

Use of DiazaCon™ as a reproductive inhibitor for monk parakeets (*Myiopsitta monachus*)

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Abstract. Feral monk parakeet (*Myiopsitta monachus*) populations have become established in the United States and other countries around the world, and can cause damage to electrical facilities. Because the monk parakeet is a highly visible species and there is often public opposition to lethal control measures, non-lethal methods, such as contraception, are being developed to help control the spread of feral populations. Two gavage studies and one *ad libitum* nesting study were conducted to assess the efficacy of DiazaCon™ as a potential contraceptive for the monk parakeet. The first gavage study compared daily dose levels of 0, 50, 75, and 100 mg DiazaCon™ (kg bodyweight)⁻¹ administered for 10 consecutive days. Cholesterol concentrations decreased significantly concomitant with a significant increase in desmosterol concentrations in the treated groups, but did not vary between sexes. Cholesterol and desmosterol concentrations did not differ significantly among DiazaCon™ groups, and cholesterol remained significantly suppressed 12 weeks after treatment. On the basis of these results, the second gavage study compared 5 or 10 consecutive days of DiazaCon™ administration at 50 mg kg⁻¹ bird⁻¹ day⁻¹. Cholesterol concentrations decreased significantly concomitant with a significant increase in desmosterol concentrations in the treated groups, but did not vary between sexes. Cholesterol and desmosterol concentrations did not differ significantly between DiazaCon™ groups, and cholesterol remained significantly suppressed 11 weeks after treatment. Parakeets in the nesting study were fed hulled sunflower seeds treated with a target dose of 50 mg DiazaCon™ kg⁻¹ bird⁻¹ day⁻¹. Birds consumed enough to receive an average dose of 34 mg kg⁻¹ pair⁻¹ day⁻¹, or 17 mg kg⁻¹ bird⁻¹ day⁻¹. Birds in the treated group laid an average of 1.6 ± 0.7 eggs per clutch compared with 3.9 ± 1.1 eggs per clutch in the untreated control group. None of the eggs laid by treated birds hatched compared with 1.1 ± 0.6 eggs per clutch hatching in the control group. Reproductive inhibition was effective for the length of the breeding season, at which time the study was stopped and no more data were collected. DiazaCon™ is a promising avian oral contraceptive that should be further investigated in a field setting with monk parakeets.

Introduction

The monk parakeet (*Myiopsitta monachus*) is a parrot whose native range extends from central Bolivia and southern Brazil to central Argentina. Highly adaptable, the species has become established in the United States as well as in Puerto Rico, the Bahamas, the West Indies, Belgium, Italy, Spain and the Canary Islands (Spreyer and Bucher 1998). Monk parakeets became established in New York in the United States in the 1960s through a combination of intentional and accidental releases. Concerned about the potential damage this introduced species might cause, the United States Fish and Wildlife Service implemented a removal program in the 1970s (Neidermyer and Hickey 1977). The program reduced the population by 44% and ended in 1975. Since then, monk parakeet populations in the United States have grown dramatically, with populations in Florida growing exponentially over the last 30 years (van Bael and Pruett-Jones 1996; Pruett-Jones *et al.* 2005).

Although the monk parakeet is considered an agricultural pest in South America, it has so far been associated with little agricultural damage in the United States (Bump 1971; Bucher

and Bedano 1976; Pruett-Jones and Tarvin 1998; Tillman *et al.* 2001). However, the monk parakeet causes substantial damage by its nest-building activities. The monk parakeet is the only parrot species to construct its own nest, which is a large, bulky structure of sticks and branches used both for breeding and roosting. Many of the nest structures are built in electric utility substations and on support structures for distribution and transmission lines (Bucher and Martín 1987; Marone *et al.* 1992; van Doorn 1997; Avery *et al.* 2002; Tillman *et al.* 2004). Nesting materials can cause short circuits directly by arcing power structures, and other outages occur when parakeets, or predators attracted by parakeet nesting activity, are electrocuted. In Florida, problems with monk parakeets at electric power facilities have occurred at least since the late 1980s, and the species also adversely affects the reliability of electrical services in other states, including New York, Connecticut and Texas (Avery *et al.* 2002, 2006).

