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Poly(methyl methacrylate) synthetic grit formulations sustain the delivery of nicarbazin, a contraceptive agent, in pest waterfowl

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Abstract

Sixty-three mallards were fed one of ten poly(methyl methacrylate) based synthetic grit formulations containing varying concentrations of a proposed wildlife contraceptive (nicarbazin), plasticizer (acetyl tributylcitrate) and/or cross-linking agent (1,4-butanediol diacrylate). Release characteristics of the contraceptive agent were monitored for the purpose of developing a contraceptive formulation for control of pest waterfowl in urban settings. The addition of plasticizer increased the erosion rate ($t_{1/2}$=0.97–2.85 days), cross-linking the polymer matrix slightly decreased the erosion rate ($t_{1/2}$=4.45–5.05 days) and increasing the concentration of the contraceptive agent increased the erosion rate ($t_{1/2}$=3.3 and 9.9 days at 60% and 7.5% active ingredient, respectively). The larger and smaller grit pieces had longer half lives at 11.0 and 11.6 days, respectively while the mid sized grit had a half life of 4.95 days. Control grit had a half life of 12.7 days based on weight loss. Analysis of blood and feces for monitoring release from the grit and approximate indirect plasma levels of the active ingredient proved feasible.

Keywords: Synthetic grit; Contraception; Poly(methyl methacrylate); Controlled release; Cross linking and plasticizer; Nicarbazin

1. Introduction

Over the last few years many areas in the United States have seen dramatic increases in ducks and geese, particularly of resident populations in urban environments. These populations have led to esthetic problems such as fecal material in public parks, and golf courses as well as the potential for health concerns [1]. Relocation programs have been used in many places, but success has been limited. Denver, Colorado recently decided to discontinue their geese relocation program as they had run out of sites that would accept any more animals [2]. Lethal programs are generally effective, but often have negative public relation consequences. Population management by contraception should be well suited for resident animals. A significant number of agents ranging from steroids to natural products have been shown to have contraceptive action. Nicarbazin (NCZ) is a coccidiostat used in broiler and egg layer chickens with a long history of safe use. However, if nicarbazin is fed to laying chickens, at intermediate concentrations the eggs fail to hatch and at higher concentrations egg laying is dramatically reduced. Nicarbazin was chosen for this study because of its

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history of safe use in chickens even though previous work at this facility had shown that 20–30 mg per day was necessary for effective action in ducks. This amount of material per day would clearly represent a most difficult case scenario for the controlled release system envisioned for use.

Nicarbazin is an equimolar complex of 4,4′-dinitrocarbanilide (DNC) and 2-hydroxy-4,6-dimethylpyrimidine (HDP). The DNC–HDP complex is necessary for absorption of DNC, the bioactive component of nicarbazin. After absorption, DNC is freed upon dissociation of the complex and is eliminated from the animal with a half life of 24–48 h. The contraceptive effect of nicarbazin rapidly diminishes with the excretion of DNC. HDP is eliminated rapidly and blood levels fall below detection limits within 24–72 h [3–5].

While reversibility is positive from a regulatory agency perspective, a continuous supply of NCZ is needed through the entire breeding season if population reduction is to be achieved. Given this scenario, a NCZ fortified bait would be feasible if the target animal consistently consumed a sufficient quantity on a daily basis. As such a scenario is extremely unlikely, we attempted to formulate a controlled release system to smooth over intake fluctuations. To minimize concerns associated with the consumption of a contraceptive bait by non-target animals, we concentrated our efforts on the development of a controlled release grit because only gizzard containing animals (i.e. birds) could absorb the NCZ. Additionally, by proper selection of grit size and placement of bait, exposure to nontarget avian species could also be minimized [6].

