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AN EVALUATION OF METHYL ANTHRANILATE, AMINOACETOPHENONE, AND UNFAMILIAR COLORATION AS FEEDING REPELLENTS TO AMERICAN KESTRELS

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ABSTRACT.—A comparison of methyl anthranilate and 4-aminoacetophenone as feeding repellents to a captive colony of American Kestrels (*Falco sparverius*) was made to determine whether aversive conditioning to these chemicals is possible in a bird of prey species. Our results suggested that, while these chemicals seemed to cause some food rejection by kestrels, they did not deter them from eating treated dead, day-old cockerels. A second study using a combination of chemical additives linked to food dyed an unfamiliar color revealed that color, and not the chemicals, was a more aversive agent. This suggested that manipulation of a kestrel's visual perception of a prey item alone had potentially more success than conditioning it to avoid a chemical additive. These results may prove useful in practical applications such as protecting game bird young at wild release sites or domestic homing pigeons associated with a particular home loft. These measures may in turn help to protect birds of prey from persecution as competitors for prey of human economic importance.

KEY WORDS: *American Kestrel*; Falco sparverius; *conditioned taste aversion*; *CTA*; *food choice*; *appetite suppressant*; *visual perception*; *aposmatic coloration.*

Metyl antranilato, aminoacetofen y la coloracion inusual como repelentes alimenticios de *Falco sparverius*

RESÚMEN.—Una comparación de metil antranilato y 4 aminoacetofen como repelentes alimenticios de una colonia en cautiverio de *Falco sparverius* fue utilizada para determinar si un acondicionamiento de aversión a estos químicos es posible en una especie de ave rapaz. Nuestros resultados sugieren que mientras estos químicos pudieron haber causado algun tipo de rechazo por los cernícalos, esto no los detuvo de alimentarse de pollos muertos de un día de nacidos. Un segundo estudio utilizando una combinación de aditivos químicos ligados a una comida teñida de un color inususal, reveló que el color y no los químicos obraron mas como agente de aversión. Esto sugirió que la manipulación de la percepción visual de una presa tuvo potencialmenmte mas éxito que el acondicionamiento para evitar los aditivos químicos. Estos resultados pueden ser útiles en la aplicación práctica como en la protección de juveniles de aves de caza en los sitios de liberación o de palomas mensajeras asociadas a ciertos sitios. Estas medidas pueden a la vez ayudar a proteger a las aves rapaces de la persecución como competidoras de presas de importancia económica.

[Traducción de César Márquez]

Although charismatic and often of high conservation priority, birds of prey are regarded as pests when taking prey of human economic interest, such as when Peregrine Falcons (*Falco peregrinus*) take domestic pigeons (*Columba livia*) (Ratcliffe 1993), Hen Harriers (*Circus cyaneus*) and other raptors kill Red Grouse (*Lagopus lagopus*) (Redpath and Thirgood 1997), and Northern Goshawks (*Accipiter gentilis*) kill Ring-necked Pheasants (*Phasianus colchicus*) (Kenward 1977). Such conflicts of interest have resulted in the illegal killing of birds of prey (Cadbury 1992, Etheridge et al. 1997). Musgrove (1997) has suggested the use of aversive conditioning to chemical deterrents as an acceptable (in the sense of Liss 1997) way of reducing Peregrine Falcon predation on pigeons and his pilot studies have shown that methyl anthranilate mixed with food causes vomiting in several falcon species, and so was presumably potentially aversive.

Limitations of methyl anthranilate application in

field situations are associated with its volatility and degradation in sunlight (Askham 1992). Isomers of aminoacetophenone appear to be up to 10 times more repellent to European Starlings (*Sturnus vulgaris*) than does methyl anthranilate (Mason et al. 1991), while the importance of intramolecular hydrogen bonds in the different isomers suggests greater repellency (Clark and Shah 1991) and lower vapor pressure (i.e., lower volatility). Thus 4-aminoacetophenone probably combines lower volatility and higher repellency when compared with methyl anthranilate (M. Baldwin pers. comm.).

The use of chemical repellents to instigate chemical aversion conditioning has been used with varying success in the control of many vertebrate pests (Mason 1997) including numerous avian species (Belant et al. 1997, Mason and Clark 1997, Clark 1998). Other workers (Reynolds and Nicolaus 1994, Reynolds 1999) have concentrated on the predation deterrent value of conditioned taste aversion (CTA) and report varying degrees of success in field application.

