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EFFECTIVENESS, BIASES AND MORTALITY IN THE USE OF APOMORPHINE FOR DETERMINING THE DIET OF GRANIVOROUS PASSERINES[1]

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Abstract. We analyze the effectiveness, biases and limitations of a poorly-known emetic, apomorphine, for studying the diet of granivorous birds. Apomorphine was tested on three Fringillids yielding an effectiveness of 43–78%, comparable to that reported for the most common emetic, tartar. The effectiveness of the emetic varied significantly among species, probably due to different physiological tolerances. Older chicks and adults were equally sensitive to apomorphine, but it was ineffective in young chicks. The action of the chemical was not independent of food type, and certain food items were underrepresented with this method. However, the importance of most food categories closely resembled the one found through dissection. Mortality caused by exposure to apomorphine was low (< 3%) and successive applications of apomorphine proved to be innocuous, thus allowing for long term studies. Yet, the effectiveness of apomorphine decreased when administered repeatedly within a short time. Apomorphine is an advisable tool for laboratory and field work on granivorous passerines because it is effective, practically harmless, provides reliable results, and can be used repeatedly and easily both with nestlings and adults.

Key words: apomorphine, diet determination, emetic, granivorous birds, regurgitation.

INTRODUCTION

Detailed knowledge of diets is critical to many studies of avian biology and ecology. Several techniques have been developed specifically for seed-eating birds: direct observation through the skin of the neck (Newton 1967, Glück 1985), mechanical methods to extract the seeds from the crops (Payne 1980, Zann and Straw 1984), constrictive ligatures around nestlings' necks (Johnson et al. 1980), and forced regurgitation (Prys-Jones et al. 1974). The best known method of forced regurgitation uses chemical emetics; antimony potassium tartrate (tartar emetic) is the most common (Prys-Jones et al. 1974, Robinson and Holmes 1982, Poulin et al. 1994). This emetic is reported to perform well in granivorous birds (Herrera 1975, Díaz 1989, Poulin et al. 1994), although high mortality associated with its use has been described (Herrera 1975, Zach and Falls 1976, Lederer and Crane 1978, Zann and Straw 1984).

Paradoxically, even though the emetic effect of apomorphine has been known since the 1960s (Chaney and Kare 1966), it has been seldom used (Schluter 1988, Díaz 1989). Unlike other emetics, apomorphine acts through stimulation of the vomit center (near the solitary tract, approximately at the level of the dorsal motor vagal nucleus), via the chemoreceptor trigger zone located at the fourth ventricle in the bulb of the spinal cord (i.e., medulla oblongata) (Chaney and Kare 1966). Díaz (1989) showed that its effectiveness does not depend on dose, sex, age or season, but little is known about the factors affecting the effectiveness, biases, and mortality inherent in this method.

Here we evaluate the effectiveness of apomorphine and its effect on several granivorous species by using both field and laboratory data. We also test the influence of age (young nestlings, grown nestlings, and adults), food type (hard vs. soft seeds) and repetitive treatments on the usefulness of this emetic. We finally examine the merits and limitations of apomorphine in comparison with other methods (tartar emetic, ligature) described in the literature.

METHODS

FIELD DATA

We conducted the field study during the breeding periods of 1990 and 1991 on an olive tree

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plantation in southern Spain. The species considered in this study (Serin *Serinus serinus,* Goldfinch *Carduelis carduelis* , and Greenfinch *Carduelis chloris*) usually breed on olive orchards from March to July. Incubation lasts 13 days for all three species and the brooding period is 14 days in Serin and Goldfinch and 15 days in Greenfinch (Valera, pers. observ.). The diet of both chicks and adults consists mainly of milky ripe weed seeds (Newton 1967, Glück 1985, Valera, unpubl. data).

Once a nest was located, a fresh saturated solution of apomorphine (0.04 g ml^{-1} water) was prepared and administered to all nestlings, while leaving one or two chicks in the nest to avoid desertion. Birds were held in hand until droplets of solution (0.03 to 0.05 ml on average) placed in the eyes were absorbed. Then, they were kept in a covered container for 20 min. Regurgitation occurred in all species within 15–20 min, but vomit never occurred after 20 min. The whole process takes one person less than 25 min (no more than 5 min for administration of apomorphine per bird and 15–20 min to allow regurgitation), and can be consecutively repeated on several birds. To determine whether regurgitation was due to the emetic and not the handling procedure and possible associated stress, we used a control group of 20 Serin nestlings (approximately 8–10 days old) and several mist-netted adults of several species (10 Serins, 10 Goldfinches, and 11 Greenfinches). All control birds had their gullets filled with seeds and were treated exactly as those receiving the emetic, except that droplets of distilled water were placed in the eyes instead of apomorphine. In no case did vomiting occur, indicating that regurgitation was due to the emetic.

