Efficacy of Cholecalciferol rodenticide against Wood Rat, Rattus tiomanicus and its secondary poisoning impact towards Barn Owl, Tyto javanica javanica

Ariff Ateed Mohd Noh (ariffateed50@gmail.com)
Universiti Sains Malaysia
Abu Hassan Ahmad
Universiti Sains Malaysia
Hasber Salim
Universiti Sains Malaysia

Article

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Abstract

Studies were conducted on the potential use of cholecalciferol as an alternative to anticoagulant rodenticides against common rat pest in oil palm plantations, i.e., wood rats, *Rattus tiomanicus,* and the secondary poisoning impact of cholecalciferol on barn owls, *Tyto javanica javanica*. The laboratory efficacy of cholecalciferol was compared with commonly used first-generation anticoagulant rodenticides (FGARs): chlorophacinone (0.005% a.i) and warfarin (0.05% a.i). The results of the study showed cholecalciferol baits recorded high mortality at 71.39% against wild wood rats in six-day feeding laboratory trial. Similarly, the FGAR chlorophacinone recorded a high mortality rate of 74.20%. Warfarin baits recorded the lowest mortality rate at 46.07% against the rat samples. The days-to-death of rat samples was in range of 6-8 days. The highest daily consumption of bait by rat samples was recorded by warfarin at 5.85±0.38 gram per day while the lowest was recorded in rat samples fed with cholecalciferol, i.e., 3.03±0.20 gram per day. Chlorophacinone-treated and control rat samples recorded consumption of about 5 gram per day. A secondary poisoning assessment on barn owls in captivity fed with cholecalciferol-poisoned rats showed after 7 days of alternate feeding, the barn owls appeared to remain healthy. All the barn owls fed with cholecalciferol-poisoned rats survived the 7-day alternate feeding test and throughout the study, up to six months after exposure. All the barn owls did not show any abnormal behavior or physical change. The barn owls were observed to be as healthy as the barn owls from the control group throughout the study.

Introduction

Introduction of anticoagulant rodenticides (ARs) in the 1950s marked a turning point in the agriculture industry, especially in oil palm (Bentley, 1972). The efficiency of ARs in dealing with rat problems became a fundamental reason for planters to switch their interest from acute poison to ARs as a default rat control measure (Wood & Chung, 2003). However, the challenges regarding AR application emerged a few years after AR establishment. In Malaysia, resistance of a major species of rats in oil palm, wood rats (*Rattus tiomanicus*) against ARs was one of the major concerns among planters. The first detection of wood rat-resistance against the first-generation anticoagulant rodenticide (FGAR) warfarin was reported in the 1980s in three different localities (Klang, Teluk Intan, Renggam) (Wood & Chung, 1990; 2003). To combat this problem, more potent ARs were used and proved to be effective against warfarin-resistant rats (Marsh, 1977; Greaves and Cullen-Ayres, 1988; Buckle, 2012). The over-dependance on toxic ARs, especially second-generation anticoagulant rodenticides (SGARs), to combat warfarin-resistant rats rose to a more complex situation where two major concerns emerged. The first concern was that the rats were observed to have developed a resistance against some of the SGARs (Ishizuka et al., 2008; Vein et al., 2011) and the second concern was that ARs were found to bioaccumulate in predators that feed on rats or other rodent pests, leading to secondary poisoning (Thomas et al., 2011).

In 1984, cholecalciferol was developed as a rodenticide by Bell Laboratories in Wisconsin and was registered as a rodenticide the same year (Marshall, 1984; Tobin et al., 1993). The rodenticide was developed to deal with the challenges encountered in attempts to control commensal rodents, e.g., house

