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## GROWTH PERFORMANCE OF NESTLING BARN OWLS, Tyto Alba javanica IN RAT BAITING AREA IN MALAYSIA

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#### ABSTRACT

The growth of nestling barn owls, Tyto Alba javanica in immature oil palm in Malaysia was investigated under rat baiting with three different rodenticides. Four treatment plots were established with three plots baited each with warfarin, brodifacoum and a protozoan based biorodenticide, Sarcocystis singaporensis plus a fourth non-baited control plot. Three rat baiting campaign were carried out during the study, the first rat baiting campaign was conducted in October 2008, the second was in March 2009 (except for biorodenticide baiting was conducted a month earlier), and the last third baiting campaign in October 2009. The baiting campaigns coincided with the breeding season of barn owl. Nestlings body measurements namely: body mass, culmen length, tarsus length, wing length and tail length were taken after the third baiting campaign, from September 2009 to January 2010. Measurements were recorded every three days from hatching up until 49 days old, i.e., several days before fledging. Nestlings in control plot showed superior for all parameter taken compared to rodenticides treated plots. Body mass of nestlings in control plot were heavier by 8.17%, 13.04%, and 6.88% compared to warfarin, brodifacoum and biorodenticide treated plots respectively. The culmen and tarsus length of nestling barn owls reached the adult size during the growth period; while culmen length in control plot was longer by 3.07%, 5.28%, and 1.41% compared to warfarin, brodifacoum and biorodenticide treated plots respectively. The tarsus length of nestlings in control plot was also longer by 2.40%, 3.08% and 3.36% compared to warfarin, brodifacoum and biorodenticide treated plots respectively. In contrast with culmen and tarsus length, wing and tail length still grew until day 49 i.e., several days before fledging. The wing and tail length in control plot was shorter by 15.77% and 13.73% compared to adult size. Teratogenic sign was shown by one nestling in brodifacoum treated plot, where its primary feathers were malformed rendering it flightless besides tail length that were very short if compared to nestlings in control plot. Wing and tail length in brodifacoum treated plot was shorter by 15.26% and 18.24%, respectively compared to control plot.

Keywords: Tyto Alba javanica, growth performance, nestling, warfarin, brodifacoum, Sarcocystis singaporensi, teratogenic sign.

#### **INTRODUCTION**

The barn owl, Tyto Alba is widely distributed around the world, occurring in all continents in a wide range of habitats, except Antarctica and the smaller Pacific Islands (Smith and Cole, 1989). There are 36 subspecies including T. Alba javanica, found in Peninsular Malaysia and also in Sumatra and Java Islands of Indonesia (Taylor, 1994). Due to its wide distribution, the barn owl has been extensively studied (Bunn et al., 1982; Newton et al., 1991; Eason et al., 2002). In Malaysia, T. Alba javanica is commonly propagated to control rats in plantation (Lenton, 1984; Smal, 1989; Hafidzi et al., 1999). Since 1970's, its distribution had rapidly expanded and the status changed from rare in the late 1960s to common (Duckett, 1984; Duckett and Karrupiah, 1990). The increase of T. Alba javanica population in the Peninsular Malaysia was associated with the phenomenal increase in oil palm acreage. This brought about rat outbreaks which translate into readily available food source. Previously, when rat damage reaches threshold levels planters usually resort to warfarin, the first generation anticoagulant rodenticide to deal with the infestation (Duckett, 1984). However prolonged exposure to warfarin, triggers resistance to the latter, prompting more planters to switch to brodifacoum, a second generation anticoagulant rodenticide introduced in the early 1980s. The use of brodifacoum has caused marked decline in rat populations in oil palm (Duckett, 1984; Wood and Fee, 2003). The downside of bodifacoum is it was proven toxic to T. Alba from field observations and laboratory studies. The potential hazard of using brodifacoum is not only due to its high potency of the active ingredient, but also the risk to barn owl as nontarget animal by direct consumption or secondary poisoning from build-up of rodenticide residues (Newton et al., 1990; Shore et al., 1999; Dowding et al., 2010). Based on that fact, several workers try to find safer rodenticide to replace brodifacoum with an equally effective but have less impact on non target organism. One such alternative is the biorodenticide based on Sarcocystis singaporensis, a protozoan pathogen that has been proven effective against rat in the rice field but does not causes harm to humans other animals, such as fish, other mammals and birds apart form rats of the genus Bandicota and Rattus (Jakel et al., 1996; Jakel et al., 2006). Although there have been many studies on the effects of chemical rodenticides on adult barn owl, very little information exist about the effects of chemical rodenticides on growth and development of nestlings. Therefore the objective of this study is to evaluate the effects of regular rat baiting on the growth performance of the nestlings barn owl, T. Alba javanica in an immature oil palm area in Malaysia.



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#### MATERIALS AND METHODS

#### Location and period of study

The study was conducted in immature oil palm at FELCRA oil palm plantation scheme in Seberang Perak  $(4^{0}02^{\circ}N, 100^{0}53^{\circ}E)$ , Perak, Malaysia from September 2008 to January 2010. The study sites constitute part of the replanting area started in mid 2007.