Doses were adjusted to age and body size (range 7–11 g for Serin, 7.5–11.5 g for Goldfinch and 7.5–12 g for Greenfinch), even though the effectiveness of apomorphine seems unrelated to dosage (Díaz 1989). Apomorphine was administered within 24 hr after the preparation of the solution to avoid loss of effectiveness (see Díaz 1989). The emetic was applied to 157 chicks: 115 Serins, 19 Goldfinches, and 23 Greenfinches.

All three species have modifications of the digestive tract (gullet or esophageal pouches) to temporarily store seeds. It is therefore easy to estimate visually the volume of food stored in the nestlings' gullets before and after the administration of apomorphine. Thus, to highlight the possible relationships between quantity of ingested food and frequency of regurgitation, we distinguished between chicks with more and less than half their esophageal pouches full of food. To assess the influence of the amount of ingested food on the quantity of vomited seeds, we ranked the quantity of food from 0 (empty pouches) to 4 (completely full pouches) before and after the use of the emetic. Visual estimations of the amount of food stored in gullets of nestlings were made independently by two of the authors. Those cases in which disagreements occurred were not used in the analyses, which accounts for differences in sample sizes in Table 1 and some of the tests.

The effectiveness of apomorphine, measured as percentage of birds vomiting, rated highest in Serin, which was the most sensitive species to this emetic (see Results). Most of the analyses (effect of age, reliability of the results, effect of seed hardness, and cumulative effects of apomorphine) were therefore performed on this species.

Serin nestlings open their eyes when they are at least 4 days old (Valera, pers. observ.), after which apomorphine can be administered. The effectiveness of the emetic in relation to age was studied by classifying chicks into young (< 7 days old) and grown (> 7 days old).

Biases in the diet composition obtained with apomorphine were evaluated by comparing the regurgitated samples (n = 24) with those from the gullet content of recently dead Serin chicks (n = 11) as a consequence of mechanical tillage. All the nestlings came from the same area and year of study to avoid any diet variation due to differences in food availability.

Samples were preserved at −10°C until analyzed. Sorting and identification was carried out under a dissecting microscope. Seeds were counted and measured with an ocular micrometer, and representation of each seed type was visually estimated to the nearest 10%.

LABORATORY EXPERIMENTS

Effects of seed hardness. During 1993 and 1994, we assessed the role of seed hardness on the effectiveness of apomorphine through laboratory experiments with two types of commercial seeds, one hard (canary, *Phalaris canariensis*) and the other soft and easy to crumble (rape, *Brassica rapa*). Although both seed types differ in other respects, we strongly believe that the most important characteristic is the difficulty to split them into separate pieces. Twenty-one adult Serins were fed with canary seeds several days before the experiment. Three weeks later the experiment was repeated on 20 adult Serins with rape seeds. Food was withdrawn the night before the birds were tested, and during the test birds were offered a dish with a known number of seeds. Birds were allowed to feed for 20 min before administration of apomorphine. The emetic was made fresh daily. After treatment, we placed each bird in a dark box for 20 min and allowed regurgitation. Then we tallied the number of vomited and ingested seeds. For each bird we measured the time necessary for the administration of the emetic after food withdrawal. The maximum difference between individuals was less than 9 min.

Cumulative effects of apomorphine. We conducted a second experiment with rape seeds to study the cumulative effect of apomorphine. Twelve adult Serins were tested daily over 10 days. The procedure was the same as described above, although in this case interindividual differences in the period of administration were always less than 7 min. Regurgitation was considered to occur if at least one seed was ejected. After taking them out of the container, the birds were closely observed for 15 min to 1 hr to assess ill effects and the recovery process.

DATA ANALYSES

To minimize biased interpretations (Rosenberg and Cooper 1990), diet data are presented in three forms: percent occurrence of each item in the diet, mean volume (expressed as a percentage of the total volume of the contents) and, finally, a general importance index (IG). IG is the product of the two former measurements, so that the higher the value the more important the food type is in the diet (Jordano and Herrera 1981, Jordano 1988).

