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8-1-2021

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Olivera, Lourdes; Pereyra, Silvia; Banchemo, Georgget; Tellechea, Guillermo; Sawchik, Jorge; Avery, Michael L.; and Rodríguez, Ethel, "Nicarbazin as an oral contraceptive in eared doves" (2021). *USDA Wildlife Services - Staff Publications*. 2486.

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## Review

## Nicarbazin as an oral contraceptive in eared doves

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## ARTICLE INFO

## Keywords:

*Zenaidura macroura*  
Reproductive control

## ABSTRACT

Eared doves (*Zenaidura macroura*) are responsible for substantial losses in cereal and oil crops as well as in dairy and feedlot production in the southern cone of South America. Various strategies have been shown to be effective in reducing damage at the farm scale, but in some scenarios, it is necessary to also incorporate population control methods due to excessive bird population size. An alternative approach to reduce pest bird populations is the use of contraceptive methods, minimizing the impact on the environment and non-target populations. Nicarbazin is registered in the United States as a contraceptive for *Branta canadensis* and *Columba livia*. The aim of this study was to measure the effect of nicarbazin on the reproductive performance of eared doves in captivity. This study included eleven caged pairs of nesting eared doves in three experimental phases (pre-treatment, treatment, recovery). Each pair was exposed to nicarbazin bait for 4 h per day. The contraceptive used was OvoControlP® (0.5% nicarbazin) ground with a millstone into particles of 0.5–3.0 mm. Daily bait consumption and reproductive variables per pair (egg laying and 14-day-old fledgling) were recorded, and levels of 4,4'-dinitrocarbanilide were measured in feces and unhatched eggs. Median consumption was 4.2 g of bait/pair/day. We observed a 62% reduction in the number of viable eggs and successful nestlings in the treatment phase in contrast to pre-treatment ( $V = 36$ ;  $p = 0.006$ ). There were no significant differences ( $V = 0$ ;  $p = 1$ ) in the number of viable eggs between the pretreatment and recovery phases. Median daily bait consumption by pairs producing zero or one nestling (4.4 and 5.0 g/pair/day respectively) was significantly higher than that of pairs that had two nestlings (3.4 g/pair) during the treatment phase ( $t = 2.0$ ;  $p = 0.002$ ). Nicarbazin was effective in reducing reproductive performance of eared doves, and its effect was reversible when the treatment finished.

## 1. Introduction

Wild birds cause economic damage to agriculture crops throughout the world. Resources impacted include aquaculture (Otieno, 2019), fruit and berry crops (Tracey et al., 2007), seeds and grain crops (Klosterman et al., 2011; De Mey and Demont, 2012) and livestock feed (Carlson et al., 2018).

Farmers use various methods to reduce bird damage, including modified agronomic practices, bird-resistant varieties (Linz et al., 2015), auditory and visual scare techniques (Bishop et al., 2003) and chemical repellents (Werner and Avery, 2017). Such non-lethal techniques applied properly may be effective in reducing damage on a farm scale. However, in some situations the depredating bird population is so great that it is necessary to complement non-lethal control with population

management practices as part of an integrated management strategy (Avery, 2014).

Lethal control is often practiced to reduce pest bird populations, but this approach has been found to be costly, ineffective and detrimental to the environment, especially to non-target species (Linz et al., 2015). An alternative approach to reduce pest birds populations would be the use of contraceptive methods that minimize the impact on the environment and on non-target populations (Avery, 2014). Field applications of nicarbazin baits will address factors such as location, timing, duration, and amount of bait deployed to reduce likelihood of exposure of non-target species to the contraceptive.

In several South American countries, eared doves (*Zenaidura macroura*) cause damage to cereal and oil-production crops (Dardanelli et al., 2016; Rodríguez et al., 2011; Bucher and Ranvaud, 2006; Robles et al.,

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<https://doi.org/10.1016/j.cropro.2021.105643>

Received 22 December 2020; Received in revised form 23 March 2021; Accepted 27 March 2021

Available online 1 April 2021

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2003; Bruggers et al., 1998) and food losses in dairies and feedlots. For example, in Uruguay losses up to 49% have been documented along crop edges in soybean fields (Bou et al., 2016). In Argentina, losses range from 5% to 40% in sunflower (Vitti and Zuil, 2012) and up to 16% in soybean crops (Scalora et al., 2013). Furthermore, eared doves consume as much as 22 g of cattle ration/bird/day in Uruguayan cattle feedlot systems (Olivera et al., in press). Consequently, eared doves represent a major constraint for agricultural producers in Uruguay and elsewhere in the region.