mice (Bull, 1983), with the existing ARs. New Zealand (Eason et al., 1993; 2010), European countries (where application was, initially, synonymous in a mixture with coumatetralyl and as in year of 2020,, the majority of the European market has cholecalciferol only baits (no coumatetralyl)) (Tobin et al., 1993; Pospischil and Schnorbach, 1994; Baldwin et al., 2016b, British Pest Control Association, 2020) and the United States (Eason et al., 2000; Baldwin et al., 2016a) are countries that have established the use of cholecalciferol as a rodenticide. Cholecalciferol is consumed by humans as a dietary supplement and can be found naturally in fish oils, egg yolk and milk fat (Horst et al., 1982). However, when the compound dosage is at toxic levels, it can raise the calcium level in blood (hypercalcaemia) by absorption from bone and small intestine and causes calcification of blood vessels (Marshall, 1984; Marsh & Tunberg, 1986; Jolly et al., 1993; Beasley et al., 1997). Heart failure usually is one of the major effects to the consumer (Eason et al., 2010). The other mechanism of cholecalciferol is the “stop feed effect’ where the toxin causes the consumer to lose their appetite, eventually resulting in death due to hypercalcaemia which occur after only fatal dose has been consumed due to a rise of calcium level above normal concentration in blood, leading to calcification of blood vessels in vital organs such as kidneys, stomach, lungs and cardiovascular system (Morgan & Rhodes, 2000). The mineralization causes blockage to blood vessels and eventually leads to heart failure and death (Jolly et al., 1993).

Cholecalciferol has been proven to be very effective against Norway rats, *Rattus norvegicus* and house rats, *Rattus rattus* (Marshall, 1984; Eason et al., 2010). However, there are no published studies regarding the efficacy of cholecalciferol against wood rats, *R. tiomanicus* in oil palm plantations, as the application of cholecalciferol is more concentrated in urban settings and poultry farms rather than in agricultural settings. In Malaysia, barn owls *Tyto javanica javanica* have been employed as natural predator, with combination of ARs to control rats in oil palm through implementation of Integrated Pest Management (IPM) program as part of commitment towards sustainable palm oil (Lam, 1982; Wood & Chung, 2003; Turner & Gillbanks, 2003). Since the incident of warfarin resistant rats occurred, the manufacturers came up with other initiative by producing more toxic ARs with longer hepatic biological half-life which rose the concern on potential of secondary poisoning towards barn owls (Khoo et al, 1991; Erickson & Urban, 2004). Various conducted studies have approve the existence secondary poisoning of ARs towards barn owl (Mendehall & Pank, 1980; Lee, 1994; Saravanan & Kanakasabai, 2004; Fisher et al., 2003; Salim et al., 2014). However, with regards to cholecalciferol, there have been studies on secondary poisoning against non-target animals. However, the information of these study is limited to very few non-target animal species such as cat and dogs (Eason et al., 1996, 2000). There is no published information on the secondary poisoning effect of cholecalciferol against barn owls.

In this study, we evaluated the laboratory efficacy of cholecalciferol and commonly used FGARs against a major rat species in oil palm plantations, i.e., wood rats, *R. tiomanicus*. A secondary poisoning assessment of cholecalciferol towards barn owls was also carried out. Since there is already established data available on secondary poisoning risk of FGARs against barn owls, we did not repeat the assessment of secondary poisoning risks of these compounds.

Methods And Materials

Laboratory trial

The aim of this study was to evaluate the efficacy of cholecalciferol (0.075% a.i) in comparison with selected ARs against wild wood rats, *R. tiomanicus*, in laboratory conditions using a no-choice feeding study. The study consisted of an acclimatization period, followed by a pre-test diet intake assessment, then a six-day (multiple rodenticide dose) test period and 21 days of post-treatment observation. The lab testing procedure followed the Malaysian Standard MS1256 – Household insecticide products-rat bait – chemical, physical and biological efficacy requirements (2nd revision). The study protocol was approved by an animal ethics committee (Approval number: USM/IACUC/2020/123/1064). Additionally, this study is reported based on instruction provided by ARRIVE guidelines (PLoS Bio 8(6), e1000412,2010).

