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# AN EVALUATION OF TWO LONG-LIFE BAITS CONTAINING DIPHACINONE FOR THE CONTROL OF FERRETS (MUSTELA FURO)

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# ABSTRACT

Two long-life baits (gel and polymer) were developed by Pest-Tech Ltd. and tested on captive ferrets. The palatability of both baits was significantly enhanced using 'natural' animal extracts, with extract palatability ranked in order of decreasing consumption as rabbit=chicken>hare=rat>fish. Final bait formulations (with animal extracts) ranked in order of decreasing consumption were gel=polymer>Pestoff<sup>TM</sup> ferret paste. The response of ferrets to gel bait containing diphacinone was highly variable, with males significantly more susceptible than females. Accordingly, large doses of diphacinone will be required to kill most ferrets in the field. In conclusion, both gel and polymer baits were superior to Pestoff<sup>TM</sup> ferret paste and have good potential for ferret control.

Keywords: ferret control, Mustela furo, diphacinone.

## INTRODUCTION

Ferrets (*Mustela furo*) are potential wildlife vectors involved in the transmission of bovine tuberculosis to domestic livestock (Ragg et al. 1995; Ragg & Walker 1996) and they also feed upon endangered indigenous wildlife (Murphy 1996). Between 1993 and 1998, Landcare Research Ltd. attempted to develop a baiting strategy for ferret. This baiting strategy was to apply a fish-based paste (Pestoff<sup>TM</sup> ferret paste) containing 0.03% diphacinone in bait stations spaced at 250 m intervals. In pilot field trials during March 1997, 100% of radio-tagged ferrets were killed, but in July 1997 only 31% of radio-tagged ferrets were killed (Spurr et al. 1997a; Spurr et al. 1997b). More widespread use of Pestoff<sup>TM</sup> ferret paste by Regional Councils has also had mixed results (S. Gooding, pers. comm.). Consequently, most ferret control is now reliant on prolonged periods of trapping (Moller et al. 1996).

This research evaluated two types of bait (polymer and gel) containing diphacinone as an alternative to trapping.

# METHODS

## Animal husbandry

Ferrets were caught from the wild using Holden treadle traps (Livecapture Traps, Waipara) baited with rabbit meat. All animals were individually housed in large wiremesh cages with a nest box and fresh water. They were fed daily on a mix of minced beef and offal (80 g) supplied by a local pet food manufacturer.

This research was undertaken with the approval of the Lincoln University Animal Ethics Committee (No. 858), and conformed to the New Zealand standards for care and use of animals for scientific purposes (Animal Welfare Advisory Committee 1995). Formulation of the bait bases

Prototype ferret baits were formulated by Pest-Tech Ltd. as either a polymer bait or an aqueous gel. A previous pilot study conducted by Pest-Tech Ltd. had demonstrated that polymer bait is readily eaten by caged ferrets (89 g/ferret/night). Further research

New Zealand Plant Protection 56:71-76 (2003)

conducted by Landcare Research Ltd. also indicated that mustelids would eat gelatinous baits (Spurr 1999). Each bait type was formulated from ingredients that were considered unlikely to degrade following the addition of preservatives and waterproofing agents. Whilst actual bait ingredients or concentrations cannot be disclosed for commercial reasons, the polymer baits were manufactured by blending all the ingredients into a homogenous loose mix with a dough mixer, and then manufactured into pellet baits weighing 1–2 g using a Christy–Norris orbital pelleter. The gel bait was manufactured from a solution of sugar and water.

#### Influence of flavour additives

Flavour additives considered likely to enhance ferret consumption were included in the new bait types at a minimum of three different concentrations. Flavour additives used were: (i) processed flavours used during the manufacture of pet food, (ii) waste cooking oils or (iii) 'natural' animal extracts from chicken, rabbit, hare, rat and fish. Natural extracts were prepared as 'emulsions' or as 'digests'. Emulsions did not require the use of heat during their preparation, whereas digests were prepared using temperatures of  $70-130^{\circ}$ C for periods of 0.5-2 h.

#### **Two-choice feeding trials**

The effect of flavour additives on bait palatability was tested in two-choice feeding trials (Grote & Brown 1971) that compared consumption of test and non-additive control bait. Paired trays were presented to ferrets on the day that the baits were manufactured. One tray contained 60 g of test bait and the other contained 60 g of the control bait. The amounts of test and control baits eaten were compared by Student's *t*-test to determine whether the addition of the flavour additive significantly enhanced bait consumption. Bait palatability was determined as the percentage of test bait eaten relative to total bait consumption. Throughout all two-choice feeding trials ferrets also received 80 g of fresh meat as a maintenance diet. All statistical analyses were made using Minitab 13 for Windows.