#### **Rat baiting and treatment**

Twenty two artificial nest boxes, made of wood, were set up in April to June 2008 in the immature palm by Felcra management. Four treatment plots were established. The area for each plot is no less than 100 ha. Three plots were baited with warfarin, brodifacoum and the biorodenticide Sarcocystis singaporensis, respectively. The fourth was left untreated and served as the rodenticide-free control plot. The average nest box density was 1 box for  $25 \pm 3.83$  ha. The first baiting campaign for all three rodenticides was carried out on 20-25<sup>th</sup> October 2008. The second baiting campaign for warfarin and brodifacoum on 10-12<sup>th</sup> March 2009, while second baiting campaign for biorodenticide was carried out on 25-27th January 2009. Third baiting campaign was carried out on 28 September to 3<sup>rd</sup> October 2009 for all three rodenticides. The baits were placed at the base of the palm tree. In the first campaign, a single round of baiting was carried out while two baiting rounds were conducted in the second and the third baiting campaign.

#### **Data collection**

48 nestlings were selected for this study: 14 from control plot, 12 from warfarin treated plot, 9 from brodifacoum treated plot and 13 from biorodenticide treated plot. They were weighed at three day interval for up to 49 days i.e., several days before fledging. The day of hatching was designated as day zero (Janiga, 1992) for monitoring growth rate. Hatched siblings were individually marked using different colored ribbons tied to the leg for age determination during later stages of growth. All observations were carried out in the nests from 5 to 7 p.m during the breeding season, i.e., from September 2009 to January 2009.

#### Nestling growth metrics

For nestling growth metrics, five measurements were taken, namely: body mass, culmen length, tarsus length, wing length and tail length. Body mass was measured using Apex A-5001, a portable digital weighing scale (accurate to 1 g); culmen length was measured using Mitutoyo Caliper, from the tip of the upper mandible to the base of the culmen, to the nearest 1 mm. Tarsus length was measured from the top of the tarsus (just below the tibio-tarsal joint) to the joint at the base of the middle toe, to the nearest 1 mm. Tail length was measured from the fold of skin between the central tail-feathers and the tip of the longest tail-feathers, to the nearest 1 cm. Wing length was measured from the bend of the folded wing to the tip of the longest primaries (Weick, 1980; Janiga, 1992).

#### Statistical analysis

Data of similar-aged nestlings from all the nest boxes were pooled to calculate the mean for different growth and to analyze the pattern of growth changes in the measured variables using Kruskal-Wallis test. Means are presented in  $\pm$  SE. For hypothesis testing P < 0.05 was considered significant.

For the growth in nestlings, logistic growth curve was used (Starck and Ricklefs, 1998), by the given equation:

$$W = A/(1 + exp(-K(t-ti)))$$

Where W = the growth variable, A = asymptote, K = the growth rate constant, t = age of nestling, and ti = the inflection point of the growth curve. The logistic growth equations were fitted to the data using the nonlinear regression procedure of the SAS package version 9.1.

#### RESULTS

#### The body mass

Of the 48 nestling barn owls measured, only 32 were successfully measured up to day 49 while the rest died. Of the 32 nestlings, 12 were from the untreated control plot, eight from the warfarin treated plot, two from the brodifacoum treated plot and ten from the biorodenticide treated plot. Since only two nestlings in the brodifacoum treated plot survived during the measurements, the growth comparisons were made based on the age of these nestling with other nestlings in the other treatment plots.

From 226 measurements for nestlings from hatching up to fledging in the untreated control control plot (n = 12), 153 measurements in the warfarin treated plot (n = 8), 67 measurements in the brodifacoum treated plot (n = 2) and 174 measurements in the biorodenticide treated plot (n = 10), nestlings grew from  $18.00 \pm 0.37$  g (n =6 ),  $18.20 \pm 0.58$  (n = 5),  $18.25 \pm 0.49$  (n = 4),  $18.20 \pm$ 0.37 (n = 5) at hatching, to a peak mass of  $631.60 \pm 12.96$ g (day 46, n = 5),  $597.30 \pm 5.24$  g (day 45, n = 3), 565.00 $\pm$  16.05 g (day 43, n = 2) and 604.67  $\pm$  16.00 g (day 45, n = 4) in control, warfarin, brodifacoum and biorodenticide treated plots, respectively (Table-1). Nestlings in control plot have a heavier body mass compared to the average adult body mass by 4.16% (545.90  $\pm$  9.04, n = 10), and lighter by 3.71%, 7.86%, 2.55% for nestlings from warfarin, brodifacoum and biorodenticide treated plots respectively compared to adult body mass. Nestlings in control plot were heavier in body mass by 8.17%, 13.04%, and 6.88% compared to warfarin, brodifacoum and biorodenticide treated plots respectively. Kruskal-Wallis test showed that there was no significant difference for body mass of nestlings in all treatments irrespective of days from day 1 to day 49 for the nestlings.