Animal trapping and laboratory feeding test

Adult *R. tiomanicus* were live-trapped in two oil palm plantations: Felda Gunung Besout 4 and Kiara Jubli Estate located in Sungkai, Perak. Traps used were drop-door cage traps (27 x 18 x 13 cm^3) and loose oil palm fruit was used as bait. Trapping sessions were conducted for two weeks and about 400 individual *R. tiomanicus* were caught. The captured rats were brought back to the laboratory in Universiti Sains Malaysia (USM) for inspection. The rats were weighed, sexed and caged individually. Only rats that weighed in the range of 85–120 g were selected for the trial. All rats sampled were subjected to an acclimatization and conditioning period for at least 14 days prior to the feeding trial. Water was given *ad libitum* and they were fed laboratory diet (broken corn). Rats that were not eating within a normal range (3–8 g per day) were removed from the study. A total of 160 rats that were screened as healthy and feeding normally during the acclimatization period were randomly divided into 40 rats per group (20 males and 20 females) and assigned into cholecalciferol (0.075% a.i), chlorophacinone (0.005% a.i), warfarin (0.05% a.i) and control. Aforementioned total rats covered total of four replications which were carried out in this study. During the feeding trial, each rat was offered only rodenticide bait based on the treatment assigned, with water given *ad libitum*. Rats in the control group were given laboratory diet. Weight of the bait was recorded before being offered to the rat, and again after 24 hours and replenished with fresh bait/diet (this was repeated for six consecutive days). At the end of feeding period, the rats were maintained with laboratory diet and observed until day 21. Parameters evaluated in this study were the mortality of the rats (%), days-to-death and average consumption of the baits by rat samples. Dead rats were dissected to determine the cause of death.

Secondary poisoning to barn owls' assessment

The study was conducted in the barn owl aviary of the School of Biological Sciences, USM, Penang. All the barn owls used in the study were captive-bred and were about one year old. All the barn owls were in captivity as part of the research of introduction of barn owls to the campus of Universiti Sains Malaysia (USM), Penang. Throughout their period in captivity, prior to the secondary poisoning study, all the barn owls were fed with healthy and rodenticide-free wild wood rats. The rats were released in a feeding arena

inside the aviary to be preyed upon by the owls. The captivity procedure and protocol of the study followed the guidelines suggested by Salim et al (2014). A total of eight barn owls were selected for this study. After being weighed, the owls were randomly assigned to the control group (four barn owls: two males and two females) and cholecalciferol group (four barn owls: two males and two females).

Rats that were offered to the barn owls were fed with 20 g of cholecalciferol bait (0.075% a.i) each day for two days in no-choice feeding. Another group of healthy rats were fed with corn and offered to barn owls of the control group. All rats had free access to water *ad libitum*. At the end of the feeding procedure, the poisoned rats and healthy rats were offered to barn owls according to the treatment group by placing them in the feeding arena of the aviary. The remnants of the baits left by rats were collected, weighed, and recorded.

The cholecalciferol-treated owls were offered a single poisoned rat (rat fed with cholecalciferol bait for 48 hours) and a non-poisoned rat on alternate days over a seven day feeding period, depicting a 50% exposure to rodenticide in a weeks' diet. Control barn owls received non-poisoned rats daily throughout the seven-day duration and throughout the study. Daily behavioral observations were carried out on all birds. Position and movements of the birds in the aviary were monitored. Each bird was inspected and monitored at pre-treatment, day 1, day 3, day 5, day 7 during treatment and day 11, 12, 14, 30 post treatment to observe for any signs of cholecalciferol poisoning. The weight of each bird was recorded during initial physical inspection and the same step was repeated again after the completed feeding period. The survival and the health status of all the barn owls were assessed up to 6 months.

Data statistical analysis

One-way ANOVA was used for data analysis. The difference in mean days taken for the treatment to kill the rat samples (days-to-death) and mean amount of bait eaten between each treatment by all rats were analyzed using one-way ANOVA. Further post-hoc Tukey HSD test was used to analyze for any significant difference between each treatment group. The amount of cholecalciferol bait consumed by rats offered to each individual barn owl was analyzed by one-way ANOVA. Lastly, weight change of barn owls after the one-week feeding trial was analyzed by non-parametric Mann Whitney U-test.

