



Progressing development of a bird repellent for kea (Stage 1)

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Manaaki Whenua

Progressing development of a bird repellent for kea (Stage 1)

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Summary

Project and Client

- Discussions between the Animal Health Board, Department of Conservation and Landcare Research during 2011/12 led to agreement for progressing development of bird repellent additives to possum baits for aerial control. Before proceeding to trials with kea, it was considered necessary to assess the effects of bird repellents, if any, on the acceptability and efficacy for possums and rats of baits for used for aerial possum and rat control. This project was undertaken jointly for the Ministry of Business, Innovation and Employment, the Animal Health Board and the Department of Conservation.

Objective

- By June 2013, progress the development of bird repellents for inclusion in RS5 baits used for possum and rodent control in kea areas, by:
 - Assessing possum and rat responses to five bird repellent treatments using d-pulegone and/or anthraquinone to identify those that can be added to RS5 cereal pellet prefeed and/or toxic baits without compromising efficacy of toxic baits containing 0.15% 1080.

Methods

- We conducted feeding trials with captive possums and ship rats to compare consumption of non-toxic prefeed and toxic RS5 cereal pellets with and without various combinations of d-pulegone (0%, 0.17%) and anthraquinone (0%, 0.1%, 0.25%).
- There were five treatment groups and one experimental control group of possums ($n = 15$ per group) and ship rats ($n = 20$ per group). Possums and rats were acclimatised to captivity in individual cages on a standard diet of pellets and fruit.
- We used two-choice feeding trials to simulate conditions in the wild where animals have a choice of food. We simulated operational use by offering animals a choice of their normal pellet diet or RS5 cereal pellets for 3 days, returned them to normal pellet diet for 5 days, and then offered choice of normal pellet diet and toxic pellets with 0.15% 1080 for 2 days. Amount of test baits eaten (corrected for spillage and changes in moisture content) and mortality were measured daily.

Results

- During prefeeding, the number of possums eating test prefeed pellets showed little change across days 1-3, except for a slight, progressive decline in groups presented with 0.25% only anthraquinone in the prefeed. Palatability and amounts of prefeed pellets eaten with 0.25% anthraquinone was less than for other treatments.
- There was no significant difference in palatability of toxic pellets between test groups of possums on the first day they were offered although, again, palatability of the test groups that had experienced 0.25% anthraquinone in prefeed tended to be lower than

the other groups. The test groups that had experienced 0.25% anthraquinone in prefeed ate significantly less toxic pellets than the other groups.

- Mortality in the various groups of possums ranged from 53–93%, and was lowest in the test groups that had experienced 0.25% anthraquinone in prefeed.
- During prefeeding, all four test groups of rats offered pellets with anthraquinone alone or in combination with d-pulegone showed a significant reduction in the number of animals eating test pellets over the three days of prefeeding. Prefeed test pellets containing anthraquinone alone or in combination with d-pulegone were also less palatable and less of them were eaten.
- Few rats ate toxic pellets other than those in the two groups that had no experience of anthraquinone.
- Mortality in the two groups of rats that did not experience anthraquinone was 71% and 75%, whereas that in the four groups offered pellets with anthraquinone alone or in combination with d-pulegone ranged from 0–35%.

Conclusions

- For both possums and rats, the only test bait that had similar palatability, consumption and mortality to the control pellets with no bird repellent was the group offered prefeed and toxic pellets with 0.17% d-pulegone.
- Responses to test pellets containing both anthraquinone and d-pulegone were usually intermediate between those with only d-pulegone and those with only anthraquinone. This suggests a possible mitigating effect of d-pulegone on aversion caused by anthraquinone.

Recommendations

- Further testing of bird repellents on kea should focus on 0.17% d-pulegone.
- Operational trials to assess impact on possum and rat number should use 0.17% d-pulegone on both prefeed and toxic baits.
- Further research should be done to assess the possible mitigating effect of d-pulegone on the development of aversion to anthraquinone because of the potentially valuable role of d-pulegone as a learned cue to the presence of anthraquinone. This would be best done by assessing the prescription tested on kea by Orr-Walker et al (2012) (i.e., prefeed 0.17% d-pulegone+0.1% anthraquinone; toxic 0.17% d-pulegone) using methodology similar to that of the Kemp (2010) operational trial.

1 Introduction

The occurrence of kea mortality during past aerial 1080 possum control has identified an urgent need to identify and test bird repellents for inclusion in baits used for possum and rodent control in kea areas. Such repellents must be effective in reducing risk of poisoning of non-target native birds but not reduce kill efficacy for possum and rat control. A feeding trial to assess the effects of bird repellents on the consumption, palatability and efficacy of cereal pellet baits for possum and rat control was undertaken by Landcare Research on behalf of the Animal Health Board (AHB), the Department of Conservation (DOC), and the Science and Innovation Group, Ministry of Business, Innovation and Employment (MBIE) (contract no. C09X007).

