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# PHYSIOLOGY, ENDOCRINOLOGY, AND REPRODUCTION

## Effect of Method of Delivering Nicarbazin to Mallards on Plasma 4,4'-Dinitrocarbanilide Levels and Reproduction

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**ABSTRACT** Nicarbazin (NCZ), a coccidiostat used in the poultry industry, has been developed as a contraceptive for resident Canada geese. We tested the efficacy of NCZ as a contraceptive using mallards (*Anas platyrhynchos*) as a model for Canada geese. Nicarbazin-treated corn was fed ad libitum for 14 d at 0, 750, 1,000, or 1,500 ppm. Plasma and egg levels of 4,4'-dinitrocarbanilide (DNC), the active anticoccidial component of NCZ, differed among treatment groups in a dose-response relationship, but plasma levels did not differ between sexes. Nicarbazin caused a decrease in egg weight, but there was no effect of NCZ on the numbers of eggs laid per female per day. Nicarbazin did not significantly impact bird health. An additional trial tested the effect of the method of NCZ delivery on plasma DNC levels. Mallards were given NCZ daily for 12 d either by gavage with a

corn oil suspension, gavage with a water suspension, peroral administration of a capsule, or feeding 500 mg of NCZ/kg of pelleted feed ad libitum. The method of delivery significantly affected plasma DNC levels, with the highest levels in the corn oil suspension group and the lowest levels in the pelleted feed group. This is likely due to decreased availability of NCZ in a pellet compared with gavage with a suspension or capsule. Mallards receiving 34.2 mg of NCZ/kg of BW when fed cracked corn coated with NCZ daily for 14 d had higher plasma DNC levels than those obtained by liquid gavage, capsule, or pelleted NCZ feed. For maximum effect in the field, NCZ should be coated onto corn. A higher concentration of NCZ is needed in pelleted feed to obtain comparable plasma DNC levels to allow for the decreased absorption of DNC.

**Key words:** Canada goose, contraception, 4,4'-dinitrocarbanilide, mallard, nicarbazin

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### INTRODUCTION

Canada goose (*Branta canadensis*) populations are expanding, and nonmigratory populations are becoming more frequent in urban areas as development provides attractive, year-round habitat (Forbes, 1993; Ankney, 1996; Gosser and Conover, 1999). This causes health and safety issues in urban areas as large numbers of geese in parks and golf courses damage grass, create hazards if they become aggressive (Conover and Chasko, 1985; Forbes, 1993), and deposit large amounts of fecal matter (Conover and Chasko, 1985; Fairaizl, 1992). Fecal material may contaminate drinking water supplies (Conover and Chasko, 1985) and cause overfertilization of lakes (Manny et al., 1975). In addition, geese are cause for concern at and around airports, where bird-aircraft strikes occur, causing serious aircraft damage and potential loss of human life (Fairaizl, 1992).

Hunting is not a feasible alternative in urban areas (Conover and Chasko, 1985; Heusmann, 1999), and the use of special hunting seasons has had limited success in controlling these resident goose populations (Heusmann, 1999). There are few places that allow goose translocation, and annual roundups have met with public resistance in some areas. Current nonlethal control methods for Canada geese include egg oiling or addling (e.g., shaking the egg). However, these methods require each nest to be located, resulting in several days of intense effort each season. In areas where nests are not easily accessible or adequate personnel are unavailable, contraception may be a reasonable alternative, requiring fewer man-hours to implement. Therefore, contraception may provide an acceptable alternative to help manage goose populations at levels that minimize damage but allow for the existence of geese (Forbes, 1993; Stout et al., 1997).

Nicarbazin (NCZ) is an anticoccidial drug routinely used in the poultry industry since the 1950s to control protozoan cecal and intestinal infections by *Eimeria* species in broiler chickens. It is an equimolar complex consisting of 4,4'-dinitrocarbanilide (DNC) and 2-hydroxy-4,6-dimethylpyrimidine (HDP). The function of HDP is to increase absorption of the material in the gut, whereas

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DNC is the active coccidiostat (Cuckler et al., 1955; Rogers et al., 1983). When fed to laying hens, NCZ affects reproduction by either reducing egg hatchability or reducing egg production (Jones et al., 1990b; Hughes et al., 1991; Chapman, 1994). Although these effects are undesirable in the poultry industry, NCZ can potentially be used as a waterfowl contraceptive.

