วารสารเกษตร 14(1) : 77 - 86 (2541)
Journal of Agriculture 14(1) : 77 - 86 (1998)

Monitoring of Ovarian Function in Captive Banteng *(Bos javanicus birmanicus)* by Determination of Faecal Progesterone

Tusanee Apichartsrungkoon[1/], *Petai Pongpiachan*[1/], *Chatri Khoohathapharak*[2/] *and Kanchai Sanwong*[2/]

Abstract : Faecal samples were obtained from 7 female and 2 male bantengs, at Chiang Mai Zoo, twice a week during hot, rainy, and cold seasons in 1995 and during rainy and cold seasons in 1997. Faecal progesterone concentrations were assayed by radioimmunoassay (RIA) technique. Progesterone profiles were found in cyclic females with 20.94 ± 4.3 days of estrous cycle length. Basal levels of progesterone concentrations were 373.6 ± 233.8 and 303.7 ± 186.7 ng/g dry faeces from the samples in 1995 and 1997 respectively. Maximum levels of progesterone concentrations were 8,195.5 ± 2,908 and 4,540 ± 1,761 ng/g dry faeces from the samples in 1995 and 1997 respectively. Progesterone profiles of pregnant bantengs were fluctuated with the progesterone levels ranged from 120 to 17,400 ng/g dry faeces. In conclusion, progesterone concentrations assayed from faecal samples can be used for roughly monitoring estrous cycle and ovarian function. Factor that may affect the faecal progesterone was diet variation which animals received.

Index words : banteng, *Bos javanicus*, ovarian function, progesterone

[1/] Department of Animal Science, Faculty of Agriculture, Chiangmai University, Chiang Mai 50200, Thailand.

[2/] Chiang Mai Zoo, The Zoological Park Organization, Chiang Mai 50200, Thailand.

INTRODUCTION

Banteng *(Bos javanicus)* is one of the most beautiful wild cattle in the area of South East Asia, from north Burma to northern edge of the Malay peninsula, Thailand, Cambodia, Laos, Java, Bali and Borneo. Banteng that found in Thailand is in subspecies of *Bos javanicus birmanicus* which also found in other countries on the Asian mainland. Banteng is bigger than native domestic cattle. The coloration of the coat is golden brown or chestnut in males and bright rufous brown or fawn - coloured in females (IUCN/ SSC, 1995). The markings of banteng includes white "stocking", white lips, white hair in the ears and a large white rump patch.

Today, Banteng are seriously endangered. The world population of *Bos javanicus birmanicus* is less than 1,000 and the population in Thailand is around 500. Srikosamatara and Suteethorn (1995) reported that the number of banteng in Thailand decreased approximately 80% during the past 25 years, from 2,300-2,500 animals in 1970 to 500 animals in 1995. Hunting, habitat degradation and diseases from domestic livestock are major reasons of a reduction in population.

Increasing in number of banteng is required urgently. A possible way is to breed more animals in zoological areas and then release them in protected natural forest. Studying of banteng reproductive function is improtant for breeding management. And progesterone determination in females is a criteria to monitor the ovarian function. However, taking blood sample from wild animals seem to be impossible. Therefore, noninvasive method by determining the concentration of progesterone in faeces might be an alternative. Faecal sample is easy to be collected without disturbing the animals. The purpose of the present study was to monitor the ovarian function of the banteng by determination of faecal progesterone.

MATERIALS AND METHODS

Seven female bantengs (cow no. 1-7), 10 - >20 years old, at Chiang Mai Zoo, were used for this study with 2 males (bull no. 1-2) as the controls. They were devided into 2 groups and kept in 2 separated restrict areas, 4 females and 2 males in one group and 3 females in another group. Their diet varied from season to season with water *ad libitum*.

Fresh faecal samples were collected from the ground and stored at -20°C until analysis. Samples were taken twice a week (3-4 days interval) during hot-dry (April-May), rainy (August-September) and cold (December -January 96) seasons in 1995 and during rainy (June-July) and cold (December-January 98) seasons in 1997.

