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Effect of supplementary feeding of vitamin K_1 on difethialone treated Indian gerbil, *Tatera indica* Hardwicke in laboratory

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Two dosages (1 and 2 mg/kg) of vitamin K_1 supplementation for 5 and 15 days were given to Indian gerbil *T. indica* fed on difethialone bait (0.0025%) for one day. The results indicated that the lower dosage could not reverse the anticoagulation process, however the period of mortality was considerably increased from 3-9 days (in control) to 5-14 days (5 days supplementation regime). Subsequently when the vitamin K_1 dosage was doubled and given for 15 days, there was 100% reversal of anticoagulation process and all the test gerbils became normal within a month of poisoning with difethialone bait.

Keywords: Anticoagulants, Baits, Difethialone, Gerbils, Plain food, Vitamin K_1 supplemented food

Rodent pest management is largely dependent on usage of toxic rodenticides. Zinc phosphide, an acute rodenticide is being extensively used for the purpose in India and elsewhere[1-3]. Besides being highly toxic to non-targets, zinc phosphide induces bait/poison shyness in a variety of pest rodent species[4,5]. With the advent of second-generation single dose anticoagulant rodenticides, the whole concept of rodent pest management has been revolutionized in recent years. They act on the blood vascular system of target animals and inhibit blood coagulation mechanism leading to excessive internal haemorrhage and death of the animals within 3-15 days even after one-day exposure. Moreover, due to their requirement in very small dosages (0.0025-0.005%) in baits, these anticoagulants tend to be relatively safer to non-target species. The anticoagulants and vitamin K_1 are known to share the same site of action by blocking the epoxide reductase enzyme system in the blood[6]. Thus process of anticoagulation of blood may be reversed by addition of larger doses of vitamin K_1[(ref.7)]. Most of the studies on effect of vitamin K_1 on anticoagulant poisoning have been conducted on larger vertebrates[8,9], however, very little information on this aspect is available on rodents. Considering the bioefficacy of difethialone against major Indian pest rodent species and the safety factor involved through antidoting, the present communication makes a maiden attempts to describe and quantify the effect of supplementary feeding of vitamin K_1 on difethialone treated Indian gerbil, *Tatera indica* Hardwicke in laboratory.

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Material and Methods

The Indian gerbils were captured from crop fields and grasslands near Jodhpur (Lat. 26°18′N Long. 73°01′E). It was ascertained that the captured rodents had no previous experience of feeding on anticoagulant baits. In all 30 healthy adult gerbils of weight range 98-120 g were used in different sets of experiment. The test gerbils were weighed and lodged in iron mesh cages (24″×12″×12″) for acclimatization. During this period the gerbils were fed pearl millet grain and water was available to them *ad libitum*. Difethialone, a second-generation anticoagulant rodenticide was selected for this study.

Five sets containing six experimental gerbils in each were housed in individual cages in the laboratory. Temperature in laboratory was maintained at 28° ±4°C. Four sets acted as treatment, whereas one set was for control/check. Each animal was exposed to difethialone baits (0.0025%) for 24 hr. During this period no other food was available to the test gerbils, however, tap water was provided *ad libitum*.

Sets I and II—Immediately after exposure of anticoagulant rodenticides, the test animals of these

two sets were fed with arachis oil (2% w/w) smeared pearl millet grains mixed with vitamin K_1 powder @ 1 mg/kg which was almost equal to the active ingredient of difethialone ingested by the gerbils. In Set I the vitamin K_1 supplemented feed was given for 5 days whereas in Set II this period was extended for 15 days.

Sets III and IV—In these sets, the vitamin K_1 doses was doubled i.e. 2 mg/kg mixed in pearl millet grains smeared with 2% arachis oil. This feed was provided to the difethialone (0.0025%) fed gerbils for 5 days (Set III) and for 15 days (Set IV).

Set V—In this set the experimental gerbils were exposed to difethialone (0.0025%) in pearl millet grains for one day and only plain bait (no vitamin K_1 supplement) was offered on subsequent occasions till death of the animals. This set acted as control/check.