Parametric tests were used when the data were normal or could be transformed into normality, otherwise nonparametric tests were applied. Repeated-measures ANOVAs were used on those variables measured on the same individual over the 10-day study period. Unless otherwise stated, tests are two-tailed and data are presented as means ± SE. Statistical analyses follow Zar (1984).

TABLE 1. Effectiveness of the emetic (percentage of individuals that vomit) in grown nestlings (>7 days old) of three granivorous species.

Species	Treated nestlings	Positive effect	Effectiveness
Serinus serinus	95	74	77.9
Carduelis carduelis	19	9	43.4
Carduelis chloris	23	12	52.2

RESULTS

EFFECTIVENESS OF APOMORPHINE

The application of the emetic to young Serin chicks (< 7 days old) showed a low effectiveness: only 2 out of 20 (10%) treated chicks regurgitated food. In contrast, 74 out of 95 (77.9%) grown Serin chicks vomited. The age difference in effectiveness was highly significant ($\chi^2_1 = 31.0$, $P < 0.001$). Besides the low effectiveness in young chicks, the quantity of seeds vomited by the two young nestlings mentioned above was very low and scarcely representative of their diet. Accordingly, subsequent data will refer only to grown chicks (> 7 days old).

Overall, the effectiveness of apomorphine in nestlings of the three Fringillids averaged 57.8%, although it varied significantly among species ($\chi^2_2 = 10.8$, $P < 0.005$): Serins were most sensitive to the emetic (77.9%), whereas Goldfinches and Greenfinches responded similarly (Table 1).

There were no differences in the frequency of vomit in relation to the quantity of seeds ingested (more vs. less than half the esophageal pouches holding food) in grown Serins ($\chi^2_1 = 2.1$, $n = 83$, $P = 0.15$) and Goldfinches (Fisher exact test, $n = 19$, $P = 0.65$), but frequency of vomit was positively affected by the amount of ingested food in Greenfinches (Fisher exact test, $n = 23$, $P = 0.04$). In addition, we found a significant correlation between the number of seeds ingested and vomited in Serins ($r = 0.43$, $n = 72$, $P < 0.001$) and in Greenfinches ($r = 0.70$, $n = 23$, $P < 0.001$), but not in Goldfinches ($r = 0.36$, $n = 18$, $P = 0.14$).

RELIABILITY OF THE RESULTS

We found significantly more species of seeds per sample in dissected nestlings than in nestlings treated with apomorphine (t-test, $t_{33} = 2.26$, $P = 0.03$) (Table 2). Most seed species occurred in higher frequencies in the samples obtained

TABLE 2. Diet of Serin nestlings using apomorphine ($n = 24$ birds) and dissection ($n = 11$ birds). Mean volume (percentage), percent occurrence (PO) and a general importance index (IG, the product of both percentages above divided by 100) of each item in the diet are shown.

	Samples obtained by dissection			Samples obtained by apomorphine		
Diet	Volume	PO	IG	Volume	PO	IG
Seed fraction						
Diplotaxis virgata	33.9	90.9	30.82	44.8	100.0	44.80
Erodium sps.	27.3	90.9	24.79	35.4	100.0	35.46
Cerastium glomeratum	3.9	90.9	3.55	1.1	67.7	0.72
Stellaria media	10.4	81.8	8.47	5.9	58.3	3.42
Cruciferae sp.	8.1	81.8	6.63	3.4	29.2	0.98
Poa annua	2.0	54.5	1.09	6.2	45.8	2.84
Biscutella sp.	1.5	36.4	0.56	0.2	12.5	0.03
Senecio vulgaris	0.1	9.1	0.01	0.6	12.5	0.07
Chamaemelum fuscatum	0.9	45.5	0.41	0.04	4.2	0.001
Linaria sp.	0.0	0.0	0.00	1.5	4.2	0.06
Others	9.1	9.1	0.83	0.4	4.2	0.01
Mean number species/sample		5.91 ± 0.65			4.37 ± 0.35	
% volume		97.3 ± 0.92			99.5 ± 0.27	
Non-seed fraction						
Fragments of plants	0.1	9.1	0.01	0.3	12.5	0.04
Olive pulp	2.4	54.5	1.30	0.0	0.0	0.00
Insects	0.3	27.3	0.07	0.1	16.7	0.02

through dissection (Table 2), although such differences were significant only for the Cruciferae ($\chi^2_1 = 6.44$, $P = 0.01$) and *Chamaemelum fuscatum* (Fisher exact test, $P < 0.01$). Similarly, the volume of seeds of the Cruciferae sp. and *Cerastium glomeratum* was significantly greater in dissected birds (Mann-Whitney U-test, $z = 2.91$ and 3.18, respectively, both $P < 0.01$). Thus, seeds of the Cruciferae sp. were the ones most underestimated using this emetic method.