Sample extraction and progesterone analysis

Faecal steroids were extracted using the technique developed by Wasser *et al.* (1993). Faecal sample was taken to dryness in oven at 45°C overnight. Then 0.1 g dry faeces was mixed with 3 ml ethanol and shaked for 30 minutes. The mixture was centrifuged at 1000X g for 10 minutes. The supernatant was then used for progesterone concentration measurement by radioimmunoassay (RIA) technique.

Progesterone analysis from extracted faecal samples by RIA, based on the method described by Pongpiachan and Apichartsrungkoon (1990). Antisera was raised in rabbit against progesterone linked with molecule of bovine serum albumin. Labeled progesterone used in the assay was [1,2,6,7 - ^{3}H] - P_4. The separation of bound/free fractions was accomplished by the use of charcoal solution. The intra-assay and inter - assay coefficient of variation were 19.4% and 11.15%, respectively. Faecal progesterone concentration was expressed in nanogram per gram dry faeces (ng/g dry faeces).

Statistical Analysis

The data were analysed using one-way analysis of variance. All results are expressed as means ± SD.

RESULTS

Estrous cycles presented by progesterone profiles were found in cyclic nonpregnant cows (cow no. 1,5,6 and 7 ; progesterone profile of cow no. 1 is shown in Figure 1) compared to the steady basal levels of progesterone profiles in bulls (bull no.1 and 2 ; progesterone profile of bull no. 2 in shown in Figure 2). Basal levels of progesterone concentrations in cyclic cows were 373.6 ±233.8 and 303.7 ± 186.7 ng/g dry faeces from the samples in 1995 and 1997 respectively. Maximum levels of progesterone concentrations were 8,195. 5 ± 2,908 and 4,540 ± 1,761 ng/g dry faeces from the samples in 1995 and 1997 respectively. Basal levels of progesterone concentrations were not significantly different between the samples collected in 1995 and in 1997, whereas maximum levels of progesterone concentrations of the samples collected in 1995 were significantly ($P<0.01$) higher than those in 1997. Mean progesterone concentrations of bull no. 1 and 2 were 537.6 ± 519.3 and 530 ± 571.9 ng/g dry faeces, respectively. Average estrous cycle length of cyclic cows was 20.94 ± 4.3 days.

During the period of study in 1995 , cow no. 2, 3 and 4 were pregnant confirmed by parturition dates which were 26/12/95, 06/03/96 and 19/04/96, respectively. These meant the cows started pregnancy in March 1995, June 1995 and

1995, respectively. In 1997, cow no.3 and 4 were pregnant again. Both of them were mated and pregnant in July 1997 and laboured on 05/04/98 and 07/04/98, respectively. Progesterone profiles of cow no. 3 and 4 are shown in Figure 3 and 4 respectively. In pregnant cows, progesterone levels were fluctuated and were not consistent with the pregnancy status. Means of progesterone concentration at peaks during pregnancy were 10,993 ± 4,202 and 4,342.5 ± 1,109 ng/g dry faeces from the samples in 1995 and 1997 respectively. The level of progesterone at peaks of the samples collected in 1995 was significantly ($P<0.01$) higher than those in 1997.

Estrous cycles were found in banteng throughout the year. However, 4 out of 5 cows started their pregnancies in June and July. Therefore, this period of the year should be their breeding season.

DISCUSSION

Mekchay (1994), studied in dairy cattle, confirmed that there was a relationship between milk and faecal progesterone concentrations with a maximum positive correlation ($r = 0.739$) for third degree polynominal on curvilinear regression; $Y_{cubi}c = -1.157 + 0.0083x - 2.25*10^{-6}x2 + 2.1*10^{-10}x^3$. This was supported by Wasser *et al.* (1993) who reported the relationship between serum and faecal progesterone concentrations in baboons. The use of faecal progesterone concentrations for ovarian activity determination and pregnancy diagnosis were supported by Mekchay (1994), studied in dairy cattle; Schwarzenberger *et al.* (1991), studied in mares ; Schwarzenberger *et al.* (1992), studied in mares; Schwarzenberger *et al.* (1993), studied in okapi and Larter *et al.* (1994), studied in dairy cattle. Therefore, only faecal samples were used in this study without the collection of blood sample to avoid animal disturbing.

