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Consumption Pattern of Bromadiolone in Presence of Alternative Food by House Mice (Mus musculus) Infesting Poultry Farms

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ABSTRACT

Poultry farms in Central Argentina are often infested by *Mus musculus* L., despite the regular application of the rodenticide bromadiolone. This failure may be explained by the consumption pattern of mice, which may prefer alternative foods available on the farms to bromadiolone baits. Here we examine the consumption pattern of bromadiolone in the presence of wheat by *M. musculus* infesting poultry farms compared with the laboratory CF1 mouse strain. Overall, the poultry farm mice had longer survival and lower total food consumption in comparison with the CF1 mice. On the first day of the experiment, rodents from both strains and sexes consumed bromadiolone in the same proportion as wheat. On the second day, female mice of both origins showed a significant decrease in the consumption of bromadiolone, while males kept that proportion constant. Despite the consumption differences between males and females, survival rates were not different. We concluded that rodents from farms behaved as if they had never been in contact with bromadiolone, since they showed the same pattern of poison consumption that the CF1 mice. Females may have associated physical upset with the consumption of bromadiolone, since they decreased its consumption relative to wheat. However, this consumption pattern did not help them to achieve higher survival than males. On poultry farms, the balanced food fed to chickens may function as an alternative food to the poison for rodents. Therefore, we propose that rodenticide should be applied during downtime, when shed are cleaned and there is no chicken feed, which could be used as alternative food.

INTRODUCTION

Throughout history, the murine rodent *Mus musculus* L. (house mouse) has been considered a harmful pest worldwide. The damages it causes in rural areas, particularly on livestock and poultry farms, include the consumption and contamination of food, structural damage to building components and equipment, production loss, and spread of diseases and ectoparasites (Berry, 1970; Brooks & Lynwood, 1978; Timm, 1987; Pratt, 1991). On poultry farms of Central Argentina, *M. musculus* usually achieve worrying densities, and nearly 100% of the farms continue to be infested, despite the regular application of the anticoagulant rodenticide bromadiolone (Guidobono *et al*., 2010). Gómez Villafañe *et al.* (2001) suggested that strategies for controlling rodents based solely on chemical methods are not sufficient to effectively control rodents on poultry farms. The lack of success can be attributed to: (1) permanent recolonization by rodents; (2) the reproductive rate of *M. musculus* is greater than its mortality; (3) the existence of resistant individuals with ability to maintain a permanent population; and/or (4) rodents consume other food sources instead of bromadiolone baits.

A previous study on poultry farms suggests that the populations of *M. musculus* could remain *in situ* due to their population dynamics (León *et al*., 2007). It was observed that *M. musculus* on the farms is reproductively active throughout the year, achieves sexual maturity with a month and a half of age, and produces litters with an average of five and seven pups (Miño, 2003). On the other hand, it may also move from other farms, probably using riparian habitats as corridors (León *et al*., 2010, 2013). More recently, a study on bromadiolone resistance reported that 8% of *M. musculus* from these farms were resistant (Guidobono *et al*., 2010).

It is currently unknown whether feeding behaviour strategies may explain the low effectiveness of mouse population control measures applied on poultry farms. Guidobono *et al.* (2010) proposed that the feeding behaviour of *M. musculus* may have contributed to increase its survival time by a mechanism that potentially allows the metabolic clearance of bromadiolone in *M. musculus* populations present on the farms (Guidobono *et al*., 2010). The authors suggested that, under rural conditions, the field control program with anticoagulants is less effective than in captivity because there are alternative foods available, which may be preferred to bromadiolone baits. However, this has not been tested yet.

Based on the results of Guidobono *et al*. (2010), the presented study analysed, under experimental conditions, the consumption pattern of bromadiolone in the presence of an alternative food by wild *M. musculus* individuals that infest poultry farms where bromadiolone is applied periodically. This consumption behaviour was compared with that of laboratory mice of the CF-1 strain not previously exposed to bromadiolone. We predicted that, if poultry farm mice developed bromadiolone aversion, they would consume a smaller proportion of bromadiolone relative to the alternative food than CF1 mice on the first day of the experiment. Furthermore, if laboratory mice are able to associate the physical upset caused by the intake of bromadiolone, its proportional consumption relative to wheat will decrease throughout the experiment.