Seed size did not seem to affect the effectiveness of the emetic, as Serins regurgitated easily the seeds of *Erodium* sps., which are significantly larger (long diameter: 3.15 ± 0.02 mm, $n = 81$) than those of the Cruciferae (long diameter: 1.67 ± 0.01 mm, $n = 58$) ($t_{137} = 48.8$, $P < 0.001$).

However, the volume of the non-seed fraction was significantly smaller in regurgitations than in samples from dissected nestlings (Mann-Whitney U-test, $z = 2.75$, $P < 0.01$). This was mainly due to the absence of olive fruit pulp, which was the only food type missing from the emetic samples. Percent occurrence of insects (16.7% vs. 27.3%) and plant fragments (12.5% vs. 9.1%) did not differ significantly in emetic and dissection samples (Fisher exact test, $P > 0.1$ in both comparisons). Although the volume of insects and plant fragments was low in Serin's diet (see Table 2), these food types still occurred in emetic samples.

Even though some food items were underrepresented in the diets obtained with apomorphine, we found a significant association between the index of global importance (IG) of emetic and dissection samples (seeds only: $r = 0.90$, $n = 11$, $P < 0.001$; all food types: $r = 0.90$, $n = 14$, $P < 0.001$).

DIFFERENTIAL EFFECT RELATED TO HARDNESS OF THE SEED

Whereas 16 out of 20 (80%) adult Serins feeding on soft seeds (rape) vomited, the emetic was effective only in 7 of 21 (33.3%) individuals feeding on hard seeds (canary). This difference in effectiveness was significant ($\chi^2_1 = 7.26$, $P < 0.01$). The effectiveness of the emetic in adult Serins fed with soft seeds was similar to that obtained for Serin nestlings fed with wild soft seeds (77.9%, see Table 1).

To assess the effect of seed hardness on the amount of vomited food, we calculated the percentage of seeds vomited in relation to the number of seeds ingested. Birds fed with soft rape seeds vomited significantly more seeds (33.5 ± 3.9%, $n = 16$ adults) than those fed with hard canary seeds (10.3 ± 3.7%, $n = 7$ adults) (Mann-Whitney U-test, $U = 10$, $P = 0.002$).

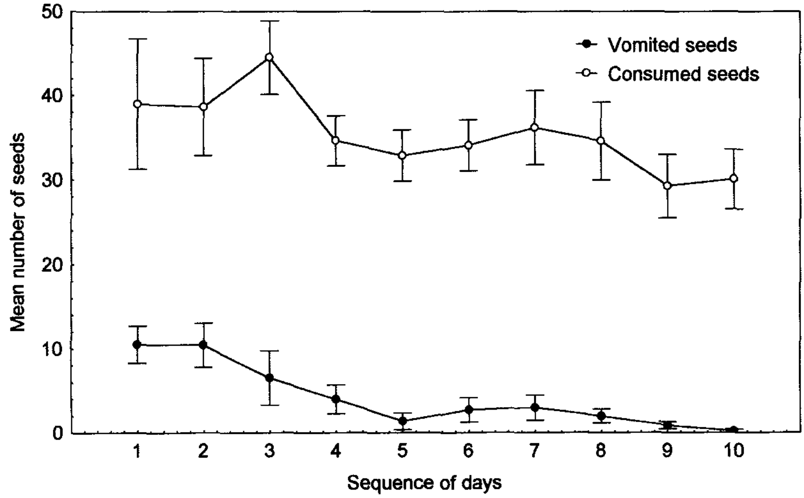

FIGURE 1. The mean (± SE) number of seeds ingested and the mean number of seeds vomited by Serin (n = 11 adults) over 10 days.

There were no differences in either the number of ingested seeds/individual (35.6 ± 5.1, $n = 21$, for canary seeds vs. 34.3 ± 4.6, $n = 20$, for rape seeds, $t_{39} = 0.18$) or in the time prior to the administration of the emetic (273.8 ± 34.8 sec, $n = 12$, for canary seeds vs. 369.6 ± 41.7 sec, $n = 12$, for rape seeds, $t_{22} = 1.76$, $P = 0.09$). Therefore, the difference in hardness of the seeds most probably accounts for the differences in the response of adults to the emetic.