In Uruguay, control strategies have been focused on preventing damage and protecting crops at the farm level, such as chemical and physical repellency methods (Rodríguez et al., 1995, 2011). Spraying problems and high cost/benefit ratios (Olivera et al., 2020) limit the use of these methods. Also, effectiveness is affected by timing and regularity of application, availability of alternative food for birds, and bird population size (Avery, 2014; Rodríguez et al., 2011). Additionally, agricultural expansion in recent decades has caused increased conflicts with depredating birds, especially eared doves (Tellechea and Rodríguez, 2016) which has reduced the possibility of effectively controlling bird damage with standard crop prevention and protection techniques. In such cases, crop protection strategies might require a population reduction component (Avery, 2014). New alternatives should be explored given the economic consequences of avian damage and the limitations to its control.

Although contraceptives have been evaluated for population management in bird species such as monk parakeet *Myiopsitta monachus* (Rodríguez and Tiscornia, 2002; Yoder et al., 2007), rock pigeon *Columba livia* (Albonetti et al., 2015; Avery et al., 2008), Canada geese *Branta canadensis* (Bynum et al., 2007) and quail *Coturnix coturnix* (Yoder et al., 2004), their effectiveness has not been investigated in eared dove.

Avian contraceptive research has focused on two compounds: 20,25-diazacholesterol (DiazaCon®) and nicarbazin (OvoControl®) (Yoder and Miller, 2006; Kirkpatrick and Turner, 1985). Nicarbazin was registered in the United States in 2005 as OvoControlG® for Canada geese and OvoControlP® for rock pigeon in 2007 (Fagerstone et al., 2008). The compound is a bimodal salt consisting of two components: 4, 4'-dinitrocarbanilide (DNC) (active ingredient) and hydroxy-4, 6-dimethylpyrimidine (HDP, adjuvant). The adjuvant is added to increase DNC absorption at the intestinal level (Cuckler et al., 1955). Nicarbazin acts inside the eggs and increases the permeability of the yolk membrane causing the yolk and the albumin to mix, interrupting embryo development (Yoder et al., 2006a). When nicarbazin was provided to rock pigeons, the number of chicks was reduced by 59% and in the case of Canada geese eggs hatching decreased by 56% (Bynum et al., 2007; Avery et al., 2008).

In a previous assessment of the possible role for chemosterilants in integrated pest bird management programs, Feare (1991) concluded that the contraceptive method was not effective in reducing immediate damage. We agree that contraception should not be viewed as a short-term solution to bird damage in crops. Instead, it is best viewed as a method for long-term population reduction which might be an effective tool as part of a multi-year integrated approach to bird damage management in Uruguay (Avery, 2014).

Recent progress in the USA and Europe on avian contraceptives (Albonetti et al., 2015; Fagerstone et al., 2010; Avery et al., 2008) and the need to add new strategies to mitigate damage caused by eared doves prompted this study. Our aim was to measure the effect of nicarbazin consumption on the reproductive performance of eared doves in captivity. Our hypotheses were that (1) nicarbazin would reduce the numbers of viable eggs and successful nestlings (14 days after hatching) relative to reproductive performance without treatment; (2) since the nicarbazin ingested by birds is transported through blood and deposited in the eggs, it would be detected in unhatched eggs; and (3) the effect of the contraceptive is reversible, so after discontinuing nicarbazin consumption the reproductive variables would be restored to

pre-treatment levels.

## 2. Materials and methods

### 2.1. Description of aviaries and cages

Experiments were conducted from April 2019 to July 2020 in two aviaries at the Ministry of Livestock, Agriculture and Fisheries (MGAP), Montevideo, Uruguay.

A quarantine aviary, with natural light conditions and controlled temperature (from 18 °C to 25 °C) was used as the first step with the aim of achieving a standard optimal physical condition in all doves and to prevent diseases. It had 22 individual cages (40.5 cm long, 23.5 cm high, 24.0 cm deep) made of steel wire mesh 1.5 by 13.0 cm in section. Each cage had two external metal feeders, a drinker, and a perch stick.

The experimental aviary, used during the second phase, also had natural light and controlled temperature (from 12 °C to 32 °C). It comprised 26 cages (1.20 m long, 1.20 m high, 0.85 m deep) made of braided netting of 1 cm × 1 cm section and steel wire floor. Each cage was provided with branches, pine needles and three types of artificial nests: platform (metal netting), plastic open (cup-shaped) and plastic closed nest (simulating a hollow). Materials for nest construction were offered daily.

### 2.2. Birds

A total of 42 adult doves were caught using two “walk in” funnel bird traps (1 m × 1 m side x 0.15 m high steel wire mesh) in an industrial dairy farm located in Durazno, Uruguay (33°20'27.58"S, 56°33'50.40"W). Birds were transported to the quarantine aviary and maintained there for 22 days.