Results

Rat no-choice feeding test

Results of feeding test are presented in **Table 1**. In general, all treatments recorded mortality rates of wood rats within the range of 71-74% within six to eight days of the feeding, except warfarin. FGAR chlorophacinone showed good control against the rats with mortality recorded at 74.20%, followed by cholecalciferol with a 71.39% mortality rate. Warfarin recorded only a 46.07% mortality rate of the rat samples. Chlorophacinone took 6.53 ± 0.66 days while cholecalciferol recorded 8.40 ± 0.67 days to result in death of the rat samples. Warfarin took 8.65 ± 0.63 days to result in deaths. No mortality was detected in the control group. One-way ANOVA analysis showed that there were no significant difference in days-

to-death of rats between the treatments, $F(3, 69)= 1.23$, $p=0.30$. The rats in the warfarin treatment group consumed the highest amount of bait at an average of 6.50 ± 0.38 g/day, while rats in the chlorophacinone treatment group recorded 5.43 ± 0.35 g/day bait consumption. The control group consumed 5.02± 0.23 g/day, and all rats in the cholecalciferol treatment group consumed the lowest amount of bait at 3.03 ± 0.20 g/day. According one-way ANOVA analysis, daily bait consumption by rats across the treatments were significantly different, $F(3, 148)= 23.84$, $p < 0.05$.

All the rats in anticoagulant rodenticide treatments exhibited external bleeding or haemorrhage from the mouth, eyes and ears, which were common signs before death. Upon dissection, dead rats showed internal bleeding in the body cavity and general loss of blood in organs such as lungs, liver and muscle. The rats in the cholecalciferol group did not show any signs of external bleeding throughout the experiment. The bodies of the dead rats treated with cholecalciferol bait were dissected and no symptoms of external and internal haemorrhage or bleeding were observed.

Table 1: Mortality rate, mean days-to-death, and bait consumption of wood rats, *Rattus tiomanicus*, in six-day feeding trial of selected rodenticides

Treatments	Mortality rate (%)	Means Days-to-death	Mean bait Consumption (g/day)
Cholecalciferol	71.39%	8.40 ± 0.23^{a}	3.03 ± 0.17^{a}
Warfarin	46.07%	8.65 ± 0.67^{a}	5.85 ± 1.34^{c}
Chlorophacinone	74.20%	6.53 ± 1.07^{a}	5.43 ± 0.44^{bc}
Control	0.00%	0.00 ± 0.00^{b}	4.95 ± 0.23^{b}

Means in column with different letters are statistically significant different ($P<0.05$) by Tukey's test

Secondary poisoning assessment

Table 2 displays the mean weight and total cholecalciferol bait consumed by rats offered to individual barn owls. Each barn owl consumed four poisoned rats with an average body weight of 93.69 ± 3.04 g. Average total bait consumed by the rats in the two days no-choice feeding before being offered to barn

owls was 9.88 ± 0.75 g. This average consumed in the two days is within the normal expected dietary consumption of 3-8 g per day. The total average bait consumed corresponding to a.i. consumed by rats per body weight was 0.080 ± 0.009 mg/g. There was no statistical significant difference ($F_{2,9}$= 1.49, P= 0.28) in mean total bait and a.i consumed ($F_{3,12}$= 2.75, P= 0.89) by rats offered to each owl according to one-way ANOVA.