Various methods of control have been used in South America and the United States, including shooting, fire, netting and

poison. These methods were largely unsuccessful in South America (Godoy 1963), and meet with public opposition in the United States. A publicly acceptable method of control is reproductive inhibition (Messmer *et al.* 1997; Stout *et al.* 1997). Monk parakeets can lay 4–12 eggs per clutch, with 6 eggs per clutch the average size (Navarro *et al.* 1992; Peris and Aramburu 1995; Spreyer and Bucher 1998). Any significant reduction in reproductive rate will likely affect population growth. Monk parakeets are an ideal species for control through reproductive inhibition as they have low dispersal rates (Martin and Bucher 1993; Spreyer and Bucher 1998), and they frequent bird feeders that potentially can be used to deliver a reproductive inhibitor.

One promising contraceptive is 20,25-diazacholesterol dihydrochloride (DiazaCon™) which prevents the conversion of desmosterol to cholesterol by inhibiting the Δ_{24} -reductase enzyme (Emmons *et al.* 1982; Yoder *et al.* 2004). DiazaCon™ was formerly registered in the United States under the trade name Ornitol to control reproduction of feral pigeons. Cholesterol is needed for the production of pregnenolone, the precursor hormone to progesterone, oestradiol and testosterone. Progesterone and oestradiol are needed for egg formation, ovulation and egg-laying; testosterone is necessary for sperm production. Reducing cholesterol reduces reproductive steroid hormone synthesis, thereby decreasing reproduction (Yoder *et al.* 2004).

Two gavage studies and a nesting study were undertaken to determine the efficacy of DiazaCon™ as a contraceptive for the monk parakeet. The purpose of the two gavage studies was to determine a minimum effective dose and the minimum length of time DiazaCon™ can be applied without reducing efficacy. The purpose of the nesting study was to quantify the effect of DiazaCon™ on egg production and hatchability in captive birds under free-feeding conditions. All experimental protocols were reviewed and approved by the National Wildlife Research Center's (NWRC) Animal Care and Use Committee, and complied with the Animal Welfare Act.

Materials and methods

All birds were trapped in southern Florida and transported to the NWRC field station in Gainesville, Florida, for testing. Parakeets were banded, weighed, and housed in groups of 10–12 in outdoor pens (9.3 × 3.1 × 1.8 m) for a minimum of six months before testing. Blood was obtained from the brachial vein for DNA analysis to determine the sex of each bird (Griffiths *et al.* 1998). Parakeets were maintained on a diet of mixed seeds, fresh fruit and lettuce.

Dose–response gavage study

Parakeets were randomly assigned to one of four treatment groups ($n = 10$ per group): (1) 0 mg kg⁻¹, (2) 50 mg kg⁻¹, (3) 75 mg kg⁻¹, or (4) 100 mg kg⁻¹. One male and one female parakeet were transferred to individual cages (45 × 45 × 45 cm) in a roofed outdoor aviary for testing. Pairs were allowed to acclimate for ≥3 weeks before treatment. DiazaCon™ was dissolved in water such that each bird received the appropriate amount of DiazaCon™ in 1 mL of water. Control birds were gavaged with water only. Birds were gavaged once a day for 10 consecutive days using a rounded 20-gauge stainless steel gavage needle.

Blood (1 mL) was drawn from the jugular vein on Days 0, 7, 14, 28, 42, 70 and 98, and birds were weighed at the time of blood collection. Plasma was analysed for desmosterol and cholesterol concentrations by high-performance liquid chromatography (HPLC) using the method of Johnston *et al.* (2003). At the end of the study, birds were euthanised, and the testes and ovaries were removed and weighed (wet weight). Only testes from the control and 100 mg kg⁻¹ groups were weighed.

Exposure period gavage study

Parakeets were randomly assigned to one of three treatment groups ($n = 10$ per group): (1) 0 mg kg⁻¹, (2) 50 mg kg⁻¹ for five consecutive days, or (3) 50 mg kg⁻¹ for 10 consecutive days. For testing, parakeets were transferred from the communal pen to individual cages (45 × 45 × 45 cm) in a roofed outdoor aviary. DiazaCon™ was dissolved in water such that each bird received the appropriate amount of DiazaCon™ in 1 mL of water. Birds were gavaged once a day according to the treatment group to which they were assigned using a rounded 20-gauge stainless steel gavage needle. Control birds were gavaged once a day for 10 consecutive days with water only.

Blood (1 mL) was drawn from the jugular vein on Days 0, 7, 14, 21 and 77, and each bird was weighed at the time of blood collection. Plasma was analysed by HPLC for desmosterol and cholesterol concentrations (Johnston *et al.* 2003). A small portion of the pretreatment blood sample was used for DNA analysis to determine the sex of each bird (Griffiths *et al.* 1998).

