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Research Notes

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ABSTRACT Nicarbazin (NCZ), a coccidiostat commonly used in the poultry industry, causes reduced hatchability and egg quality in layer hens at a concentration of 125 ppm (8.4 mg/kg) in the feed. Although this effect is undesirable in the poultry industry, NCZ could provide a useful wildlife contraception tool for waterfowl, particularly urban geese. We tested the absorption of NCZ in chickens (*Gallus gallus*), mallards (*Anas platyrhynchos*), and Canada geese (*Branta canadensis*) gavaged with 8.4 mg of NCZ/kg per bird each day for 8 d. Plasma levels of 4,4′-dinitrocarbanilide (DNC) differed significantly among species. Peak plasma DNC levels were 2.87 ± 0.15 μg/mL, 2.39 ± 0.15 μg/mL, and 1.53 ± 0.15 μg/mL in chickens, mallards, and Canada geese respectively. It took 6 d to obtain peak DNC levels in chickens as opposed to 8 d in mallards and Canada geese. The half life of DNC in plasma was 1.43 d in chickens, 0.72 d in mallards, and 1.26 d in Canada geese. Mallards eliminated 100% of plasma DNC 4 d post-treatment, whereas Canada geese eliminated 100% of plasma DNC 8 d post-treatment. Chickens had only eliminated 99% of plasma DNC 8 d post-treatment. Mallard plasma DNC levels were highly correlated with Canada goose plasma DNC levels. This research showed mallards are an ideal model species for the Canada goose for future reproductive studies on NCZ in a laboratory setting. However, levels higher than 8.4 mg/kg must be fed to waterfowl in order to obtain a plasma level comparable to chickens.

(*Key words*: absorption, contraception, 4,4′-dinitrocarbanilide, half-life, nicarbazin)

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INTRODUCTION

Nicarbazin (NCZ) was developed in the 1950s as a coccidiostat for broiler chickens (*Gallus gallus*). Used at 125 ppm in feed, it improves weight gain and feed efficiency (Ott et al., 1956; Newberne and Buck, 1957). Nicarbazin is an equimolar complex consisting of 4,4′-dinitrocarbanilide (DNC) and 2-hydroxy-4,6-dimethylpyrimidine (HDP). The HDP strictly helps with absorption of the material, whereas the DNC is the active compound (Cuckler et al., 1955; Rogers et al., 1983). Metabolic studies show that HDP is cleared within 24 h in the urine, whereas DNC remains in the blood for approximately 4 d before clearing in the feces (Wells, 1999). When accidentally fed to layer hens, NCZ has negative side effects, including severe mottling of the egg yolk (the appearance of white or clear spots in the yolk) and reductions in hatchability and egg production (Jones et al., 1990; Hughes et al., 1991; Chapman, 1994). These effects occur to varying degrees beginning with dietary concentrations of 50 ppm. They becoming severe at 125 ppm and are reversed once the compound is withdrawn from the feed (Jones et al., 1990).

Resident Canada goose (*Branta canadensis*) populations are increasing across the US (Forbes, 1993; Ankney, 1996; Gosser and Conover, 1999). As a result, conflicts between humans and wildlife are increasing. Large numbers of geese damage grass, foul water supplies, and become aircraft strike hazards (Conover and Chasko, 1985; Fairaizl, 1992; Forbes, 1993). Because these geese are nonmigratory, hunting is rarely a feasible solution to reducing population numbers to acceptable levels (Conover and Chasko 1985; Heusmann, 1999). Translocation of geese has not proven to be effective (Hall and Groninger, 2002), and the public may find annual roundups objectionable. Contraception provides one publicly acceptable means of reducing overpopulated resident goose flocks (Stout et al., 1997).