A formaldehyde resorcinol mestranol polymer grit was successfully used as a controlled release agent for Japanese quail and pigeons [7,8]. However, in this study, only micrograms per day of release were required to achieve results. This level of release would be insufficient for NCZ where milligrams per day are required. Other long-acting systems including oil suspension injections [9] and cornstarch tablets [10] have been evaluated, but these would not be applicable here as the injection of large populations is impractical and the corn starch tablets only lasted a few hours. Bone cement, an acrylic polymer, has been used for controlled release of antibiotics via implanted beads [11–14]. These studies strongly suggest that the acrylic polymers do not constitute a health hazard. Preliminary studies in our labs with chickens, ducks and geese showed that control bone cement grit pieces (with no extractable agents) were still present, though eroded, up to 60 days after oral dosing. Given these promising preliminary results, this study was undertaken to determine the effect of grit size, NCZ concentration, and addition of plasticizer or cross linking agents on the release of nicarbazin. Assuming that the erosion of grit and subsequent release of NCZ was proportional to blood DNC levels, blood plasma DNC levels were monitored over time following oral dosing with a variety of NCZ containing grit formulations. Additionally, feces samples were collected at the same time as blood samples to determine if a correlation between blood and fecal DNC levels was present. If so, a less invasive method of monitoring individual dosing could be achieved.

2. Materials and methods

2.1. Materials

Ingredient used to prepare the grit include nicarbazin (Kolfolk, Rancho Santa Fe, CA), monomethyl methacrylate(MMA) (Jorgensen Laboratories, Inc. Loveland, CO), benzoyl peroxide and 1,4-butanediol diacrylate, (Aldrich, St. Louis, MO), acetyl tributylcitrate (Morflex, Greensboro, NC), and gel capsules (Torpac, Fairfield, NJ).

2.2. Methods

2.2.1. Bird feeding and housing

Female mallard ducks (Whistling Wings, Hanover, IL) were group housed with water, duck chow (Layena Checkers, Purina, Denver, CO) and cracked corn ad libitum. Ducks were exposed to a 14/10 h (light/dark) light cycle with temperatures of 18–22 °C and ambient humidity.

2.2.2. Grit preparation

The initiator (benzoyl peroxide) was dissolved in the methyl methacrylate monomer plus plasticizer (acetyl tributylcitrate) or crosslinking agent (1,4-butanediol diacrylate), as indicated per Table 1. This

Table 1
Grit formulations used in the study

Treatment %NCZ-gel size	NCZ (%)	MMA (%)	Initiator (%)	Plasticizer (%)	Crosslinker (%)
60-3	60	37.5	2.5	–	–
30-3	30	67.5	2.5	–	–
15-3	15	82.5	2.5	–	–
7.5-3	7.5	90	2.5	–	–
30-1	30	67.5	2.5	–	–
30-5	30	67.5	2.5	–	–
30-15pl-3	30	52.5	2.5	15	–
30-30pl-3	30	37.5	2.5	30	–
30-15xl-3	30	52.5	2.5	–	15
30-30xl-3	30	37.5	2.5	–	30
0-3	0	97.5	2.5	–	–

solution was added to the NCZ powder and thoroughly mixed to give a uniform suspension. This suspension was poured into a disposable syringe which was used to fill #1(0.50 ml), #3(0.30 ml) and #5(0.13 ml) gel cap bottoms. The gel cap tops were placed on and the caps set aside to complete polymerization. The gel caps were dissolved in warm water to yield smooth grit pieces.

2.2.3. Gavaging

Table 2 lists the treatments, weights, gel cap sizes, number of capsules administered, and mg of NCZ for each of the various treatment groups. The grit pieces were orally administered to the individual animals over several minutes with water as a lubricant.

2.2.4. Blood and feces collection

At 3 day intervals, or until NCZ levels were below detection limits, 1 ml samples of blood and a sample of feces (ca. 1–5 g) were collected from each animal. The blood samples were centrifuged at 16 000×*g* for 5 min, and the plasma was removed and stored at −20 °C until analyzed. The feces samples were stored in 20 ml liquid scintillation vials at −20 °C until analyzed.