Table 2: Mean weight and total cholecalciferol bait consumed by rats offered to individual barn owls

Barn owl		No. rats consumed	Mean weight of rat (g)	Mean total bait consumed by rats (g)	Mean total a.i. consumed by rats per body weight (mg/g)
Code (sex)	Weight (g)				
234 ( )	450	4	97.50 ± 6.89	10.5 ± 1.19	0.081 ± 0.007
230 ( )	434	4	102.75 ± 7.41	7.75 ± 0.63	0.057 ± 0.004
236 ( )	470	4	85.25 ± 3.04	11.25 ± 1.44	0.098 ± 0.011
220 ( )	473	4	89.25 ± 3.33	10.00 ± 1.58	0.085 ± 0.016
Mean	457.00	4	93.69 ± 3.04	9.88 ± 0.75	0.080 ± 0.009

Results of the toxicity effect of cholecalciferol on barn owls fed with four poisoned rats each in the seven-day feeding period are shown in **Table 3**. In general, all treated owls did not show any behavioral or physical abnormality. The behavior of all the barn owls after consuming the poisoned rats were examined through their movement, especially the flying activities in the aviary. The flying activities of the barn owls after consumption of four poisoned rats did not change from the pre-treatment condition and the treated owls were as active as the barn owls from control group throughout the feeding and observation period. All barn owls did not show any change in their feeding activities, the treated owls fed normally on the rats offered, even in the post feeding period. During the observation, all owls spent more time on the perching point rather than on the ground, indicating normal barn owl behaviour which did not exhibit the common

signs of cholecalciferol poisoning in birds where according to Swenson *et al.* (2013) can be identified as following behaviour; subdued behavior, weight loss, and an inability to fly.

The physical characteristics of the treated birds after examination were similar to the control group which were fed with healthy rats. All the barn owls gained weight during the seven-day feeding period except for barn owl code **220** which experienced a slight weight reduction compared to its initial weight. Mean weight increase of barn owls after the one-week feeding trial was recorded at 10.50±7.86 g for the cholecalciferol group while the control group owls recorded a higher weight gain at an average of 12.75±1.25 g.

All treated barn owls survived the seven-day feeding period. There were no toxicity symptoms observed during the feeding period and post-feeding period up to six months after the treatment as all the barn owls were observed to be as healthy as barn owls in the control group. All the barn owls were later successfully released in the university campus as part of our soft release method to introduce acclimatized barn owls. The Mann Whitney test showed that there was no statistical difference ($U(N_{cholecalciferol}=4, N_{control}=4) = 8.00, z= 0.00, p> 0.05$) in weight change between both treated and untreated owls.

Table 3: Toxicity effects of cholecalciferol on barn owls fed with four poisoned rats in the seven days feeding period

Treatments	Owl code	Sex	Initial weight	Weight at day 7 (g)	Weight change after 1 week (g)	Fate after 6 months	Toxicity symptoms
	230	F	434	439	+5	S	
Cholecalciferol	220	F	473	464	-9	S	Not detected
	234	M	450	476	+26	S	
	236	M	470	490	+20	S	
Mean			456.80±9.14	467.25± 10.81	10.50±7.86	-	
	101	F	455	467	+12	S	
Untreated	102	F	490	500	+10	S	
	103	M	482	495	+13	S	Not detected
	104	M	481	497	+16	S	
Mean			477.00±7.60	489.75± 7.65	12.75±1.25	-	

Remarks: All means are statistically no significant difference according to non-parametric Mann Whitney U test (P>0.05)

Discussion

The feeding test results of cholecalciferol against wood rats, *R. tiomanicus*, showed that cholecalciferol is efficacious to control a major rat species in oil palm plantations compared to other ARs, particularly FGARs. Cholecalciferol is known to be effective against Norway rats (*Rattus norvegicus*) and house mice (*Mus musculus*). Marshall (1984) reported that all tested Norway rats (n = 145) and house mice (n = 100) died after consuming cholecalciferol baits with a 750 ppm (0.075%) concentration, with an average 3.9 to 6.1 days (mice) and 3.3 to 4.7 days (Norway rats) to result in death, though the number of days of the feeding trial was not mentioned in the report. Similarly, Eason et al. (2010) reported that cholecalciferol baits at a higher concentration at 0.8% (originally used to control possums, *Trichosurus vulpecula*) was effective against Norway rats and house rats (*Rattus rattus*). The authors ran a feeding trial of choice and no-choice feeding for two days on 35 rats consisting of ship rats (*n* = 20) and Norway rats (*n* = 15). Mortality was recorded in 34 of the 35 rats (97%) in an average of four days.