2 Background

A review by Spurr (2008) suggested d-pulegone and anthraquinone as high priorities for investigation, based on positive results in trials using these compounds to deter a range of bird species from feeding on crops (Clapperton et al. 2011). Subsequently, captive kea (*Nestor notabilis*) offered baits containing d-pulegone and anthraquinone were shown to eat significantly less of such baits than untreated baits (Orr-Walker et al. 2012). DOC then conducted a small-scale field trial, which showed no significant difference in the estimated kill rate of possums and rodents between the standard operating protocol for aerial 1080 poisoning with RS5 baits and an experimental protocol using baits that also incorporated d-pulegone and/or anthraquinone (Kemp 2010).

A series of discussions between DOC, AHB and Landcare Research during 2011/12 led to agreement for progressing development of bird repellent additives for possum baits for aerial control. After Landcare Research and an internal DOC review of bird repellents, five potential strategies to protect kea were identified using different combinations and/or concentrations of d-pulegone and anthraquinone on either or both of prefeed and toxic baits, chosen by considering likely efficacy and cost. Once these strategies have been evaluated for their potential effects on possums and rats, DOC will undertake a parallel trial to confirm which strategies are most effective in preventing kea from eating (nominally) lethal quantities of RS5 baits.

It is critical that the inclusion of bird repellents in baits for possum and rodent control does not reduce the efficacy of the baits for the target animals. This project therefore assessed possum and rat responses to five bird repellent treatments using d-pulegone and/or anthraquinone added to RS5 cereal pellet prefeed and/or toxic baits.

3 Objective

By June 2013 progress the development of bird repellents for inclusion in RS5 baits used for possum and rodent control in kea areas, by:

- Assessing possum and rat responses to five bird repellent treatments using d-pulegone and/or anthraquinone to identify those that can be added to RS5 cereal pellet prefeed and/or toxic baits without compromising efficacy of toxic baits containing 0.15% 1080.

4 Methods

We conducted feeding trials with captive possums (*Trichosurus vulpecula*) and ship rats (*Rattus rattus*) to compare consumption of non-toxic prefeed and toxic cereal pellets with and without various combinations of d-pulegone (0%, 0.17%) and anthraquinone (0%, 0.1%, 0.25%) representing various strategies agreed in discussion with AHB and DOC for repelling native birds, particularly kea, from eating toxic baits. These included anthraquinone-only treatments, partly in case the volatility of d-pulegone created operational problems related to duration of bait storage (the decay rate of d-pulegone is being measured in a separate project for DOC).

There were five treatment groups and one experimental control group of possums ($n = 15$ per group) and rats ($n = 20$ per group) (Table1).

Table 1 Concentrations of bird repellents in pellets offered to test groups of possum and rats

Test group	Prefeed pellets		Toxic pellets (0.15% 1080)	
	d-pulegone + anthraquinone		d-pulegone + anthraquinone	
1	0	0	0	0
2	0.17%	0	0.17%	0
3	0	0.25%	0	0.25%
4	0.17%	0.1%	0.17%	0.1%
5	0.17%	0.25%	0.17%	0.25%
6	0	0.25%	0.17%	0.25%

Animal Control Products (Castlecliff, Wanganui) manufactured all baits. Baits were 12-g green-dyed RS5 cereal baits. Cinnamon was added to both prefeed (0.15%) and toxic baits (0.3%). Bird repellents were mixed into baits during manufacture (i.e. baits were not surface coated). Before trials began, and sometimes when toxic pellets were first offered, concentrations of d-pulegone, anthraquinone, cinnamon and 1080 were assayed by the Landcare Research toxicology laboratory using approved protocols, including those developed for d-pulegone and anthraquinone (Booth 2010).

We used two-choice feeding trials to simulate conditions in the wild where animals have a choice of food. We also simulated operational use by prefeeding animals for 3 days, returned them to normal diet for 5 days, and then offered toxic bait for 2 days. Wild-caught possums and ship rats were acclimatised to captivity in individual cages on a diet of standard pellets and fruit, weighed, and then offered for 3 days their normal pellet diet and an appropriate amount of prefeed test pellets, as per Table 1. Animals were then returned to their normal pellet diet only for 5 days then offered for 2 days a choice between their normal pellet diet and toxic baits with 0.15% 1080, as per Table 1. During test periods, the amounts of normal pellet diet and test pellets eaten (corrected for spillage and changes in moisture content) and mortality were measured daily. In addition to weighing, test baits were inspected visually to check whether any bait had been eaten. At the start of the trial test groups were balanced for sex and body weight, as far as possible. Because d-pulegone is volatile and has a distinctive minty-like smell, testing with baits containing d-pulegone was done in separate rooms.

Originally, rat and possum trials were to be run simultaneously. However, the rat trials were delayed by 2 weeks because the original supplier withdrew from the supply contract at short notice. The rat trials were run in three sets as rats became available, with some rats in each set as experimental controls (Group 1). The possum trials were also delayed because of an error in the order for bait, which meant one of the test baits had to be reordered. Possums were therefore tested in two sets, again with some possums in each set as experimental controls (Group 1).