2.2.5. DNC analysis

2.2.5.1. Feces procedure

2.2.5.1.1. *Sample extraction* A 1.0 g fecal sample (wet weight) was extracted with 5.0 ml of a 1:1 (v/v) solution of acetonitrile–dimethylformamide (ACN–DMF). The mixture was shaken on a horizontal shaker for 10 min with an oscillation rate of 250 strokes/min. The sample was then centrifuged for 5 min at 1470×*g*. The supernatant was transferred to a 10 ml volumetric flask. The extraction was repeated

Table 2
Formulation and treatment used in the study

	mg. per grit	Gel cap #	Pieces given	NCZ, mg total
60%NCZ	293	3	6	1056
30%NCZ	234	3	6	483
15%NCZ	282	3	6	252
7.5%NCZ	226	3	7	102
30%NCZ	448	1	4	536
30%NCZ	144	5	12	516
0%NCZ (control)	230	3	6	0
30%NCZ15%pl	286	3	6	515
30%NCZ30%pl	288	3	6	518
30%NCZ15%xl	268	3	6	482
30%NCZ30%xl	270	3	6	486

a second time. The extracts were combined and the solution was brought to 10.00 ml total volume. An aliquot was filtered with a 0.45 m Teflon filter into an LC vial and capped immediately.

2.2.5.1.2. *Chromatography* Samples were analyzed on a Hewlett-Packard Series 1050 HPLC which consisted of a quaternary pump, and automatic injector, a thermal control module and a variable wavelength detector. The system was interfaced via a GPIB interface to a Hewlett-Packard Vectra X/M Series 4-5/150 computer running Hewlett-Packard Chemstation software. Samples were separated on a 250×4.6 mm Keystone ODS/H C_{18} (5 mu particle size) column (Keystone, Bellfonte, PA, USA). A Keystone 150×4.6 mm ODS/H guard column was used. Samples were eluted from the column using ACN–water (70:30% v/v) as mobile phase, at 1.0 ml/min. Forty microliters were injected on the column with an auto sampler. The column was maintained at 35 °C, and samples were detected with a UV detector at λ=347 nm.

2.2.5.2. Plasma procedure

2.2.5.2.1. *Sample extraction* Then, 200 μl of acetonitrile were added to a 100 μl aliquot of plasma and vortex mixed. The mixture was centrifuged for 5 min at 16 000×*g* in a micro centrifuge. The supernatant was decanted into a LC vial containing a 350 μl glass liner and cap.

2.2.5.2.2. *Chromatography* Plasma samples were analyzed by high-performance liquid chromatography (HPLC) using the same instrument and conditions as used for feces except that an ACN–water (60:40%, v/v) mobile phase was used and the injection volume was 60 μl. For feces and plasma, the minimum level of detection (MLOD) was defined as the quantity of DNC required to generate a chromatographic response greater than three times the baseline noise at the retention time of DNC in the control chromatogram. This generally equated to 0.02–0.04 ppm over the period of analysis of these samples.

2.2.6. Gizzard examination

On day 33 post dosing, 14 birds were euthanized, their gizzards removed and the gizzard contents recovered for analysis. The normal grit (i.e. sand, pepples) and remaining synthetic grit pieces were recovered by repeated soaking of the gizzard contents in water and decanting away the hydrated organic materials.

2.2.7. Statistical analysis

Three univariant analyses of variance were used to examine the effects of concentration of NCZ, additives, and grit size on the half lives of the grit pieces in mallards. Due to unequal samples sizes, type 3 sums of squares were used (General Linear Model; SAS, 1997). Multiple comparisons of means were made using Fisher's exact test (LSD with lines option in SAS. SAS, 1997. SAS/STAT Software: Changes and Enhancements Through Release 6.12, SAS Institute Inc., Cary, NC pp. 1167).

3. Results

Each of the treatment groups were initiated with six birds, but bleeding and feces collection were discontinued on a given bird when the plasma DNC analyses fell below MLOD. The half-lives for treatment groups were calculated from the plasma DNC concentrations for all birds in each treatment group (Fig. 1) and were based on a first order decay of the grit. The ln[DNC] vs. time graphs gave reasonable correlations; R^2 values ranged from 0.8 to 1.0. The mean R^2 was 0.86 and the plots of 31 out of 50 birds had R^2 values greater than 0.9. No standard deviations are shown on Figs. 2–5 as each curve represents a typical single animal. As the birds regurgitated approximately half the total gavaged grit

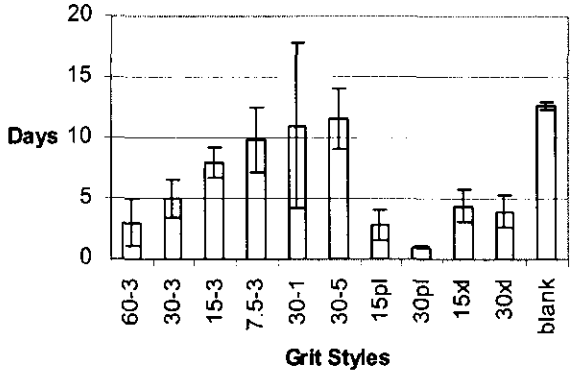

Fig. 1. Half life values for the 11 NCZ treatment groups.