CUMULATIVE EFFECTS OF APOMORPHINE ON ADULTS

The effectiveness of apomorphine after successive administrations was tested by applying the emetic daily to 12 adult Serins over 10 days. One individual never vomited and therefore was excluded. Variation in the number of ingested seeds or in the period necessary for the administration of the emetic after food withdrawal might have influenced the pattern of vomited seeds over days. Thus these two variables were included in the analysis. Whereas neither the number of ingested seeds nor the period of administration differed across days (repeated-measures ANOVA, $F_{9,81} = 1.37$, $P = 0.21$; $F_{9,81} = 1.29$, $P = 0.25$, respectively), the number of vomited seeds did decrease significantly over days ($F_{9,81} = 5.47$, $P < 0.001$) (Fig. 1), suggesting that the effectiveness of apomorphine declines in successive applications.

MORTALITY OF NESTLINGS AND ADULTS

Nests were checked several days after administration of the emetic. Eighty-four Serin nestlings, 12 Goldfinch nestlings and 14 Greenfinch chicks were monitored. Mortality was recorded only in one Serin nest, where all three treated young were found dead. If we assign such deaths to the emetic, the overall mortality rate is 2.7%. There were no deaths during the application of the emetic and handling of birds, with the exception of a young Goldfinch that died of asphyxia when it failed to expel the big seeds from its gullet.

Considering our laboratory experiments (the ones shown here and unpublished data, on the whole 12 adult Serins, 4 adult Greenfinches and 2 adult Goldfinches tested consecutively over 10 days), no adult birds died, and all birds recovered completely and started feeding again within an hour after administration of the emetic.

DISCUSSION

The mean effectiveness found in this study (57.8%) is within the range described for similar species treated with apomorphine: 71% for African granivorous passeriformes (Schluter 1988), 43% for various small granivorous species (Díaz 1989) and 56% for Fringillids (Díaz 1989). However, effectiveness of the emetic differed among the three species studied. Serin was most sensitive to the action of apomorphine, yielding a higher effectiveness (77.9%) than the maximum value found by Díaz (1989) (70% for Linnet, *Carduelis cannabina*). Emetic samples of Goldfinch and Greenfinch nestlings obtained in the same year and study area suggest that their diet contents do not differ substantially from that of Serins. Although Goldfinch and Greenfinch feed on fewer seed species than Serin (6 and 4 vs. 11, respectively), they include only one seed species different from those consumed by Serin (amounting to less than 3% of volume in both cases). Finally, *Erodium* sps. is their most important food item, amounting to over 75% volume (Valera, unpubl. data). Thus, differences in effectiveness of the emetic are probably related to differences in physiological tolerance. Furthermore, interspecific differences arise in the relationship between the quantity of food in the gullet and both the effectiveness of the emetic and the quantity of vomited seeds. Whereas such associations are evident in Greenfinches, they do not exist in Goldfinches and only partially in Serins.

Díaz (1989) found similar effectiveness of apomorphine in juveniles and adults of several species. Our results for adult and young Serins, and the comparison between our value for nestlings of Goldfinch (43.4%) and the data for adults offered by Díaz (1989) (41.8%), suggest that chicks and adults are equally sensitive to the emetic. However, apomorphine is ineffective in young chicks (< 7 days old), presumably because of the incomplete development of the gastric and esophageal muscles.

Apomorphine is as effective as other emetics. Prys-Jones et al. (1974) found that 50% of the granivores treated with tartar emetic (antimony potassium tartrate) regurgitated samples, and Poulin et al. (1994) obtained recognizable food in 76% of samples from granivorous birds treated with tartar. Díaz (1989) described apomorphine as less effective than tartar in sparrows and buntings, but both had similar effectiveness in finches and larks. On the other hand, unlike tartar emetic, apomorphine seems to be ineffective when administered to insectivorous birds such as Great Tit *Parus major* and Blue Tit *P. caeruleus* (Pulido and Díaz 1994, Valera, pers. observ.).