Data for palatability (amount of test pellets eaten/[amount of test pellets + normal diet pellets eaten]) and amounts eaten were modelled using the lme (linear mixed effects) procedure in the statistical computing environment R (version 2.15.2). Random effects were animal ID (as there were repeated measures on the dependent variables). Fixed effects were treatment group (a factor), experimental day (a continuous variable) and sex (a factor) and their two-way interactions. Minimum adequate models were obtained by initially fitting a full model (with all main effects and interactions) and sequentially dropping non-significant factors until all terms in the model were significant. All data were $\log_{10}+1$ transformed to stabilise the residual variances.

5 Results

5.1 Possums

5.1.1 Mortality

Mortality of possums is shown in Table 2. Comparison of test groups showed weak evidence for an effect of treatment on mortality (Fisher’s Exact Test for count data, $P = 0.067$). The two test groups with the lowest mortality were those exposed to pellets with 0.25% anthraquinone during prefeeding.

Table 2 Mortality of possums in test groups offered toxic pellets with various combinations of bird repellents as noted in Table 1

	Test group					
	1	2	3	4	5	6
Alive	2	2	7	1	1	4
Dead	13	13	8	14	14	11
Total	15	15	15	15	15	15
% Mortality	87	87	53	93	93	73

5.1.2 Responses during prefeeding

The proportions of possums in each test group that ate detectable amounts of test pellets during days 1–3 of prefeeding are shown in Figure 1. Possums showed little change across days 1–3 in proportions eating test prefeed pellets, except for a slight, progressive decline in groups 3 and 6.

In terms of the palatability of the test prefeed (i.e. amount of test pellets eaten/[amount of test + normal diet pellets eaten]), the patterns shown by test groups 3 and 6 were significantly different from the other test groups ($P = 0.012, 0.034$, respectively), with palatability declining over the 3 days of prefeeding whereas palatability to the other test groups remained roughly constant (Figure 2).

The same trends were shown by analysis of the amounts of test pellets eaten per kilogram body weight. Consumption by test groups 3 and 6 differed significantly from the other test groups ($P = 0.033, 0.041$, respectively) particularly on day 3 and by males ($P = 0.041$) (Figure 3).

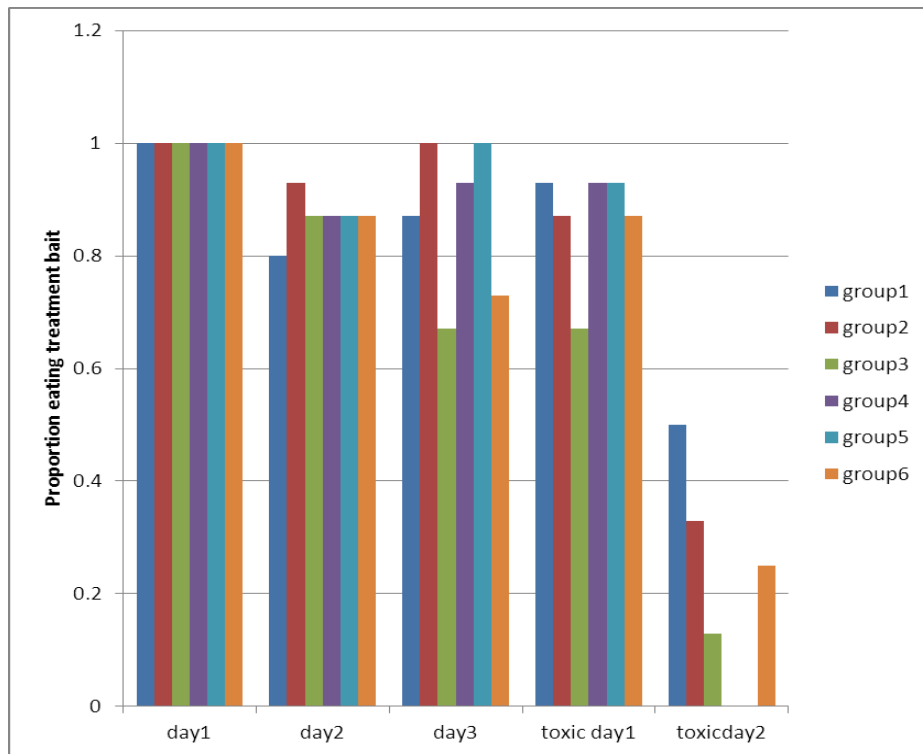


Figure 1 Proportion of possums in test groups eating detectable amounts of test pellets on days 1–3 of prefeeding and days 1–2 of toxic pellet presentation.