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Age	Body mass (g)			
(days)	Control	Warfarin	Brodifacoum	Biorodenticide
1	$18.00 \pm 0.37 \ ns$	$18.20 \pm 0.58$	$8.25 \pm  0.48$	$18.20 \pm 0.37$
7	$73.67 \pm 4.58 \text{ ns}$	$84.00 \pm 2.94$	$9.00 \pm 3.52$	$81.00 \pm  1.15$
14	$223.67 \pm 11.99$ ns	$216.00\pm13.50$	$218.33 \pm 7.62$	$215.00\pm11.53$
22	$379.80 \pm 10.26$ ns	$359.33 \pm 16.72$	$355.50\pm21.56$	$364.75 \pm 15.46$
28	$449.40 \pm 10.16 \text{ ns}$	$436.00 \pm 9.30$	$428.50\pm20.56$	$434.75 \pm 15.17$
34	$546.80 \pm 13.46$ ns	$536.67 \pm 8.38$	$525.50\pm24.57$	$546.00\pm14.71$
43	$615.00 \pm 7.75 \text{ ns}$	$588.00\pm16.04$	$565.00\pm16.03$	$590.50\pm10.60$
49	568.60 ± 15.58 ns	$525.67 \pm 18.24$	$503.00 \pm 13.03$	532.00 ± 15.35

Table-1. Body mass (mean  $\pm$  SE) of nestling barn owls in rodenticide treated areas.

Generally, absolute rates of growth varied throughout the nestling period (Figure-1) and the most rapid rates occurred between days 10 - 35. Although the growth constant did not vary and ranging from 0.146 to 0.150 g per day, but the asymptote calculated using SAS Version 9.1 indicates the highest asymptote was found in nestlings from the control plot, followed by the

biorodenticide, warfarin, and brodifacoum treated plots, respectively. The highest increase in body mass differ from one treatment to another, whereby the control plot was recorded on day  $19.20 \pm 0.26$ , in warfarin day  $18.87 \pm 0.31$ , in brodifacoum on day  $17.99 \pm 0.40$ , in biorodenticide on day  $18.75 \pm 0$  (Table-2/ Figure-1).

**Table-2.** Body mass (mean  $\pm$  SE) obtained from logistic growth equations fornestling barn owls in rodenticide treated areas.

Treatment	<i>A</i> (g)	$\boldsymbol{K}$ (day <sup>-1</sup> )	ti (days)
А	$613.5\pm5.98$	$0.150\pm0.004$	$19.20\pm0.26$
В	$585.8\pm 6.62$	$0.146\pm0.005$	$18.87\pm0.31$
С	$554.9 \pm 8.72$	$0.145\pm0.006$	$17.99 \pm 0.40$
D	$586.1 \pm 6.60$	$0.147\pm0.005$	$18.75\pm0.30$

Remarks: A is the asymptote, K is the growth constant, and ti is the inflection point

#### The culmen length

The culmen length of nestling barn owls grew from  $7.50 \pm 0.10$  mm ( n = 6),  $7.60 \pm 0.10$  mm ( n = 5),  $7.50 \pm 0.13 \text{ mm}$  ( n = 4),  $7.60 \pm 0.10 \text{ mm}$  ( n = 5) in control warfarin, brodifacoum, and biorodenticide treated plots respectively at hatching to  $22.70 \pm 0.20$  mm (n = 65,  $22.00 \pm 0.17 \text{ mm}$  (n = 3),  $21.50 \pm 1.00 \text{ mm}$  (n = 2) and  $22.38 \pm 0.37$  mm (n = 4) for corresponding treatment plots at day 49. The culmen grew full length to reach the adult size (22.80  $\pm$  0.17, n = 10) during the growth period when measurement were taken at day 49. Culmen length in the control plot was longer by 3.18%, 5.58%, and compared to warfarin, brodifacoum and 1.43% biorodenticide treated plots respectively. Kruskal-Wallis test showed there was no significant difference for culmen length of nestlings in all treatments irrrespective of days, from day 1 to day 49 for the nestlings.

Patterns in growth of culmen were similar to that of body mass that exhibits a sigmoidal curve, where the culment grew slowly in the first 10 days and then rapidly to day 35, slowing down again to day 49. The asymptote of culmen length tend to be similar for nestlings in all treatments ranging from  $22.926 \pm 0.23$  mm,  $22.160 \pm 0.20$ mm,  $22.040 \pm 0.37$  mm and  $22.878 \pm 0.23$  mm for control, warfarin, brodifacoum and biorodenticide treated plots, respectively. The growth constant ranging from 0.110  $\pm$ 0.004 mm, 0.105  $\pm$  0.004 mm, 0.102  $\pm$  0.005 mm and  $0.112 \pm 0.005$  mm per day in control, warfarin, brodifacoum and biorodenticide treated plots, respectively. The highest increase in culmen length was also quite similar from one treatment to another, ranging from day  $10.768 \pm 0.31$ , day  $10.171 \pm 0.28$ , day  $10.125 \pm 0.46$ , and day  $10.500 \pm 0.39$  in nestlings from control, warfarin, brodifacoum and biorodenticide treated plots, respectively (Table-4/ Figure-2).