The health routine at the quarantine phase included providing water with the following drugs: sulfametazine and sulfaquinoxaline sodium 2.0 mL.L<sup>-1</sup> for six days (Nitro Sulfa Aviar, Laboratorios Sur, Montevideo, Uruguay); metronidazole 4.6 g.L<sup>-1</sup> for five days (formulated by veterinary “Garibaldi”, Montevideo, Uruguay); piperazine adipato 4.0 g.L<sup>-1</sup> one day (Piper Vetcross, Portinco S.A, Montevideo, Uruguay); and vitamins and amino acids 1.0 mL.L<sup>-1</sup> for two days (Promotor L, Calier, Montevideo, Uruguay). Food and water were offered *ad libitum*. Diet in this period consisted of a feed ration made of equal parts of wheat, sorghum, and cracked corn grains.

During the experiment, doves were fed a diet containing 13.33% of sorghum, 13.33% cracked corn, 13.33% wheat and 13.33% sunflower grains, each: 6.67% of foxtail-millet, 6.67% millet, 6.67% birdseed, 6.67% rapeseed, 6.67% peeled oats, 6.67% flax, 6.67% poultry pellets (Molino San José, San José, Uruguay). Magnesium biocalcium (CaCO<sub>3</sub> 96%; Mg<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> 1%; ZnSO<sub>4</sub> 1.7%; BioLab, Montevideo, Uruguay) was added to the mixed feed ration (8.75 g.Kg<sup>-1</sup>).

All procedures with birds were carried out in accordance with Uruguayan Law N°18,611 regarding the use of experimental animals. The protocols were approved by the Ethics Committee for the Use of Experimental Animals of the General Directorate of Agricultural Services (MGAP), form 01. No birds died in the course of the experiments.

### 2.3. Contraceptive bait

The product used was OvoControlP®, registered for rock pigeons in all states of United States of America (except New Hampshire), by Innolytics LLC (Rancho Mirage, United States). The formulated cylindrical granules (ca.7.0 mm long and 5.0 mm diameter) contained 0.5% nicarbazin. Pellets were cracked with a millstone (OrientSun Model N° 500 hand mill) into particles of 0.5–3.0 mm.

The amount of contraceptive bait offered was estimated based on the recommendation for rock pigeons (Avery et al., 2008). As the bait was cracked, it comprised variable particles sizes. Consequently, two additional grams of the contraceptive bait were added to the estimated dose

to anticipate that the eared doves might have preferences for some specific particle sizes (Olivera et al., in press).

#### 2.4. Variables measured

The experimental unit was the dove pair. Contraceptive consumption ( $C$ ) was estimated per pair and per day as  $C = W_{i_{cor}} - W_f$ .  $W_f$  feeder final weight.  $W_{i_{cor}}$ : feeder initial weight as a function of the variation measured in an extra feeder (ef) without access to birds. This was used to record fluctuations in weight due to loss or gain of humidity, calculated as:  $W_{i_{cor}}: (W_i * W_{f_{ef}}) / W_{i_{ef}}$ , being  $W_i$ : initial weight of the bird feeder,  $W_{i_{ef}}$ : initial extra feeder weight,  $W_{f_{ef}}$ : final extra feeder weight.

For every dove pair, the following reproductive variables were recorded: number of laid eggs, viability of each egg and number of flying 14-day-old nestlings. The amount of 4,4'-dinitrocarbanilide (DNC) was quantified in fecal matter when eggs were laid and in unhatched eggs. Samples were stored at  $-18^\circ\text{C}$ . DNC quantification was done according to Stahl and Johnston (2002) and Stahl et al. (2003).

#### 2.5. Experimentation phases

##### 2.5.1. Pairing and maintenance

Individuals were sexed following Bucher et al. (1981) and by polymerase chain reaction (PCR) amplification at Institute for Biological Research "Clemente Estable" (IIBCE) using wing feathers. Twenty-one male and female pairs were randomly arranged and their behavior was observed to determine their compatibility. In case the pair was not compatible, new pairings were arranged. In some cases, we put two females and one male together in a cage. In other cases, we connected two cages with one pair each, until suitable mates were found. Then, the extra female was removed from the cage and compatible pairs were maintained for the experiment.

Reproductive behaviors such as male vocalizations, mutual grooming, or material collection for nest construction were daily monitored. After four months in captivity, 11 pairs started their reproductive phase successfully. Four pairs laid eggs that were not viable and six pairs did not lay eggs throughout the study period.