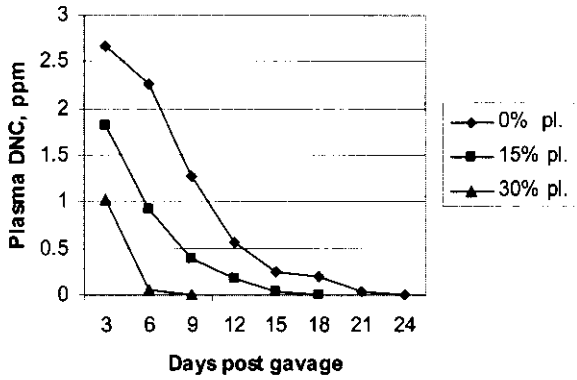

Fig. 2. Effect of plasticizer addition to #3 grit with 30% NCZ.

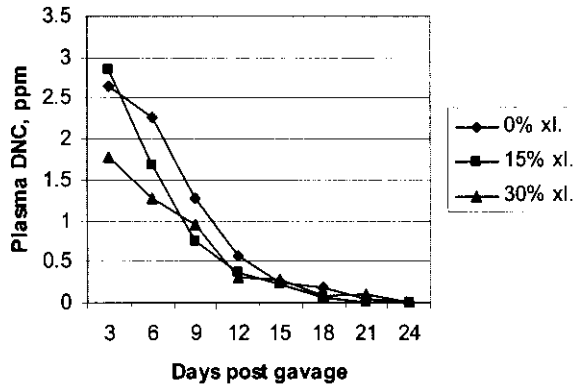

Fig. 3. Effect of cross linker on NCZ release from #3 grit pieces with 30% NCZ.

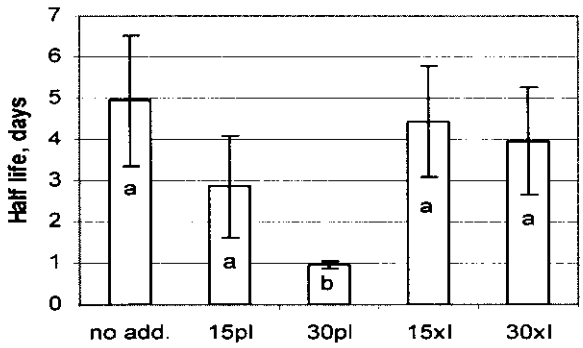

Fig. 4. Additives effect on half lives of #3 sized grits, 30% NCZ with none, 15, or 30% additions of plasticizer or crosslinker. (Bars labeled with the same letter are not significantly different, $\alpha = 0.05$).

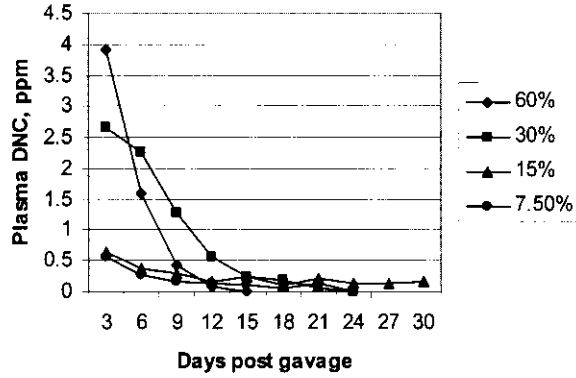

Fig. 5. NCZ concentration effect on release from #3 grit.

pieces, individual birds retained a variable and generally unknown number of grits. For half life determination, the actual number of grit in any given bird was not required.