In a previous study using mallards as a model for Canada geese, hatchability was 55% for control mallards compared with 26% for mallards treated with 34 mg of NCZ/kg of BW (Yoder et al., 2006). Because the initial testing indicated that 500 ppm (33.75 mg/kg of BW) was the minimum dose needed to affect reproduction, this study used higher doses of NCZ. Because geese in the field would be given NCZ on treated feed rather than by gavage, we chose to feed NCZ coated onto cracked corn ad libitum for this study.

Many contraceptive studies rely on initial testing using gavage procedures to determine a dose level for further testing. Therefore, we wanted to determine how plasma DNC levels compared when NCZ is given either as a bolus dose or fed continuously throughout the day. We monitored plasma DNC levels for mallards given NCZ either by liquid gavage, capsule, or in the daily feed ration.

The objectives of this study were to determine plasma and egg DNC levels when NCZ was fed ad libitum coated onto cracked corn and whether plasma DNC levels differed between male and female mallards. Further objectives included assessing the effect of NCZ on reproduction by monitoring egg production and hatchability. We also wanted to determine whether NCZ negatively affected general health by monitoring bird weight, hematocrit, and hemoglobin.

## MATERIALS AND METHODS

### *Comparison of Delivery Methods*

The experimental protocol was reviewed by the Colorado State University and National Wildlife Research Center's Animal Care and Use Committees and complied with the Animal Welfare Act. The experiment consisted of 4 treatment groups as follows: NCZ in capsules, 2 females and 4 males; NCZ suspended in corn oil, 2 females and 5 males; NCZ suspended in water, 3 females and 4 males; and NCZ in feed, 3 females and 4 males. Mallards (Whistling Wings Inc., Hanover, IL) were randomly assigned to treatment groups and cages and were housed individually. Males were included to determine whether NCZ absorption differed between sexes. All birds were approximately 52 wk of age at the beginning of the study, and a 17L:7D light cycle was maintained throughout. Mallards were maintained on a game bird layer diet (Purina Mills Inc., St. Louis, MO) that included 3.25 to 4.25% Ca, 0.5% P, and 16% CP.

All treatment groups received 34 mg of NCZ/kg of BW daily for 12 d. Capsules were loaded with 25% NCZ on wheat middlings (Phibro Animal Health Inc., Fairfield,

NJ), such that each capsule contained 34 mg of pure NCZ. Corn oil and water suspensions were made by vortexing 25% NCZ on wheat middlings in the appropriate medium to achieve an even suspension, such that 2 mL of suspension contained 34 mg of pure NCZ. Mallards in the pelleted feed group received a daily ration of 68 g of pelleted feed treated at 500 mg of NCZ/kg of feed.

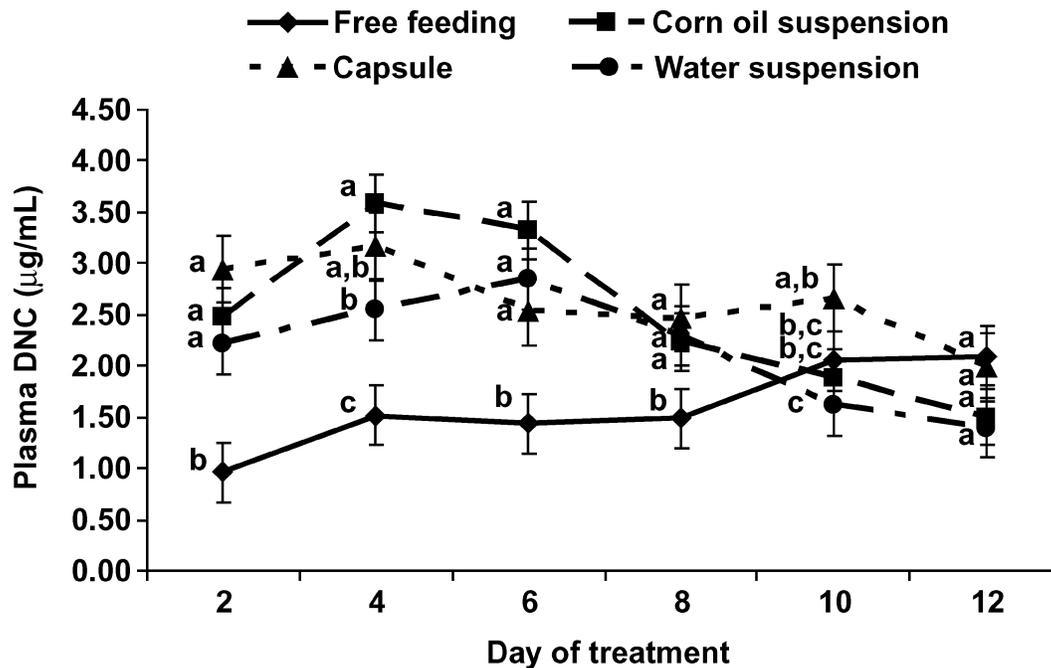
A total of 3 mL of blood was obtained from the brachial vein on d 2, 4, 6, 8, 10, and 12 of treatment. Blood was centrifuged, and the plasma was stored at  $-70^{\circ}\text{C}$  until analysis of DNC concentration using HPLC (Primus et al., 2001).