##### 2.5.2. Pre-treatment phase

The purpose of this phase was to measure the reproductive variables of the species, represented by 21 pairs, without contraceptive effect. It began when the first egg was laid. Six days later presentation of the contraceptive started. This was to ensure that the levels of nicarbazin in the blood would be effective the next time the females ovulated. The phase ended when the flying nestlings were 14 days old. At egg laying, fecal matter produced by each pair in one day was collected for DNC analysis from pans placed under their cage.

##### 2.5.3. Treatment phase

The objective of treatment phase was to measure the effect of contraceptive consumption on reproductive variables. It started when the chicks from the previous phase (pre-treatment) were taken out of the parental cage. The contraceptive bait supply to each pair continued from the previous phase. Food was offered at 9:30 a.m. in two plastic feeders (8.5 cm in diameter and 4.5 cm high), with 8 g of contraceptive each. During a 4 hr-period the contraceptive was available without any other alternative food. At 01:30 p.m. the remaining bait was collected and weighed as was spillage (feeder final weight). Subsequently, 11 g of maintenance feed were offered. This amount was then increased to 14 g when the pre-treatment chicks were 10 days old. Additionally, an extra plastic feeder with 8 g of contraceptive was added without access to birds in order to record fluctuations in weight due to loss or gain of humidity.

The contraceptive bait supply was discontinued with the laying of at least one egg. If no eggs were laid for 21 days after the end of the pre-treatment phase, the contraceptive routine was discontinued, and the

treatment phase was ended. Fecal samples were taken for DNC analysis at egg laying or at the end of treatment phase.

If eggs from the treatment phase hatched, chicks were removed from the cage when they were 14 days old and the treatment phase ended. Non-viable eggs were removed from the nest five days after the calculated hatching date and the amount of DNC was measured.

##### 2.5.4. Recovery phase

The recovery phase began when nestlings from the previous phase were removed from the parental cage or the treatment phase was concluded. It ended when the chicks were 14 days old. Only untreated food was offered during this phase. At egg laying, fecal samples were taken for DNC analysis.

#### 2.6. Statistical analysis

The effect of the contraceptive was analyzed by calculating the median of reproductive traits and contrasting pre-treatment vs. treatment and pre-treatment vs. recovery. Wilcoxon range test was used to determine significant differences among treatments, in number of eggs and successful nestlings. Consumption median were compared with Kruskal-Wallis test, according to the number of nestlings in the treatment phase.

Associations among traits were also investigated. We were interested in the relationship between the median of daily and accumulated consumption of contraceptive and the number of successful nestlings produced. We tested linear correlations with Spearman and assessed the fit to other functions such as the second-degree polynomial. In addition, the association between DNC residues in feces and eggs, with the consumption of contraceptive was examined. In all cases, the Spearman's rank correlation coefficients were calculated.

### 3. Results

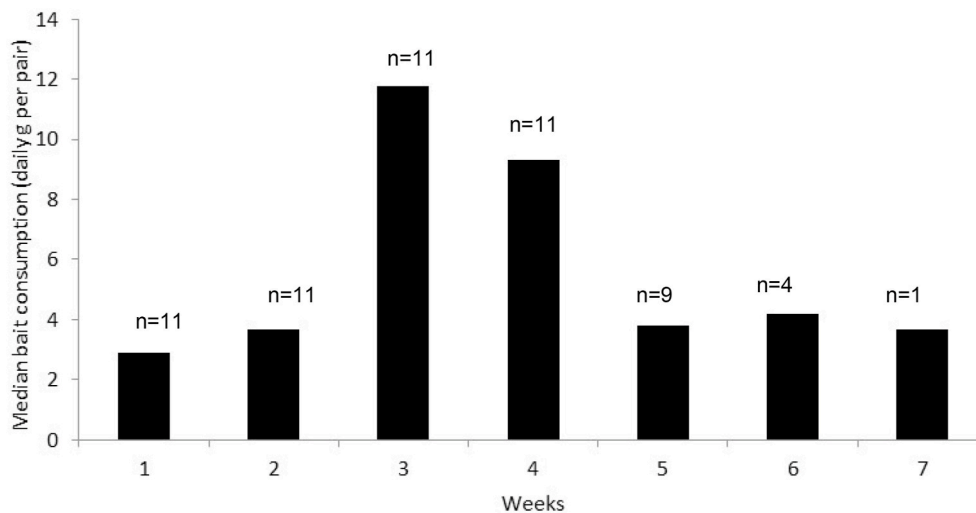
Levels of daily and accumulated consumption of nicarbazin bait varied among pairs of doves, in amount consumed and number of days the doves were exposed to the bait (Table 1). During the 7-week treatment period, median consumption of nicarbazin bait was 3.8 g/pair/day, ranging from 2.9 to 11.8 g/pair/day, with a coefficient of variation of 50.1% (Fig. 1). Since the contraceptive supply was interrupted at egg laying, the length of this period varied among pairs. Two pairs laid eggs in the fourth week after the contraceptive bait offer started. Five pairs did not lay eggs until the fifth week and two others completed their laying at the sixth week after supplying the contraceptive. Three pairs of doves did not lay eggs and therefore, the provision of contraceptive continued until the end of the treatment phase.