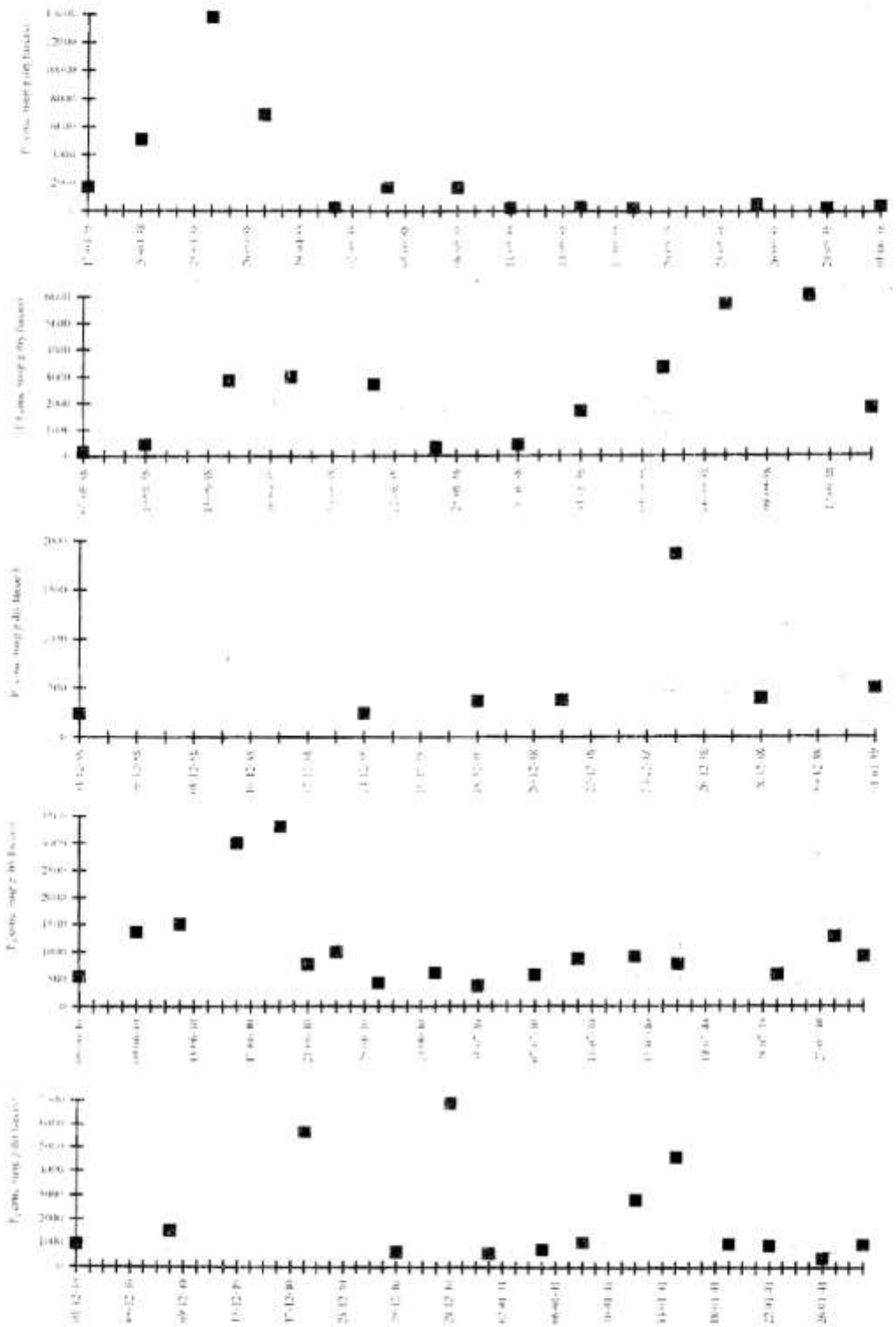

Figure 1 Faecal progesterone concentrations of cow no. 1 during hot, rainy and cold seasons in 1995 and during rainy and cold seasons in 1997 respectively.

