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Influence of vitamin K-rich plant foods on anticoagulant baiting efficacy in wild House Mice, wild Norway Rats, and wild Black Rats

G. W. WITMER[1] and P. W. BURKE

Rodents introduced to islands have caused the extinction of many species of animals. Anticoagulant rodenticides are relied on to eradicate rodents from these islands, but if the rodents are eating plant materials that contain high amounts of vitamin K (the antidote to anticoagulants) anticoagulant rodenticides may not be effective. In a laboratory trial, individually caged Norway Rats *Rattus norvegicus*, Black Rats *R. rattus* and House Mice *Mus musculus* were fed fresh plant material high in vitamin K (Collards [0.62 mg vitamin K per 100 g] and Brussels Sprouts [0.19 mg vitamin K per 100 g]) for a period of 7 days. When presented later with anticoagulant rodenticides (0.0025% brodifacoum pellets or 0.005% diphacinone pellets) along with the diet of plant material, 94% of the rodents died. We conclude from this study that the presence of green feed rich in vitamin K does not reduce the effectiveness of anticoagulant rodenticides. However, we add a word of caution on one of the findings of our study. While we think the low efficacy (75%) we found in the case of brodifacoum and Black Rats was probably an artifact of small sample size in that treatment group, the result warrants further investigation.

Key words: anticoagulants, efficacy, invasive rodent, *Mus*, *Rattus*, rodenticide, Vitamin K

INTRODUCTION

Introduced, invasive rodents pose a serious threat to the native flora and fauna of islands worldwide (Towns *et al.* 2006). Rats and mice can be very prolific on islands where they have few, if any, predators, and their omnivorous foraging has lead to the endangerment or extinction of numerous island species (Moors and Atkinson 1984; Alcover *et al.* 1998). Most seabirds that nest on islands have not evolved to deal with predation and are very vulnerable to introduced rodents (Dowding and Murphy 2001). There has been a concerted worldwide effort to eradicate introduced rats (and to a lesser extent, introduced house mice) from islands with numerous successes (e.g., Veitch and Clout 2002; Towns and Broome 2003; Howald *et al.* 2007; Witmer *et al.* 2007). Research by the United States Department of Agriculture's National Wildlife Research Center (NWRC) has resulted in the recent registration of a 0.005% diphacinone bait pellet and a 0.0025% brodifacoum bait pellet to be used for aerial broadcast baiting of conservation areas to manage or eliminate introduced rats (Witmer and Eisemann 2007).

At the 2nd National Invasive Rodent Summit (Witmer and Eisemann 2005), a question was raised in a discussion session of rodent eradication specialists: what effect would the consumption of plants rich in vitamin K by the introduced rodents on an island have on the efficacy of anticoagulant rodenticides? No one knew the answer and the group identified this as a research need. There are several chemical formulations of vitamin K and the vitamin is required in metabolic activity in the liver in which essential blood-clotting proteins are synthesized (Robbins 1993; Tasheva 1995). Vitamin K1 is phytomenadione and it is produced by green plants. Vitamin K2 compounds (1–13, depending on the number of isoprenoid residues) are menaquinones and they are produced by bacteria. Vitamin K3 is menadione; this form is produced commercially and is added to animal feeds. The vitamin K requirements of vertebrates are met by dietary intake and microbial synthesis in the digestive system, but also by "recycling" of vitamin K in the liver (Hadler and Buckle 1992; Robbins 1993). Vertebrates require a constant supply of vitamin K to remain healthy and, in fact, the more vitamin K ingested by vertebrates, the more that is excreted (Harrington *et al.* 2007).

The United States Department of Agriculture (USDA) provides a listing of the vitamin K content of a large number of food types (www.nal.usda.gov/fnic/foodcomp/Data/SR21/nutrlist/sr21w430.pdf). The list makes it clear that certain leafy green vegetables are particularly rich in vitamin K. One such green leafy plant that contains high levels of vitamin K is the common dandelion (*Taraxacum officinale*). This plant species is native to Europe, but its invasive capabilities have allowed it to become cosmopolitan in distribution in the world (Gleason 1952). It is only one of thousands of invasive plant species have become established and naturalized in Australia (Groves 2002), Hawaii (Staples and Cowie 2001), and New Zealand (Clout and Lowe 2000). Most of the islands that rodents have successfully invaded have diverse green plants available

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year-round as food. Invasive rodents are omnivorous and opportunistic in their feeding habits (King 1990, Menkhorst and Knight 2001, Witmer *et al.* 2006), but where they have become established on temperate islands, they must feed primarily on plant materials in the fall and winter when animal foods are much less available (Innes 1990).