The number of eggs decreased marginally in the treatment phase compared to pre-treatment ( $V = 6$ ;  $p = 0.09$ ). The number of viable eggs and successful nestlings produced under treatment decreased 62% compared to pre-treatment ( $V = 36$ ;  $p = 0.006$ ) (Table 2). On the other

**Table 1**  
Median consumption of contraceptive bait.

	Accumulated consumption (g per pair)	Median consumption (daily g per pair)	Period of consumption (days)
Median	208	4.2	35
Maximum	327	5.9	43
Minimum	131	2.5	27
Coefficient of variation (%)	29.1	23.9	17.6

Eleven pairs of eared doves (*Zenaida auriculata*) were offered 16 g of contraceptive Ovocontrol® daily for 4 h in the morning with no alternative food. Accumulated consumption is the total intake of the pair during the treatment period. Median consumption was calculated considering each pair. Period of consumption is the number of days that the contraceptive was consumed.



**Fig. 1.** Median daily consumption of contraceptive Ovocontrol® bait (g per pair). Eleven pairs of eared doves (*Zenaida auriculata*) were offered 16 g of contraceptive for 4 h in the morning without another food. The number of pairs in the treatment is indicated above each bar.

**Table 2**

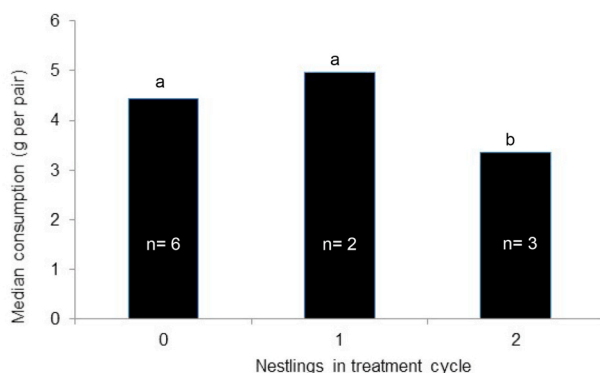
Reproductive variables of pairs of eared doves (*Zenaida auriculata*) in the experiment.

	Pre -treatment	Treatment	Recovery
Pairs (n)	11	11	11
Pairs that did not lay eggs	0	3	0
Number of eggs laid	21 <sup>a</sup>	16 <sup>a</sup>	21 <sup>a</sup>
Number of viable eggs	21 <sup>a</sup>	8 <sup>b</sup>	18 <sup>a</sup>
Number of unviable eggs	0	8	3
Number of successful nestlings	21 <sup>a</sup>	8 <sup>b</sup>	18 <sup>a</sup>

A reproductive cycle before the administration of the contraceptive Ovocontrol® (pre-treatment), during (treatment) and after its administration (recovery). Different letters in the same row indicate significant differences ( $\alpha$  0.05). Successful nestlings are those that reach 14 days of age.

hand, the number of eggs in the recovery phase did not differ from pre-treatment ( $V = 0$ ;  $p = 1$ ). Also, the number of successful nestlings decreased marginally in the recovery phase compared to pre-treatment ( $V = 6$ ;  $p = 0.07$ ).

Consumption of treated bait by pairs of doves that produced zero or one nestling was significantly higher than that observed in pairs that had two nestlings in the treatment phase (Fig. 2;  $t = 2.0$ ;  $p = 0.002$ ). There was no difference in consumption of treated bait between pairs with zero



**Fig. 2.** Median daily consumption of contraceptive Ovocontrol® bait (g per pair) and standard deviation as a function of the number of nestlings produced in the treatment phase. The number of pairs of eared doves (*Zenaida auriculata*) in each category is indicated. Different letters indicate significant differences ( $\alpha \leq 0.05$ ).

and one nestling in the treatment phase ( $p = 0.24$ ).