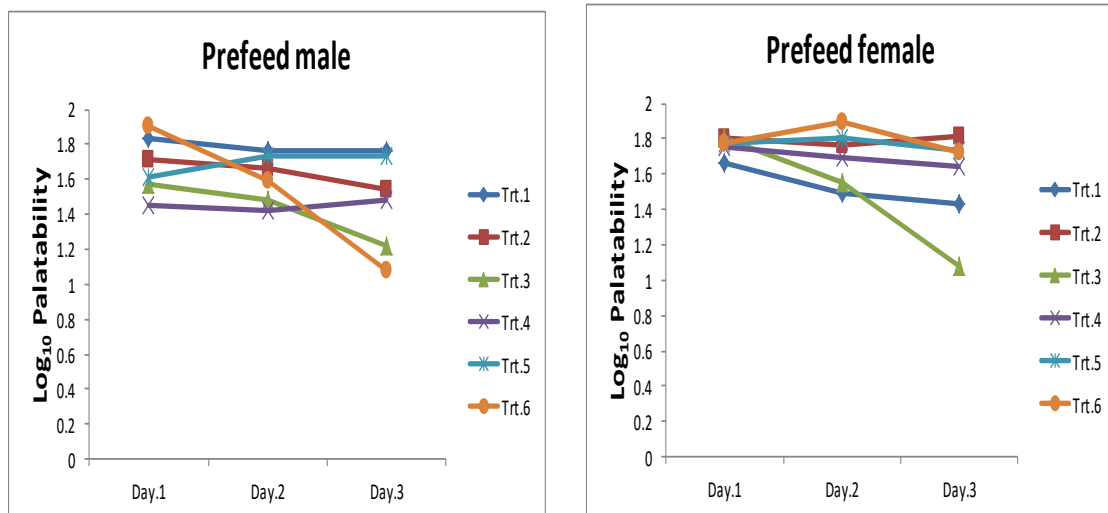


Figure 2 Palatability of test baits to male and female possums in each test group during days 1–3 of prefeeding.

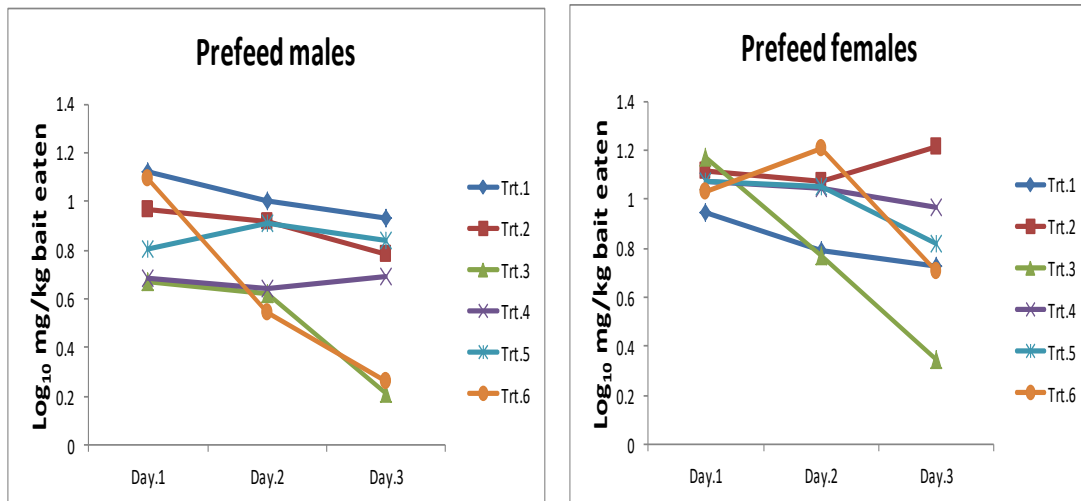


Figure 3 Amount of pellets eaten (per kilogram body weight) by male and female possums in each test group on days 1-3 of prefeeding.

The proportions of possums in each test group that ate detectable amounts of toxic test pellets during days 1–2 of presentation are shown in Figure 1. The proportions largely mirrored those on day 3 of prefeeding.

In terms of the palatability of the toxic test pellets (i.e. amount of toxic test pellets eaten/[amounts of toxic test + normal diet pellets eaten]), there was no significant difference between test groups on day 1, although again that of test groups 3 and 6 tended to be lower than the other test groups (Figure 4). The apparent decline in palatability on day 2 largely represented avoidance of test baits by sublethally poisoned animals.