Nesting study

Pairs were housed in groups of 10–12 in outdoor pens (9.3 × 3.1 × 1.8 m), and we identified pairs of birds based on their propensity to perch near one another and allopreen. Pairs were randomly assigned to either a control group or a DiazaCon™ group and transferred from the communal pens to individual cages (1.8 × 1.2 × 1.2 m) in a roofed outdoor aviary for testing. Each cage had a nesting platform (30 × 30 cm), and short sticks and branches were supplied *ad libitum* as nesting material. Pairs were allowed to acclimate for ≥3 weeks before treatment.

Bait was prepared by dissolving DiazaCon™ in water, mixing the solution with hulled sunflower seeds, and allowing the mixture to dry overnight. The final mixture of seeds contained 0.05% DiazaCon™ (w/w). The target dose was 50 mg kg⁻¹ and was based on each bird weighing 110 g, on average, and consuming 11 g of seed per day. Each pair was offered 30 g of sunflower seeds for five consecutive days. Seed was placed in a small food cup, which was placed on a larger pan to facilitate weighing back of uneaten seed to determine consumption and to estimate the actual dose of DiazaCon™ received.

Nest building and nest status were observed daily until the first egg was laid. Thereafter, nests were checked weekly to determine the number of eggs laid and/or hatched. The study was terminated in mid-August when it was determined that the birds were no longer reproducing.

Statistical analysis

All data except egg production, number of chicks hatched, and gonad weights were analysed as a mixed-effects model (PROC MIXED: SAS Institute Inc. 2003). Data were analysed for treatment, week, and treatment-by-week interaction effects. Where

appropriate, data were also analysed for differences between sexes. Egg production, number of chicks hatched, and gonad weights were analysed using Wilcoxon's rank sum test.

Results

Dose-response gavage study

Desmosterol and cholesterol concentrations varied by treatment ($P < 0.0001$) and week ($P < 0.0001$), and there was a significant treatment-by-week interaction ($P < 0.0001$). Desmosterol concentrations increased (Fig. 1), and cholesterol concentrations decreased (Fig. 2) in the treated groups compared with the control group. Cholesterol concentrations remained markedly suppressed 12 weeks after treatment. There were no differences among DiazaCon™ groups with respect to cholesterol suppression. Desmosterol and cholesterol concentrations did not vary between sexes ($P = 0.3513$ and 0.5998 , respectively). The overall means of desmosterol concentrations, excluding the pretreatment period, were 21.4 ± 6.4 (mean \pm s.e.m; $n = 60$), 739.5 ± 68.5 ($n = 51$), 637.0 ± 56.7 ($n = 47$) and 703.8 ± 45.3 ($n = 55$) $\mu\text{g mL}^{-1}$ in the 0, 50, 75 and 100 mg kg^{-1} groups, respectively. The overall means of cholesterol concentrations, excluding the pretreatment period, were 809.7 ± 45.3 ($n = 60$), 328.0 ± 32.6 ($n = 51$), 371.7 ± 42.7 ($n = 47$) and 310.5 ± 36.8 ($n = 55$) $\mu\text{g mL}^{-1}$ in the 0, 50, 75 and 100 mg kg^{-1} groups, respectively.

Testicular and ovarian weights did not vary by treatment ($P = 0.4498$ and 0.2385 , respectively). Mean testicular weights were 0.03 ± 0.01 g in both the control and 100 mg kg^{-1} treatment group ($n = 6$ and 3, respectively). Mean ovarian weights were 0.05 ± 0.02 ($n = 3$), 0.08 ± 0.01 ($n = 5$), 0.08 ± 0.00 ($n = 2$) and 0.06 ± 0.01 g ($n = 6$) in the 0, 50, 75 and 100 mg kg^{-1} groups, respectively. During the study, a total of 10 birds died (all males); four birds died in both the 50 mg kg^{-1} and 75 mg kg^{-1} groups, and two birds died in the 100 mg kg^{-1} group. One male in the 75 mg kg^{-1} group died on Day 10 of dosing, likely a result of aspiration of the gavage material. The remainder of the birds died 10–34 days after treatment. No mortality was observed in the control group.