Since the symptom of difethialone poisoning appears in the gerbils after 3-4 days of poisoning and lasts upto 14 days, two feeding regimes of 5 and 15 days were experimented for these trials. Consumption pattern and symptoms of poisoning, such as blood discharge from nose, eyes, anus, ear etc., dullness in activity and paralyses of limbs and death of experimental rodents were monitored for 30 days immediately after feeding on difethialone baits.

Results and Discussion

Difethialone has proved its potency as an effective anticoagulant rodenticide against rodent pest species in India at a relatively lower dosages (0.0025%)[10-12] in comparison to other rodenticides of the same generation viz., bromadiolone, brodifacoum, flocumafen etc., which are effective at 0.005% in baits[13,14]. In the present investigation also, difethialone (0.0025%) yielded 100% mortality of Indian gerbils after one day exposure (Table 1). Effect of vitamin K_1 supplementation on difethialone fed Indian gerbils is discussed below.

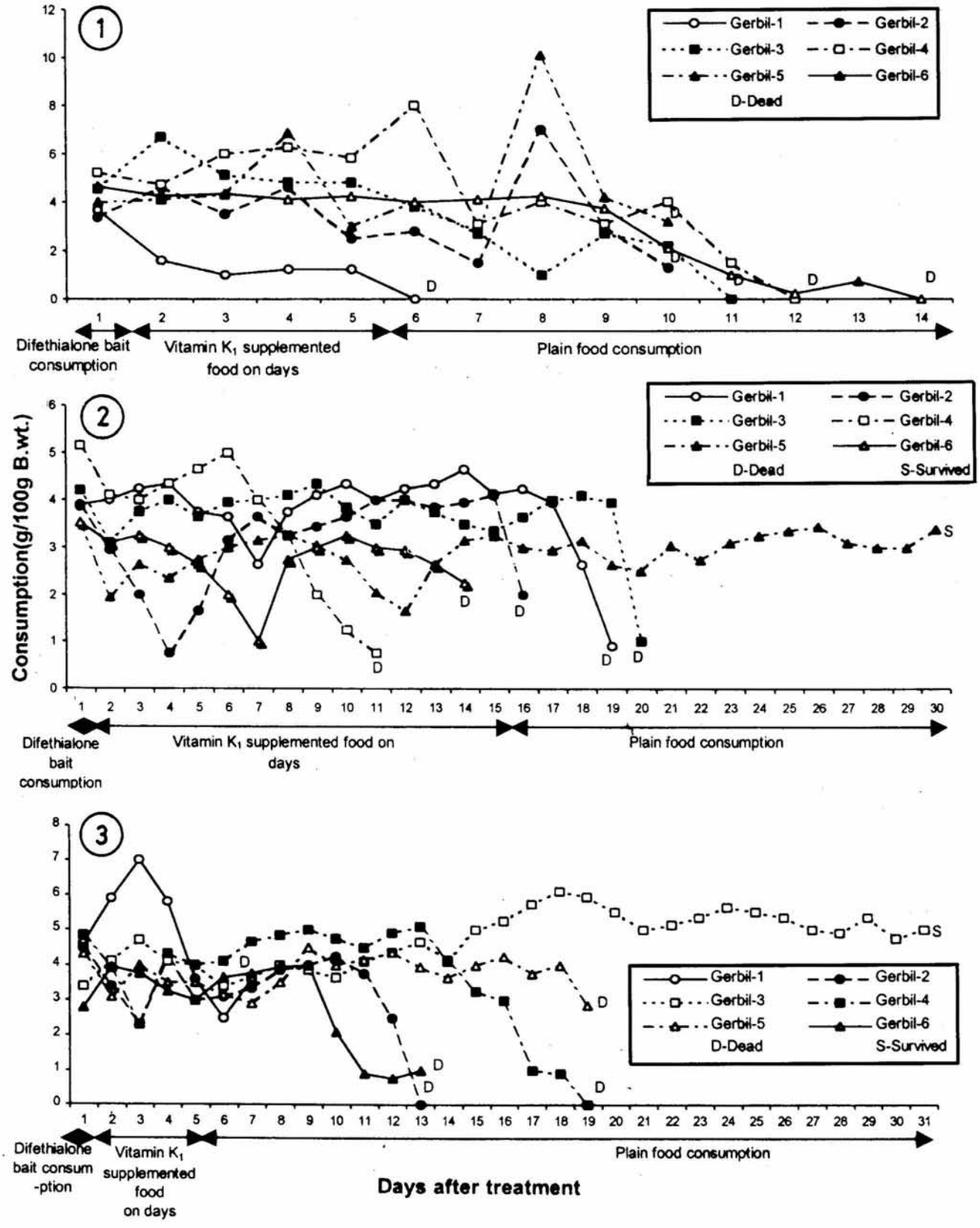
Set I—Despite an intake of 4.25±0.28g difethialone treated baits, the test gerbils in this set consumed vitamin K_1 supplemented feed at the rate of 4.12±0.67g/day with a total intake of 20.38±3.53 during 5 days (Table 1). Additional supply of vitamin K_1 @ 1mg/kg for five days could not help the poisoned gerbils to recover which was evident from the drastic fall in plain food consumption during post treatment period (2.93±0.58g/day) resulting in mortality of all the test gerbils within two weeks of poisoning. Analysis of consumption and mortality data for individual test animal revealed that one animal (gerbil no. 1) consumed significantly lower amounts of vitamin K_1 supplemented food for 4 days (1-1.60g) and died on 5th day only, whereas other five test animals consumed 2.80-8g vitamin K_1 treated food during this period (Fig. 1). These observation indicated that vitamin K_1 supplementation @ 1mg/kg for five days doubled the mean duration of death (10.17 days) as compared to control (5.5 days), but was not enough to reverse the anticoagulation effect of difethialone.