### *Reproductive Trial*

The experiment consisted of 4 female and 2 male treatment groups as follows: 0 ppm of NCZ (Phibro Animal Health Inc.), 12 females; 0 ppm of NCZ, 5 males; 750 ppm of NCZ, 12 females; 1,000 ppm of NCZ, 13 females; 1,000 ppm of NCZ, 5 males; and 1,500 ppm of NCZ, 13 females. Mallards (Whistling Wings Inc.) were randomly assigned to treatment groups and cages, and were housed individually. Because males would consume treated feed in the wild, they were included to determine the effect of NCZ on male health. All birds were 30 wk of age at the beginning of the study, and a 17L:7D light cycle was maintained throughout. Mallards were maintained on a game bird layer diet (Purina Mills Inc.) that included 3.25 to 4.25% Ca, 0.5% P, and 16% CP. Untreated male mallards were placed with control and treated female mallards, and treated males were placed with untreated females 2 to 3 times each week. Pairs were observed until copulation occurred, and then males were removed.

Doses were made by overcoating cracked corn with 0, 750, 1,000, or 1,500 ppm of NCZ using 5% corn oil and 5% milk powder. Feed was formulated such that a mallard eating 65 g of feed daily would receive 50.4, 67.2, or 100.8 mg of NCZ/kg of BW in the 750, 1,000, or 1,500 ppm groups, respectively. Each mallard was offered 75 g of treated feed daily for 14 consecutive days. Any feed remaining after 24 h was collected, dried in an oven dryer at  $93.3^{\circ}\text{C}$  overnight, and weighed to determine actual dose received.

Prior to treatment, we randomly selected 7 females from each group for blood sampling. The same 7 females from each group were used for blood sampling throughout the study, and blood samples were drawn from all males. A total of 3 mL of blood was obtained from the brachial vein once pretreatment; on d 4, 7, 10, and 14 of treatment; and on d 2 and 5 posttreatment. Two microhematocrit tubes per blood sample were filled and analyzed for hematocrit (Dein, 1986). A 20- $\mu\text{L}$  subsample of blood was analyzed for hemoglobin content using a cyanmethemoglobin method (525-A, Sigma Chemical Co., St. Louis, MO). The remainder of the blood was centrifuged, and the plasma was stored at  $-70^{\circ}\text{C}$  until analysis of DNC concentration using HPLC (Primus et al., 2001). All birds were weighed at the time of bleeding.



**Figure 1.** Plasma 4,4'-dinitrocarbanilide (DNC) levels of mallards given 500 ppm (34 mg/kg of BW) nicarbazin either by gavage in a corn oil suspension, gavage in a water suspension, peroral administration of a capsule, or treated pellets fed ad libitum daily for 12 d. Means within the same time period with different superscripts are significantly different. Means are from the LSMEANS option in PROC MIXED, and means separations were carried out using PDMIX800.