# Comparison with Pestoff<sup>™</sup> ferret paste

Non-toxic formulations of the new baits (with flavour additives) and Pestoff<sup>™</sup> ferret paste were presented to ferrets in a randomised Latin-square design. Each ferret received 60 g of test bait in a no-choice feeding trial. Ferrets also received 80 g of fresh meat as a maintenance diet. The amounts of bait eaten were tested by ANOVA using bait type and flavour additive as factors. Pair-wise comparisons between bait types were performed using Fishers LSD test.

#### Storage stability

The palatability of the final bait formulations was evaluated shortly after manufacture and again after bait had been stored for one month at an average temperature of 15°C. The amount of test bait eaten during these feeding trials was compared by ANOVA using duration of storage and the bait type as factors.

### **Toxicity of diphacinone**

Twenty caged ferrets were presented 60 g of gel bait containing 0.06% wt/wt diphacinone, 60 g of non-toxic control and 80 g of fresh meat on five successive days. To improve precision, dose-related mortality from this study was combined with previous data (Ogilvie et al. 1995; Ogilvie et al. 1996; Spurr et al. 1997a) increasing the total number of ferrets in the database to 147 animals. While it has been suggested that a sample size of 120 animals is the minimum necessary for a reliable LD<sub>50</sub> estimation, samples of greater than 240 animals may be required for accurate LD<sub>90</sub> dose estimates (Robertson et al. 1984). The ferrets used in the earlier toxicity trials conducted by Landcare Research Ltd. were acclimatised in a similar manner to the ferrets in our toxicity study. POLO (Russell & Pearce 1971) was used to calculate the LD<sub>50</sub> and LD<sub>90</sub> dose estimates using probit analysis (Finney 1971). POLO also compared the intercepts and slopes of different regression curves (e.g. male versus female). In the earlier toxicity trials, some ferrets were dosed over multiple nights and others given one large dose. Accordingly, we also used POLO to investigate whether the dosing regime affected the acute toxicity of diphacinone.

## RESULTS

# Influence of flavour additives

Whilst some of the waste oils and processed flavours improved bait consumption, the rabbit and chicken 'natural' animal extracts had the greatest influence on the palatability of the polymer and gel baits (Table 1).

Animal extract	Bait type	Effect on bait	Bait eaten	Percentage
Chicken emulsion	Polymer	Е	18.9	79
Rabbit emulsion	Polymer	E	29.4	80
Hare emulsion	Polymer	NS	14.6	57
Rat emulsion	Polymer	E	15.2	62
Freeze-dried rat emulsion	Polymer	NS	5.4	33
Chicken digest	Gel	E	38.5	100
Rabbit digest	Gel	E	28.9	100
Hare digest	Gel	NS	15.3	30
Tuna oil	Gel	NS	5.9	26

TABLE 1: Mean consumption of bait containing animal extracts	TABLE 1: Mean	consumption	of bait	containing	animal	extracts.
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<sup>1</sup>E=Enhanced – the addition of the flavour additive significantly improved palatability (P<0.05). <sup>2</sup>NS=No significant effect of the flavour additive (P>0.05).

## Comparison with Pestoff<sup>™</sup> ferret paste

Bait type influenced consumption (P<0.001; Table 2), with polymer and gel baits both eaten in significantly greater amounts than the Pestoff<sup>TM</sup> ferret paste. Gel (with rabbit extract) had the highest mean consumption although there was no significant difference between chicken or rabbit extract (P=0.74).

Bait type	Animal extract	Mean bait consumption (g)
Polymer	Chicken	$21.8 \pm 5.1$
Polymer	Rabbit	$20.2 \pm 5.1$
Gel	Chicken	$38.3 \pm 8.2$
Gel	Rabbit	$40.7 \pm 7.8$
Pestoff <sup>TM</sup> ferret paste	Fish	6.3 <u>+</u> 2.2

# TABLE 2: Amount of non-toxic bait eaten by captive ferrets. Values are the mean <u>+</u> SE.

### Storage stability

Both the chicken and rabbit gel were eaten in significantly smaller amounts after they had been stored for one month (P=0.005; Table 3), suggesting that either the antioxidant, bactericide or fungicide did not totally prevent biodegradation or that one or more bait ingredients had reacted to produce an unpalatable residue. The polymer bait did not significantly lose palatability during storage (P=0.65).

TABLE 3:	Amount (g) of gel and polymer bait eaten shortly after manufacture
	and then after one month of storage. Values are the mean $\pm$ SE.