A review of the scientific literature provided very little insight into the question posed earlier. The only relevant published work we located was not with commensal rodents, but rather with Indian Gerbils *Tatera indica*. With that species, Chaudhary and Tripathi (2004) reported that small amounts of vitamin K (1 mg/kg for 5 or 15 days) did not reverse the effects of the anticoagulant difethialone, but that a higher dose (2 mg/kg for 15 days) did reverse the anticoagulant effects. Most literature on vitamin K effects on rodents and anticoagulant control methods address the vitamin K requirement of anticoagulant-resistant rats. Resistance to anticoagulants is controlled by a single gene and has become relatively common around the world where these rodenticides have been used repeatedly for long periods of time (Partridge 1980a). Resistant rats have a 4–20 times higher requirement for vitamin K (Partridge 1980a; MacNicoll and Gill 1993; Markussen *et al.* 2003). Generally, they cannot "recycle" enough vitamin K material and must get it through other sources such as diet and copraphagy (i.e., re-ingestion of faeces; Partridge 1980b; Hadler and Buckle 1992). Several researchers noted that some commercial livestock feedstuffs are supplemented with a form of vitamin K and that the availability of these foodstuffs to rats may lessen the effectiveness of rodenticide baits (Partridge 1980b; MacNicholl and Gill 1993). Commercial rat chows such as Formulab 5008 and Formulab 5001 (PMI Nutrition International, Inc., Brentwood, Missouri) each have small amounts of vitamin K (menadione form) added as a nutritional supplement; 3.2 ppm and 0.5 ppm, respectively.

In this study, we determined if a rodent diet containing green vegetables rich in vitamin K would reduce the efficacy of two commonly-used anticoagulant rodenticides, brodifacoum (a single-feeding, second-generation anticoagulant) and diphacinone (a multiple-feeding, first-generation anticoagulant). The green vegetables used in the study were Collards (0.62 mg vitamin K per 100 g food according to USDA) with rats and Brussels Sprouts (0.19 mg vitamin K per 100 g food) with mice, based on brief pre-trial food preferences. Because of the very high vitamin K content of the green vegetables selected for the trial, we considered this trial a "worst case scenario." That is, if the rodenticides are still effective while the rodents are feeding on these plants, they should be effective regardless of what green plants the rodents are feeding on in a natural or human-altered environment. We hypothesized that the rats receiving vitamin K-rich plant foods would not have the same high mortality rate after anticoagulant exposure as rats fed the anticoagulant bait, but without the vitamin K supplement.

METHODS

Wild-caught Norway Rats *Rattus norvegicus* and House Mice *Mus musculus* were obtained from the property of private landowners (with permission) in the Fort Collins, Colorado, area. Wild-caught Black Rats *R. rattus* were obtained from Wildlife Services operations personnel in the Phoenix, Arizona, area. Trials were conducted in an indoor animal research room (mice) or an outdoor rodent building (rats) at the NWRC, Fort Collins, Colorado. Conditions in the indoor room were maintained at 22.2°C, a relative humidity of 15%, and a 12 hr on/12 hr off light cycle. Conditions in the outdoor building (cement pit with 1 m of soil and a metal roof overhead) were ambient. In September, the ambient conditions in Fort Collins, Colorado, consist of maximum temperatures of 29.4°C, minimum temperatures of 2.2°C, a daily average temperature of 14.4°C, relative humidity of 12–20%, and 14 hr of light and 10 hr of darkness.