In our study, cholecalciferol produced similar results as chlorophacinone against wood rats, i.e., mortality rate and days-to-death, despite cholecalciferol having a lower daily dosage consumption than chlorophacinone. Based on the oral LD_{50} of both compounds on Norway rats, LD_{50} of cholecalciferol (43.6 mg/kg) was higher than chlorophacinone (20.5 mg/kg) (Marshall, 1984; PMEP, 2001; Eason et al., 2010). This shows that the rats are more susceptible to chlorophacinone than cholecalciferol due to the lower LD_{50} value. Hence, the high concentration of cholecalciferol in formulated baits compared to other anticoagulants was necessary since only a high concentration of the compound can make the formulated rodenticide effective against target pests (Kaukeinen et al. 2000). Based on a publication by Lund (1971), one day feeding of chlorophacinone with concentration of 0.025% was able to result in 60% mortality against mice (n = 20) and the mortality achieved was more than 70% after two days feeding of the bait. 95% mortality was achieved after 21 days of multiple feeding against the mice. According to publication results by Marshall (1984) mentioned earlier, all tested mice (n = 100) died after fed baits containing 0.075% cholecalciferol. However, a contradiction to the results reported by Marshall (1984) was reported by Hix (2009a) where 0.075% of cholecalciferol was only sufficient to kill rats but not mice. Hix (2009b) stated that based on feeding trials, both rat and mice can be controlled with higher dosage at 0.4% cholecalciferol.

The poor performance by warfarin compared to cholecalciferol was expected as reflected in the mortality rate, as the mortality of rats in the warfarin treatment did not even reach half of the total sample of rats tested. Even though the amount of bait consumed per day by rats in the feeding trial was the highest among all treatments, it only resulted in 44% of mortality of the rat samples, though warfarin took a shorter time than cholecalciferol to result in mortality of rats. The poor performance of warfarin has been reported in many publications due to resistance which was recorded since the 1980s against wood rats in Malaysia (Wood & Liau, 1977; Wood & Chung, 1990; 2003). However, there have been no proper laboratory trials conducted against wood rats except by Lee and Mustafa (1983). The researchers reported that warfarin compound was effective against 80–95% of wood rat samples (n = 20) but it took 6–8 days feeding of the baits. This showed that warfarin was still effective against wood rats at that time, as the initial reports of wood rat resistance against warfarin was only documented in 1983 and began to be considered a serious problem after 1985 when three different localities in Malaysia had the same resistance problem (Wood & Chung, 1990). The results of Lee and Mustafa (1983) are in contrast with our current study where the rats consumed 31.10 ± 1.96 mg/kg a day for 6 days feeding but were unable to achieve 50% mortality; indicating that the species can tolerate the toxin compared to the situation of 36 years ago. There is no species-specific LD_{50} of warfarin against wood rats, but the information is available for house mice and Norway rats. A higher acute oral LD_{50} of warfarin is reported for house mice at 374 mg/kg, while for Norway rats it is between 58–323 mg/kg, depending on the strain (Hagan & Radomski, 1953; Erickson & Urban, 2004). In comparison, cholecalciferol has a lower LD_{50} recorded against mice and Norway rats at 42.5 and 43.6 mg/kg respectively, reflecting that rodents are more susceptible to cholecalciferol compared to warfarin.