Mallards (*Anas platyrhynchos*) are the recommended test species for many U.S. EPA reproductive toxicity studies (EPA, 1996). Mallards will continue to produce eggs in the laboratory if the eggs are removed daily. Their reproductive cycles can be easily manipulated by changing the light cycle. Husbandry of mallards is better known than the husbandry of other wild species. Therefore, we chose to compare the absorption of NCZ in chickens, mallards,

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Abbreviation Key: DNC = 4,4′-dinitrocarbanilide, HDP = 2-hydroxy-4,5-dimethylpyrimidine, NCZ = nicarbazin.

and Canada geese. Our objectives were to 1) determine the relative absorption and half-life of NCZ; 2) determine a starting dose for further experimentation on waterfowl using NCZ as a contraceptive; and 3) determine if mallards could be used as a model species for Canada geese in the laboratory for further NCZ reproductive studies.

MATERIALS AND METHODS

The experiment consisted of 6 Rhode Island Red chickens[2] (763 ± 254 g [mean ± SEM]), 6 mallards[3] (896 ± 299 g [mean ± SEM]), and 6 wild caught Canada geese (3,500 ± 1,750 g [mean ± SEM]). Birds were maintained on a layer ration[4] that consisted of 3.25 to 4.25% calcium, 0.5% phosphorus, and 16% CP. Chickens and mallards were housed in cages individually, whereas geese were housed one pair per animal room. Birds were maintained on a 12L:12D light cycle, and were allowed 2 wk to acclimate prior to dosing. The test dose was based on an average chicken (1.0 kg) consuming 125 μg of NCZ[5]/g of feed (125 ppm) at an average rate of 67 g/d. A 1.0-kg chicken consuming 67 g of the feed daily would receive 8,375 μg of NCZ, or 8.4 mg of NCZ. Therefore, a dose of 8.4 mg/kg was chosen for this study. Nicarbazin doses were prepared by adding 25% NCZ on wheat middlings to No. 3 gelatin capsules,[6] such that each bird would receive 8.4 mg of NCZ/kg per capsule. Birds were gavaged with 1 capsule/d for 8 d. We drew 2 mL of blood from either the jugular or brachial vein once pretreatment, every 2 d during treatment, and every 2 d for 8 d posttreatment. Blood samples were drawn 4 h after gavage. The blood was centrifuged and the plasma stored at −70°C until analysis by HPLC for DNC concentration (Primus et al., 2001). The experimental protocol was reviewed by the National Wildlife Research Center's Animal Care and Use Committee and complied with the Animal Welfare Act.

We analyzed the data as a mixed effects model (Proc Mixed; SAS Inst., Inc., 2001) using $P \leq 0.05$ to determine species, day, and species × day interaction effects. Least squares means were used to compare DNC means among species and days. To determine the half-life of NCZ in the plasma, only data from treatment d 8 to 8 d posttreatment were used. A natural logarithmic transformation was performed on DNC levels to obtain a more linear relationship. A linear regression (Proc Ref) was then performed to obtain regression equations for each species. These equations were used to determine the half-life of NCZ in plasma. A correlation analysis (Proc Corr) was used to determine the correlation of DNC levels between species during the absorption and elimination phases using species means for each day.

[2]Denver, CO.
[3]Whistling Wings, Inc., Hanover, IL.
[4]Ranchway Feeds, Fort Collins, CO.
[5]Phibro Animal Health, Inc., Fairfield, NJ.
[6]Torpac, Inc., Fairfield, NJ.