MATERIALS AND METHODS

Experimental design

Two experiments were conducted: one with *M. musculus* rodents captured on poultry farms (henceforth farm rodents) located in the county of Exaltación de la Cruz, Buenos Aires province, Argentina (34º18' S, 59º14' W), and the other with mice of the CF-1 strain born in captivity (henceforth CF1 mice).

Farm rodents were captured on five farms where bromadiolone (0.005%) had been applied for at least three years. Mice were captured using Sherman live traps for small rodents baited with a mixture of oats, peanuts, and bovine fat. Species, sex, reproductive status, and body measures (length and weight) of each captured rodent were registered. Only *M. musculus* adults were selected for the experiment. Individuals of other species, as well as mice weighing less than 10 g and pregnant females, were released at capture site.

During the experiment, mice were individually housed in cages (30 cm x 25 cm x 20 cm), equipped with a bottle of water and a feed pan, and were maintained at environmental temperature and natural photoperiod throughout the experiment.

Rodents were randomly assigned to one of the following three experimental groups: Group B, receiving bromadiolone daily (6 g); Group W (control group), receiving wheat grains daily (6 g); and Group BW, receiving both bromadiolone (6 g) and wheat grains (6 g) daily in separate feed pans. All three groups received water *ad libitum*.

The bromadiolone product used in the experiment was GlexRat™, the same brand applied on the poultry farms, consisting of red granule baits containing (hydroxy-4'cumarinyl-3')-3-phenyl-3-(bromo-4-biphenyl-4')-1-propane-1 at 0.005 g per 100 g, a bittering agent [denatonium benzoate], colouring, and inert compounds qs). In order to easily distinguish bromadiolone granules from wheat grains, the latter were stained with natural green food colouring based on chlorophyll. *M. musculus* has poor colour perception (Latham and Mason, 2004) and there is no evidence that it feels attraction for chlorophyll. During the experiment, non-consumed food was carefully collected and weighed daily to evaluate daily consumption. Rodents that died during the experiment were necropsied to confirm the presence of anticoagulation signs caused by bromadiolone consumption, such as massive internal haemorrhages and dark spots in the large intestine.

Before the experiment, mice underwent an acclimatization period of 10 days, during which they never ate an amount of food equal or greater than 6 g per day. This amount of food was considered *ad libitum*. During acclimatization, each rodent was fed with wheat grains dyed with red or green food colouring according with the food they were going to receive during the experiment (Group B: red grains; Group W: green grains; Group BW: a mix of green and

red grains). A total of 76 farm mice were placed for acclimatization; however, 12 escaped and 4 died, and therefore 60 mice were used during the experimental phase, which lasted 10 days (day of placement and nine days of registration of consumption and survival): 9 females and 10 males in Group B, 11 females and 10 males in Group BW, and 10 rodents of each sex in Group W. Mice weight at the beginning of the experiment was 16 g ± 4 g, both for males and females.

The experiment conducted with CF1 mice differed slightly from that with farm rodents. The CF1 mice were 2 months old and included 45 males, with average weight of 27 g, and 45 females with average weight 24 g. Fifteen rodents of each sex were assigned to each experimental food group (B, W and BW) and none of them died or escaped during the acclimatization period.

Data analyses

Consumption

In order to analyse individual total feed consumption per treatment, we fitted two general linear mixed-effect models (GLMM) to the data using the *nlme* library of the R Package (R Development Core Team, 2014). The first model included total food consumption as response variable and treatments (Groups B; W and BW), origin (farm or CF1), sex, and day (1 to 5th day; the following days were not analysed due to the strong unbalance among treatments caused as a result of mortality) as fixed effects. For individuals of Group BW, a second model was fitted, with the proportion of bromadiolone consumption relative to total food offer (with angular transformation) as response variable, and origin (farm or CF1), sex and day (1 to 5th day) as fixed effects.