Figure-1. Logistic growth curve of the changes in body weight of nestling barn owls under rat baiting campaign in oil palm area in Malaysia.

Age	Culmen length (mm)				
(days)	Control	Warfarin	Brodifacoum	Biorodenticide	
1	$7.50\pm0.10\ ns$	$7.60\pm0.10$	$7.50\pm0.13$	$7.60\pm0.10$	
7	$8.92\pm0.15~ns$	$9.00\pm0.16$	$9.00\pm0.15$	$9.00\pm0.18$	
14	$13.00 \pm 0.29 \text{ ns}$	$12.83\pm0.17$	$12.67\pm0.17$	$13.17\pm0.17$	
22	$18.30\pm0.20~\text{ns}$	$18.00\pm0.29$	$18.00\pm0.50$	$18.38\pm0.23$	
28	$20.60\pm0.30~ns$	$19.33\pm0.33$	$19.50\pm0.50$	$20.75\pm0.14$	
34	$21.00\pm0.45~ns$	$20.33\pm0.17$	$20.25\pm0.25$	$21.25\pm0.25$	
43	$21.90\pm0.10~\text{ns}$	$21.33\pm0.17$	$20.75\pm0.75$	$22.00\pm0.20$	
49	$22.70 \pm 0.20$ ns	$22.00 \pm 0.17$	$21.50 \pm 1.00$	$22.38 \pm 0.37$	

Table 3. Culmen length (mean  $\pm$  SE) of nestling barn-owls in rodenticide treated areas.

**Table-4.** Culmen length (mean  $\pm$  SE) obtained from logistic growth equationsfor nestling barn owls in rodenticide treated areas

Treatment	<i>A</i> (g)	$\boldsymbol{K}(\mathrm{day}^{-1})$	ti (days)
А	$22.926\pm0.23$	$0.110\pm0.004$	$10.768\pm0.31$
В	$22.160\pm0.20$	$0.105\pm0.004$	$10.171\pm0.28$
С	$22.040\pm0.37$	$0.102\pm0.005$	$10.125\pm0.46$
D	$22.878\pm0.23$	$0.112\pm0.005$	$10.500\pm0.39$

Remarks: A is the asymptote, K is the growth constant, and ti is the inflection point

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Figure-2. Logistic growth curve of the changes in culmen length of nestling barn owls under rat baiting campaign in oil palm area in Malaysia.

#### The tarsus length

The tarsus length of nestlings grew from  $16.33 \pm 0.33 \text{ mm}$  (n = 6),  $16.70 \pm 0.30 \text{ mm}$  (n = 5),  $16.00 \pm 0.41 \text{ mm}$  (n = 4),  $16.20 \pm 0.37 \text{ mm}$  (n = 5) in control, warfarin, brodifacoum, and biorodenticide treated plots, respectively at hatching to  $86.40 \pm 0.81 \text{ mm}$  (n = 5),  $84.33 \pm 1.20 \text{ mm}$  (n = 3),  $83.75 \pm 1.00 \text{ mm}$  (n = 2) and  $83.50 \pm 1.32 \text{ mm}$  (n = 4) for the corresponding treatment plots in day 49 (Table-5). Same like culmen, tarsus length of chicks grew

and reached adult size  $(86.45 \pm 0.20, n=10)$  during the growth period. Tarsus length in the control plot was longer by 2.45%, 3.16% and 3.47% compared to warfarin, brodifacoum and biorodenticide treated plots, respectively. The tarsus length in control plot was longer than rodenticides treated plot. However, there was no significant different irrespective of days when tested with Kruskal-Wallis analysis of variance.

Age	Tarsus length (mm)			
(days)	Control	Warfarin	Brodifacoum	Biorodenticide
1	$16.33 \pm 0.33$ ns	$16.70\pm0.30$	$16.00\pm0.41$	$16.20\pm0.37$
7	$23.00\pm0.37~ns$	$22.60\pm0.24$	$22.33 \pm 0.67$	$22.50\pm0.31$
14	$46.00\pm2.08\ ns$	$46.33\pm0.88$	$45.33 \pm 1.45$	$44.33 \pm 1.45$
22	$67.00\pm1.58~ns$	$67.67 \pm 0.33$	$69.00 \pm 1.00$	$69.00\pm0.91$
28	$78.40 \pm 1.21 \text{ ns}$	$78.00\pm0.58$	$78.00\pm3.01$	$77.50 \pm 1.19$
34	$83.00 \pm 1.22$ ns	$81.67 \pm 1.20$	$80.00\pm2.51$	$80.25\pm0.85$
43	$85.40 \pm 1.21 \text{ ns}$	$83.00\pm1.00$	$83.00\pm2.00$	$82.50 \pm 1.04$
49	$86.40 \pm 0.81$ ns	84.33 ± 1.20	83.75 ± 2.76	83.50 ± 1.32