To date, however, largescale replicated trials of aversive conditioning on a bird of prey species remain untried. Accordingly, this paper reports on assessment of methyl anthranilate and 4-aminoacetophenone as feeding repellents to American Kestrels, a useful raptor model (Bird 1982) and also whether aversive conditioning to these chemicals is possible in this species.

METHODS

Thirty-three adult male, captive-bred American Kestrels at the Avian Science & Conservation Centre (ASCC) of McGill University were housed individually in open-fronted, wooden cages (60 × 40 × 48 cm) during April 1998 in an ambient temperature room on a 14 hr/10 hr light/dark regime. A rope perch was attached diagonally across each cage and floors were lined with waxed paper to facilitate daily cleaning.

Before the experiment began all birds were examined, weighed, randomly ascribed to cages, and then left to condition for 3 d. Caged kestrels were each fed two day-old cockerel chicks per d at 0900 H each morning and uneaten food was removed at 1600 H. Cockerel chicks are the staple diet used throughout the McGill colony and kestrels fed *ad lib* normally eat 1–1.5 cockerels/d. After the conditioning period, the kestrels were fasted for 1 d before each experiment was begun.

Mason et al. (1991) report that isomers of aminoacetophenone are at least an order of magnitude more repellent to European Starlings than is methyl anthranilate. Therefore, 1% (m/m) 4-aminoacetophenone and 10% (m/m) methyl anthranilate in 85% ethanol were chosen for investigation. Test food was prepared daily by spraying cockerel chicks with one of these solutions until all the perinatal down was saturated, or with 85% ethanol only in the case of controls. The ethanol was then allowed to evaporate for 1 hr before the chicks were packaged and stored under refrigeration. Treated chicks and controls were visually indistinguishable to human experimenters. However, cockerels were discretely labelled by amputation of distal toes and half of the lower mandible. This label was randomly alternated between test and control cockerels for each d of the food choice experiments.

Two variables were scored in the feeding trials. One of these was "first choice" (i.e., the cockerel which a kestrel moved directly to and took hold of from perching). In the test situation this is not necessarily the food item which the kestrel eventually consumed but was considered analogous to a wild kestrel perch-hunting, the species' most frequent hunting technique (Bildstein and Collopy 1987, Varland and Klaas 1991). The second variable scored was the amount of food eaten by each kestrel between 0900–1600 H each day.

It is difficult to measure quantitatively the amounts of cockerels consumed by caged kestrels. After thawing, water evaporates from partly consumed cockerels, which usually also become contaminated with kestrel feces. Therefore, taking fresh weights of intact cockerels and leftovers is not a reliable way of calculating food consumed. However, cockerels are remarkably constant in size; mean fresh mass in this study was 41.04 ± 0.61 g, (CV = 0.87%, $N = 30$). This remains so for the proportions of their body parts. The head and neck is 0.17 of a whole cockerel, eviscerated torso 0.40, yolk sac 0.16, other viscera 0.09, each pectoral limb 0.02, thighs 0.06, and feet 0.01 each. Food consumption could, therefore, be accurately assessed as proportions of "day-old cockerel units." When feeding, kestrels sometimes first plucked some of the perinatal down from a cockerel and then began eating the head. It is possible that in this way they avoided ingesting chemical additives.

Experiment 1. Kestrels were divided randomly into three groups of 11 birds each. No significant differences between groups was found for the body mass of the kestrels ($\bar{x}$ = 113.71 ± 0.46 g, $N = 33$, CV = 6.02%). The first group of 11 was used to test the reaction to food treated with methyl anthranilate, a second the reaction to food treated with 4-aminoacetophenone, and the final control group assessing voluntary food intake.

A two-choice experimental procedure (Mason et. al. 1989) was followed for the first two groups. Kestrels were given a choice of one treated (treated with either repellent) and one untreated day-old cockerel, each day for 4 d. Each day at 0900 H, the two cockerels were placed in each cage, and within approximately 15 min after food introduction the first choice selection by the kestrels was recorded. At 1600 H, food remains were removed and assessed to determine the amount of food consumed in "day-old cockerel units." Kestrels in the control group were each fed daily with two control cockerels and food consumption similarly measured. On day 5, all birds were reweighed, given two untreated cockerels each and the opportunity to bathe. Total food consumed was measured for each bird at the usual time.

