July 2001

Determination of 4,4′-Dinitrocarbanilide (DNC), the Active Component of the Antifertility Agent Nicarbazin, in Chicken, Duck, and Goose Plasma

Thomas M. Primus  
U.S. Department of Agriculture/Animal and Plant Health Inspection Service/National Wildlife Research Center

Dennis J. Kohler  
USDA/APHIS/WS National Wildlife Research Center, dennis.kohler@aphis.usda.gov

Margaret A. Goodall  
U.S. Department of Agriculture/Animal and Plant Health Inspection Service/National Wildlife Research Center

Christi Yoder  
U.S. Department of Agriculture/Animal and Plant Health Inspection Service/National Wildlife Research Center

Doreen Griffin  
U.S. Department of Agriculture/Animal and Plant Health Inspection Service/National Wildlife Research Center

See next page for additional authors

Follow this and additional works at: https://digitalcommons.unl.edu/icwdm_usdanwrc

Part of the Environmental Sciences Commons

Primus, Thomas M.; Kohler, Dennis J.; Goodall, Margaret A.; Yoder, Christi; Griffin, Doreen; Miller, Lowell A.; and Johnston, John J., "Determination of 4,4′-Dinitrocarbanilide (DNC), the Active Component of the Antifertility Agent Nicarbazin, in Chicken, Duck, and Goose Plasma" (2001). USDA National Wildlife Research Center - Staff Publications. 564.  
https://digitalcommons.unl.edu/icwdm_usdanwrc/564

This Article is brought to you for free and open access by the U.S. Department of Agriculture: Animal and Plant Health Inspection Service at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in USDA National Wildlife Research Center - Staff Publications by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.
Determination of 4,4'-Dinitrocarbanilide (DNC), the Active Component of the Antifertility Agent Nicarbazin, in Chicken, Duck, and Goose Plasma

Thomas M. Primus,‡ Dennis J. Kohler,† Margaret A. Goodall,‡ Christi Yoder,‡ Doreen Griffin,† Lowell Miller,‡ and John J. Johnston†

Analytical Chemistry Project and Infertility Project, U.S. Department of Agriculture/Animal and Plant Health Inspection Service/National Wildlife Research Center, 4101 LaPorte Avenue, Fort Collins, Denver, Colorado 80521

4,4'-Dinitrocarbanilide (DNC) was extracted from chicken, duck, and goose plasma and isolated by reversed-phase high-performance liquid chromatography. DNC was detected by ultraviolet absorbance at 347 nm and quantified by comparison to a calibration standard. Recovery data were determined by analyzing DNC-fortified control plasma. The mean recovery of DNC in fortified chicken plasma samples was 99.7 ± 1.9% for 0.18 and 9.1 ppm DNC, and in fortified duck and goose plasma samples was 99.5 ± 4.9% and 101.4 ± 4.5%, respectively, for 0.18, 9.1, and 18 ppm DNC.

Keywords: 4,4'-Dinitrocarbanilide; nicarbazin; high-performance liquid chromatography; plasma; chicken; duck; goose

INTRODUCTION

Canada geese are commonly thought of as migrating birds. However, the number of nonmigrating (resident) Canada geese is increasing (1). Generally, people usually accept a few Canada geese as pleasant. However, as the number of resident geese increases, problems such as the fouling of water supplies, lawns, beaches, and golf courses with excreta, overgrazing of grassy areas, and flocks feeding on crops such as corn, soybeans, rice, lettuce, and wheat occur more frequently (2). Recommended management techniques for Canada geese and their associated problems include use of scaring devices, dogs to chase geese, prevention of nesting, installation of barriers, reducing feeding practices by the public, adjusting landscaping practices, relocating birds, and utilizing hunting practices (3). Reducing Canada geese populations in resident flocks would help to alleviate many of the problems associated with this species. Thus, an antifertility agent used on a limited basis for a growing pest species such as the Canada goose may reduce numbers to a desirable and manageable level.

Nicarbazin is an FDA-approved drug used for the treatment and prevention of coccidiosis in broiler chickens. Nicarbazin is an equimolar complex of 4,4'-dinitrocarbanilide (DNC) and 4,6-dimethyl-2-pyrimidinol (HDP). When nicarbazin was accidentally fed to breeder chickens, decreased egg hatchability was observed. The active component of nicarbazin, DNC, is responsible for the decreased hatchability of eggs. The National Wildlife Research Center is evaluating nicarbazin as a potential antifertility agent for Canada geese. It is hoped that correlations between nicarbazin diet concentration, nicarbazin dose, blood DNC levels, and hatchability will permit the determination of efficacious, yet safe, Nicarbazin diet concentrations for multiple avian species. To help bridge our future findings with the extensive Nicarbazin database for chickens, chickens as well as Canada geese and mallard ducks were used as test species. To achieve our research goals, analytical methods needed to be developed for the quantification of DNC in plasma.

Initially chickens, mallards, and Canada geese were dosed with nicarbazin at 8.4 mg/kg and plasma samples were collected, frozen, and analyzed. Plasma data for chicken have been reported for DNC and HDP but no method was published (currently unpublished proprietary data). The Analytical Chemistry Project (ACP) at the National Wildlife Research Center (NWRC) developed and validated a method for the determination of DNC in the plasma of chickens, Canada geese, and mallards.