In Malaysia, secondary poisoning is one of the main issues with regards to AR usage since in oil palm plantations (one of the main agriculture sectors in Malaysia) barn owls are utilized as a biological control agent in order to reduce dependency on chemical practices such as rodenticides (Wood & Chung, 2003). Uncontrolled application of ARs can result in deleterious effects on barn owls as the diet of the owls are highly dependent on rats which make up about 99% of prey (Lenton, 1984; Hafidzi et al., 1999; Salim et al., 2014). There are various reports on secondary poisoning risks of ARs against non-target animals, including barn owls (*Tyto alba*). However, there is a lack of information on the effects of cholecalciferol on barn owls, though the compound is considered less toxic to bird species which appear to be less sensitive than mammals from the species tested in the literature (Eason et al., 2000; Erickson & Urban, 2004; Eason & Ogilvie, 2009). Marshall (1984) stated that oral LD_{50} of mallard duck was as high as 2000 mg/kg while Eason et al. (2000) proved that even at 2000 mg/kg dosing, no deaths were recorded out of the 6 ducks tested. On the other hand, Eason et al. (2000) stated that 3 out of 4 and 1 out of 4 of total domestic chickens and canaries tested died when given the same dose. Meanwhile in the same study, 10 of 16 weka (*Gallirallus australis*) which voluntarily ingested 0.1% cholecalciferol bait exhibited no signs of toxicity.

In addition to lower primary toxicity of cholecalciferol as reported in the aforementioned acute toxicity bird studies, there are published secondary toxicity studies of cholecalciferol towards a range of animals. Eason et al. (1996, 2000) reported that feral cats showed no signs of poisoning after being fed with poisoned-possum carcasses for five to six days. However, dogs are quite susceptible to cholecalciferol regardless of primary or secondary exposure. Gunther et al. (1988) stated that all four tested dogs died after two dogs consumed a high dose of 540g bait (20 mg ai/kg) or equal to one fourth of LD_{50} while the other two were given half of that amount, 10 mg ai/kg or equal to one eighth of the LD_{50}. The author also reported that all four dogs developed signs of poisoning before death such as lethargy, weakness and anorexia within 48 hours and recorded death after 65 to 77 hours of treatment. Furthermore, the paper also mentioned that further investigation reported that all four dogs developed hypercalcemia and hyperphosphatemia which was concluded through detection of gastrointestinal hemorrhage, myocardial necrosis and mineralization of vascular walls.

Secondary exposure toxicity to dogs was reported by Eason et al. (2000) where the signs of toxicity detected on tested dogs fed on five poisoned-possum carcasses from acute group (the possum were dosed prior to the feeding period with LD95 dose of 0.8% cholecalciferol-treated cereal bait before humanely euthanized after 48 hours of dosing) were observed having partial anorexia and lethargy after 4 to 14 days of feeding. Despite being affected by the treatment, the author also reported that all affected dogs recovered gradually after about 14 days of exposure with the appetite and body weight returning to pre-treatment conditions without any veterinary intervention. According to similar study but against lower concentration of cholecalciferol (commonly used as rodenticide) conducted by Marshall (1984) there were no sign of toxicosis observed on six beagle-type dogs after being fed with poisoned-carcasses of Norway rats, which died after fed on 0.075% cholecalciferol bait prior to the feeding trial. In our present study, we let the rats voluntarily consume the 0.075% cholecalciferol bait for two days and recorded an

average 0.08 mg/g a.i per body weight. This is far lower than the dose given by Eason et al. (2000) to the possums before feeding them to cat and dogs but similar to concentration used by Marshall (1984) because in this study we used cholecalciferol bait for rodents (0.075% a.i.) where the a.i concentration was ten times lower than possum baits (0.8% a.i.). The barn owls which consumed the poisoned rats did not display any signs of toxicity from secondarily consuming cholecalciferol such as typical behaviour aberration. For instance, the behaviours that are commonly observed in poisoned barn owls are less flying activity, passive manner and spending more time on the ground rather than on perching point, along with reduction of weight as stated by Hasber *et al*(2014) as symptoms of barn owls affected due to secondary poisoning of AR rodenticides.