**Table-5.** Tarsus length (mean  $\pm$  SE) of nestling barn-owls in rodenticide treated areas.

The tarsus length grew slowly in the first seven days and then rapidly to day 30, slowing down again to

day 50. The tarsus length reached asymptote around 30 to 35 days after nestling hatched, ranging from  $86.93 \pm 0.43$ 

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mm,  $84.44 \pm 0.45$  mm,  $84.39 \pm 1.03$  mm and  $83.66 \pm 0.52$  mm for control warfarin, brodifacoum and biorodenticide treated plots, respectively. The growth constant ranging from  $0.14 \pm 0.0043$  mm,  $0.15 \pm 0.003$  mm,  $0.15 \pm 0.006$  mm and  $0.15 \pm 0.004$  mm in control, warfarin, brodifacoum and biorodenticide treated plots, respectively.

The highest increase in tarsus length differs from day  $13.34 \pm 0.15$ , day  $12.86 \pm 0.16$ , day  $13.03 \pm 0.30$ , and day  $12.90 \pm 0.18$  in control, warfarin, brodifacoum and biorodenticide treated plots, respectively (Table-6/ Figure-3).



Figure-3. Logistic growth curve of the changes in tarsus length of nestling barn owls under rat baiting campaign in oil palm area in Malaysia

Treatment	<b>A</b> (g)	$\boldsymbol{K}(\mathrm{day}^{-1})$	ti (days)
А	$86.93 \pm 0.43$	$0.14\pm0.003$	$13.34\pm0.15$
В	$84.44\pm0.45$	$0.15\pm0.003$	$12.86\pm0.16$
С	84.39 ± 1.03	$0.15\pm0.006$	$13.03\pm0.30$
D	$83.66 \pm 0.52$	$0.15\pm0.004$	$12.90\pm0.18$

**Table-6.** Tarsus length (mean  $\pm$  SE) obtained from logistic growth equationsfor nestling barn owls in rodenticide treated areas

Remarks: A is the asymptote, K is the growth constant, and ti is the inflection point

#### The wing length

The wing length of nestlings grew from 1.48  $\pm$  0.03 cm (n = 6), 1.50  $\pm$  0.04 cm (n = 5), 1.50  $\pm$  0.04 cm (n = 4), 1.44  $\pm$  0.05 cm (n = 5) in control, warfarin, brodifacoum, and biorodenticide treated plots, respectively at hatching to 26.02  $\pm$  0.21 cm (n = 5), 25.93  $\pm$  0.20 cm (n = 3), 22.05  $\pm$  0.36 cm (n = 2) and 26.07  $\pm$  0.23 cm (n = 4) for the corresponding treatment plots at day 49 (Table-

7). Unlike tarsus and culmen that reached the adult size during the growth period, wing length still grew up to day 49, several days before fledging, and wing length was shorter by 15.77%, 16.06%, 28.62%, and 15.60% in control, warfarin, brodifacoum and biorodenticide treated plots, respectively compared to adult size ( $30.89 \pm 0.14$ , n=10). Wing length in brodifacoum treated plot was shorter by 15.26% compared to control plot.

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Age	Wing Length (cm)			
(days)	Control	Warfarin	Brodifacoum	Biorodenticide
1	$1.48\pm0.03\ ns$	$1.50\pm\ 0.05$	$1.50\pm\ 0.04$	$1.44\pm\ 0.05$
7	$3.30\pm0.04\ ns$	$3.24\pm\ 0.05$	$3.27 \pm \ 0.03$	$3.20\pm\ 0.02$
14	$6.80\pm0.12\ ns$	$6.90 \pm \ 0.03$	$6.90 \pm \ 0.21$	$6.90\pm\ 0.17$
22	$14.96 \pm 0.14$ ns	$14.87\pm\ 0.17$	$14.60 \pm \ 1.20$	$14.80\pm\ 0.26$
28	$20.94\pm0.26\ ns$	$21.07\pm\ 0.18$	$19.30\pm\ 2.91$	$20.83 \pm \ 0.17$
34	$24.08 \pm 0.19 \text{ ns}$	$23.77\pm\ 0.09$	$20.75\pm\ 3.97$	$23.97 \pm \ 0.17$
43	$25.56\pm0.19~ns$	$25.07 \pm \ 0.17$	$21.55\pm~4.16$	$25.53\pm\ 0.15$
49	$26.02 \pm 0.21$ ns	$25.93 \pm \ 0.20$	$22.05\pm\ 4.36$	$26.07 \pm \ 0.23$

**Table-7.** Wing length (mean  $\pm$  SE) of nestling barn-owls in rodenticide treated areas.

The asymptote reached by the wing length were  $26.28 \pm 0.11$  cm,  $25.86 \pm 0.13$  cm,  $22.15 \pm 0.23$  cm and  $26.24 \pm 0.10$  cm in control, warfarin, brodifacoum and biorodenticide treated plots, respectively. For the growth constant, ranging from  $0.167 \pm 0.003$  cm,  $0.169 \pm 0.003$  cm,  $0.174 \pm 0.005$  cm and  $0.165 \pm 0.002$  cm per day in control, warfarin, brodifacoum and biorodenticide treated plots, respectively. The highest increase in wing length was differ from one treatment to another, ranging from

day 20.18  $\pm$  0.11, day 19.99  $\pm$  0.13, day 18.41  $\pm$  0.24, and day 20.12  $\pm$  0.10 in control, warfarin, brodifacoum and biorodenticide treated plots, respectively.