Rats and mice in this study were maintained in individual cages and fed rat chow and apple slices daily. All received water *ad libitum*. Each cage had a den box and bedding material. For each rodent species, there were three groups of 10 animals randomly assigned to each of the three treatments except for Black Rats for which only a total of 10 animals were available for the study. An effort was made to balance the number of males and females in each group, but that depended on the ratio of males and females available at the start of the study. The three treatments were 1) green vegetable plus brodifacoum rodenticide bait, 2) green vegetable plus diphacinone rodenticide bait, and 3) green vegetable plus rodent chow (control). The green, vitamin K-rich vegetables tested in a pre-trial preference session were Brussels Sprouts, Collards, and Kale. These had been identified as being very high in vitamin K by the USDA (www.nal.usda.gov/fnic/foodcomp/Data/SR21/nutrlist/sr21w430.pdf). In the pre-trial feeding, three types of green vegetables rich in vitamin K were offered to rodents overnight and the amount consumed was monitored by weighing before and after the pre-trial.

A two-choice, 10-day exposure rodenticide trial was conducted. The weight, sex, cage

number, and treatment group of each rodent was recorded before the initiation of the study. Rodents were fed rat chow and the green vegetable for 7 days prior to rodenticide exposure. We decided that 7 days of feeding on the green leafy vegetables would be an adequate period for the rodents to establish a baseline level of vitamin K because vertebrates do not store vitamin K, must continually replenish their vitamin K supply from dietary sources, and excrete any excess amounts ingested (Harrington *et al.* 2007). They continued to receive water *ad libitum* throughout the study. On day 8, all food was removed from all cages. Groups 1 (0.0025% brodifacoum pellets) and 2 (0.005% diphacinone pellets) received a weighed amount of rodenticide along with the fresh green vegetable. For rats, this was 40 g of rodenticide; for mice, it was 15 g. The rodenticide bait was replenished each day, as needed, so that it was always available to the rodents during the next 10 days. Fresh green vegetable was replenished each day, as needed, so that it was also available to the rodents during the next 10 days. Group 3 rodents (control) continued to receive the green vegetable and measured amounts of rat chow, starting with 40 g for rats and 15 g for mice and replenished as needed. Additionally, all rodents in this study received a small amount of vitamin K precursor (menadione) in their laboratory rat chow (Lab Diet's Formulab Diet 5008). Spillage under rack cages was gathered each day and weighed. At the end of the 10-day two-choice trial with rodenticides, all food was removed and weighed. All rodents went back to the maintenance diet of rodent chow and were observed for a 10-day post-exposure period.

All rodents were examined twice daily by the study director or his designee and the condition of the rodents and any mortalities were recorded. Dead rodents were placed in individual, labeled zip-lock bags and refrigerated for later necropsy. When necropsied, they were examined for signs of anticoagulant poisoning as described by Stone *et al.* (1999). All rodents were euthanized and incinerated at the end of the study.

The EPA requires a rodenticide efficacy (i.e., mortality rate) of 90% in individually-caged rodent trials before it will consider issuing a registration for commercial use of the material as a legal rodenticide in the United States (Schneider 1982). We compared our observed mortality rates with the EPA standard of 90% (as the expected mortality rate), using Chi Square contingency tests (SAS Institute 2003). We also used a two-way ANOVA test (SAS Institute 2003) to compare days to death by rodent species (Norway rat, black rat, house mouse) and by rodenticide type (diphacinone, brodifacoum). We used a probability level of $P < 0.05$ to determine significance of the statistical test result.

RESULTS AND DISCUSSION

The green vegetables fed to rats in the study were Collards (0.62 mg vitamin K per 100 g food according to USDA) and those fed to mice were Brussels Sprouts (0.19 mg vitamin K per 100 g food), based on the food preference pre-trial. For perspective, the highest amount of vitamin K listed by the USDA in a fruit (plums) was 0.03 mg per 100 g and in a meat (duck) was 0.004 mg per 100 g. After adjusting for vegetable weight loss due to evaporation, House Mice ate an average of 0.89 g (SD = 0.89) of Brussels Sprouts per day, Norway Rats ate an average of 13.3 g (SD = 4.0) of Collards per day, and Black Rats ate an average of 12.3 g (SD = 1.2) of Collards per day. While the control animals gained weight over the course of the trial, the animals on rodenticide baits generally lost weight (Table 1). This occurs because rodents feeding on anticoagulant rodenticides continue feeding for a number of days, but eventually at a reduced rate as they near death from internal hemorrhaging.

Table 1. Weight change, mortality rate and time to death in rats and mice fed different rodenticide treatments, along with fresh plant material.