Egg production and egg weight were monitored daily. For birds included in plasma DNC analysis, eggs laid the day of blood collection were also analyzed for DNC levels. Eggs for DNC analysis were broken open, and the degree of mottling was assessed. Mottling was assessed using the following scale: 0 = no mottling, 1 = mild mottling, 2 = moderate mottling, and 3 = severe mottling. The shells were removed, and the remainder of the egg was homogenized using a handheld blender (Cuisinart, East Windsor, NJ) and was analyzed for DNC content by HPLC (Johnston et al., 2002). Because egg production was severely reduced in all groups during treatment, no eggs were incubated.

### Statistical Analyses

**Delivery Methods.** Data were analyzed as a mixed effects model (PROC MIXED; SAS Institute Inc., 2003), and significance was defined as  $P \leq 0.05$ . Data were analyzed for sex, delivery method, treatment day, and all interaction effects. Means separations were carried out using PDMIX800 (Saxton, 1998).

**Reproductive Trial.** We divided the study into 5 periods among groups for all statistical analyses as follows: pretreatment (1 to 14 d), treatment 1 (1 to 7 d), treatment 2 (8 to 14 d), posttreatment 1 (d 2 posttreatment), and posttreatment 2 (d 5 posttreatment). All data were analyzed as a mixed effects model (PROC MIXED; SAS Institute Inc., 2003), and significance was defined as  $P \leq 0.05$  for all analyses. Data were analyzed for sex, treatment, period, and all interaction effects. Means separations were carried out using PDMIX800 (Saxton, 1998). Food con-

sumption decreased significantly in all groups including the control group during the treatment period, resulting in a severe decline in egg production. Because we had no data for the treatment periods for egg mottling scores, mottling data were not analyzed. Correlations were determined between plasma and egg DNC levels and also among plasma or egg DNC levels and numbers of eggs laid, egg weight, BW, hemoglobin, and hematocrit.

## RESULTS

### Delivery Methods

Plasma DNC levels did not differ between sexes ( $P = 0.9283$ ) but differed among delivery methods and treatment days, and a significant method-by-day interaction existed (Figure 1). Peak DNC levels were  $3.58 \pm 0.28$ ,  $3.17 \pm 0.33$ ,  $2.85 \pm 0.29$ ,  $2.10 \pm 0.29$  µg/mL in the corn oil suspension, capsule, water suspension, and pelleted feed groups, respectively. Males received an average dose of  $25.8 \pm 1.8$  mg/kg of BW compared with an average dose of  $34.3 \pm 2.1$  mg/kg of BW for females ( $P = 0.0031$ ) in the pelleted feed group.

### Reproductive Trial

Plasma and egg DNC levels differed among treatment groups and periods, and a significant treatment-by-period interaction existed (Table 1). There were no differences in plasma DNC levels between male and female mallards ( $P = 0.5854$ ). Peak plasma DNC levels were  $2.69 \pm 0.41$ ,  $3.64 \pm 0.32$ , and  $5.38 \pm 0.43$  µg/mL in the 750,

**Table 1.** Plasma and egg 4,4'-dinitrocarbanilide (DNC) levels across sexes for mallards fed cracked corn treated with nicarbazin at 0, 750 (30.8 mg/kg of BW), 1,000 (34.2 mg/kg of BW), or 1,500 ppm (49.1 mg/kg of BW) ad libitum daily for 14 d