Teratogenic signs showed by a nestling in brodifacoum treated plot, where up to 49 days old it had malformed primary feathers rendering it flightless (Figures 4 and 5). No nestlings in control, warfarin and biorodenticide treated plots shown teratogenic sign as showed by nestling in brodifacoum treated plot.



Figure-4. Normal nestling.





Figure-5. Teratogenic sign showed by nestling in brodifacoum treated plot that has no primary feathers.

#### The tail length

The tail length of nestlings grew from  $0.00 \pm 0.00$  cm in all treatment at hatching to  $11.62 \pm 0.17$  cm (n = 5),  $11.47 \pm 0.15$  cm (n = 3),  $9.50 \pm 1.00$  cm (n = 2) and  $11.85 \pm 0.21$  cm (n = 4) for control, warfarin, brodifacoum and biorodenticide treated plots at day 49 (Table-9). Same like wing length, the tail length still grew up to day 49. Tail

length was shorter 13.73%, 14.85%, 29.47%, 13.88% in control, warfarin, brodifacoum and biorodenticide treated plots, respectively compared to adult size ( $13.47 \pm 0.14$ , n = 10). The tail length of nestlings in brodifacoum treated plot also shorter by 18.24% compared to nestling in control plot.

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<b>Table-8.</b> Wing length (mean $\pm$ SE) obtained from logistic	c growth equations
for nestling barn owls in rodenticide treated	areas

Treatment	<i>A</i> (g)	$\boldsymbol{K}$ (day <sup>-1</sup> )	<i>ti</i> (days)
А	$26.28\pm0.11$	$0.167\pm0.003$	$20.18\pm0.11$
В	$25.86 \pm 0.13$	$0.165\pm0.003$	$19.99\pm0.13$
С	$22.15\pm0.23$	$0.174\pm0.005$	$18.41\pm0.24$
D	$26.24\pm0.10$	$0.165\pm0.002$	$20.12\pm0.10$

Remarks: A is the asymptote, K is the growth constant, and ti is the inflection point



Figure-6. Logistic growth curve of the changes in wing length of nestling barn owls under rat baiting campaign in oil palm area in Malaysia

Table-9.	Tail length	$(mean \pm SE)$	) of nestling	barn-owls in	rodenticide	treated areas.
		X	/ //			

Age	Tail length (cm)				
(days)	Control	Warfarin	Brodifacoum	Biorodenticide	
1	$0.00 \pm 0.00 \text{ ns}$	$0.00 \pm 0.00$	$0.00\pm~0.00$	$0.00 \pm 0.00$	
7	$0.35 \pm 0.02 \text{ ns}$	$0.46 \pm 0.05$	$0.37 \pm 0.05$	$0.43 \pm 0.02$	
14	$1.23 \pm 0.09 \text{ ns}$	$1.23 \pm 0.03$	$1.27 \pm 0.03$	$1.30 \pm 0.06$	
22	$4.78 \pm 0.07 \text{ ns}$	$4.83 \pm 0.08$	$4.80\pm\ 0.10$	$4.80 \pm 0.16$	
28	$7.44\pm0.10\ ns$	$7.03\pm\ 0.14$	$7.50\pm\ 0.10$	$7.25 \pm 0.09$	
34	$9.68 \pm 0.12$ ns	$9.37 \pm 0.18$	$8.85 \pm 0.65$	9.43 ± 0.20	
43	$10.94 \pm 0.12$ ns	$10.87 \pm 0.15$	$9.20\pm\ 0.80$	$10.87 \pm 0.13$	
49	$11.62 \pm 0.17$ ns	$11.47 \pm 0.15$	$9.50 \pm 1.00$	$11.60 \pm 0.17$	

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The nestling's tail length reached asymptote between 40 - 45 days with the constant growth rate ranging from  $0.19 \pm 0.004$  g per day,  $0.17 \pm 0.004$  g per day,  $0.24 \pm 0.005$  g per day and  $0.17 \pm 0.004$  g per day for control, warfarin, brodifacoum and biorodenticide treated plots, respectively. The highest increase in tail length

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differs from one treatment to another, ranging from day  $24.15 \pm 0.16$ , day  $25.03 \pm 0.19$ , day  $21.82 \pm 0.12$ , and day  $24.61 \pm 0.16$  in control, warfarin, brodifacoum and biorodenticide treated plots, respectively (Table-10/ Figure-5).