As mentioned above, there have been assessments of secondary poisoning risks of several ARs against barn owls. Mendenhall and Pank (1980) stated that consumption of rats poisoned with SGAR compounds such as bromadiolone, brodifacoum and difenacoum by barn owls caused hemorrhaging effect after a one-week feeding trial. Gray et al. (1994) recorded that at least one out of four barn owls did not survive the feeding test of mice poisoned with brodifacoum and difenacoum while two of four owls did not survive after consuming flocoumafen-poisoned mice. In Malaysia, Lee (1994) stated that not only SGARs such as bromadiolone, brodifacoum and flocoumafen, but FGAR compounds such as warfarin also caused high degree of toxic effects on barn owls. The author fed four barn owls with poison rats treated with SGAR bait which resulting in death of three of four owl samples after two weeks of the exposure, while in the same study against another group of barn owls (n = 4) fed with poison rats treated with FGAR compound, warfarin have caused death on two of four tested barn owls after three weeks of exposure to the poisoned rats. A study conducted by Salim et al. (2014) against two group of four barn owls where each group of barn owls were fed with chlorophacinone and bromadiolone treated rats. The result showed that one of four tested owl samples from each group were observed with following sign of poisoning, coarse breathing, reduce of weight and flying activity, hemorrhage at the beak and hematoma (bromadiolone) after consumed three poisoned rats in a week of feeding period.

The secondary toxicity effect of ARs is not only reported in barn owls, but also in other non-target raptors. Lutz (1986) recorded an increase in blood coagulation after Eurasian buzzards (*Buteo buteo*) were fed with bromadiolone-poisoned mice for 10 days. Grolleau et al (1989) observed that 27 Eurasian buzzards (*Buteo buteo*) exhibited bleeding after feeding on bromadiolone-poisoned voles for three days. In the same study with mammals, 10 tested ermines (*Mustela erminea*) were observed to be bleeding after being fed with bromadiolone-poisoned vole for three to five days. Twenty American kestrels exhibited external bleeding after feeding on chlorophacinone-poisoned voles for one to three days (Radvanyi et al., 1988) while an increase in blood coagulation time was reported in Eurasion buzzards after fed poisoned mice (Riedel et al., 1991). Several predators such as black kites, red kites, short toed snake-eagles, and golden eagles showed flocoumafen contamination, as reported by Sanchez-Barbudo et al. (2012) in an opportunistic study on carcasses. Warfarin is generally considered less hazardous to non-target animals. Minks, least weasels and dogs have recorded deaths and survivors exhibited external bleeding signs after eating prey poisoned with warfarin (Prier & Derse, 1962; Evan & Ward, 1967; Townsend et al., 1984).

One of the reasons our secondary poisoning assessment on barn owls did not include other ARs used in the feeding trial was due to the fact that data on the poisoning of barn owls are already established from past publications. Moreover, barn owls are a protected species in Malaysia under Act 716, Wildlife Conservation Act 2010. Thus, only a limited number of samples were permitted for this study and there was no necessity to run a higher number of samples simply to confirm known poisoning effects from other ARs. Past publications such as those mentioned above, have reported the toxic effects of ARs, both FGARs and SGARs, on non-target barn owls via secondary poisoning.

Conclusion

Based on the results of this study, cholecalciferol is potentially a good alternative rodenticide to control rats in oil palm plantations. Cholecalciferol can be considered as a better choice compared to FGARs as from this study there were no barn owl deaths seen after consuming 4 poisoned rats over a 7-day period. A secondary poisoning assessment on barn owls fed with poisoned rats showed that after 7 days of alternate feeding, the barn owls appeared to remain healthy (based on weight and behavioural assessment) and there were no mortalities recorded throughout the study period. In current times where environmental concern is a pressing worldwide issue, practices that are less detrimental to the environment need to be a primary option for plantation operators, while also complying with standards set by the Roundtable on Sustainable Palm Oil (RSPO) and Malaysian Sustainable Palm Oil (MSPO) in order to maintain a sustainable practice in oil palm.

Declarations

DATA AVAILABILITY STATEMENT

Not applicable.

Authors' contributions

Ariff Ateed Mohd Noh contributed to rat and barn owl sampling; trial execution; data analysis; original draft; writing, review and editing. Abu Hassan Ahmad contributed to supervision; validation; writing, review and editing. Hasber Salim contributed to conceptualization; supervision; validation; visualization; writing, review and editing.

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