Group (ppm)	Period <sup>1</sup>	Plasma DNC (µg/mL)			Egg DNC (µg/g)		
		n	Mean	SE	n	Mean	SE
0	PRE	12	0.00 <sup>abc</sup>	0.39	5	0.00 <sup>a</sup>	1.98
	TRT1	22	0.01 <sup>b</sup>	0.32	2	0.00 <sup>abc</sup>	3.12
	TRT2	23	0.03 <sup>b</sup>	0.31	0		
	POST1	11	0.04 <sup>abcde</sup>	0.40	1	0.01 <sup>abcd</sup>	4.40
750	POST2	11	0.00 <sup>abc</sup>	0.40	3	0.08 <sup>abc</sup>	2.55
	PRE	7	0.00 <sup>abcde</sup>	0.51	3	0.00 <sup>ac</sup>	2.56
	TRT1	13	2.69 <sup>fg</sup>	0.41	2	6.88 <sup>abcd</sup>	3.13
	TRT2	14	2.35 <sup>gh</sup>	0.40	0		
1,000	POST1	7	0.76 <sup>abcde</sup>	0.51	1	10.60 <sup>bde</sup>	4.41
	POST2	7	0.05 <sup>abcde</sup>	0.51	2	8.08 <sup>bcd</sup>	3.13
	PRE	12	0.00 <sup>abc</sup>	0.39	5	0.00 <sup>a</sup>	1.98
	TRT1	21	3.64 <sup>f</sup>	0.32	0		
1,500	TRT2	24	3.54 <sup>f</sup>	0.31	1	22.80 <sup>e</sup>	4.42
	POST1	12	1.11 <sup>de</sup>	0.39	2	18.69 <sup>e</sup>	3.12
	POST2	12	0.06 <sup>abc</sup>	0.39	3	8.78 <sup>d</sup>	2.55
	PRE	7	0.00 <sup>bce</sup>	0.51	3	0.08 <sup>abc</sup>	2.55
1,500	TRT1	12	5.38 <sup>i</sup>	0.43	0		
	TRT2	12	4.85 <sup>i</sup>	0.43	0		
	POST1	6	1.29 <sup>adh</sup>	0.54	0		
	POST2	7	0.04 <sup>abcde</sup>	0.52	4	16.65 <sup>e</sup>	2.25

<sup>a-i</sup>Means within columns with different superscripts are significantly different. Means are from the LSMEANS option in PROC MIXED, and means separations were carried out using PDMIX800.

<sup>1</sup>PRE = pretreatment d 1 to 14; TRT1 = treatment d 1 to 7; TRT2 = treatment d 8 to 14; POST1 = d 2 posttreatment; POST2 = d 5 posttreatment.

1,000, and 1,500 ppm groups, respectively. Peak egg DNC levels were  $10.60 \pm 4.41$ ,  $22.80 \pm 4.42$ , and  $16.65 \mu\text{g/g}$  in the 750, 1,000, and 1,500 ppm groups, respectively. Mallards received an average dose of  $30.8 \pm 1.2$ ,  $34.2 \pm 1.3$ , and  $49.1 \pm 2.4 \text{ mg/kg}$  of BW in the 750, 1,000, and 1,500 ppm groups, respectively.

There was no effect of NCZ on the number of eggs laid per female per day ( $P = 0.8179$ ), but there was a significant period effect (Table 2), with fewer eggs laid during both treatment periods and posttreatment period 1. Egg weights differed among treatment groups and periods, and a significant treatment-by-period interaction existed (Table 3).

Bird weights differed between sexes, with males weighing more than females (Table 4). Body weights differed among periods, with weights decreasing during the treatment periods and posttreatment period 1 (Table 5),

**Table 2.** Changes over time across treatment groups for eggs laid per female per day for female mallards fed cracked corn treated with nicarbazin ad libitum daily for 14 d

Period <sup>1</sup>	n	Mean	SE
PRE	655	0.54 <sup>a</sup>	0.03
TRT1	245	0.29 <sup>b</sup>	0.04
TRT2	191	0.01 <sup>c</sup>	0.04
POST1	141	0.18 <sup>d</sup>	0.04
POST2	518	0.44 <sup>e</sup>	0.03

<sup>a-e</sup>Means within columns with different superscripts are significantly different. Means are from the LSMEANS option in PROC MIXED, and means separations were carried out using PDMIX800.

<sup>1</sup>PRE = pretreatment d 1 to 14; TRT1 = treatment d 1 to 7; TRT2 = treatment d 8 to 14; POST1 = d 2 posttreatment, POST2 = d 5 posttreatment.

but there was no effect of NCZ on BW ( $P = 0.0861$ ). Hemoglobin concentrations and hematocrit differed between sexes, with females having a lower hemoglobin concentration and hematocrit than males (Table 4). Hemoglobin concentrations and hematocrit differed among periods (Table 5), with the lowest levels occurring during

**Table 3.** Egg weights (g) for female mallards fed cracked corn treated with nicarbazin at 0, 750 (30.8 mg/kg of BW), 1,000 (34.2 mg/kg of BW), or 1,500 ppm (49.1 mg/kg of BW) ad libitum daily for 14 d

