during repellent exposure.

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

345 To test our prediction regarding the influence of subject body condi-
 346 tion, we measured the condition (body mass \times tarsus length $^{-1}$) and
 347 enumerated intestinal parasites (*Eimeria* spp., *Capillaria* spp., other
 348 nematodes) from collected fecal samples [30] for each tested gobbler
 349 (i.e. independent of test groups; $n = 16$). Body condition and parasite
 350 data were not available for individual hens that were paired for our feed-
 351 ing experiments. These indices of body condition were correlated with
 352 4-day average test consumption and relative test consumption [4-day
 353 average TiO_2 consumption \times (4-day average TiO_2 consumption +
 354 untreated consumption) $^{-1}$] of food treated with the UV cue during
 355 the test of conditioned avoidance.

356 **3. Results**

357 **3.1. Exposure to an UV-absorbent, postingestive repellent**

358 Hens in the control group consumed 216.1 ± 21.0 g of untreated
 359 oats during the exposure phase of our study; their average, pre-test
 360 consumption of untreated oats was 193.3 ± 22.5 g. In contrast,
 361 hens exposed to oats treated with 0.5–4% anthraquinone exhibited
 362 75–98% repellency during repellent exposure (Table 1). Hen repellency
 363 (y) was a function of anthraquinone concentration (x): $y = 10.746$
 364 $\ln(x) - 12.029$ ($r^2 = 0.94$, $P = 0.030$). We therefore predicted a
 365 threshold concentration of 5,300 ppm anthraquinone (i.e. 80% repel-
 366 lency), or 47.0 ± 18.3 mg anthraquinone \times kg body mass $^{-1}$, for hens
 367 offered treated oats.

368 Gobblers exposed to oats treated with 0.5–4% anthraquinone exhib-
 369 ited 78–99% repellency during repellent exposure (Table 1). Gobbler

t1.1 **Table 1**
 t1.2 Feeding repellency of oats treated with an anthraquinone-based repellent (Avipel®;
 t1.3 Arkion Life Sciences, New Castle, DE, U.S.A.) among wild turkeys, *Meleagris gallopavo*.
 t1.4 Actual anthraquinone concentrations among oat seed treatments were quantified using
 t1.5 high performance liquid chromatography. The method detection limit (MDL) of our anal-
 t1.6 yses was 0.50 μg anthraquinone/g. Percent repellency represents daily consumption of
 t1.7 repellent-treated oats relative to average pre-treatment consumption of untreated oats
 t1.8 among five groups of females ($n =$ four cages of paired hens per group) and four groups
 t1.9 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

370 repellency (y) was a function of anthraquinone concentration (x):
 371 $y = 9.921 \ln(x) - 2.260$ ($r^2 = 0.93$, $P = 0.034$). We therefore pre-
 372 dicted a threshold concentration of 4000 ppm anthraquinone, or
 373 13.7 ± 8.3 mg anthraquinone \times kg body mass $^{-1}$, for gobblers offered
 374 treated oats.

375 On average, hens and gobblers consumed 114 ± 88 mg and
 376 48 ± 15 mg of anthraquinone when exposed to oats treated with 4%
 377 anthraquinone, respectively. In comparison, average consumption
 378 among hens and gobblers was 204 ± 34 mg and 129 ± 38 mg anthra-
 379 quinone, respectively, when exposed to oats treated with 0.5%, 1%, or 2%
 380 anthraquinone. Thus, conditioned food avoidance was positively related
 381 to the amount of the postingestive repellent consumed during the one-
 382 day exposure.

383 **3.2. Conditioned avoidance of an UV-absorbent feeding cue**

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

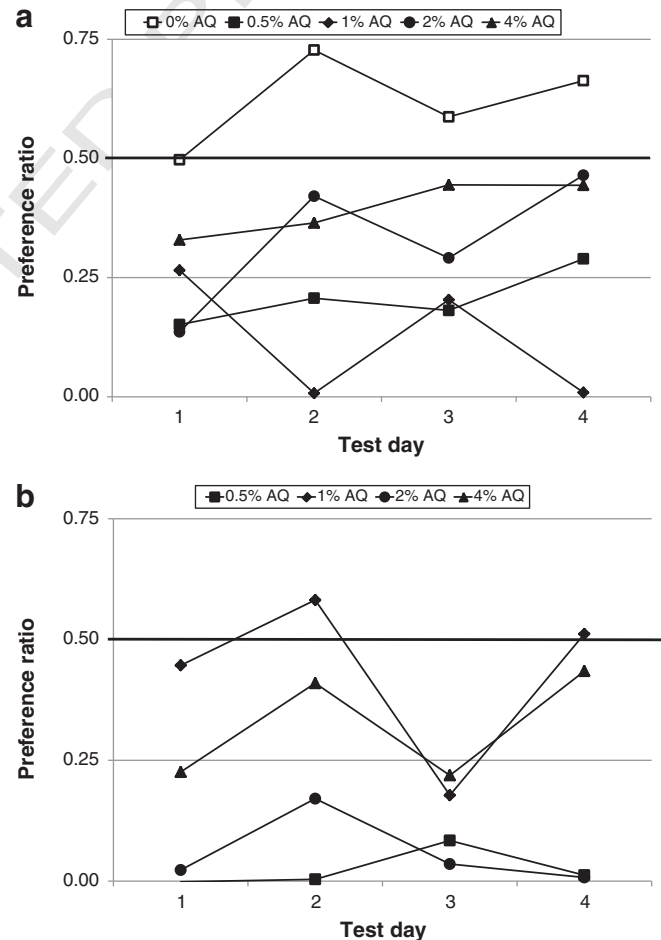


Fig. 1. Preference ratios for (a) five test groups of female wild turkeys (*Meleagris gallopavo*; $n =$ four cages of paired hens per group) and (b) four test groups of male wild turkeys ($n =$ four individually-caged gobblers per group) offered untreated food and food treated with an UV-absorbent cue (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.

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 \times tarsus length⁻¹), intestinal parasite infection (abundance \times 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	<i>Eimeria</i> spp.	<i>Capillaria</i> 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
<i>Eimeria</i> spp.	-0.213			
<i>Capillaria</i> 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

505 turkeys contributes to a broader avian data set and will enable subse-
 506 quent investigations regarding the sensory physiology and behavioral
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