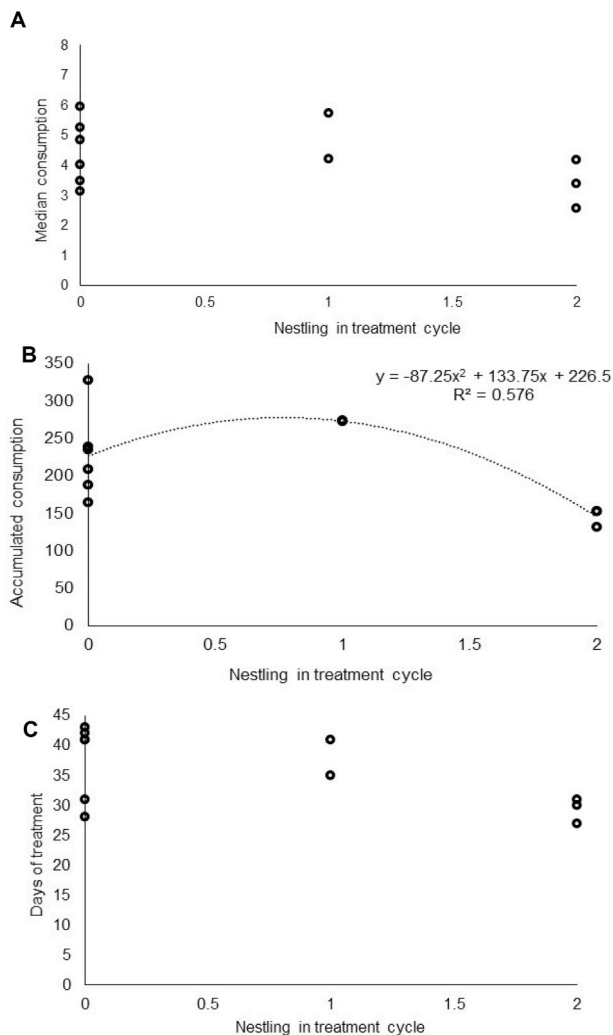
No relationship was found between successful nestlings in the treatment phase and median contraceptive consumption using either linear regression ( $\rho = -0.32$ ;  $p = 0.34$ ) or a second-degree polynomial function ( $R^2 = 0.29$ ;  $p = 0.24$ ; Fig. 3A). The number of nestlings did not have a linear association with the accumulated consumption of contraceptives ( $\rho = -0.50$ ;  $p = 0.11$ ), which was better explained with a second-degree polynomial function ( $R^2 = 0.58$ ;  $p = 0.03$ ; Fig. 3B). The length of the period (days) in which the birds consumed contraceptive showed a non-significant linear correlation with the number of successful chicks ( $\rho = -0.55$ ;  $p = 0.08$ ). However, a second-degree polynomial function was not significant ( $R^2 = 0.40$ ;  $p = 0.13$ ; Fig. 3C).

The contraceptive residues (DNC - 4,4'-dinitrocarbanilide) recovered from feces and unhatched eggs (Fig. 4) did not show a linear association with the median contraceptive consumption ( $\rho = 0.18$ ;  $p = 0.59$  for DNC in feces;  $\rho = 0.30$ ;  $p = 0.62$  for DNC detected in eggs).

#### 4. Discussion

Under our study conditions, nicarbazine reduced reproductive success in caged eared doves. The numbers of viable eggs and successful nestlings (14 days) in the treatment phase were significantly lower than those produced by the same birds in the previous phase without treatment. The magnitude of the reduction on successful nestlings in our study was similar to that described by Bynum et al. (2007) and Avery et al. (2008), who reported reductions in nestling numbers of 59% for rock pigeons and up to 56% in eggs hatching in Canada geese, respectively. Nicarbazine, used as contraceptive, has an effect on egg development at the time of onset of follicle development (Avery et al., 2008; Yoder et al., 2006a). Yoder et al. (2006a) found that nicarbazine increases the activity of lipoprotein lipase, reducing the amount of very low-density lipoprotein deposited in the follicle and consequently decreases the production of eggs and their weight.

The dose of nicarbazine that Avery et al. (2008) found to be effective for rock pigeons ranged between 90 and 120 mg  $\text{kg}^{-1}$  body mass/day. Based on the manufacturer's suggestions, we expected the dose of nicarbazine for eared dove would be approximately 1/3 of that used in rock pigeons, equivalent to 11–15 mg of nicarbazine/dove/day. This dose could be achieved by the consumption of 2–3 g/bait/dove/day. In fact, the proposed intake level was similar to the actual median consumption registered per pair, which was 4.2 g of bait/pair/day, assuming that both individuals ate similar amounts. Based on previous aviary evaluations and the feeding behavior of eared doves, we assumed that this amount of



**Fig. 3.** Associations between the numbers of successful nestlings produced in the treatment phase and contraceptive Ovocontrol® bait consumption variables for the eleven pairs of eared doves (*Zenaidia auriculata*) that ingested the product daily in the morning without any other food alternative. A- Second degree polynomial function with the median consumption per pair. B- Second degree polynomial function with the accumulated consumption of total contraceptive. C- Second degree polynomial function with the number of days that the contraceptive was consumed in the treatment phase.