Group (ppm)	Period <sup>1</sup>	n	Mean	SE
0	PRE	55	50.7 <sup>ab</sup>	1.2
	TRT1	3	41.4 <sup>cdef</sup>	2.8
	TRT2	0		
	POST1	3	51.7 <sup>abgh</sup>	2.8
750	POST2	3	55.3 <sup>a</sup>	2.8
	PRE	51	48.5 <sup>bgh</sup>	1.3
	TRT1	2	41.6 <sup>cdef</sup>	3.4
	TRT2	0		
1,000	POST1	3	46.5 <sup>bcdgh</sup>	2.8
	POST2	4	43.3 <sup>cde</sup>	2.5
	PRE	57	47.2 <sup>cgh</sup>	1.2
	TRT1	1	46.9 <sup>abcdegh</sup>	4.6
1,500	TRT2	1	31.9 <sup>f</sup>	4.6
	POST1	4	41.5 <sup>def</sup>	2.4
	POST2	8	41.6 <sup>de</sup>	1.8
	PRE	49	50.4 <sup>abg</sup>	1.3
1,500	TRT1	2	37.3 <sup>ef</sup>	3.4
	TRT2	1	50.4 <sup>abcdegh</sup>	4.7
	POST1	0		
	POST2	6	45.4 <sup>cdh</sup>	2.2

<sup>a-h</sup>Means within columns with different superscripts are significantly different. Means are from the LSMEANS option in PROC MIXED, and means separations were carried out using PDMIX800.

<sup>1</sup>PRE = pretreatment d 1 to 14; TRT1 = treatment d 1 to 7; TRT2 = treatment d 8 to 14; POST1 = d 2 posttreatment; POST2 = d 5 posttreatment.

**Table 4.** Differences between sexes and across treatment groups for BW, hemoglobin, and hematocrit for mallards fed cracked corn treated with nicarbazin ad libitum daily for 14 d

	Females			Males		
	n	Mean	SE	n	Mean	SE
Bird weight (g)	216	874.0 <sup>a</sup>	15.9	80	1063.8 <sup>b</sup>	26.2
Hemoglobin (g/dL)	179	15.1 <sup>a</sup>	0.3	70	19.1 <sup>b</sup>	0.4
Hematocrit (%)	188	40.7 <sup>a</sup>	0.5	70	48.8 <sup>b</sup>	0.9

<sup>a,b</sup>Means within rows with different superscripts are significantly different. Means are from the LSMEANS option in PROC MIXED, and means separations were carried out using PDMIX800.

the posttreatment periods. There were no differences in hemoglobin concentrations and hematocrit among treatment groups ( $P = 0.7028$  and  $P = 0.8249$ , respectively).

Overall, plasma DNC levels were negatively correlated with the number of eggs laid per female per day and BW (Table 6). Plasma DNC levels were positively correlated with egg DNC levels during treatment period 1 and post-treatment period 2. Plasma DNC levels were negatively correlated with BW during treatment period 2. Plasma DNC levels were negatively correlated with BW in the 750 ppm group and positively correlated with hemoglobin in the 750 and 1,500 ppm groups. Egg weight and the number of eggs laid per female per day were negatively correlated with plasma DNC in the 1,000 ppm group.

## DISCUSSION

### Delivery Methods

Differences in plasma DNC levels obtained by utilizing different delivery methods can be explained by differences in the bioavailability of DNC with each method. The increased anticoccidial activity of NCZ compared with DNC alone is believed to be due to the formation of smaller DNC crystals in the gut (Rogers et al., 1983). The aqueous environment of the gut causes HDP to leach out of the NCZ complex. Although DNC crystals tend to aggregate in aqueous environments, HDP prevents DNC from aggregating in larger crystals by keeping the DNC crystals more separated than if DNC is fed alone (Rogers et al., 1983). The average diameter of wetted DNC crystals formed from DNC alone is 0.53  $\mu\text{m}$ , compared with an average diameter of 0.11  $\mu\text{m}$  obtained from the wetted NCZ complex. (Rogers et al., 1983). Because of the smaller crystal size from the NCZ complex, the DNC crystals have a larger surface area, resulting in greater absorption.