Mortality caused by emetics can be high and may depend on the species involved, dosage, and stressful effects of handling (Zachs and Falls 1976, Lederer and Crane 1978, Ford et al. 1982). For instance, high mortality rates caused by tartar emetic have been reported for Ovenbirds *Seiurus aurocapillus* (12.5%; Zach and Falls 1976) and House Sparrows *Passer domesticus* (20%; Lederer and Crane 1978). Poulin et al. (1994) reported the lowest mortality value for tartar emetic (2%; although mortality could be higher depending upon recapture rates) and showed that some adjustment in emetic concentration and dosage reduced mortality. In the case of apomorphine, there is little comparative information available. Schluter (1988) did not record mortality, and some recaptured birds showed no apparent ill effects. Our data indicate that mortality caused by apomorphine is very low (< 3%), an important advantage in comparison with other emetics. Apomorphine can be administered repeatedly and innocuously, whereas successive applications of tartar emetic within a relatively short period of time may result in death (Zachs and Falls 1976). Although our data show that the effectiveness of apomorphine decreases in successive applications, few studies will require such frequent use of an emetic. Our results are therefore conservative and we would expect no decrease in the effectiveness of apomorphine if administered less frequently, as in long-term field studies.

As described in this study, apomorphine also can be easily applied to grown nestlings, and is an improvement over the most widespread method, ligature, in several respects. First, mortality rate for apomorphine (< 3%) is lower than using ligatures on bigger birds: 1.9–8.5% for Spotless Starling *Sturnus unicolor* (Peris 1980, Pascual and Peris 1992), and 5% for Gray Catbird *Dumetella carolinensis* and Brown Thrashers *Toxostoma rufum* (Johnson et al. 1980). Ligature has other problems: disgorging of food (Orians 1966, Johnson et al. 1980), food removed by adult birds from the nestlings' mouths (Robertson 1973), and biases related to food size (small food items may slip past the ligatures, see Orians 1966, Walsh 1978, Johnson et al. 1980). Rosenberg and Cooper (1990) recommend that ligatures not be used alone. Therefore, apomorphine can be an alternative method, as long as it is not used with very young chicks. In any case, ligatures also are often reported to be difficult to fit on young nestlings (Orians 1966, Willson 1966).

Perhaps the most important factor when using an emetic is the reliability of the results. Our results suggest that samples obtained with apomorphine describe a less diverse diet than that actually consumed by Serin. Furthermore, certain food categories either do not appear (i.e., olive pulp) or are vomited less frequently and in smaller amounts (i.e., the Cruciferae sp. seeds).

As to regurgitated seeds, hardness, rather than size, seems to be the cause of the differences in frequency of occurrence and volume. During the breeding season, Fringillids feed mainly on milky ripe seeds, usually removing the seed coat, whereas the Cruciferae sp. are the only seeds ingested with a hard and leathery coat. Our laboratory experiments with adult Serins confirm the role of seed hardness in the frequency of vomit and in the amount of vomited seeds: vomit is significantly more frequent and the percentage of vomited seeds significantly higher if the birds have ingested seeds that are soft and easy to crumble. The non-seed fraction also seems to be underestimated, especially regarding olive pulp. Olive pulp was found in most dissected nestlings as a viscous mass which is probably difficult to vomit in pieces and too big to be fully expelled. Although this food item could not be quantified using apomorphine, its occurrence in the diet of the nestlings was not completely inconspicuous, as small drops of oil appeared during the analysis of some samples.

In short, it could be argued that the main disadvantage of apomorphine is that it may be dependent on the type of food consumed and may therefore result in qualitative bias. Similar biases also have been described for the most widespread emetic, tartar (Zachs and Falls 1976, Gavett and Wakeley 1986). However, with the exception of olive pulp, the relative importance of each food item is approximately the same in emetic and dissection samples. Therefore, apomorphine should provide an acceptable representation of the overall diet of granivorous species.

In species with a well-developed crop, the crop contents are thought to be the most unbiased representation of a bird's diet (Hartley 1948). Moreover, the gullets of many granivorous species often contain large samples of seeds eaten recently (Newton 1967, Payne 1980). Under such conditions, the use of the right technique can improve our knowledge of avian diets. Díaz (1989) recommended apomorphine because of its easy use, consistency of effectiveness among individuals, and its high efficacy for specific families of birds.

Our results show additional and important merits of this technique (low mortality, ability to use on grown nestlings, potential for repeated usage, and acceptable representation of the overall diet), as well as possible limitations (qualitative bias in the representation of some food types, variation in effectiveness). All in all, however, this study clearly indicates that apomorphine has a considerable potential for the analysis of diets of granivorous birds.

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