Methods for the analysis of nicarbazin typically focus on the residue determination of DNC in eggs and chicken muscle tissue. The HDP component of the complex increases the adsorption of DNC into the circulatory system. The HDP that is adsorbed into the blood stream is excreted at a much faster rate than DNC (4, 5). Most residue methods for DNC include a sample cleanup. Most cleanup steps are accomplished by liquid–liquid extraction, solid-phase extraction columns, or online columns prior to the analytical column in the HPLC analysis (6–9). Others have used liquid chromatography/mass spectrometry (LC/MS) to avoid sample extract cleanup (10–12). Unfortunately, LC/MS is not a widely available technique. To accomplish our research goals, we developed a simple HPLC method for the quantification of DNC in avian plasma. This method uses very small solvent volumes for the extraction with high sample throughput. The resulting data will be used to determine target Nicarbazin dose levels for field studies to develop nicarbazin as an antifertility agent in Canada geese.
HPLC (Hewlett-Packard 1090M high-performance liquid chromatography) was added to 0.100 mL of control plasma and vortex mixed. Likewise, for samples fortified at the 9.1 or 18 ppm DNC levels, standard solutions (1000 μg/mL) were prepared by accurately weighing 50 mg of DNC reference standard into a 50-mL volumetric flask (Supelco, Bellefonte, PA), which had been placed into a HPLC vial and capped. An injection of 60 μL was completed for each sample and quality control sample into the HPLC and the concentration of DNC was determined versus calibration standards.

**Sample Preparation.** A 100-μL aliquot of each plasma sample was transferred into a 1.5-mL plastic Eppendorf microcentrifuge tube. The samples were diluted with 200 μL of acetonitrile and vortex mixed. The samples were centrifuged in an Eppendorf microfuge (Eppendorf Netheler-Hinz, Hamburg, Germany) for 5 min. The supernatant was accurately transferred into a 350-μL glass insert (Supelco, Bellefonte, PA), which had been placed into a HPLC vial and capped. An injection of 60 μL was completed for each sample and quality control sample into the HPLC and the concentration of DNC was determined versus calibration standards.

**Fortification of Control Plasma.** A DNC concentrated standard solution (1000 μg/mL) was prepared by accurately weighing 50 mg of DNC reference standard into a 50-mL volumetric flask. The DNC was diluted and diluted to volume with DMF. Fortification standard solutions were prepared by dilution of the concentrated standard solution to 200, 100, and 2 μg/mL in DMF in 10-mL volumetric flasks. For 0.18 μg/mL fortified samples, 10.0 μL of the 2.0 μg/mL standard solution was added to 0.100 mL of control plasma and vortex mixed. Likewise, for samples fortified at the 9.1 or 18 μg/mL, 10.0 μL of the 100 or 200 μg/mL standard solution was added to 0.100 mL of control plasma and vortex mixed. We then proceeded with the extraction procedure as described above.

**Chromatographic System.** Samples were analyzed by a Hewlett-Packard 1090M high-performance liquid chromatograph (HPLC) equipped with a Hewlett-Packard diode array ultraviolet-visible detector. The HPLC parameters utilized are listed in Table 1. The DNC chromatographic response was identified by comparison with the retention time and UV-visible spectra of a standard and quantified with the use of external calibration standards. A Hewlett-Packard computer work station with chromatographic software and printer were used to collect, process, store, and print the chromatographic data.
Method Limit of Detection (MLOD). The method limit of detection (MLOD) was estimated from the mean chromatographic response of three control plasma samples for each type of plasma and the response of a control plasma fortified at 0.18 g/mL DNC (at least four replicates were fortified for each type of plasma). The MLOD was defined as the concentration of DNC required to generate a signal equal to 3× the baseline noise (measured peak-to-peak) observed in the control sample. The MLODs for DNC in chicken, duck, and goose plasma were 0.033, 0.027, and 0.035 µg/mL, respectively.

Bias and Repeatability. Replicate control plasma samples were fortified with DNC and assayed according to the procedures in this method. The mean recoveries of DNC from chicken, duck, and goose plasma were 97.7 ± 1.9%, 99.5 ± 4.9%, and 101.4 ± 4.5%, respectively. The recovery and precision data are shown in Table 2.
Method Performance. The usefulness of the method was demonstrated by the analyses of chicken, duck, and goose plasma collected during a study to assess the DNC plasma levels of birds dosed with Nicarbazin at 8.4 mg/kg for 8 days followed by 8 days of no treatment. Plasma samples were drawn every other day over the duration of the study. Analysis of 230 plasma samples required 11 working days. The mean recoveries of DNC fortified (0.18 and 9.1 µg/mL) chicken, duck, and goose plasma were 97.5 ± 3.7%, 97.7 ± 5.7%, and 101.4 ± 6.7%, respectively, for this study. A portion of the data is presented in Figure 2. The data points represent the average DNC concentration in the plasma for three individual birds in each test group. For all 3 species, DNC plasma levels reached a plateau after 6 days of treatment. DNC plasma levels rapidly decreased following cessation of nicarbazin treatment. Within 4 days, duck and goose DNC plasma levels decreased to less than the MLOD, whereas chicken required 6 to 8 days to reach this level. A goose plasma sample collected on day 6 from a bird treated with 8.4 mg/kg and assayed with the method is shown in Figure 1C.

CONCLUSIONS

This methodology developed for the determination of DNC in avian plasma proved to be reliable, efficient, and simple, with high sample throughput. The same method was used for plasma from three different species. The mean recovery of DNC in fortified avian plasma samples was 99.6 ± 4.3% for 0.18, 9.1, and 18 ppm DNC.

ACKNOWLEDGMENT

We acknowledge the assistance of the Induced Infertility Project personnel of the National Wildlife Research Center for collecting plasma samples.

LITERATURE CITED


Received for review February 23, 2001. Revised manuscript received June 21, 2001. Accepted June 21, 2001. Funding for this project was provided through a cooperative agreement with Koffolk, Inc. Mention of commercial products is for identification only and does not constitute endorsement by the United States Department of Agriculture.