Nicarbazin suspended in corn oil is likely protected from "prewetting," keeping crystal sizes small. In addition, NCZ is distributed throughout the suspension, making NCZ more available to the gut. The corn oil suspension produced the highest peak DNC levels of any of the delivery methods tested. A water-based suspension would be expected to produce lower plasma DNC levels, because the NCZ is already in an aqueous environment prior to ingestion, and the results of this study confirmed this. Nicarbazine delivered in a capsule produced plasma DNC levels higher than the water-based suspension but lower than the corn oil suspension. Likely, this is because NCZ is dry upon ingestion but is subsequently exposed to the aqueous environment in the gut. This may result in crystal sizes intermediate to those in the corn oil and water-based suspension groups.

The lowest plasma DNC levels were obtained by feeding pelleted feed. Pellets have a lower exposed surface area than suspension- or capsule-based delivery methods. Because of this, less NCZ is immediately available for uptake in the gut, and lower plasma DNC levels result. Male mallards received only 26 mg/kg of BW, rather than the predicted 34 mg/kg of BW dose, but females received a 34 mg/kg of BW dose. Although the lower dose level in the males may have contributed to lower plasma DNC levels overall, no significant differences were found in plasma DNC levels between sexes.

During the reproductive trial, mallards fed 1,000 ppm of NCZ on cracked corn received an average dose of 34 mg/kg of BW. Because mallards in the delivery method trials also received 34 mg/kg of BW doses and both trials used 25% NCZ on wheat middlings, plasma DNC levels can be compared. Mallards receiving cracked corn had higher peak plasma DNC levels than birds receiving treatment via any of the other 4 delivery methods tested (Figure 1, Table 1). This may be due to 2 factors. First, coating

**Table 5.** Changes over time across treatment groups and sexes for BW, hemoglobin, and hematocrit for mallards fed cracked corn treated with nicarbazin ad libitum daily for 14 d

	PRE <sup>1</sup>			TRT1			TRT1			POST1			POST2		
	n	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE
Bird weight (g)	76	1031.6 <sup>a</sup>	22.7	74	888.9 <sup>b</sup>	22.8	73	853.2 <sup>c</sup>	22.9	36	833.9 <sup>d</sup>	26.2	37	977.0 <sup>e</sup>	26.0
Hemoglobin (g/dL)	31	17.8 <sup>a</sup>	0.5	74	17.6 <sup>a</sup>	0.4	72	16.0 <sup>b</sup>	0.4	35	13.5 <sup>c</sup>	0.5	37	14.7 <sup>d</sup>	0.5
Hematocrit (%)	38	43.8 <sup>a</sup>	0.8	74	43.6 <sup>a</sup>	0.8	73	42.7 <sup>b</sup>	0.8	36	41.7 <sup>c</sup>	0.8	37	41.9 <sup>bc</sup>	0.8

<sup>a-e</sup>Means within rows with different superscripts are significantly different. Means are from the LSMEANS option in PROC MIXED, and means separations were carried out using PDMIX800.

<sup>1</sup>PRE = pretreatment d 1 to 14; TRT1 = treatment d 1 to 7; TRT2 = treatment d 8 to 14; POST1 = d 2 posttreatment; POST2 = d 5 posttreatment.

**Table 6.** Correlations across treatment groups among plasma and egg 4,4'-dinitrocarbanilide (DNC) levels and the number of eggs laid per female per day, egg weight, and bird weight for mallards fed cracked corn treated with nicarbazin ad libitum daily for 14 d

	Plasma DNC		Egg DNC	
	n	r	n	r
Eggs laid per female per day	185	-0.30165	37	0.28105 <sup>1</sup>
Egg weight	38	-0.23008 <sup>1</sup>	37	-0.38437
Bird weight	252	-0.19641	37	-0.33811

<sup>1</sup>Correlations were not significant at  $P < 0.05$ . All other correlations were significant.

NCZ onto cracked corn gives more surface area than a pellet, resulting in more NCZ being immediately available to the gut. Second, corn oil was used to help NCZ adhere to the cracked corn, and this likely protected the NCZ from prewetting, resulting in formation of smaller DNC crystals in the gut.