Treatment	N	Start weight (g) Mean	Start weight (g) SD	Weight change (g) Mean	Weight change (g) SD	Mortality rate (%)	Time to death (days) Mean	Time to death (days) SD
Norway Rats								
Brodifacoum	10	207.38	49.89	-10.44	12.45	100	6.40	2.50
Diphacinone	10	236.66	54.30	-9.01	14.00	100	6.30	1.19
Control	10	234.69	78.57	17.39	10.36	0	N/A	N/A
Black Rats								
Brodifacoum	4	175.75	6.56	-24.35	9.23	75	10.00	2.45
Diphacinone	4	184.63	38.70	-14.13	5.14	100	8.75	2.86
Control	2	160.55	1.15	1.90	7.10	0	N/A	N/A
House Mice								
Brodifacoum	10	19.35	4.15	-2.36	2.25	100	10.20	3.29
Diphacinone	10	18.50	5.60	0.61	4.76	90	7.78	2.05
Control	10	15.90	4.68	2.74	3.76	0	N/A	N/A

Efficacy was high (94% overall) for both brodifacoum and diphacinone across all three species of commensal rodents (Table 1). It was 90-100% effective in all cases except with brodifacoum and Black Rats (75% efficacy). In all cases, the efficacy levels were not significantly different ($P > 0.12$) than the 90% required by the EPA. The lowest observed efficacy rate ($P = 0.12$) was with brodifacoum and Black Rats. In this case, the efficacy of rate of only 75% may have been an artifact of the small number of black rats (4) in that treatment group. Pitt *et al.* (2008) used 10 Black Rats per treatment group in their rodenticide efficacy trials and achieved an efficacy rate of 90% for black rats with the same commercial brodifacoum pellets that we used in our study. We do not believe that genetic resistance to anticoagulants was a factor with our Black Rats because we had a high efficacy rate (100%) with diphacinone, the other anticoagulant used in the trials. None of the control rodents died during the course of the study.

Rats ate 9.5–13.2 g of the rodenticide baits per day, while mice ate 2.5–2.8 g of the baits per day. The average time to death was 6.3–10.2 days and always longer for rodents feeding on brodifacoum bait (Table 1). The statistical analysis of the time to death revealed a significant difference between species with Norway Rats having a shorter time to death than Black Rats or House Mice ($P = 0.003$). There was no significant difference between rodenticides and time of death ($P = 0.109$) and the interaction between species and rodenticide was also not significant ($P = 0.371$). The results of our study suggest that Norway Rats are more susceptible to anticoagulant rodenticides than are Black Rats or House Mice. In previous studies of the efficacy of a broad array of commercial rodenticides, we found that many more were effective with Norway Rats than with House Mice (Witmer 2007a, b). Fisher (2005) also reported a lower susceptibility of House Mice to anticoagulants than rats in New Zealand.

Most (90%) of the dead treatment rodents showed signs of internal haemorrhaging upon necropsy, although a much smaller portion (40%) showed external signs. External signs were generally slight bleeding from the nose. The amount of vitamin K in these vitamin K-rich green plants was not sufficient to overcome the lethal effects of the two anticoagulant rodenticides used in this study.

All rodents in this study received a small amount of vitamin K precursor (menadione) in their laboratory rat chow (Lab Diet's Formulab Diet 5008). The menadione content of this rodent chow is 3.2 ppm (0.0032%). In previous rodenticide trials with wild Norway Rats and wild House Mice, we found several anticoagulant rodenticides to be very efficacious even when the same rodent chow was used as the alternative food in the two-choice trials (Witmer 2007a, b). We mention this point because some researchers have suggested that that the availability of vitamin K supplements in animal feedstuffs may lower anticoagulant rodenticide efficacy at livestock facilities infested with anticoagulant-resistant rats and mice (MacNicoll and Gill 1993). Of course, with invasive rodent eradication efforts on remote islands, supplemental animal feed would not be an issue. In some cases, however, those remote islands might contain vitamin K-rich plants. The finding of this study suggest that managers do not need to worry about decreased efficacy of anticoagulant rodenticides from vitamin K-rich plant consumption when those rodenticides are used to control or eradicate introduced rodents on islands. However, we add a word of caution on one of the findings of our study. While we think the low efficacy (75%) we found in the case of brodifacoum and Black Rats was probably an artifact of small sample size in that treatment group, the result warrants further investigation.

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