Set II—In this set the vitamin K_1 supplementation was continued for two weeks resulting in increased total intake of the supplemented feed (45.38±4.59g), however mean consumption of this feed per day was at par (3.27±0.22g) with previous set. Similar trend was noticed in plain food consumption also during post treatment periods (Table 1). Data presented in Fig. 2 showed that two test rodents (No. 4 and 6) died on 11th and 14th day of poisoning due to blood loss during the vitamin K_1 administration period. Remaining

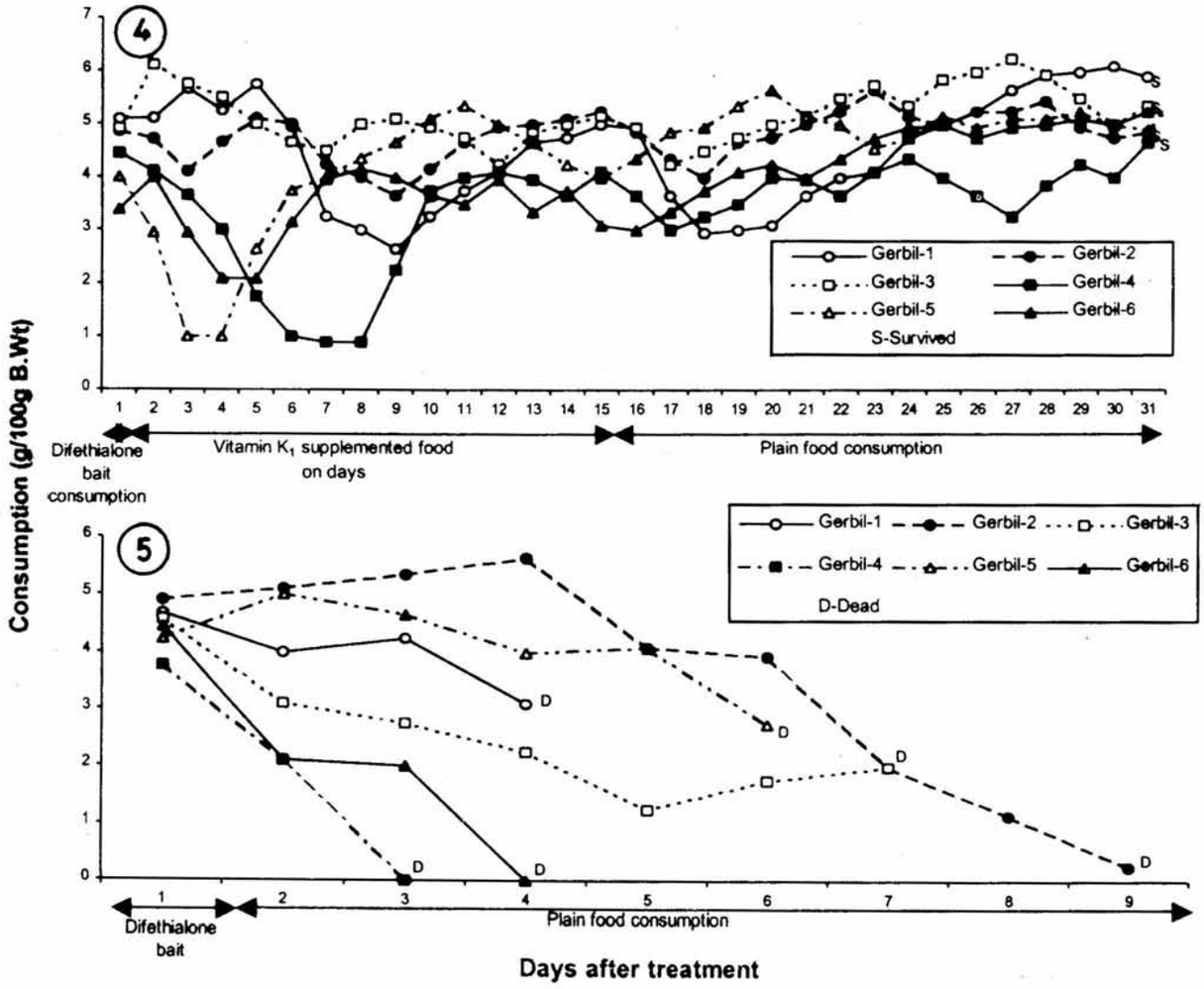
Table 1 — Effect of supplementary feeding of vitamin K_1 on difethialone fed *Tatera indica* Hardwicke

[*Values expressed in g/100 g body weight are mean±SE]

Experiment Set	Difethialone Bait (0.0025%) consumption (g/100g B.wt.) Mean ± SE	Post treatment food consumption*				Mortality % (n/N)	Mean days to death	Survival % (n/N)
		Vitamin K_1 supplemented food		Plain food				
		Mean consumption/day	Mean total consumption	Mean consumption/day	Mean total consumption			
Set-I	4.25±0.28	4.12±0.67	20.38±3.53	2.93±0.58	14.72±1.91	100 (6/6)	10.17 (5-14)	Nil (0/6)