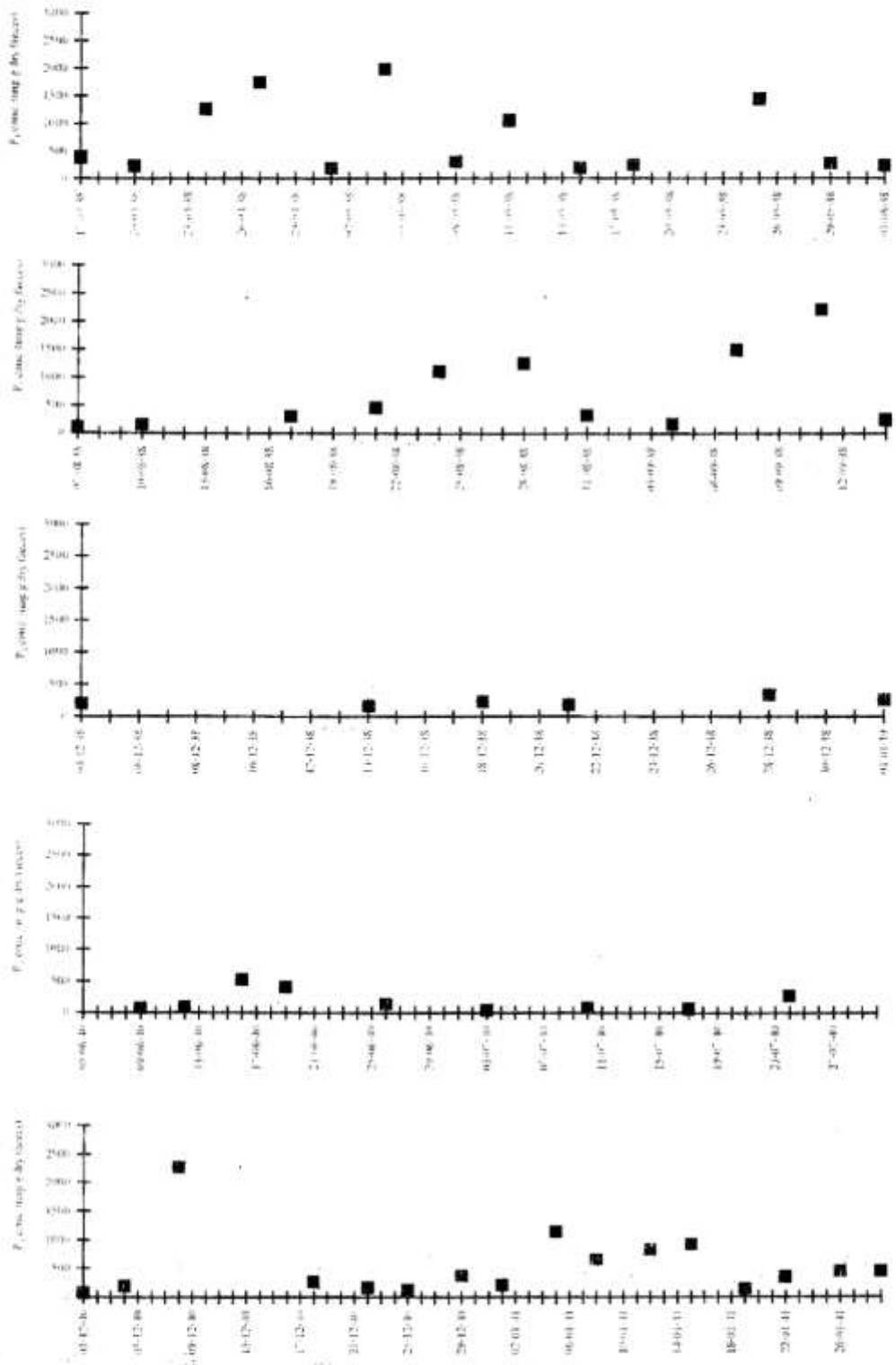

Figure 2 **Faecal progesterone concentrations of bull no. 2 during hot, rainy and cold seasons in 1995 and during rainy and cold seasons in 1997 respectively.**