Initially, a model with all possible interactions among fixed factors was fitted to the data, and then the most complex non-significant term was sequentially removed until the simplest significant model that explained most of the deviation was obtained. The significance of each term was assessed by examining the estimated p value of the likelihood ratio statistical test, associated with the change in deviation in the model obtained by the removal of each term (Pinheiro and Bates, 2000). The DGC multiple comparison test (Di Rienzo *et al*., 2002) was used to detect differences among treatments. Since food consumption was recorded daily for a same individual, a random factor (individuals) was included in both models. Because there was heteroscedasticity in both models, variances were modelled using the varPower function in the R library *nlme* (Zuur *et al*., 2009). Additionally, temporal autocorrelation (using the corAR1 function in the R library *nlme*) was included in both models. The goodness-of-fit of the models was assessed by visual investigation of residual plots. We used Akaike's information criterion (AIC), and the dimension-consistent Bayesian Information Criterion (BIC), which measure goodness-of-fit and model complexity (Zuur *et al*., 2009), to identify the model(s) that minimized information loss from a set of candidate models.

Survival

Survival times among the eight treatments, arising from the combination of origin, sex, and feeding of bromadiolone or bromadiolone and wheat (Groups B and BW, respectively) were compared. The control (group W) was not included in the comparison because the test requires a minimum of one dead animal per treatment. Post-hoc contrasts were performed using Gehan-Wilcoxon test (Gehan, 1965). In accordance with Bonferroni adjustment, a significant level of 0.017 was considered. This value was obtained by dividing the p-value of the survival test by the number of contrasts.

RESULTS

Consumption

The mice fed wheat maintained their body weight during the entire experiment, while those receiving bromadiolone and wheat lost 9% of their body weight, and those fed bromadiolone lost 13%.

Total food consumption was different between sexes, and such differences changed according to the origin of the animals ($F_{Origin\ x\ Sex}$: 12.58, $p<0.001$, Table 1). Among CF1 mice, males had higher mean daily food intake than females (4.95±0.1 g and 4.31±0.1 g, respectively; $p<0.05$). However, no significant differences between farm males and females were recorded (3.52±0.12 g and 3.86±0.12 g, respectively; $p>0.05$).

Total consumption pattern (change in food intake over days) differed among treatments ($F_{Day\ x\ Treatments}$: 9.93, $p<0.001$, Table 1). The control animals fed wheat (Group W) did not present any variations in the amount of food ingested during the five days analysed (Fig. 1A). Animals fed only bromadiolone (Group B) had a significantly lower consumption on day 1. Daily consumption increased on days 2 and 3, reaching the same level as the control group; however, from day 4, it began to decline again, reaching the minimum on day 5 (Fig. 1A). Meanwhile, animals of Group BW had a mean daily consumption similar to the control group during the first three days, whereas food intake decreased significantly on day 4, and dropped even lower by day 5 (Fig. 1A).