RESULTS AND DISCUSSION

Mallards and chickens had the most rapid rate of increase in plasma DNC levels at d 2 (Figure 1), whereas Canada geese had the slowest rate of increase. After treatment d 2, chickens had the most rapid rate of increase, whereas mallards and Canada geese had more gradual rates of increase. Plasma DNC levels peaked at d 6 in chickens (2.87 ± 0.15 μg/mL) and were not significantly changed on d 8. This is similar to the peak plasma DNC level of 3.2 μg/mL for chickens free fed 100 ppm NCZ treated feed (Johnston et al., 2002). The slightly higher plasma DNC levels in the free feeding study are likely due to differences in absorption obtained with free feeding vs. a bolus dose given by gavage. The plasma DNC level of 3.2 μg/mL correlated with a peak egg DNC level of 5.98 μg/g. A minimum egg level of 6 μg/g is needed to reduce hatchability in chickens (Jones et al., 1990). Because reproductive effects occur in laying chickens at 125 ppm (8.4 mg/kg) (Jones et al., 1990; Johnston et al., 2002), a minimum plasma level of 2.9 μg/mL will be needed in waterfowl to obtain similar reproductive effects. The highest plasma DNC levels were obtained at d 8 in mallards (2.39 ± 0.15 μg/mL) and Canada geese (1.53 ± 0.15 μg/mL). Future dose-response studies in waterfowl should use doses higher than 8.4 mg/kg (125 ppm) to achieve plasma DNC levels ≥2.9 μg/mL.

These data show the need for dose-response work in the species of interest because of species differences in uptake of NCZ. A plateau of plasma DNC levels was not observed in mallards or Canada geese as it was in chickens (Figure 1). Therefore, continued treatment either would provide higher plasma levels in mallards and Canada geese, or cause a plateau in plasma DNC levels at the peak levels observed in this study. Chickens are bred to be highly efficient at absorption for maximum weight gain. Mallards and geese may be less efficient at absorption, or may metabolize and excrete NCZ more rapidly than chickens, and these factors may account for the lower plasma DNC levels. Although tissue distribution of DNC was not investigated in this study, it seems reasonable that DNC distribution would be similar in all 3 species. However, one species may store more DNC in certain tissues than the other species.

The most rapid clearance rates were observed in mallards and Canada geese. At 2 d post-treatment, 52, 84, and 64% of plasma DNC had been cleared in the chicken, mallard, and Canada goose, respectively. Mallards cleared 100% of DNC from the plasma by 4 d post-treatment. Canada geese cleared 99% of plasma DNC by 6 d post-treatment, and 100% by 8 d post-treatment. Chickens cleared 94% of plasma DNC by 6 d post-treatment, and 99% by 8 d post-treatment.

There were significant day ($P = < 0.0001$), species ($P = 0.0013$), and day × species interaction effects ($P < 0.0001$). The regression equations significantly explained the model variation ($P < 0.0001$) and were used to calculate the half-lives of plasma DNC. The half-life of DNC was 1.43 d ($\ln[\text{DNC}] = 1.2237 - 0.47126[\text{day}]$), 0.72 d