Set-II	4.03±0.24	3.27±0.22	45.38±4.59	2.83±0.41	16.23±9.09	83.33 (5/6)	16.0 (11-20)	16.67 (1/6)
Set-III	4.07±0.33	3.82±0.26	19.09±1.29	3.56±0.35	43.94±17.35	83.33 (5/6)	14.0 (7-18)	16.67 (1/6)
Set-IV	4.45±0.27	4.04±0.32	60.58±4.80	4.72±0.21	70.87±3.21	Nil (0/6)	Nil (-)	100 (6/6)
Control Set-V	4.43±0.16	-	-	2.65±0.53	13.12±3.95	100 (6/6)	5.5 (3-9)	Nil (0/6)



Figs 1-3 — Consumption of difethialone bait, Vitamin K_1 supplemented food and plain food by *T. indica* in different experimental sets. [1: Experimental set-I, 2: Experimental set-II, 3: Experimental set-III]



Figs 4-5— Consumption of difethialone bait, Vitamin K_1 supplemented food and plain food by *T. indica* in (4) Experimental set-IV; and (5) Control set

four rodents (1,2,3,5) showed steady pattern of consumption up to 15 days. After cessation of vitamin K_1 supplement three of the four rodents (No. 1,2 and 3) died on 19th, 16th and 20th day after poisoning, respectively, however, gerbil No.5 recovered from anticoagulant effect and became normal during fourth week. This indicated that increase in period of vitamin K_1 supplement does have some positive effect on recovery of rodents from anticoagulant poisoning.