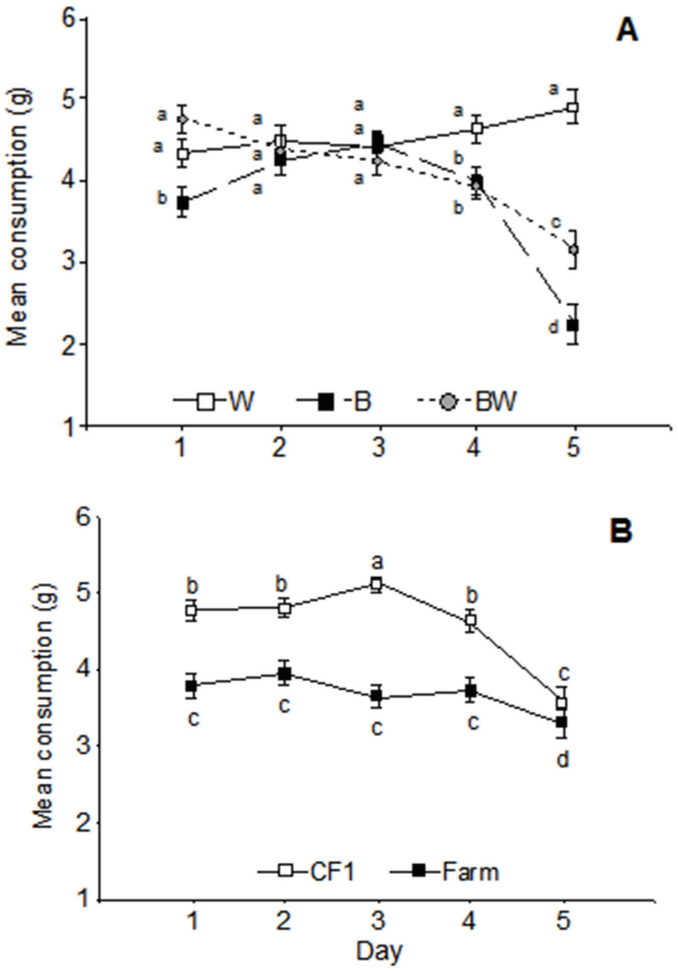

Figure 1 – Mean of the total consumptions by day for (A) *Mus musculus* fed with wheat (W), bromadiolone (B), or both (BW), by averaging the origin (poultry farms and CF1 mice) and sex (males and females); and (B) *M. musculus* from both origins averaging the three kinds of food received and the sex. Bars indicate standard error. Different letters indicate significant differences among means (p<0.05).

Table 1 – General Linear Model results for the variation in total daily food consumption of *Mus musculus*.

Factor	Numerator df	Denominator df	F-value	p-value
Intercept	1	557	3807.29	<0.0001
Day[1]	4	557	11.56	<0.0001
Origin[2]	1	144	44.22	<0.0001
Treatment[3]	2	144	13.02	<0.0001
Sex[4]	1	144	0.71	0.4017
Day x Origin	4	557	5.69	0.0002
Day x Treatment	8	557	9.93	<0.0001
Origin x Sex	1	144	12.58	0.0005

[1]Day of the experiment (1 to 5)
[2]Origin of mice (poultry farms or CF-1 laboratory strain)
[3]Kind of food offered (bromadiolone, wheat, or both)
[4]Sex of mice (male or female)
Day, origin, treatment and sex were used as fixed effects.

Furthermore, the consumption pattern also varied according to the origin of rodents ($F_{Day \times Origin}$: 5.69, p < 0.001, Table 1). On each day, mean consumption by CF1 mice was higher than that of farm rodents (Fig. 1B). CF1 mice had the highest consumption on day 3, after which consumption decreased, with lowest value recorded on day 5 (Fig. 1B). Consumption by farm rodents did not change during the first four days, but on day 5, a significant decrease in the average food intake was recorded (Fig. 1B).

For Group BW, the proportion of bromadiolone consumed per day significantly varied depending on the sex of the animal ($F_{Sex \times Day}$: 2.72, p = 0.031, Table 2). The proportion of bromadiolone consumed by males did not significantly vary during the five days of the experiment. However, a significant decrease in the proportion of poison consumed by females was recorded from day 2 (Fig. 2). The final model did not include neither the origin factor nor its interactions with other factors because they were not statistically significant.

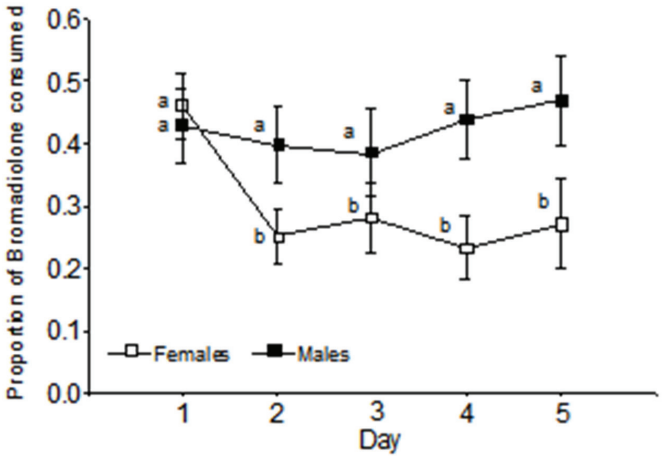

Figure 2 – Bromadiolone proportion relative to the total food ingested by females and males of *Mus musculus* per day, during the first 5 days of experiment averaging the origin (mice from poultry farms and CF1 mice) and the food they received during the experiment (wheat, bromadiolone or both). Bars indicate standard error. Different letters indicate significant differences among means (p<0.05).