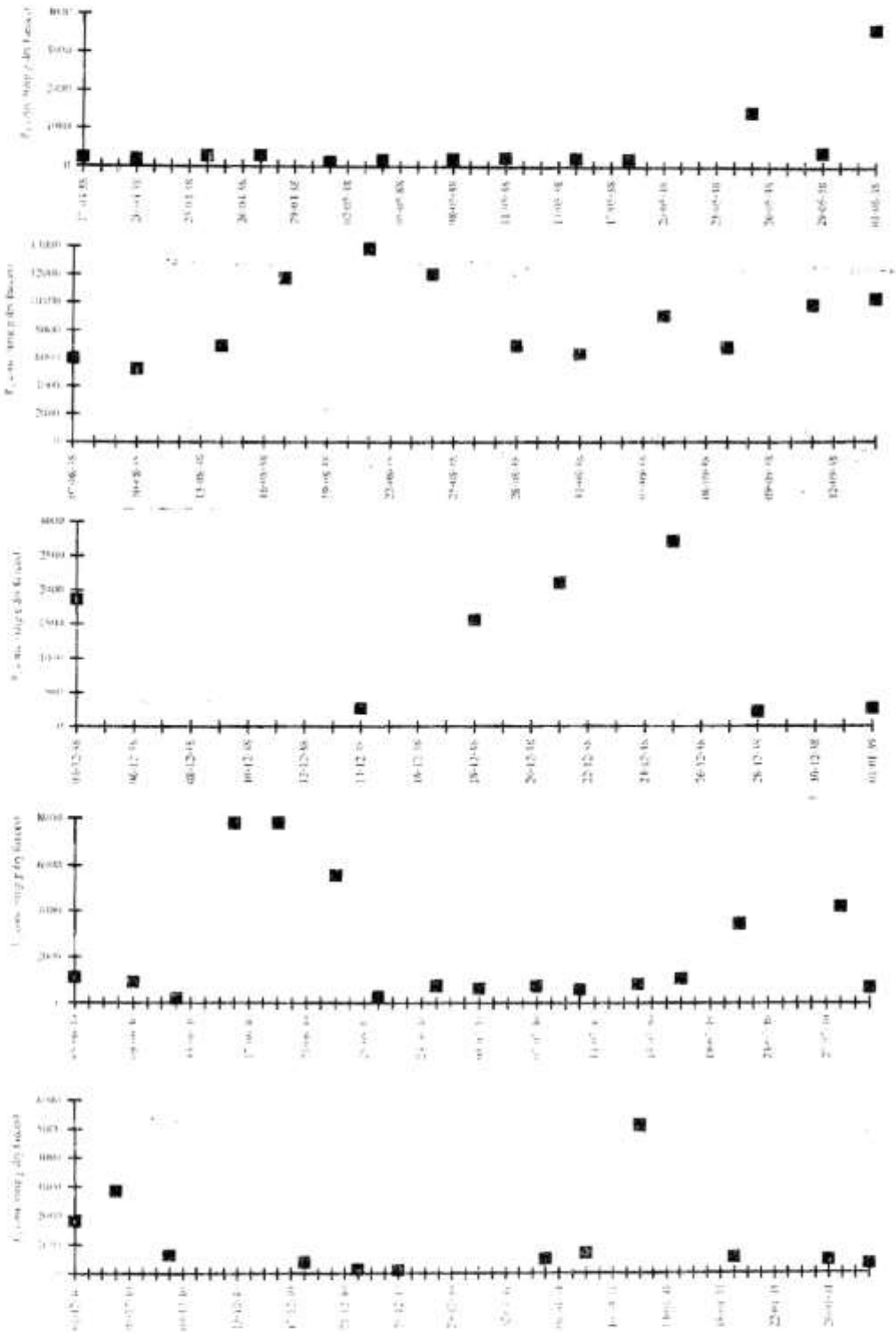

Figure 3 Faecal progesterone concentrations of cow no. 3 during hot, rainy and cold seasons in 1995 and during rainy and cold seasons in 1997 respectively.