3.1. Influence of plasticizer on plasma drug levels

As shown in Fig. 2, the slope associated with the grit containing 15% plasticizer was greater than the grit without plasticizer and the slope for the grit containing 30% plasticizer was greater than the slope for the 15% plasticizer grit. The half-lives were 4.95, 2.85 and 0.97 days for the 0, 15 and 30% plasticizer grits, respectively.

3.2. Influence of crosslinker on plasma drug levels

The effect of cross linking agent concentration on release rate was less clear than the plasticizer (Fig. 3). Compared to the grit with no cross linking agent, the addition of cross linking agent increased the half life from 4.95 to 5.05 days for the grit containing 30% crosslinking agent, and decreased the half life to 4.45 days for the 15% treatment. The results of the ANOVA treatment on the additive added grit sets are shown in Fig. 4. The degradation rates (half lives) for grits with crosslinking agent (15 and 30%), the 15% plasticizer, and the grit with no additives were not statistically different from each other. The half life of the grit containing 30% plasticizer was statistically less than the others in the additive set ($\alpha = 0.05$).

3.3. Influence of drug loading on plasma drug levels

Increased concentrations of the active ingredient in the grit were associated with increased initial release rates and decreased longevity (Fig. 5). The grits containing higher concentrations of NCZ clearly delivered higher plasma levels of DNC as evidenced by the decreasing initial levels in the birds treated with grit containing 60, 30, 15 and 7.5% NCZ. The longevity of NCZ release increased as the NCZ concentration decreased; half-lives of 3.29, 4.95, 7.97 and 9.88 days were noted for the grits containing 60, 30, 15 and 7.5% NCZ, respectively. Additionally, the release profiles for the grit containing 15 and 7.5% active ingredient were considerably more linear than the profiles for the higher loading of active ingredient. The results of the statistical treatment on concentration effect are shown in Fig. 6. The control and 7.5% grits, the 7.5 and 15% grits, and the 30 and 60% grits are not statistically different from each other, but the same inverse relationship of half life and concentration of NCZ is affirmed.

3.4. Influence of grit size on plasma drug levels

The relationship between grit size and release rates was more difficult to assess (Fig. 7) because a number of birds regurgitated some of the grit pieces on days 0 and 1. For grit containing 30% NCZ, 37.5% of the administered #1 size grit pieces were retained while 50% of the #5 size pieces were

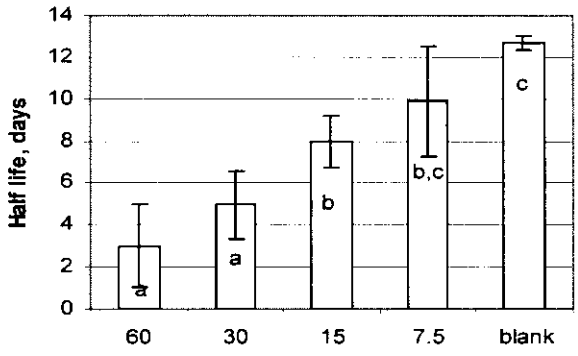

Fig. 6. Effect of NCZ concentration on half lives of #3 grit pieces.

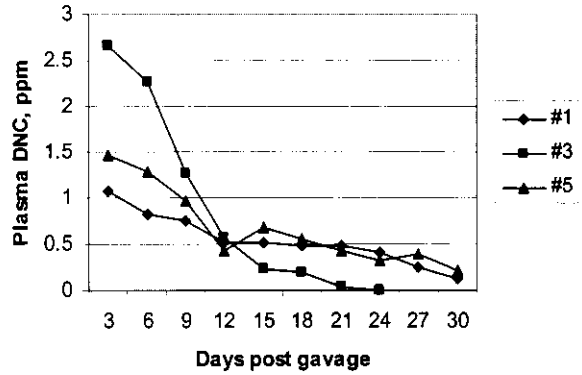

Fig. 7. Effect of grit size on release of NCZ with 30% NCZ.