Experiment 2. In nature, predators may learn to avoid unpalatable prey animals which are aposematically (warningly) colored (Mathews 1977, Turner 1977), some even

Table 1. Numbers of kestrels in methyl anthranilate treated group ($N = 11$) and 4-aminoacetophenone treated group ($N = 11$) chosing treated day-old cockerels for days 1–4 ($n = 11$). *P*-values refer to significance of a χ^2 test with df = 1.

	Day 1		Day 2		Day 3		Day 4	
Treatment	Number	*P*	Number	*P*	Number	*P*	Number	*P*
Methyl anthranilate group								
With methyl anthranilate	4		1		2		4	
Untreated	7	0.104	10	0.007	9	0.035	7	0.336
4-aminoacetophenone group								
With 4-aminoacetophenone	6		2		6		4	
Untreated	5	0.763	9	0.035	5	0.763	7	0.336

possessing an innate ability to avoid certain colors (Lindstrom et al. 1999). The second experiment tested whether aversive conditioning in kestrels is facilitated by linking an unfamiliar color to a potentially aversive chemical. In this experiment, 10% 4-aminoacetophenone, which was 10 times the concentration used in Experiment 1, was used. This higher concentration was chosen in order to give the maximum likelihood of achieving a conditioned response to the chemical additive. Day-old cockerels are usually pale yellow and this color was masked by adding green or blue food dyes to the ethanol mixture sprayed onto them.

Kestrels from Experiment 1 were rested and well fed for 1 week in large flight cages. Twenty-two kestrels from the first experiment and 11 additional kestrels were then weighed and reintroduced to test cages and ascribed to three random groups as in Experiment 1. Cross-sampling assured that kestrels exposed to a particular chemical in the first experiment were not in the second. After a 1 d fasting, they were offered four day-old cockerels each day for 3 d according to one of the following three regimes: (1) Control group—two cockerels dyed with green (green control) and two dyed with blue (blue control) food coloring; (2) Green + 4-aminoacetophenone group—two cockerels dyed green then treated with 10% 4-aminoacetophenone (green + 4-aminoacetophenone) and two dyed blue (untreated blue); (3) Blue + 4-aminoacetophenone group—two cockerels dyed green (untreated green group) and two dyed blue then treated with 10% 4-aminoacetophenone (blue + 4-aminoacetophenone group). First choice and total food consumed was measured each day as in Experiment 1 and kestrels were re-weighed after 4 d.

A further experiment showed that kestrels did not discriminate between undyed cockerels and those dyed with yellow food color.

Results

Experiment 1. Significantly more (χ^2 test, df = 1) kestrels chose untreated day-old cockerels on day 2 for both the methyl anthranilate ($P = 0.007$) and 4-aminoacetophenone ($P = 0.03$) groups and also on day 3 for methyl anthranilate ($P = 0.03$; Table 1). On day 1 and 4, treated and untreated cockerels were chosen at random and there was no significant differences in the number of kestrels choosing the two types of food.

Although it seemed that kestrels ate more untreated than treated food, treated food is also eaten, apparently peaking on day 3 for both treatment groups (Fig. 1). There was no evidence of vomiting caused by ingestion of treated food. What is clear, however, was that total food consumed by kestrels in the treatment groups tracked closely that voluntarily consumed by the control group. Had the chemical additives deterred kestrels from eating treated food, then it might have been expected that total food consumption by treatment kestrels would have been less than for controls. Analysis of variance showed this to be the case ($P = 0.0$ for treatment effect, repeated measures analysis of variance with df = 2,115) with rank order of food consumed in the sequence: control group > methyl anthranilate treatment group > 4-aminoacetophenone treatment group.

Presumably, the lower rates of food consumption of test groups was caused by kestrels avoiding treated food and so limiting their food intake. Paired *t*-tests confirmed this to be so (Table 2), however, no significant difference (ANOVA, $P = 0.901$, df = 2,29) in body mass between the three groups could be detected at the end of the trial on day 5. It seemed, therefore, that although kestrels in the treatment groups limit their food intake, they did not completely avoid treated food (Fig. 1) nor compromise their body reserves. Further comparison of post-test (day 5) intake of untreated day-old cockerels showed no difference between treatment groups and controls (ANOVA, $P = 0.199$; df = 2,28; 2 missing data items). These results together suggested that although the two test chemicals