Figure-7. Logistic growth curve of the changes in tail length of nestling barn owls under rat baiting campaign in oil palm area in Malaysia.

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Treatment	<i>A</i> (g)	$\boldsymbol{K}$ (day <sup>-1</sup> )	ti (days)
А	$11.28\pm0.08$	$0.19\pm0.004$	$24.15\pm0.16$
В	$11.46\pm0.10$	$0.17\pm0.004$	$25.03\pm0.19$
С	$9.35\pm0.09$	$0.17\pm0.004$	$24.61\pm0.16$
D	$11.74\pm0.05$	$0.24\pm0.005$	$21.82\pm0.12$

**Table-10.** Tail length (mean  $\pm$  SE) obtained from logistic growth equations for nestling barn owls in rodenticide treated areas

Remarks: A is the asymptote, K is the growth constant, and ti is the inflection point

### DISCUSSIONS

Growth in bird nestlings has been frequently described using weight versus age curves. Growth in birds generally follows a sigmoidal curve, where a small initial increase is followed by a period of relatively rapid growth before leveling off. Information of most interest is the form of the growth curve, its final magnitude or asymptote, and the rate at which it traversed (O'Connor, 1984). It has long been known that proportional differences must exist among growing anatomical parts as

a result of genetic, physiological or environmental variation (Starck and Ricklefs, 1998). The constant growth rate in body mass of nestling of barn owls, T. alba javanica in untreated control plot was similar as previously reported by Lenton (1984) i.e., K = 0.150-0.162, and with African subspecies T. alba affinis (K = 0.151) (Wilson et al, 1987), but higher than Indian barn owl, T. alba stertens (K = 0.132) (Nagarajan et al., 2002). In rodenticides treated plot, body mass of nestlings is lower than in control plot. This was probably due the stable

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number of rat prey in control plot where the adult barn owl can deliver enough food to females and nestlings regularly, in contrast to rodenticide treated plots where rat population experience a crash due to baiting campaign. A study done by Wood (1984) and Liau (1990) found that in unbaited area, the rat population varied between 200 and 600 per ha, with slow fluctuations. However, rat population drop to less than 150 per ha after baiting, and only established six month after control (Wood and Fee, 2003).

The body mass of nestlings in rodenticide treated plots especially brodifacoum reached a lower asymptote than control plot. Although some nestlings survived to fledging age, others were found dead in the nest box. This is because in rodenticide treated plots the rat populations were not as abundant as in the control plot and encourage the males to travel farther and take a longer time to bring rat prey to the nestling. When food is limited the older nestling will out compete the younger siblings for food depriving the latter of food bringing down the average body mass. A study by Durant and Handrich (1998) showed that nestlings have the same body mass when food given is reduced by 17% when compared to nestlings fed enough and show the same pattern for linear growth and fledging. However, when food is reduced to more than 30% than usual, the nestlings showed lower fat accumulation when compared to normal fed nestlings (Lacombe et al., 1994).

The culmen and tarsus length in control plot grew rapidly in the first 3 weeks and reached the adult size in the growth period, quite similar to that reported by Wilson et al (1987) and Nagarajan et al. (2002) where they reported higher growth rates for these bodily parts for young nestlings in Central Mali and India and can reach adult size before fledging. The rapid growth of culmen was also reported in the spotted owl (Athene brama brama) (Kumar, 1983). Faster growth of these body parts may be a direct reflection of the use of these organs during the nesting period and immediately after fledging (Holcomb and Twiest, 1968). Rapid leg growth rates as evidenced by tarsus and talon growth rate are another feature of growth in nestling barn owls. Rapid growth of the legs considered by Nagarajan et al. (2002) as a selective advantage in competition within broods and also important in post fledging foraging activities, such as collection and handling of food items. In contrast to culmen and tarsus, wing and tail length was shorter than adult size until fledging. Wilson et al. (1987) and Nagarajan et al. (2002) reported that tail and wing will continue to grow after fledging. The tail and wing would still increase in length after the bird had left the nest and the full ability of young barn owls to catch their own prey would not be achieved until some time had elapsed after leaving the nest (Wilson et al., 1987).

Besides receiving less food than the nestlings in the control plot, nestlings in rodenticide treated plots also face the risk of secondary poisoning by rodenticide residues. If the parents bring home rats that had consumed baits, the young would be exposed to the ingested rodenticide especially brodifacoum, risking them to secondary poisoning. Brodifacoum acts by inhibiting the normal synthesis of vitamin K in the liver (Hadler and Shadbolt, 1975), resulting in an increase in blood clotting time to the point where haemorrhaging occurs (Eason *et al.*, 2002). A study showed that the potential hazard of using brodifacoum is not only due to its high potency of the active ingredient, but also the risk to non-target animal either by direct consumption or from build-up of rodenticide residues from indirect consumption of baits (Shore *et al.*, 1999; Dowding *et al.*, 2010). Mendenhall and Pank (1980) reported that five of six *T. Alba* fed with rats poisoned with brodifacoum died. If the larger adults succumb from rodenticide poisoning the risk to the nestlings would be definitely greater.