Table 2 – General Linear Model results for the variation in the proportion of poison consumed by individuals of *Mus musculus* fed with bromadiolone and wheat.

Factor	Numerator df	Denominator df	F-value	p-value
Intercept	1	187	205.75	<0,0001
Sex[1]	1	49	4.22	0.0453
Day[2]	4	187	2.63	0.0357
Sex x Day	4	187	2.72	0.0308

[1]Sex of mice (male or female)
[2]Origin of mice (poultry farms or CF-1 laboratory strain)
Sex and day were used as fixed effects. Angular transformation was applied to the response variable (proportion of poison consumed).

Survival

Significant differences were observed among survival times of all treatments (χ^2 = 56.3, p < 0.001, df = 7, n = 100). No rodent in the control group (W) died during the experiment, whereas mice in Group B died significantly earlier than in Group BW (WW = 867, p

= 0.002, n = 100, Fig. 3). CF1 mice died significantly earlier than farm rodents (WW = 1828; $p < 0.001$; n = 100, Fig. 3). No significant differences were detected between sexes (WW = 179; p = 0.53; n = 100). All the rodents that died presented bleeding signs.

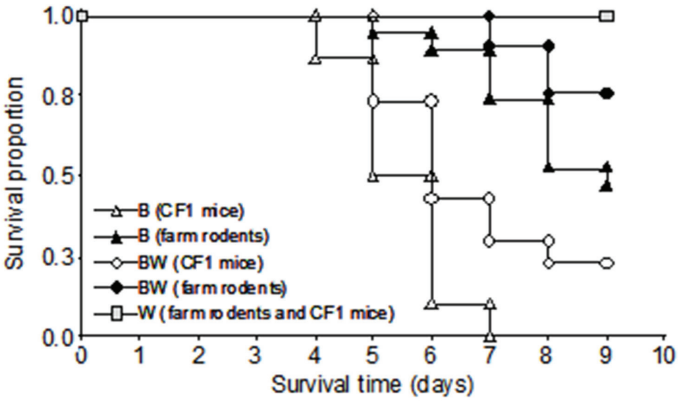

Figure 3 – Survival proportion against time between the beginning and the end of the experiment, in *Mus musculus* from poultry farms (farm rodents) and laboratory mice of the CF-1 strain (CF1 mice) fed with wheat (W), bromadiolone (B) or both kinds of food (BW). Sexes were pooled because they did not differ significantly.

DISCUSSION

We designed this experiment to analyse the consumption pattern of bromadiolone by *Mus musculus* infesting poultry farms in the presence of an alternative food, because we believe that this can help to understand the reason of their persistence in poultry farms despite the periodic application of bromadiolone. We predicted that if the rodents were able to associate physical upset with the intake of bromadiolone, its proportional consumption relative to wheat would decrease throughout the experiment. Our results revealed that this was true for female mice caught on the farms as well as for females of the CF-1 strain. Female mice decreased the proportion of bromadiolone consumption from day 2, and was maintained low for the following days. On the contrary, males of both origins maintained the same proportion of bromadiolone consumption throughout the experiment. However, the different consumption strategies of males and females did not seem to have any effect on survival rates under these experimental conditions, since both sexes showed similar survival curves when fed bromadiolone and wheat baits simultaneously. Other authors, who offered only bromadiolone to rodents, did not find any differences in survival times of males and females either (Guidobono *et al.*, 2010). In contrast, in another study, it was found that females were less susceptible than males to the effects of anticoagulants (Ashton *et al.*, 1987). We believe that these consumption strategy differences between males and females must have some evolutionary advantage for the species. It is possible that the feeding strategy of females in natural situations, such as in poultry farms, is more efficient than in the captive conditions of the present experiment, since the proportion of poison relative to total food offer in our experiment was 50% higher than that applied on the farms. Further studies on the feeding strategies of male and female mice, offering a wider range of alternative foods or lower poison proportions, are necessary to elucidate whether the strategy of bromadiolone consumption by females is more successful for survival than the strategy of males.