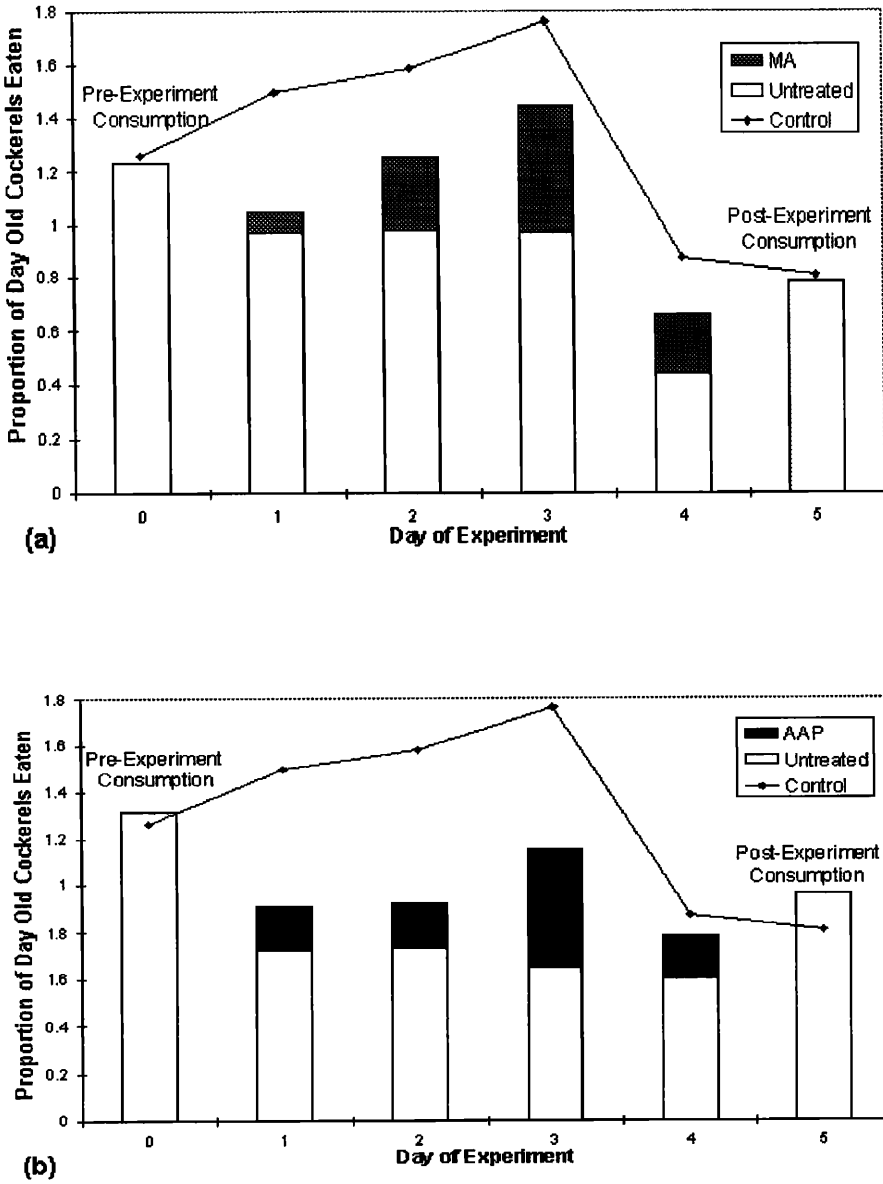

Figure 1. Consumption data for American Kestrels ($N = 11$) in (a) the methyl anthranilate treated group and (b) the 4-aminoacetophenone treated group compared with voluntary food intake of control groups ($N = 11$).

were at least avoided, 4-aminoacetophenone more so than methyl anthranilate, they were not truly aversive. Aversive chemicals would cause kestrels to avoid a particular food type even at the expense of compromising basal energy requirements and further cause them to avoid eating that food type even after chemical treatment had ceased.

Experiment 2. Only green- and no blue-colored day-old cockerels were consumed during the experiment. All but two kestrels chose green cockerels as their first choice in all groups; the remaining two birds chose not to eat at all. The green cockerels eaten by kestrels in the control and blue + 4-aminoacetophenone groups were all untreated; effectively, therefore, this second group acted as a further control. Moreover, kestrels in the

Table 2. Summary of a series of paired *t*-tests (each with df = 10) comparing consumption of methyl anthranilate treated *vs.* untreated and 4-aminoacetophenone treated *vs.* untreated food by American Kestrels. $N = 11$ kestrels in each treatment group and, where significant differences were found, the mean consumption of untreated food was greater than that of the treated food.