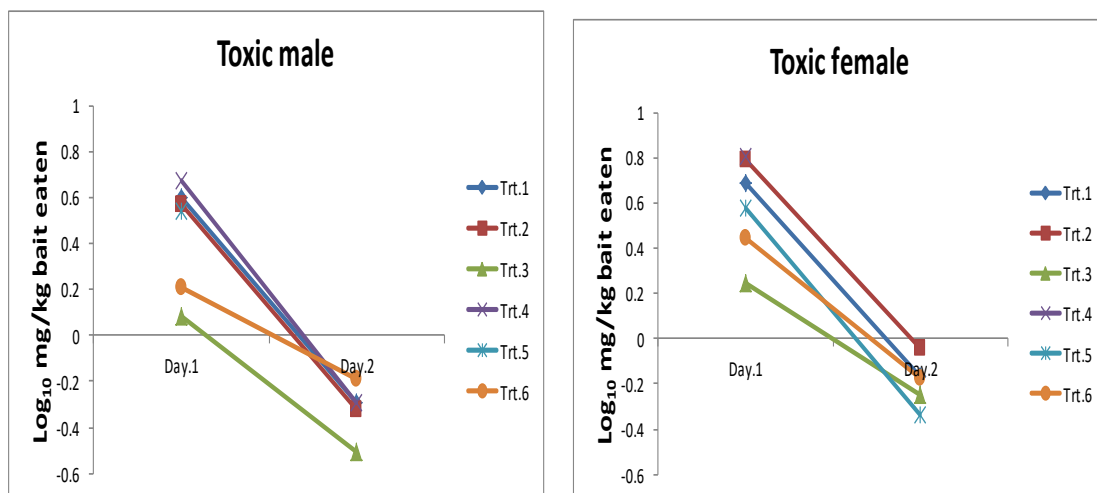


Figure 4 Palatability of pellets to male and female possums in each of the test groups on days 1–2 of toxic pellet presentation.

The amounts of toxic bait eaten per kilogram body weight were lower for test groups 3 and 6 than for the other groups ($P = 0.002, 0.044$, respectively) (Figure 5).

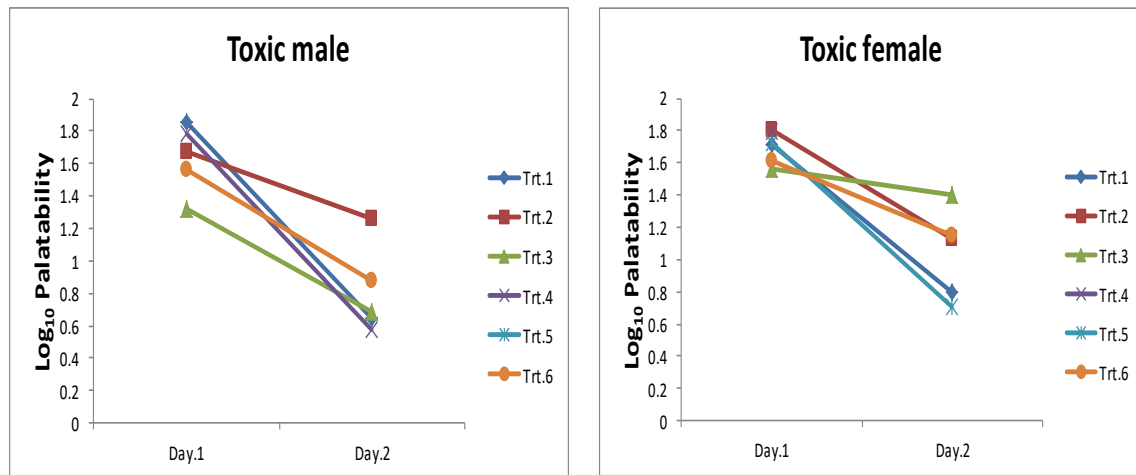


Figure 5 Amounts of toxic pellets eaten (per kilogram body weight) by male and female possums in each of the test groups on days 1–2 of toxic pellet presentation.

5.1.4 Sex differences in responses

In a few of the comparisons, there was a significant difference in response between males and females. Across all test groups, males ate slightly less of the test prefeed ($P = 0.041$) and the toxic pellets ($P = 0.073$) than females.

5.1.5 Assessing the nature of avoidance

Consistent individual differences in the behaviour of possums were evident, as shown by the significant positive correlations across all test groups between the palatability of test bait on the last day of prefeeding and the palatability of toxic bait on the first day of its presentation, and for amounts of test bait eaten (palatability $r = 0.81$, $P = 0.05$; amounts eaten $r = 0.809$, $P = 0.05$).

5.2 Rats

5.2.1 Mortality

Mortality of rats is shown in Table 3. There were highly significant differences in mortalities between test groups rats ($\chi^2 = 46.27$, $P < 0.001$). Test groups 1 and 2 had significantly higher mortality than the other groups, which did not differ among themselves.

Table 3 Mortality of ship rats in test groups offered toxic pellets with various combinations of bird repellents as noted in Table 1

	Test group					
	1	2	3	4	5	6
Alive	6	5	18	17	13	20
Dead	15	15	2	3	7	0
Total	21	20	20	20	20	20
% Mortality	71	75	10	15	35	0

5.2.2 Responses during prefeeding

The proportions of rats in each test group that ate detectable amounts of test pellets during days 1–3 of prefeeding are shown in Figure 6. All the test groups other than groups 1 and 2 showed a significant drop in number of animals eating treatment pellets over the 3 days of prefeeding.

In terms of palatability of the test prefeed (i.e. amount of test pellets eaten/[amount of test + normal diet pellets eaten]), test pellets 1 and 2 were significantly more palatable than the other test pellets. Palatability of test pellets declined significantly over the 3 days of prefeeding for all test groups except 1 and 2 (Figure 7).