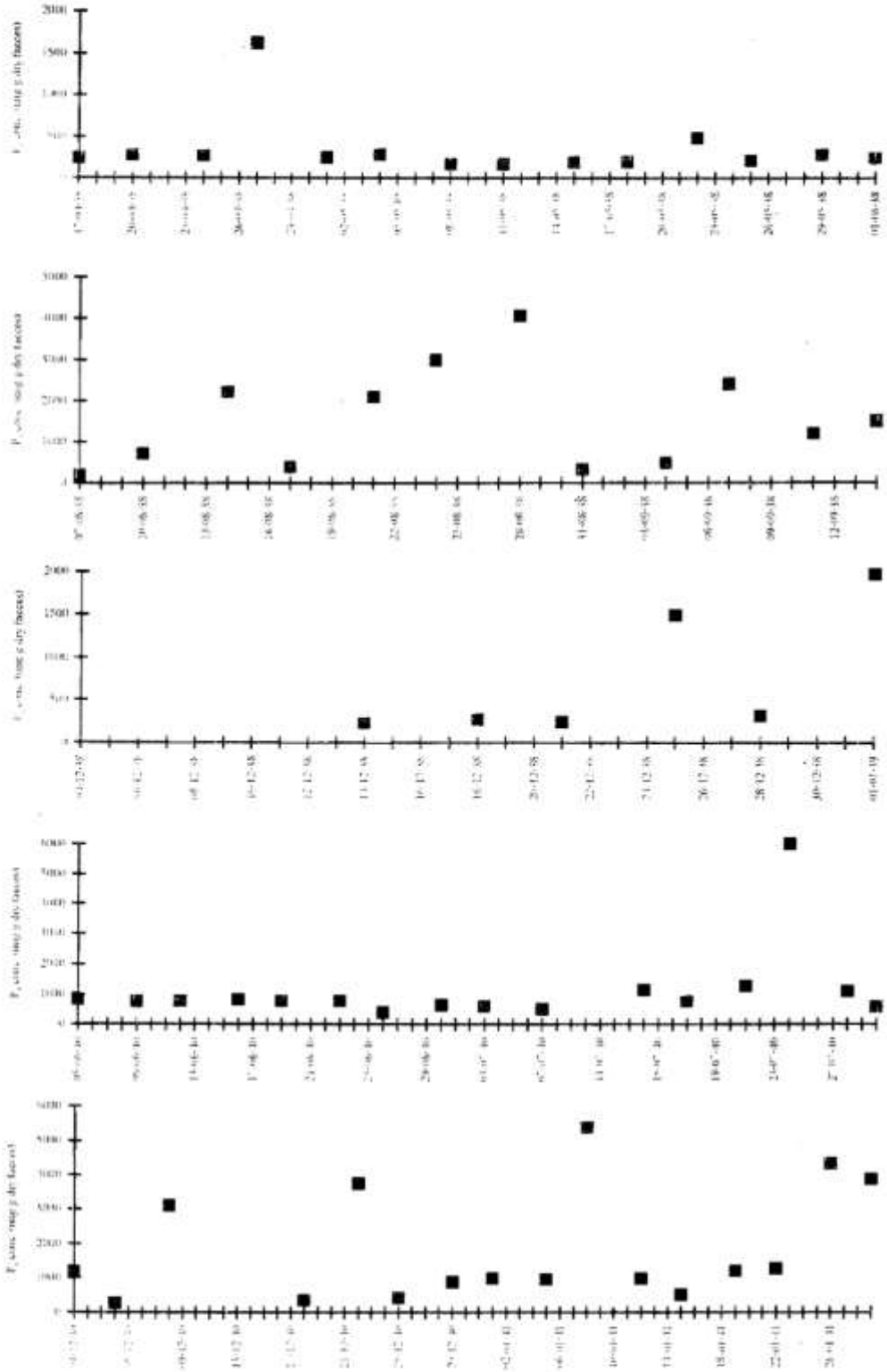

Figure 4 Faecal progesterone concentrations of cow no. 4 during hot, rainy and cold seasons in 1995 and during rainy and cold seasons in 1997 respectively.

There were progesterone profiles showed in cyclic and pregnant female bantengs compared to the absent profiles of the males. However, the progesterone concentrations were not consistent with the ovarian status, particularly in pregnant cows. This may due to the variation of diet that animals received in different season. Wasser *et al.* (1993) reported that faecal progestogen concentrations decreased as dietary fibre increased. They suggested that indexing faecal progestogens by cholestanone (cholesterol metabolite which was positive correlated with dietary fibre) improved the correlation between serum and indexed faecal progestogens.

Breeding season of banteng in the study was June and July which was supported by Fowler (1978) who reported that breeding season of banteng lasted from June until August. However, Mahannop (1990) reported that banteng in natural forest may not have a certain breeding season due to the distrubing of man.

ACKNOWLEDGEMENTS

This study was supported by grant from Chiangmai University (in 1997). We also thank the staff of Chiang Mai Zoo for the collection of samples and Mr. C. Tonginn for technical assistance.

REFERENCES