Sarcocystis singaporensis is highly host-specific and only lives in the boid snake (*Python reticulatus*) and rodents of the genera *Rattus* and *Bandicota*. The infection of rats is by the sporozoites which eventually invades the muscles to from characteristics cyst in the striated muscles. After inoculation of a lethal quantity of sporocysts, the number of merozoites, the infective stage of the pathogen increase enormously around day 11 post infection especially in the lungs. This induces a fatal pneumonia (Jakel *et al*, 1996).

Warfarin, the first generation anticoagulant, is less toxic than brodifacoum. It is not persistent, and readily metabolized and excreted, and is not retained in the liver beyond 2-4 weeks, while brodifacoum is retained in the liver for 6-12 months (Eason *et al.*, 2002). Several studies have shown that birds were almost completely resistant to the effects of warfarin (Papworth 1958). The same indication was also shown by the tawny owl (*Strix aluco*) when given mice that have consumed warfarin on alternate days for three months with no death or apparent behavioral changes (Townsend *et al.*, 1981). Lenton (1984) estimated barn owl nestlings need to consume at least ten medium sizes rats (80g) before a lethal level is reached.

In these study nestlings in rodenticide treated plots, especially brodifacoum showed shorter and lighter measurements in all five anatomical features: body mass, culmen, tarsus, wing and tail length. Previous workers showed that some birds have shorter anatomical parts and lighter in body mass if they are exposed to pesticides or if lived in polluted area. The screech owls (Otus asio) administered with fluoride at 40 ppm resulted in a significantly smaller egg and shorter tarsus length (Hoffman et al., 1985). The nestlings of the great (Parus major) that lives at large non-ferrous smelter and exposed to large amounts of heavy metals have a body mass significantly reduced at the most polluted site although tarsus length, wing length and haematocrit values did not differ significantly among study sites (Janssens et al., 2003). The nestling zebra finch (Taeniopygia guttata) that were orally dosed with monosodium methanearsonate (MSMA) for 20 days from hatching to fledging showed high mortality if given 24 mg/g, while surviving nestlings showed accumulation of arsenic in blood and specific





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tissues, and decreased tarsus length and wing length upon fledging (Albert *et al.*, 2008).

Teratogenic effect was also evidenced in one of the nestling in brodifacoum treated area where its primary feathers were malformed rendering it flightless besides tail length that were very short if compared to nestlings in control plot. Several pesticides studies also found teratogenic effect on growth and development of birds. The chicken embryos were exposed *benzo* [a] pyrene (BP) via the yolk sac route resulted in retarded growth, as reflected by lower embryonic body weight besides reduced bill length. Abnormal survivors also showed remarkably twisted legs with shortening of the bones, abdominal oedema, haematomas, blisters and a short neck (Anwer and Mehrotra, 1988). Fry (1995) also reported petroleum organochlorine, organophosphate, hydrocarbons, heavy metals, and polychlorinated biphenyls (PCBs) disrupt physiological effects at several levels on birds, including direct effects on breeding adults as well as developmental effects on embryos. The effects on embryos include mortality or reduced hatchability, failure of chicks to thrive (wasting syndrome), and teratological effects producing skeletal abnormalities and impaired differentiation of the reproductive and nervous systems through mechanisms of hormonal mimicking of estrogens. The eggs of Mallard (Anas platyrhynchos) that were treated by Phenyl phosphonothioic acid-o-ethyl-O-[4-nitrophenyl] ester (EPN) resulted in impaired embryonic growth and was highly teratogenic: 37-42% of the surviving embryos were abnormal with cervical and axial scoliosis as well as severe edema. Brain weights were significantly lower in EPN-treated groups at different stages of development including hatchlings. Hatchlings from EPN treated eggs were weaker and slower to right themselves compared to untreated hatchlings (Hoffman and Sileo, 1984).

#### CONCLUSIONS

Nestlings in rodenticide free area showed consistently heavier body mass and longer in culmen, tarsus, wing and tail length compared to rodenticide treated plots. This was associated with nestlings in rodenticides free getting sufficient food during the growth stage. The food shortage in rodenticide treated plots affect the growth of nestling and exposed to a greater risk of death especially for nestlings less than 20 days old if food shortage continues. Nestlings in brodifacoum treated area did not only face the risk of food shortages but also the risk of secondary poisoning as a result of consuming bait ingested rats. Even one nestling has teratogenic signs where it has no primary feathers in its wings rendering it flightless and the size of the tail is shorter than nestling in rodenticide free area. However, nestlings in warfarin and biorodenticide treated plots have comparable anatomical parts except body mass if compared to rodenticide free area, an indication that there was no apparent evidence of secondary poisoning effect of warfarin and biorodenticide on nestling of barn owls.

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