Set III—Mean daily consumption of vitamin K_1 supplemented food and mortality pattern were almost similar to those recorded in set II. Increased dosage of vitamin K_1 (2 mg/kg) fed to poisoned gerbils for 5 days registered total intake of vitamin K_1 treated food upto 19.09±1.29 g and that of plain food was 43.94±17.35 g (Table 1). Animal wise analysis of data (Fig. 3) showed that all the six rodents showed steady pattern of consumption except one gerbil (No. 1), which registered a significant decline in feed consumption and died on seventh day of poisoning. Mortality pattern of gerbils even after consuming higher dosages of vitamin K_1 supplement for 5 days was almost similar to that of set II as all the rodents except No.3 died within 7-18 days. Mean days to death was increased two and a half times (14.0 days) as compared to control (5.5 days) (Table 1). The surviving rodents steady recovery, which was evident from the pattern of plain bait consumption after 5 days of vitamin K_1 supplementation.

Set IV—When the dosages (2 mg/kg) as well as the period of vitamin K_1 supplement was increased (15 days) the test rodents registered complete reversal from anticoagulant poisoning. Here, all the six rodents showed steady pattern of consumption of vitamin K_1

bait all through. Average daily intake of vitamin K_1 supplemented food and plain food was at par ie, 4.04 ± 0.32 and 4.72 ± 0.21 g, respectively. Similarly there was no significant difference in mean total consumption of both types of food offered to the poisoned gerbils for 15 days each (Table 1). In case of two experimental gerbils (Nos. 4 and 5) the consumption of vitamin K_1 supplemented feed was no doubt dropped on 5th and 3rd day, but on subsequent days it increased and maintained a steady pattern. The consumption of plain food too remained steady (2.95-6.0 g/100g body wt. per day) even after the cessation of vitamin K_1 supplemented bait after 15 days and all the test gerbils recovered from toxic effects of difethialone within a month of poisoning (Fig. 4).

Control—In the control set, where the gerbils were not provided with vitamin K_1 supplement, all the test gerbils succumbed to the poison bait after single day exposure of difethialone (0.0025%). Mean consumption of plain food after poisoning was expectedly decreased to 2.65 ± 0.53 g/day (Table 1). Symptoms of anticoagulant poisoning were visible quite early and all the test rodents died between 3 and 9 days (Fig. 5).

The results showed that supplementary feeding of vitamin K_1 at a dose equivalent to the active ingredient (a.i.) of difethialone ingested by the test gerbils was not sufficient for the recovery of poisoned animals even if it was administered for 15 days. The period of mortality was no doubt prolonged considerably (11-20 days as against 3-9 days in control). On doubling the vitamin K_1 dosage (2 mg/kg) with an enhanced feeding period of 15 days, the gerbils registered complete reversal from the toxicosis caused due to feeding on difethialone baits. Hadler and Buckle[15] have also reported that for reversal of anticoagulation frequent administration of vitamin K_1 is necessary until the anticoagulant was cleared from the system.

The results clearly indicated that with the additional supply of vitamin K_1 the process of prothrombinopenia i.e. deficiency of prothrombin factors in blood set in by difethialone may have antagonized after the vitamin K_1 competitively joins the active sites, resulting into reversal of anticoagulation. Brodifacoum poisoning in dogs was antidoted with vitamin K_1 therapy either through feeding or injection daily for three weeks @ 2 mg/kg[8]. Similarly Reitmeyer *et al.*[16] reported improvement in coagulation time in anticoagulant toxicated dogs after a maintenance dose of vitamin K_1 @ 1-2.6 mg/kg twice daily for 3-10 weeks. Nee *et al.*[17] analysed the induction of prothrombin synthesis by K vitamins with that of brodifacoum treated rats for reversing the excessive oral anticoagulation.