retained. It was apparent that nearly all birds retained significant quantities of grit as 83% of birds treated birds treated with #3 and #1 grits and 100% of birds treated with #5 grits exhibited initial DNC plasma concentrations greater than 1 ppm. However, 50, 17 and 100% of birds treated with the #1, #3 and #5 grits, respectively maintained plasma DNC concentrations greater than the MLOD through day 33 post gavage. The calculated half lives were 11.00, 4.95, and 11.57 days for the #1, 3, and 5 grits, respectively. The statistical results for size are shown in Fig. 8. The half lives for the #1 and #5 grits were statistically longer than the #3 sized grit ($\alpha = 0.05$)

3.5. Recovery from birds at the end of the study

Thirty-three days post gavaging, 12 birds that still

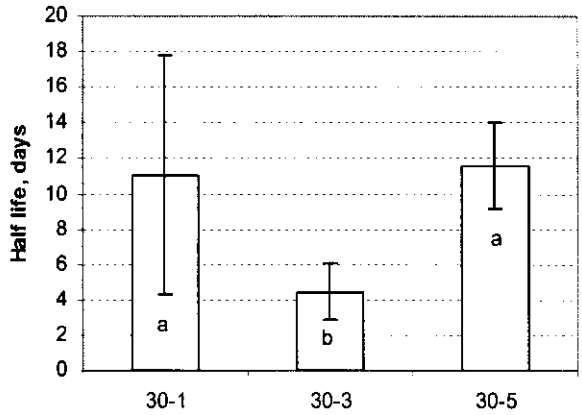

Fig. 8. Effect of grit size on half lives.

had measurable DNC in their plasma along with two control birds were euthanized and their gizzard contents examined. Synthetic grit pieces were recovered from all 14 gizzards. Overall, more pieces were recovered from the birds receiving the #3 and #5 grit (30% nicarbazin) treatments than the #1 size, but the total masses (35–85 mg) were about the same. For birds treated with #3 size grits of differing concentrations, no grit containing 60% NCZ survived to the end of the study; three large pieces of 30% NCZ grit weighing 73 mg were recovered. Two birds treated with 15% NCZ grits were opened and contained 78 mg and 125 mg of grit. One bird treated with 7.5% NCZ grit was opened and yielded two large pieces weighing 162 mg. Among the control birds (treated with #3 grits containing 0% NCZ), one contained six small grit pieces weighing 95 mg while the second contained two intact pieces totaling 85 mg. The birds treated with cross linked grit yielded many (up to 20) medium to small pieces totaling 55 mg.

3.6. Correlation of feces and plasma drug levels

Feces samples were taken from most birds at the same time as the blood samples. In general, feces DNC concentrations were greater than the corresponding blood sample. When fecal DNC concentrations were greater than 10 ppm, 17 out of 18 plasma DNC concentrations were 1 ppm or greater. Conversely, when the fecal DNC concentrations were less than 10 ppm, 9 out of 12 of the plasma DNC concentrations were less than 1 ppm (Fig. 9).

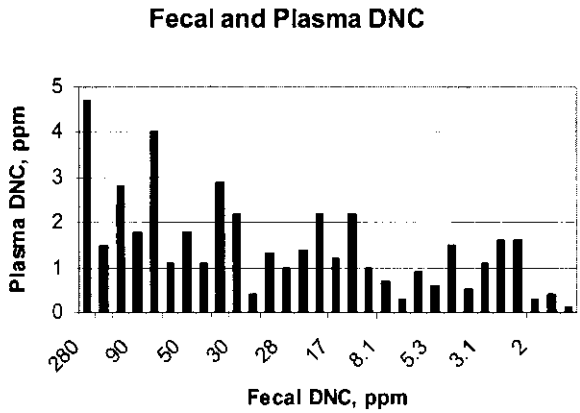

Fig. 9. Comparison of fecal and plasma DNC levels.

4. Discussion

As stated earlier, an exact accounting of the actual number of grit pieces in all birds was not possible. However in the case of the #1 size grit, the recovered pieces were easily identified and of the 24 initially given, 15 were recovered leaving nine in the animals. A review of the plasma DNC data for the #1 size grit treatment showed two birds with almost no plasma DNC on the first bleeding (day 3) and three birds values with a mean of 0.88 ppm and standard deviation of 0.07 ppm and a fourth at 1.1 ppm indicating that the three birds had two grit pieces each and the fourth had three grits. Based on the release rate, approximately 8 mg of NCZ was released each day. This amount of daily NCZ should yield a plasma DNC value of approximately 1 ppm based on other studies on ducks at this facility. Similar analysis of the data showed the same patterns with similar standard deviations in each of the other nine NCZ treatment types.