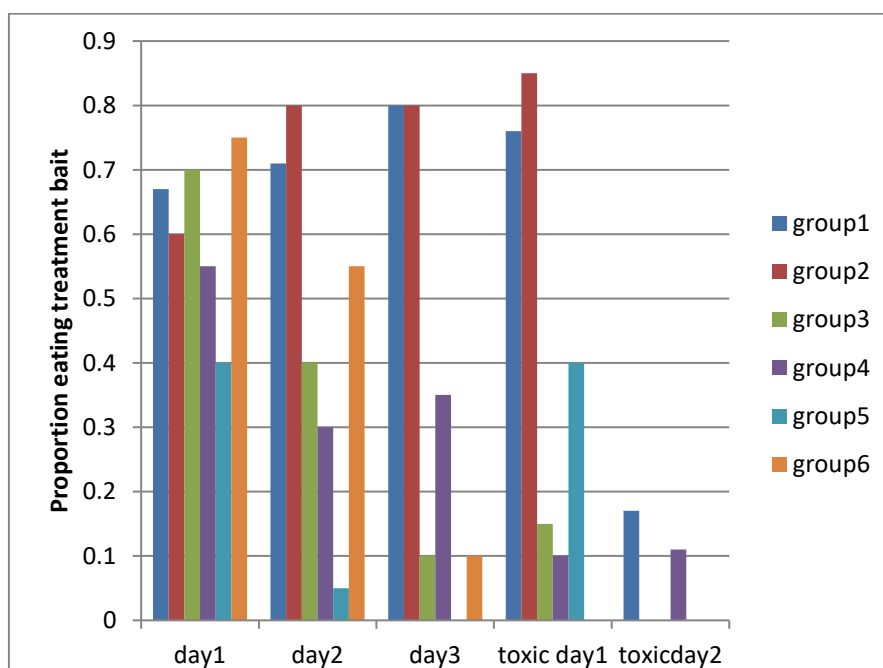


Figure 6 Proportion of ship rats in test groups eating detectable amounts of test pellets on days 1–3 of prefeeding and days 1–2 of toxic pellet presentation.

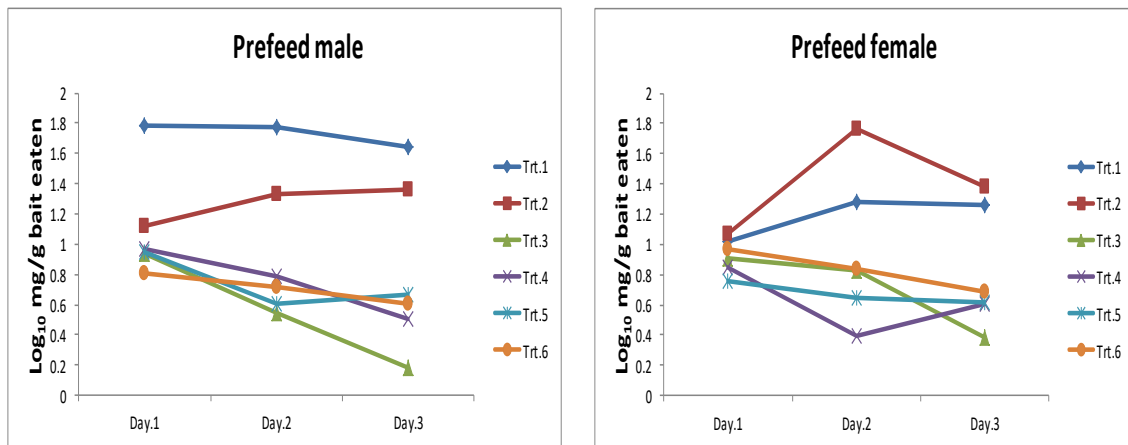


Figure 7 Palatability of test baits to male and female ship rats in each test group during days 1–3 of prefeeding.

The same differences and trends were found in the amounts of test pellets eaten per gram body weight (Figure 8).

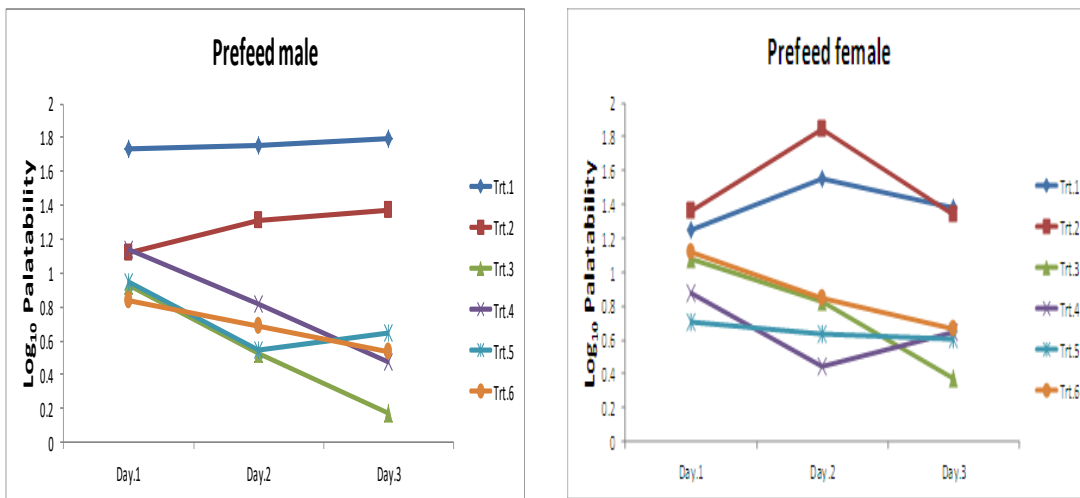


Figure 8 Amount of pellets eaten (per gram body weight) by male and female ship rats in each test group on days 1–3 of prefeeding.