Bait type	0 months	1 month
Chicken gel	$38.3 \pm 8.2$	$24.0 \pm 6.3$
Rabbit gel	$40.7 \pm 7.8$	$16.6 \pm 4.2$
Chicken polymer	$21.8 \pm 5.1$	$18.6 \pm 4.7$
Rabbit polymer	$20.2 \pm 5.1$	$19.2 \pm 4.2$
Pestoff <sup>TM</sup> ferret paste	$6.3 \pm 2.2$	$0.8 \pm 0.17$

#### Toxicity of diphacinone

The mortality of ferrets after eating various doses of diphacinone is shown in Figure 1. Using the combined database, POLO estimated a  $LD_{s_0}$  of 9.03 mg/kg (95% C.I. estimate 4.65-19.56) and a  $LD_{90}$  dose of 242.18 mg/kg (95% C.I. 81.67-1721.67). Pair-wise comparisons indicated that males were more susceptible to diphacinone ( $LD_{s_0}=3.6$  mg/kg) than females ( $LD_{s_0}=19.1$  mg/kg; P=0.002). Dosing regime did not affect the acute toxicity of diphacinone, with poison administered as a large single dose ( $LD_{s_0}=10.7$  mg/kg) ( $LD_{s_0}=7.5$  mg/kg; P=0.81).

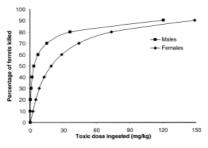


FIGURE 1: Percentage of female and male ferrets killed after ingesting diphacinone.

**Note:** Subsequent to publication, it has been discovered that unpublished reports from Landcare Research (Spurr et al. 1997a, 1997b) were incorrectly interpreted. Thus, data from some ferrets were included twice, and accordingly, the combined  $LD_{50}$  value quoted above is not correct. However, a revised analysis still indicates differences in susceptibility between male and female ferrets. Spurr et al. from Landcare Research have submitted a manuscript for publication in another journal giving the acute oral  $LD_{50}$  to ferrets of diphacinone in fish paste.

Gel containing diphacinone had lower palatability than non-toxic gel, with mean consumption of toxic bait peaking on the third day and then decreasing (Table 4).

Day of trial	Palatability of bait (%)	Mean toxic bait eaten (g)
Day 1	38.5	$15.8 \pm 4.3$
Day 2	39.1	$16.5 \pm 4.4$
Day 3	41.4	$23.4 \pm 5.1$
Day 4	30.5	$13.8 \pm 3.5$
Day 5	12.4	$8.2 \pm 3.2$

TABLE 4: Palatability (compared with non-toxic gel bait) and consumption of gel containing 0.06% diphacinone over 5 days. Values are the mean  $\pm$  SE.

# DISCUSSION

Whilst the gel and polymer baits are a significant improvement over Pestoff<sup>™</sup> ferret paste, most ferrets still ate the bulk of the fresh meat in preference to bait formulations. This suggests that bait consumption could be low where there is a surfeit of natural foods (e.g. rabbit). Bait palatability may be enhanced by improving the quality of prey odours (Clapperton 1996), and possibly by including other derivatives from animals, such as amino acids and fats (Bartoshuk et al. 1971; Beauchamp et al. 1977).

Unfortunately, the palatability of the gel decreased after one month of storage. Accordingly, further research is required to identify a preservative that will give this product a 12-month shelf life. At this stage, the polymer bait is the preferred long-life bait type.

Baits containing 'natural' animal extracts were significantly more palatable than bait formulated from processed compounds. None of the proprietary flavours that are routinely included in pet foods significantly enhanced bait consumption and only a few manufactured compounds (including some waste oil) increased the amount of ferret bait eaten.

Susceptibility to diphacinone was extremely variable with some males poisoned with as little as 0.4 mg/kg diphacinone, whereas others survived doses exceeding 60mg/kg. The low toxicity of diphacinone to some animals is likely to have contributed to the inconsistent kills previously reported with Pestoff<sup>TM</sup> ferret paste (Spurr et al. 1997a; Spurr et al. 1997b). A LD<sub>90</sub> of 242 mg/kg equates to 400 g of bait (at a concentration of 0.06%). Consumption of toxic gel by captive ferrets peaked on the third night and it is unlikely that wild ferrets would eat this much bait before the manifestation of poisoning symptoms. Another limitation of diphacinone is the low susceptibility of females compared to males. To control vertebrate pests with high intrinsic rates of population increase, it is essential to kill the females. However, the data on diphacinone toxicity should be interpreted with caution because of the small sample size and associated large variability in the lethal dose estimates.

#### ACKNOWLEDGEMENTS

We acknowledge the Animal Health Board Ltd. who provided the funding for this project.

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