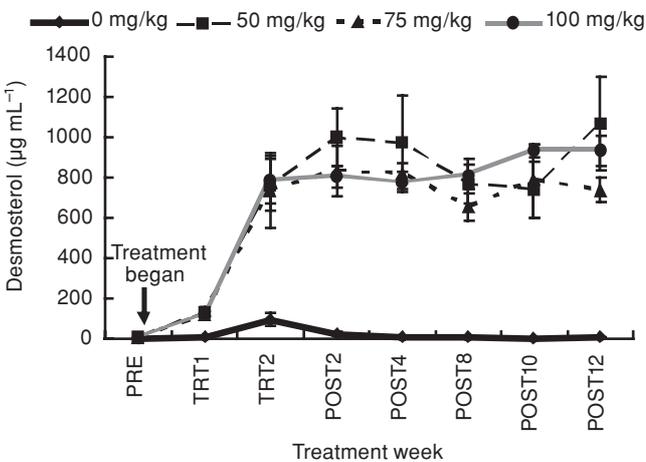


Fig. 1. Desmosterol concentrations in plasma of monk parakeets (*Myiopsitta monachus*) treated with either 0, 50, 75 or 100 mg DiazaCon™ (kg bodyweight)⁻¹ once per day for 10 days. Error bars represent the standard error of the mean.

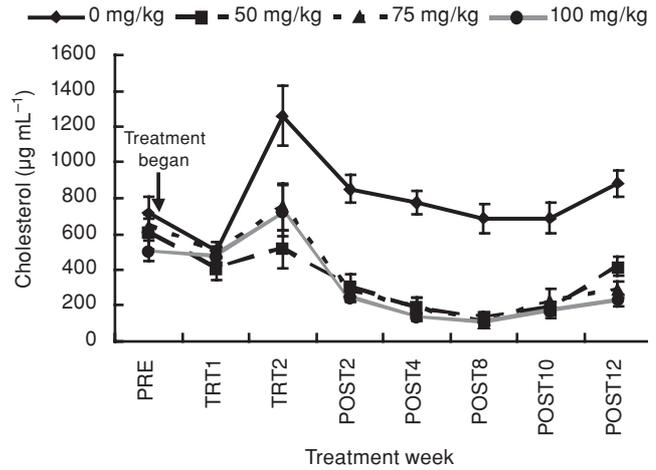


Fig. 2. Cholesterol concentrations in plasma of monk parakeets (*Myiopsitta monachus*) treated with either 0, 50, 75 or 100 mg DiazaCon™ (kg bodyweight)⁻¹ once per day for 10 days. Error bars represent the standard error of the mean.

Exposure period gavage study

Desmosterol and cholesterol concentrations varied by treatment ($P < 0.0001$ and 0.0032 , respectively) and week ($P < 0.0001$), and there was a significant treatment-by-week interaction ($P < 0.0001$). Desmosterol concentrations increased (Fig. 3), and cholesterol concentrations decreased (Fig. 4) in the treated groups compared with the control group. Cholesterol concentrations remained suppressed 11 weeks after treatment. There was no difference between DiazaCon™ groups with respect to cholesterol suppression. Desmosterol and cholesterol concentrations did not vary between sexes ($P = 0.9394$ and 0.9119 , respectively). The overall means of desmosterol concentrations, excluding the pretreatment period, were 5.0 ± 1.9 ($n = 40$), 542.8 ± 42.6 ($n = 37$) and 535.0 ± 59.5 ($n = 36$) $\mu\text{g mL}^{-1}$ in the control, 5-day and 10-day treatment groups, respectively. The overall means of cholesterol concentrations, excluding the pretreatment period, were 867.4 ± 37.9 ($n = 40$), 542.3 ± 41.9 ($n = 37$) and 508.4 ± 43.8 ($n = 36$) $\mu\text{g mL}^{-1}$ in the control, 5-day and 10-day

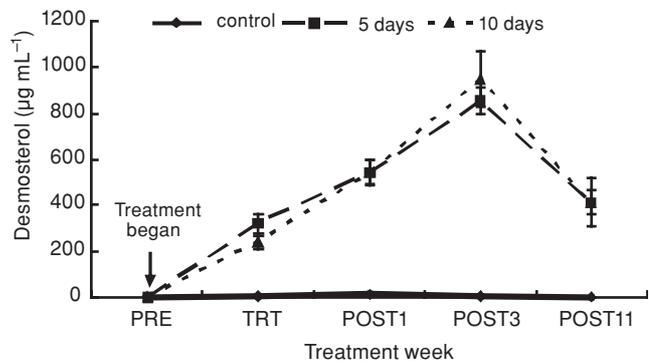


Fig. 3. Desmosterol concentrations in plasma of monk parakeets (*Myiopsitta monachus*) treated with 50 mg DiazaCon™ (kg bodyweight)⁻¹ for 5 or 10 consecutive days, or 0 mg DiazaCon™ (kg bodyweight)⁻¹ for 10 consecutive days. Error bars represent the standard error of the mean.