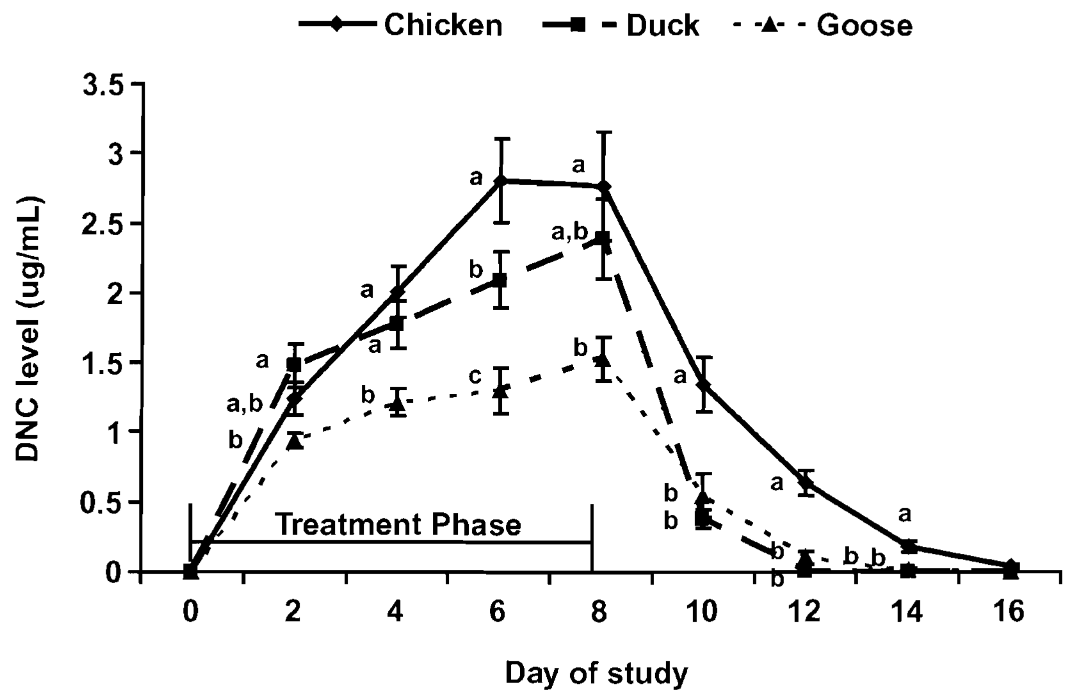

FIGURE 1. Plasma 4,4′-dinitrocarbanilide (DNC) levels in chickens (n = 6), mallards (n = 6), and Canada geese (n = 6) treated with 8.4 mg/kg of nicarbazin for 8 d at the National Wildlife Research Center, Fort Collins, CO, in June 1999. Means with the same letter are not significantly different ($P > 0.05$).

(ln[DNC] = 0.82374 – 0.96777[day]), and 1.26 d (ln[DNC] = 0.32682 – 0.55135[day]) in chickens, mallards, and Canada geese, respectively.

Both the mallard and the chicken proved to be very good models for the Canada goose in terms of absorption of NCZ. However, the characteristics of DNC elimination from plasma make the mallard a better model than the chicken. Average DNC levels during the absorption phase were significantly correlated between chickens and mallards ($P = 0.0069$, r = 0.97), chickens and Canada geese ($P = 0.0095$, r = 0.96), and mallards and Canada geese ($P = 0.0002$, r = 1.0). Average DNC levels during the elimination phase were significantly correlated between chickens and mallards ($P = 0.0159$, r = 0.94), chickens and Canada geese ($P = 0.0018$, r = 0.99), and mallards and Canada geese ($P = 0.0034$, r = 0.98).

Future reproductive studies should be conducted using the mallard as the model for the Canada goose because both belong to the order Anseriformes. This will allow testing of various effects of NCZ on reproduction and health parameters that can be extrapolated to plan a field study with Canada geese. The regression equation for mallard and goose plasma DNC levels allows for prediction of Canada goose plasma DNC levels based on mallard levels. This is helpful in estimating a dose to be used in a field setting with Canada geese.

These results have both positive and negative implications for developing NCZ as an avian contraceptive tool. Because higher plasma DNC levels were obtained in chickens than in mallards or Canada geese at the same dose, higher dose levels in feed likely will be needed to obtain a comparable plasma DNC level in waterfowl. Because DNC clears rapidly, a continuous baiting protocol will be needed in the field in order to provide effective contraception for birds throughout the breeding season. Such a protocol will make an operational baiting program more difficult. The rapid clearance time of DNC allows for a rapid reversal of reproductive effects. This is advantageous if migratory nontarget species were to consume NCZ bait 1 or 2 times. Another advantage of NCZ is the flexibility to affect different reproductive parameters based on changing doses. A lower dose affects hatchability, but still allows the female to lay eggs (Jones et al., 1990). Because the female will likely incubate eggs at least until the expected hatching date, it makes the possibility of renesting less likely. A higher dose causes cessation of egg laying (Jones et al., 1990). Nicarbazin is promising as a contraceptive agent that can be used in a comprehensive management plan to help manage resident Canada goose flocks.

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