	Day 1		Day 2		Day 3		Day 4	
Treatment	*t*	*P*	*t*	*P*	*t*	*P*	*t*	*P*
Methyl anthranilate treated *vs.* untreated food								
	33.39	0.0	7.1	0.0	3.78	0.0036	1.04	0.32*
4-aminoacetophenone treated *vs.* untreated food								
	3.92	0.003	3.27	0.009	0.65	0.53*	2.99	0.014

* Not significant.

green + 4-aminoacetophenone group preferred to eat 4-aminoacetophenone treated green cockerels rather than untreated blue cockerels (Fig. 2). Although there was a trend (control group > blue + 4-aminoacetophenone group > green + 4-aminoacetophenone group) in the total amount of food consumed, analysis of variance showed this as not significant ($P = 0.35$, df = 2,29). In all cases, however, food intake was very low, averaging less than 0.5 day-old cockerels per kestrel per d, and, as mentioned, some kestrels refused to eat during the entire experimental period. Although most

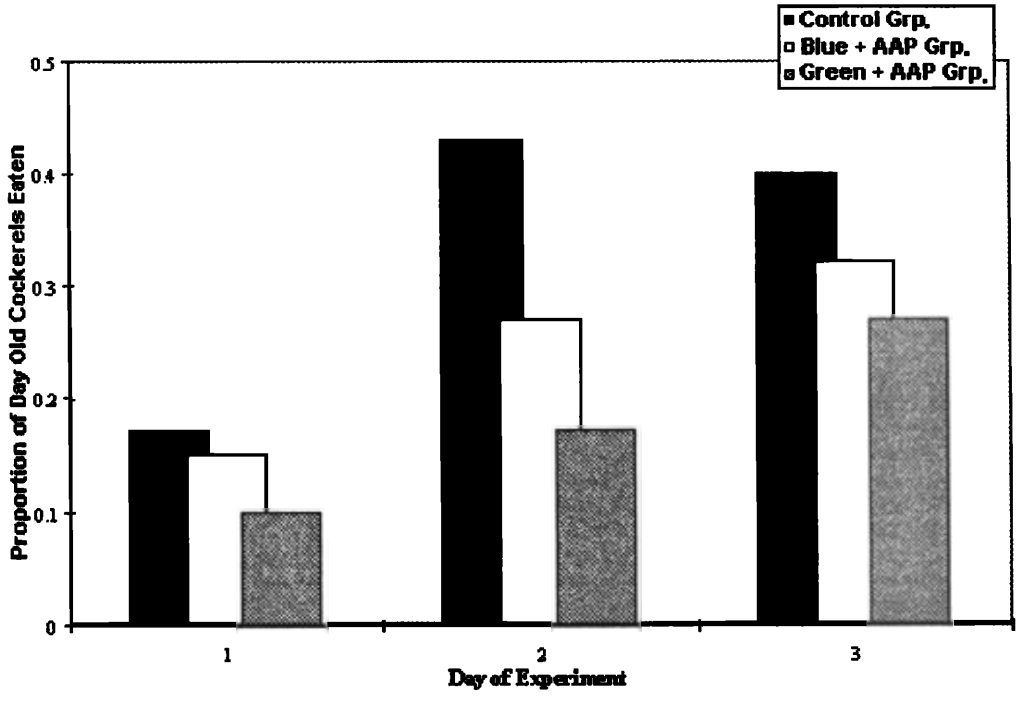

Figure 2. Comparison of total amounts of green-dyed day-old cockerels consumed by American Kestrels in Experiment 2. Treatment 1—control group or kestrels offered untreated green and blue day-old cockerels; treatment 2—blue + 4-aminoacetophenone group or kestrels offered untreated green and blue day-old cockerels and day-old cockerels treated with 4-aminoacetophenone; and treatment 3—green + 4-aminoacetophenone group or kestrels offered untreated blue and green day-old cockerels and day-old cockerels treated with 4-aminoacetophenone. In no case were blue-dyed day-old cockerels eaten.

kestrels lost weight during the trial, no significant differences in the amount of weight loss could be found by analysis of variance ($P = 0.54$, df = 2,29). Because food intake was low throughout and to avoid fatality due to starvation, the experiment was terminated after 3 d.