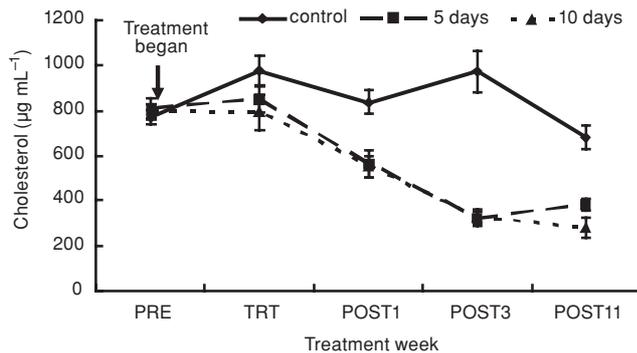


Fig. 4. Cholesterol concentrations in plasma of monk parakeets (*Myiopsitta monachus*) treated with 50 mg DiazaCon™ (kg bodyweight)⁻¹ for 5 or 10 consecutive days, or 0 mg DiazaCon™ (kg bodyweight)⁻¹ for 10 consecutive days. Error bars represent the standard error of the mean.

treatment groups, respectively. During the trial, three birds (two males, one female) died in each of the treatment groups ≥ 14 days after treatment, whereas no mortality was observed in the control group. Parakeets in the 5-day treatment group died 26–42 days after treatment, whereas birds in the 10-day treatment group died 14–28 days after treatment.

Nesting study

No eggs hatched in the treated group ($n = 10$ clutches) compared with 1.1 ± 0.6 eggs per clutch ($n = 10$ clutches) in the control group ($P = 0.0470$). Control birds averaged 3.9 ± 1.1 eggs per clutch ($n = 10$ clutches) compared with 1.6 ± 0.7 eggs per clutch ($n = 10$ clutches) for treated birds ($P = 0.0644$). The amount of seed consumed varied among days ($P < 0.0001$), but not between groups ($P = 0.2794$). Feed consumption was lowest on the first day, averaging 11.0 ± 1.0 g per pair ($n = 20$), compared with 17.0 ± 0.9 g per pair ($n = 80$) on the remaining four days. The average daily DiazaCon™ dose per pair was 33.7 ± 2.2 mg (kg bodyweight)⁻¹ ($n = 50$), or 16.8 ± 1.1 mg (kg bodyweight)⁻¹ bodyweight per bird. The average daily dose was calculated based on food consumption per pair and an assumed average pair weight of 220 g. No birds died during the nesting trial.

Discussion

In the first gavage trial, DiazaCon™ decreased plasma cholesterol concentrations ~50% by the second week of treatment in all treatment groups. Cholesterol was still suppressed $\geq 50\%$ at 12 weeks after treatment, although plasma concentrations were beginning to increase. Desmosterol concentrations remained elevated and did not appear to be decreasing at 12 weeks after treatment. These results show DiazaCon™ can suppress cholesterol for the length of a breeding season, thereby reducing reproduction.

In the second gavage trial, DiazaCon™ decreased plasma cholesterol concentrations by 67% in both treatment groups by the third week after treatment. By 11 weeks after treatment, cholesterol concentrations were still suppressed by 60% in the group receiving DiazaCon™ for 10 days, compared with 44% in the group receiving DiazaCon™ for 5 days. Desmosterol concentrations were decreasing by 11 weeks after treatment in

both treatment groups. These results indicate that feeding DiazaCon™ for 5–10 days reduces plasma cholesterol sufficiently to affect reproduction for the length of a breeding season, and that the effects are reversible.

These results are consistent with the results of a previous study (Yoder *et al.* 2004), and indicate that DiazaCon™ blocks the conversion of desmosterol to cholesterol (Dieter and Scallen 1969; Emmons *et al.* 1982). Experiments to test the effect of DiazaCon™ on the Δ^{24} -reductase enzyme, the enzyme responsible for the conversion of desmosterol to cholesterol, have yet to be conducted.