Thus the present findings revealed that lethal action of anticoagulant could be reversed by feeding vitamin K_1 supplement for 15 days at 2 mg/kg dose which was almost double the a.i. of anticoagulant ingested by the target rodents.

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References

1 Htun P T & Brooks J E, Laboratory evaluation of the toxicity and efficacy of zinc phosphide used as rodenticide against lesser bandicoot, *Bandicota bengalenses*, *PANS*, 25(3) (1979) 246.

2 Mohan Rao A M K & Prakash I, Rodenticideal evaluation of zinc phosphide against house mouse, *Mus musculus bacterium*, *Indian J Pl Prot*, 13 (1985) 61.

3 Prakash I, Bait shyness and poison aversion, In *Rodent Pest Management* (CRC Press, Boca Raton, Florida, USA) 1988 321.

4 Prakash I & Mathur R P Rodenticides, in *Management of rodent pests* (Indian Council of Agricultural Research, New Delhi) 1987 61.

5 Prakash I & Jain A P, Bait shyness of two gerbils, *Tatera indica* Hardwicke and *Meriones hurrianae* Jerdon, *Ann Appl Biol*, 69 (1971) 169.

6 Silverman R B, A model for the molecular mechanism of anticoagulant activity of 3-substituted 4-hydroxy coumarins, *J Am Chem Soc*, 102 (1980) 5421.

7 Coon W W & Willis P W, Some aspects of pharmacology of oral anticoagulants, *Clin Pharmacol Therap*, 11 (1970) 312.

8 MacKintosh C G, Laas F J Godfrey M E R & Turner K, Vitamin K_1 treatment of brodifacoum poisoning in dogs, *Proc 13th Vertebrate Pest Conference*, edited by Crabb A C and Marsh R E (Montorey, California, USA) 1988, 86.

9 Park B K & Leck J B, A comparison of vitamin K antagonism by warfarin, difenacoum and brodifacoum in rabbits, *Biochem Pharmacol*, 31 (1982) 3635.

10 Chaudhary V, Tripathi R S & Rana B D, Bio-efficacy of Difethialone — an anticoagulant rodenticide against Indian desert gerbil, *Meriones hurrianae* (Abstract), *Proc XX Annual Conference of the Society of Toxicology (STOX)* (Vapi, India) 2001, 36.

11 Sheikher C & Sood P, Acceptance and bioefficacy of difethialone as rodenticide and its suitability in the fields, *Indian J Agric Sci*, 70(5) (2000) 312.

12 Sridhra S, Ravindra Babu T & Ajay P, *Laboratory and field evaluation of difethialone*, All India Coordinated Research Project on Rodent Control, (UAS, Bangalore) 2000, 25.

13 Jain A P, Mathur M & Tripathi R S, Bio-efficacy of flocoumafen against major desert rodent pests, *Indian J Pl Prot*, 20 (1992) 81.

14 Mathur R P, Kashyap N & Mathur M, Bio-efficacy of anticoagulant rodenticide. in *Rodents in Indian Agriculture*, edited by I, Prakash & P K Ghosh (Scientific Publishers, Jodhpur) 1992, 577.

15 Hadler M R & Buckle A P, Forty-five years of anticoagulant rodenticides—Past, Present and Future trends, *Proc 15th vertebrate Pest conference*, edited by J E Borrecco and R E Marsh (New Port Beach, California, USA) 1992, 149.

16 Reitemeyer S, Kohn B, Giger U & Brunnberg L, Rodenticide intoxication in 20 bleeding dogs: diagnosis and therapy, *Kleintierpraxis* (Bib. Cit.) 46 (2001) 549.

17 Nee R, Doppenschmidt D, Donovan D J & Andrews, T C, Intravenous versus subcutaneous vitamin K_1 in reversing excessive oral anticoagulation, *Am J Cardiol*, 83 (1999) 286.