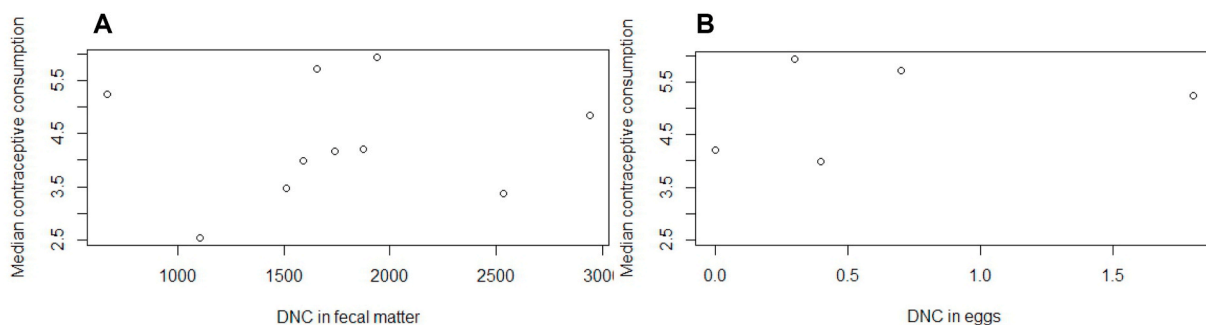
contraceptive would represent a realistic consumption in the field tests.

This study demonstrated that the accumulated consumption was the variable that best explained the contraceptive effect as measured by the number of nestlings. Cumulative consumption rose when doves increased their daily intake of nicarbazin and/or elevated the number of days that birds consumed the contraceptive. The necessary dose of nicarbazin in the blood must be ensured at the time of egg development. DNC may remain in the bird's body and might be metabolized by the liver and excreted within four days. HDP is metabolized faster and is completely eliminated in less than 24 h through the urine (Johnston et al., 2001). Therefore, nicarbazin should be ingested prior to ovulation and through continuous consumption in subsequent days (or weeks) in order to maintain the target blood concentration of nicarbazin at ovulation. In studies with chickens (*Gallus gallus*), four doses and different delivery periods were tested, the highest dose (150 ppm in feed) reduced the reproductive rate by 33% after six days of treatment and by 85% after 14 days of treatment (Johnston et al., 2001). The lowest doses (0.25 ppm in feed) tested by Johnston et al. (2001) reduced chick production by 67% after 14 days of nicarbazin treatment. In that study, DNC values measured in blood were higher at the end of the treatment. Therefore, the lowest doses that chickens consumed for 14 days were more effective than the highest doses consumed for six days. To obtain an effect on reproductive variables, it might be necessary to attain an effective dose of contraceptive consumption prior to ovulation. Thus, it is necessary that the population of birds returns repeatedly to consume the contraceptive bait. It would be informative to conduct a study with individually identified eared doves to ensure this occurs on a regular basis.

Our results showed that pairs of doves that produced one nestling consumed the same amounts of contraceptive in the treatment phase than pairs with no nestling. This fact could be related to the experimental design. Individual consumption was estimated from two birds in a cage and perhaps in some situations, the male consumed more contraceptive bait than the female. The contraceptive effect in males has not been studied as thoroughly as in females. Yoder et al. (2006a) reported a decrease in the number of sperm cells with high intracellular calcium content in male doves consuming nicarbazin compared to control males. These results indicated that the nicarbazin may act as a weak calcium channel blocker in the male dove's testis.

Contraceptive consumption by doves varied among weeks. This might be explained by the feeding of nestlings in the cage. They were in the parent cage for two weeks during pre-treatment period. Even though the intake of maintenance feed was increased, the consumption of contraceptive increased markedly in the days prior to the removal of the nestlings from the cage. The nestlings were fed by the parents and therefore, the demand for maintenance feed and contraceptive increased as the nestlings grew.

We also confirmed that the effect of nicarbazin was reversible. When



**Fig. 4.** Associations between median contraceptive Ovocontrol® consumption and residue variables of 4,4'-dinitrocarbanilide (DNC). The contraceptive was given to eleven pairs of eared doves (*Zenaidia auriculata*) which ingested the product daily in the morning hours without any other alternative food. A- Association with residues in feces at the time of laying the eggs under the effect of the contraceptive. B- Relationship with the residues recovered in the unhatched eggs of the treatment phase; when the pairs laid two nonviable eggs, they were analyzed together (n = 5 pairs, total 8 eggs).

the treatment ceased, the reproductive variables did not differ between the recovery and pre-treatment phases. Similar findings were reported by Avery et al. (2008); Yoder et al. (2006b) and Johnston et al. (2001), consistent with the high excretion rates of nicarbazin.