Plasma and faecal concentrations of testosterone, progesterone and oestradiol were evaluated using radioimmunoassay in this study. However, the results are not reported here because a component present in treated birds interfered with the assay. Both desmosterol and DiazaCon™ interfered with the assay used when tested. Because DiazaCon™ elevates desmosterol concentrations this may be one possible source of cross-reactivity. In addition, DiazaCon™ metabolites may also cross-react with the antibodies used in the radioimmunoassay kit.

DiazaCon™ can be associated with adverse health effects and mortality (Sturtevant and Wentworth 1970; Yoder *et al.* 2004). In particular, DiazaCon™ is associated with myotonia and has been used to experimentally induce myotonia for research. Studies show desmosterol accumulates in muscle membranes (Ramsey *et al.* 1978; Chalikian and Barchi 1982a; Reddy *et al.* 1982), and erythrocyte membranes (Butterfield and Watson 1977; Ashraf *et al.* 1984). Desmosterol may increase membrane fluidity, thus impairing cell function (Ashraf *et al.* 1984). Changes in fluidity can affect membrane proteins and enzymes, which in turn can affect cellular function (Langdon *et al.* 1977; Chalikian and Barchi 1982b). Cataracts and decreased renal concentrating capacity were observed in rats (Peter *et al.* 1973), and changes were observed in adrenocortical cells of Syrian hamsters (Yates *et al.* 1968). Toxicity likely is a result of the cellular changes associated with changes in cell membrane structure, with severity of effects being dependent on the magnitude of the changes.

Mortality was associated with DiazaCon™ in pigeons (Sturtevant and Wentworth 1970), quail (Powell 1966; Yoder *et al.* 2004) and house sparrows (Sanders and Elder 1976). Pigeons exhibited ruffled feathers, crouching, shivering and increased liver weights, liver transaminase activity, and white blood cell counts (Sturtevant and Wentworth 1970). Quail exhibited weight loss, listlessness, difficulty breathing, and loss of muscle control (Yoder *et al.* 2004).

In our study, mortality occurred in each of the gavage trials but not in the nesting trial when birds were allowed to feed freely. During the gavage studies, parakeets exhibited difficulty breathing, generalised weakness and listlessness. Necropsies performed at the University of Florida veterinary school in Gainesville revealed no significant abnormalities. Although toxicity may be greater when DiazaCon™ is given as a bolus dose rather than being spread out over the day, the target dose in the field should be less than 50 mg kg⁻¹. Birds fed treated seed at a target dose of 50 mg kg⁻¹ consumed only enough seed to receive a 17 mg kg⁻¹ dose, and experienced no mortality, indicating that this was a safe dose.

Mortality during the two gavage trials was predominantly among males. In the salt form, DiazaCon™ is water soluble, but once it has been absorbed in the gut it becomes water insoluble. Because DiazaCon™ is lipophilic, females in a reproductive state may be less susceptible to toxic effects. In reproductive females most lipoproteins are targeted primarily to the egg, whereas in males and non-breeding females lipoproteins are targeted to cells. Any lipophilic compounds ingested by breeding females are therefore likely to end up sequestered in the egg yolk rather than cells, potentially minimising harmful effects to the female. The major component of egg yolk is very low-density lipoprotein, which is produced in the liver in response to oestradiol stimulation. Some DiazaCon™ may be preferentially deposited in the very low-density lipoprotein particles rather than accumulating in the liver, leading to a lower risk of toxicity in females during the breeding season. In non-laying birds, DiazaCon™ will accumulate in the liver to a greater degree, and thus has the potential for greater toxicity.

A DiazaCon™ dose approximately one-third less than the target dose of 50 mg kg⁻¹ effectively completely inhibited reproduction in the nesting study, although treated birds still laid eggs. This was likely owing to the timing of treatment as egg laying began shortly after treatment started. Treating birds 2–3 weeks earlier would likely cause complete cessation of egg production.

DiazaCon™ is a promising contraceptive tool for monk parakeets, and is associated with no ill health effects at a dose less than 50 mg kg⁻¹. In addition, it needs to be fed for only 5–10 days to affect reproduction for the length of a breeding season. The effects are reversible, which allows management flexibility. Used as part of an integrated management program, DiazaCon™ could stem the rapid growth of monk parakeet populations, thereby reducing the adverse impacts caused by this exotic species.

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