DISCUSSION

The use of deterrent feeding chemicals may be loosely divided into those which cause food to be unpalatable, can be detected by the predator either directly or by associated visual cues and so emulate aposematic protection, and those tasteless substances which cause feelings of sickness and so evoke a conditioned taste aversion response to a particular food (Clark 1997, Reynolds 1999).

Kestrels in our experiments seemed to be able to discriminate and avoid day-old cockerels treated topically with 4-aminoacetophenone and methyl anthranilate when they were novel. However, in the absence of alternate adequate food, kestrels ate cockerels treated with these chemicals, presumably to maintain their caloric needs. There was, therefore, no evidence to suggest that kestrels were prepared to starve and so compromise body condition. As a contrast, McKay et al. (1999) showed that lasting aversion to dead trout could be conditioned in cormorants (*Phalacrocorax carbo*) fed previously on trout treated with carbochal. Although Musgrove (1997) showed that methyl anthranilate mixed into chopped meat caused vomiting in large falcons, we found no evidence of such violent reaction in kestrels where methyl anthranilate and 4-aminoacetophenone was applied topically to food. Both chemicals seemed, therefore, to be unpalatable rather than truly aversive to kestrels and so a conditioned taste aversion response did not seem possible.

Interestingly, the kestrels' aversion to unfamiliar color, particularly blue, was stronger than that to the chemical additives. The test kestrels are familiar solely to food of one type, yellow day-old cockerels. Blue-dyed cockerels were such a deterrent to some kestrels that, rather than eat them, they preferred food treated with 4-aminoacetophenone. Further, although they would eat green-dyed cockerels, their food intake was low. It seemed, therefore, that unfamiliar color, and not the chemicals, was a more aversive agent.

It may be said that these domestic kestrels feeding on dead cockerels are not a proper test of wild circumstances. However, the findings in our study appear to be compatible with previous studies on captive kestrels and analogous to studies on wild kestrels and the innate avoidance of certain colors by other predators (Lindstrom et al. 1999). In laboratory studies, Mueller (1987) showed that while American Kestrels developed long-term preferences for particular types of prey, they would still sample novel prey if it was still within the limits of what occurred in nature. He further inferred from the literature on laboratory and field studies that such specific search images are also formed by free-ranging kestrels and other birds of prey.

A more serious consideration is whether predatory birds conditioned to avoid dead prey, would transfer that avoidance behavior to live alternatives. Whether kestrels conditioned to avoid dead cockerels will transfer this behavior to live prey needs further study. It may, however, be feasible to condition free-living raptors to avoid a potential prey by treating live prey with chemical deterrents. It is reasonable to assume that in a field application with ample alternative prey available, the application of methyl anthranilate and 4-aminoacetophenone, or indeed some other proven agent, to a group of potential prey animals may condition a response in the predator causing it to avoid the treated group and hunt elsewhere. However, as our results suggest, if prey abundance is locally limiting and no alternative available, then treatment of prey with methyl anthranilate or 4-aminoacetophenone, at least at the concentrations tested nor higher concentrations, is not likely to deter predation. Methyl anthranilate is an oily liquid at normal temperatures and 4-aminoacetophenone a crystalline solid. Both chemicals were tested at what appeared to be maximum possible levels (10% m/m), but consumption of treated food by kestrels still occurred. Higher levels applied to, say, game birds or pigeons in the field may cause impairment of feather maintenance, increased time spent preening, and possibly increased vulnerability to factors such as cold or wet weather. Research into the repercussions of the chemicals applied to the plumage of the prey animals they are meant to protect would have to be undertaken. Perhaps, an alternate solution would be to feed potential prey items chemicals which would render their flesh unpalatable to their avian predators, emulating more closely naturally unpalatable and therefore protected animals. In addition, it may even be more efficient to use visual cues such as sufficiently novel colors, rather than chemicals, to at least initially

deter raptors from taking prey of human economic importance. However, the degree to which a color remains novel to a particular predator in wild circumstances is a complicated question (see Allen and Clarke 1968).

Given that the use of dead baits poses problems of transference of avoidance to live prey, this raises an important question concerning in what situations, if any, such management practices would best be aimed. The chemical protection of wild adult prey would not be feasible due to the difficulty and cost of catching them and then applying a sufficient amount of the deterrent chemicals. A more practical application of chemical deterrents may be their use in the protection of game bird young at wild release sites or domestic homing pigeons associated with a particular home loft. Protection from a single, attentive raptor, in these circumstances, could possibly be insured by the combination of an aversive chemical and associated aposmatic visual cues.

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