5.2.3 Responses to toxic bait

The proportions of rats in each test group that ate detectable amounts of toxic test pellets during days 1–2 of presentation are shown in Figure 6. Clearly, few rats ate toxic pellets other than those in test groups 1 and 2. The palatability of toxic test pellets was significantly higher for test groups 1 and 2 than the other groups (Figure 9), with lowest palatability in groups 3 and 6. The amounts of toxic test baits eaten showed the same differences, with highest consumption by test groups 1 and 2 and lowest by groups 3 and 6 (Figure 10).

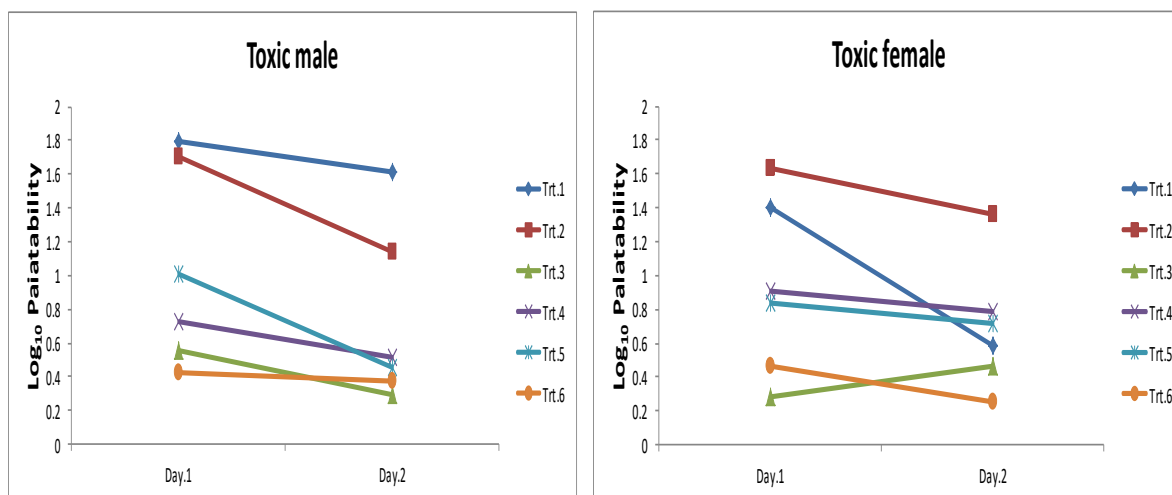


Figure 9 Palatability of pellets to male and female ship rats in each of the test groups on days 1–2 of toxic pellet presentation.

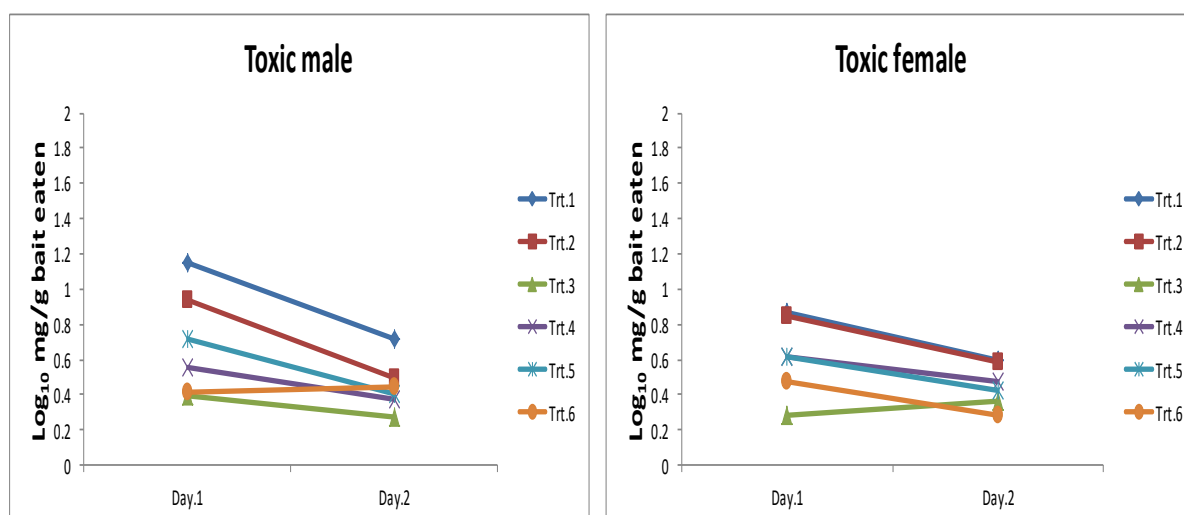


Figure 10 Amounts of toxic pellets eaten (per gram body weight) by male and females ship rats in each of the test groups on days 1–2 of toxic pellet presentation

5.2.4 Sex differences in responses

Differences in responses between males and females were only apparent with prefeed pellets. The test prefeed was more palatable to males than females ($P = 0.016$), and males ate more of it than females ($P = 0.001$).