Nevertheless, the number of viable eggs and number of successful nestlings were lower in the recovery phase compared to the pre-treatment phase. This could be explained by an effect of the reproductive seasonality of the doves, rather than by a residual effect of the contraceptive (Maldonado et al., 2020). Although evidence has been reported that at similar latitudes, eared doves can reproduce throughout the year, greater breeding activity was observed in spring and summer (Bucher and Orueta, 1977). In addition, male testosterone plasma concentrations were analyzed throughout the year by Maldonado et al. (2020), who found two peaks in October and February and the lowest testosterone plasma concentrations between March and July. In our study, five dove pairs completed their treatment phase and started the recovery in March and April. This overlap with the season of less intense breeding activity may have contributed to the fact that three of these pairs were the ones producing an unviable egg each.

Measuring contraceptive consumption requires experiments in captivity. In our study some pairs did not reproduce normally in captivity. The percentage of pairs that laid at least one egg was 71%, and 52% produced at least one successful nestling. We found no published accounts of eared doves bred in captivity, although there are reports in *Zenaida macroura* (Burger et al., 1983) and *Zenaida asiatica* (Burkepile et al., 2002). Burger et al. (1983) does not mention the percentage of pairs that did not reproduce. In a three-year study, Burkepile et al. (2002) found a mean of 75% pairs laid at least one egg and 59% had at least one successful nestling.

We did not find an association between contraceptive residues in unhatched eggs and contraceptive consumption. The concentrations we obtained from unhatched eggs were lower than those reported by Johnston et al. (2001) and Avery et al. (2008). This difference could be explained because in some cases only the shell could be analyzed. The birds tended to remove the egg from the nest and sometimes the entire yolk could not be recovered. In addition, there were pairs that consumed contraceptive but did not produce eggs. Therefore, their data were not included in the analysis.

The association between contraceptive residues in feces and contraceptive consumption was not significant. In our study, the contraceptive consumption was variable among days and weeks, which might indicate that residues from a one-day sample of fecal matter may be a poor indicator of the average consumption of the entire period. Yoder et al. (2006b) found that ducks eliminated the active ingredient in a non-uniform way when they ingested it in a single daily dose. Therefore, the rate of excretion would depend on whether they consumed small amounts of contraceptive or a large intake in a short time. In addition, we did not collect some feces that were in inaccessible places in the cage (nest, floor). Future studies should consider plasma blood residues that might better correlate with contraceptive consumption, as reported by Avery et al. (2008). These authors used DNC from blood samples collected at the end of the treatment period after the second egg was laid. However, the critical time at which egg viability occurs might have occurred weeks before sample collection. In addition, drawing blood provides stress for the eared doves which could have negative effects on the reproductive success of the pair.

## 5. Conclusion

Nicarbazin is an effective contraceptive in caged eared doves. To achieve this goal, an intake of 4.2 g of bait/day (approximately 2 g/bird) is necessary in captivity. The use of nicarbazin baits should begin before ovulation and continue until the stage of egg development in order to maintain an effective titer in the blood. The contraceptive effect proved reversible and reproductive variables returned to pre-treatment levels when the treatment ended.

We recognize that our results are preliminary, but they provide a solid step toward development of an effective management alternative for this economically important species. Our next set of experiments will measure contraceptive bait acceptance and consumption in the field, followed by evaluation of the efficiency of the contraceptive in reducing reproductive variables in eared dove breeding colonies. Future research might also include a cost-benefit analysis (e.g., Peer et al., 2003) of contraceptive use for reducing crop losses by eared doves.

## 6. Management implications

Since dove population size is regulated by the amount of available food (Bucher, 1986), this population management measure must be accompanied by other techniques directed at reducing food availability for the birds. Practices such as closing food storage sites or protect them using a bird proof net when not used to feed livestock might aid. In our opinion, the use of an oral contraceptive would be complementary tool to the existing measures to mitigate the damage caused by this species. It should be investigated within the context of an integrated crop protection strategy, including reduction of available food, prevention measurements and protection at the farm level.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

This study was financial by Agricultural Technology Promotion Fund (grant number FPTA N° 352) in National Research Agricultural Institute (INIA). Lourdes Olivera had a doctoral fellowship from National Research and Innovation Agency (ANII- grant numbers POS-NAC\_2016\_1\_130028). We want to thank Erick Wolf from Innolytics LLC (USA) for donating the contraceptive product. We are grateful to General Directorate of Agricultural Services (MGAP) for its support in the experiments. We thank Dr. Elly Navajas for reviewing the manuscript and for her valuable contributions.

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