On the other hand, our results did not support the prediction that the mice captured on farms where bromadiolone was being applied would be able to recognize it when they were offered bromadiolone baits for the first time in captivity. In our experiment, both CF1 mice (which had never been exposed to bromadiolone) and farm rodents (caught on farms where bromadiolone was applied) consumed the same proportion of poison baits on the first day of experiment. Therefore, farm rodents behaved naively, as if they had never had contact with bromadiolone. This may be attributed to: (1) the inefficiency of design of the control programme applied on the farms where mice were caught, or 2) the fact that farm rodents did not develop aversion to bromadiolone. The reason why the pest control programme failed was not due to poison ineffectiveness, since 100% of CF1 mice receiving only bromadiolone died. Moreover, bromadiolone is a second-generation anticoagulant, whose effectiveness on *M. musculus* has been well proven (Meehan, 1978; Redfern & Gill, 1980; Rowe *et al.*, 1981). The control programme on poultry farms may have failed because mice can easily access chicken feed (located on trays at ground level), and therefore, they would not need to resort to the poison baits to feed.

The other possibility is that farm rodents had contact with bromadiolone on the poultry farms, but they did not develop aversion to it. Actually, it is generally considered that second-generation anticoagulants like bromadiolone do not induce aversion in mice because symptoms appear after a few days of ingesting these poisons. However, there is evidence that some of these anticoagulants can trigger symptoms inducing aversion one day after being ingested (Smith *et al.*, 1994). In this experiment, the latter would reinforce the idea that female *M. musculus* did not have contact with the poison when they were on the poultry farms because

females from both origins (CF1 and farms) consumed the same proportion of bromadiolone on the first day, and furthermore, both decreased bromadiolone consumption on day 2.

Mus musculus infesting poultry farms may not develop aversion to bromadiolone for several reasons. Firstly, the attractants of the poisoned baits may not be efficient enough in the presence of chicken food, and to elucidate this, chicken feed should be offered as an alternative food instead of wheat grains. Chicken feed is mainly made of corn. Here, wheat was used to analyse the consumption pattern of bromadiolone in the presence of an alternative food because we wanted to minimize the influence of other variables, such as bait shape and size. As bromadiolone baits are made of wheat grains, the only difference between bromadiolone and the alternative food used was the bromadiolone cover on the baits. Secondly, although in low concentrations, chicken feed contains vitamin K_3 (menadione: 1.0-1.5 mg/kg food), which can counteract the effect of anticoagulants (MacNicoll and Gill, 1993; O'Reilly, 1976). Thirdly, rodents infesting poultry farms may be resistant to bromadiolone. As we mentioned before, 8% of the mice population on the same farms where rodents were sampled in our experiment were shown to be resistant (Guidobono *et al.,* 2010).

The lower survival rate of CF1 mice compared with farm rodents, both when fed only with bromadiolone and bromadiolone and wheat, may be due to the fact that CF1 mice always consumed more food than farm rodents in all three treatments (bromadiolone, wheat or both). The effects of bromadiolone depend on the cumulative dose ingested. Thus, since CF1 mice ingested more food per day, the lethal dose of bromadiolone was possibly achieved earlier than in farm rodents.

In conclusion, the consumption pattern of mice from farms applying rodent control programmes using bromadiolone was not consistent with those expected for mice that develop aversion to that poison, since they presented the same pattern of bromadiolone consumption as CF1 mice. On the other hand, it would seem that females associate bromadiolone with the physical upset caused by intoxication, since they reduced the proportion of bromadiolone consumed, as well as the total consumption. However, this consumption pattern was not reflected in the female survival rates under these experimental conditions.

Finally, these results allow us to recommend some management practices to control *M. musculus* populations on poultry farms. Since the balanced feed fed to chickens is accessible to rodents, it would serve as an alternative food to the poison baits. Therefore, we propose that rodenticides should be applied during down time, when chicks or chickens are not present, feeders are empty, and poultry houses are being cleaned.

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