5.2.5 Assessing the nature of avoidance

Most rats in test groups 3 and 6 did not eat any toxic test bait (Figure 6). This could have been because they were avoiding the bait, the repellent, the toxin or some combination of those. To investigate this further, we used surviving rats from groups 3 and 6 that had not eaten any detectable amount of toxic bait and so had, in effect, only eaten prefeed bait with 0.25% anthraquinone ($n = 14$ for each group). Rats in test groups 3 and 6 had no exposure during the trial to d-pulegone and so, if they had avoided toxic baits in the earlier trial primarily because of the smell or taste of anthraquinone, they might have been expected to accept baits containing only d-pulegone.

To test this, 2 weeks after the end of the previous trials, during which time rats had been on their normal pellet diet, those rats were retested. Half of each group was offered a choice for 3 days of normal diet pellets and non-toxic test pellets with (a) 0.17% d-pulegone or (b) 0.17% d-pulegone+0.1% anthraquinone.

Only one rat in each group ate any of the test pellets. This contrasts markedly with the acceptance of non-toxic and toxic baits with 0.17% d-pulegone by rats in test group 2. Thus rats in test groups 3 and 6 appear to have developed a generalised aversion to RS5 pellets as a result of their consumption of pellets containing anthraquinone.

Consistent individual differences in the behaviour of rats were also evident, as shown by the significant positive correlations across test groups between the palatability of test bait on the last day of prefeeding and the palatability of toxic bait on the first day of its presentation, and for amounts of test bait eaten (palatability $r = 0.948$, $p = 0.004$; amounts eaten $r = 0.954$, $p = 0.003$).

6 Conclusions

For possums, although there was only weak evidence of a difference in mortalities among the test groups, the pellets presented to test groups 3 and 6 were generally less palatable and less of them were eaten than those offered to the other test groups. Test groups 3 and 6 were each exposed to 0.25% anthraquinone in prefeed. The decline over days in palatability and consumption of prefeed test pellets by possums in groups 3 and 6 and their subsequent poorer responses to toxic bait suggest prefeeding had produced an aversion to RS5 pellets, whose strength varied among possums (as shown by the correlation between feeding on the last day of non-toxic prefeed pellets and day 1 of toxic pellets).

Data also suggested a weaker aversion to test pellets was shown by groups 4 and 5 which each received both anthraquinone and d-pulegone in the non-toxic prefeed pellets. Results from groups 4 and 5 were often intermediate between those of groups 1 and 2 and groups 3 and 6. Whether or not d-pulegone has a mitigating effect on aversion developed to anthraquinone requires further investigation.

Results for rats were generally similar to those of possums, although the aversion developed to test pellets containing anthraquinone was much more pronounced, such that few rats in groups 3–6 ate any toxic bait. The stronger aversion by rats was reflected in significantly lower mortality in test groups 3–6 (that all had anthraquinone in prefeed and toxic test pellets) than in group 2 (d-pulegone only) and group 1 (no added bird repellents). Palatability and consumption of test pellets by rats in groups 3–6 declined over the 3 days of non-toxic prefeeding, whereas those of groups 1 and 2 remained stable.

As with possums, data suggested a weaker aversion to test pellets by rat test groups 4 and 5, which each received both anthraquinone and d-pulegone in the non-toxic prefeed pellets. Results from groups 4 and 5 were often intermediate between those of groups 1 and 2 and groups 3 and 6.

One caveat to the findings of these trials on captive possums and rats is their applicability to operational use. The earlier trials with kea, which recommended the use of 0.17% d-pulegone and 0.1% anthraquinone in prefeed followed by 0.17% d-pulegone in toxic bait, were followed up by a small operational trial (Kemp 2010). That trial recorded similar mortality of possums and rats using baits with and without bird repellents. It included separate areas treated with d-pulegone in prefeed and toxic bait and with the combination of d-pulegone and anthraquinone tested on captive kea. Similarly, some studies demonstrating repellency of various compounds to birds in captivity failed to find such an effect when trialled on field crops (e.g. Esther et al. 2011).

7 Recommendations

- Further testing of bird repellents on kea should focus on 0.17% d-pulegone.
- Operational trials to assess impact on possum and rat percentage kills should use 0.17% d-pulegone on both prefeed and toxic baits.
- Further research should be done to assess the possible mitigating effect of d-pulegone on the development of aversion to anthraquinone. This would be best done by assessing the prescription tested on kea by Orr-Walker et al. (2012) (i.e. prefeed 0.17% d-pulegone+0.1% anthraquinone; toxic 0.17% d-pulegone) using methodology similar to that of the Kemp (2010) operational trial.

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