### Reproductive Trial

Peak plasma and egg DNC levels were in the range predicted to have a contraceptive effect in waterfowl. A minimum plasma level of 2.9  $\mu\text{g}/\text{mL}$  is needed to induce reproductive effects in waterfowl (Jones et al., 1990b; Yoder et al., 2005). Plasma DNC levels in this study ranged from 2.7 to 5.4  $\mu\text{g}/\text{mL}$ . Despite decreased food consumption, mallards in the 1,000 and 1,500 ppm groups received enough NCZ to obtain adequate plasma DNC levels to induce contraceptive effects. Reduced egg hatchability in chickens was observed at a minimum egg DNC level of 6  $\mu\text{g}/\text{g}$  (Jones et al., 1990b), and similar levels are likely needed in waterfowl eggs to prevent hatchability. Egg DNC levels in this study ranged from 10.6 to 22.8  $\mu\text{g}/\text{g}$ , indicating that even 750 ppm of NCZ in feed could reduce egg hatchability.

The mallards utilized in this study were accustomed to a pelleted waterfowl diet, and did not accept a cracked corn diet, even in the control group. Egg production and BW decreased as a result of the decreased food intake but began to return to pretreatment levels during post-treatment period 2.

Treatment with NCZ resulted in a decrease in egg weight. This is consistent with the results of previous studies on chickens (Jones et al., 1990a,b; Hughes et al., 1991). Mean egg weights were  $50.7 \pm 1.1$ ,  $47.8 \pm 1.2$ ,  $46.0 \pm 1.1$ , and  $49.4 \pm 1.3$  g in the 0, 750, 1,000, and 1,500 ppm groups, respectively. During treatment period 1, egg weights were reduced by 10% in the 1,500 ppm group. There were no eggs laid in the control group during treatment period 2 to compare with the treatment groups. Egg weights decreased by 10 and 19.7% in the 750 and 1,000 ppm groups, respectively, during posttreatment period 1. During posttreatment period 2, egg weights were reduced by 21.7, 24.8, and 17.9% in the 750, 1,000, and 1,500 ppm groups, respectively. In the 1,000 ppm group, egg weight decreased as plasma DNC increased.

Male mallards in this study had a higher hematocrit and hemoglobin concentration than females. In geese,

administration of estrogen causes a decrease in red blood cell counts (Hunsaker, 1968). Although androgen has no effect on hematocrit in geese, it increases the red blood cell count in chickens and quail (Burton and Smith, 1972; Nirmalan and Robinson, 1972). Although there are differences among species, males tend to have a higher hematocrit than females (Sturkie, 1986). Because hemoglobin concentration is related to hematocrit (Campbell, 1995; Fudge, 2000), males also have a higher hemoglobin concentration than females (Sturkie, 1986).

Increased plasma DNC was associated with decreases in egg production and BW in this study. This is consistent with the finding that egg production in chickens decreases as the NCZ dose in feed increases (Jones et al., 1990b; Hughes et al., 1991). At levels  $\geq 100$  ppm of NCZ, feed efficiency and weight gain are decreased in chickens (Bartov, 1989a,b; Sorribas et al., 1993).

Plasma and egg DNC levels high enough to induce contraceptive effects were obtained during this study at  $\geq 1,000$  ppm of NCZ in feed. Although NCZ-treated cracked corn produced the highest plasma DNC levels, and would be more readily accepted by geese than pelleted feed, it is also associated with more nontarget hazards to songbirds. Because of this, it is recommended that NCZ be coated onto whole corn instead, because the size of corn kernels will exclude most nontarget songbirds. Geese are accustomed to eating corn in fields during the winter months, therefore, they are more likely to accept corn than pelleted feed with which they have no prior experience. Geese in parks used to being fed by humans may be more likely to accept pelleted feed than geese not normally fed by humans. If pelleted feed is to be used in the field, a higher amount of NCZ must be incorporated into the pellet to allow for the decreased absorption of DNC. OvoControl G is a pelleted NCZ feed registered by the Environmental Protection Agency in 2005 for control of reproduction in resident Canada geese. It contains 2,500 mg of NCZ/kg of feed, requiring a goose to consume 50 g daily to achieve an approximate dose of 31.25 mg of NCZ/kg of BW (Bynum et al., 2006).

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