The addition of plasticizer increased the release rate of the active ingredient by a factor of 2–4 over grit containing no plasticizer. The addition of plasticizer to a grit could be useful where the delivery of medicant is desired over a 4–7 day period, but daily administration is not practical.

The lack of a consistent result for the inclusion of crosslinking agent was likely related to the brittle nature of the grit pieces and the inclusion of bubbles in the structure due to the more exothermic reactions of polymerization. As evidenced by the large number of small grit pieces recovered in the gizzards after 33 days, the grit pieces containing crosslinking agent were more easily fractured but still resistant to erosion. The inclusion of less crosslinker or cooling to moderate the reaction rate might have given grit pieces less prone to fracture and a resultant longer half life or the 30% NCZ loading may have been the dominate factor in all but the 30% plasticizer grits.

The relationship between drug loading and half life was inverse and linear with an r^2 of 0.99 for the lower three loadings and 0.90 overall. For most applications, a more constant release rate throughout

the lifetime of the formulation is desirable. This scenario is best approached by grits containing 15% or less active ingredient which would exhibit release for several weeks.

The longer half lives for the larger and smaller grits were influenced by the extended tails of the time vs. plasma drug curves which could be due to subtle differences in the mechanics of action by the gizzard interacting with the different sized grits. The initial grit pieces were cylinders while all recovered grits at the termination of the experiment on day 33 were flattened oblong disks. These findings suggest that the smaller #5 grit pieces would be the preferred size in terms of overall retention, longevity in the gizzard, and likelihood of ingestion by the target bird.

The fecal–plasma correlation was done to determine if NCZ release from grit could be monitored from feces as a substitute for blood DNC concentrations. While the linear correlation coefficient was low (0.42), the general indicative value of fecal numbers as to the existence of DNC in the animals seemed good. This approach should permit researchers to estimate grit retention and subsequent drug release under field conditions with no trauma to animals. Variability in feces DNC concentrations was due to a number of factors including the composition of the feces samples as they ranged from mostly fluid to nearly all solids, the possible nonlinear relationship between intestinal levels of NCZ and NCZ uptake into the blood stream (i.e. the higher initial release of the grit pieces may have overwhelmed the absorption mechanism), and variations in water intake and gastric emptying particularly relative to time of fecal sampling. In spite of this variability, these findings suggested that the determination of fecal DNC concentrations was more sensitive (due to low uptake by the bird and therefore relatively high fecal values) than plasma DNC for monitoring NCZ release from grit. Additionally, fecal DNC and plasma DNC concentrations were correlated at least at a nominal value of 1 ppm in the plasma at a yes/no level. As fecal collection was less labor intensive and less traumatic to birds, this approach appeared to be well suited for monitoring NCZ release and absorption in grit treated birds under both field and laboratory conditions.

Unpublished studies at this facility have shown nicarbazin to be unpalatable to ducks above levels of 250 ppm on the feed. Further, the social and feeding behavior of even resident animals leads to inconsistent feeding from bait stations and the use of grit as a supplemental source of drug rather than primary may prove to be the best utilization of the synthetic grit with this particular application. Additional studies for acceptance of grits in different shapes, sizes, and coatings are being planned.

5. Conclusions

Poly(methyl methacrylate) grit has been shown to be a promising controlled release system for the delivery of a potential avian contraceptive to ducks. Modification of additives offered a means of obtaining a wide range of release rates and grit longevity. Plasticizers greatly increased the release rate, while cross linking agents had a mild retarding effect. Percent loading of active ingredient increased absorption/release at high levels, while low loading levels gave slower release. This controlled release system would likely offer even greater longevity for contraceptive agents requiring lower dosage rates. Particle size slightly favored the smaller and larger grit pieces. Analysis of fecal material allowed a noninvasive semi-quantitative inference of release of NCZ by synthetic grit and uptake in plasma.

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Mention of commercial products is for identification only and does not constitute endorsement by the US Department of Agriculture.

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