Fowler, M.E. 1978. Zoo and Wild Animal Medicine. W.B. Saunders Company, Philadelphia, U.S.A.

IUCN/SSC (1995) Asian Wild Cattle, Conservation Assessment and Management Plan Workshop. p. 51-52.

Larter, N.C., R. Rajamahendran and K. Sivakumaran. 1994. Immunoreactive faecal progestins as indicators of reproductive status. Vet. Rec. 134 : 474-475.

Mahannop, A. 1990. Banteng. Natural and Pet Magazine. 2(12) : 31- 33 (in Thai).

Mekchay, S. 1994. Use of the faecal progesterone test for pregnant determination. Master Special Problem, Dept. of Animal Science, Faculty of Agriculture, Chiang Mai University (in Thai).

Pongpiachan, P. and T. Apichartsrungkoon. 1990. Assessment of reproductive performance in dairy cattle by radioimmuuoassay. J. Agriculture 6 (1) : 21-40 (in Thai).

Schwarzenberger, F., E. Mostl, E. Bamberg, J. Pammer and O. Schmehlik. 1991. Concentrations of progestagens and oestrogens in the faeces of pregnant Lipizzan Troter and Thoroughbred mares. J. Reprod. Fert. Suppl. 44 : 489 - 499.

Schwarzenberger, F., E. Mostl, E. Bamberg, and G. von Hegel. 1992. Monitoring of corpus luteum function by measuring progestogens in faeces of non-pregnant mares *(Equus caballus)* and Przewalski mares *(Equus przewalskii)*. Anim. Reprod. Sci. 29 : 263-273.

Schwarzenberger, F., M. Patzl, R. Francke, A. Ochs, R. Buiter, W. Schaftenaar and W. De Meurichy. 1993. Faecal progestagen evaluations to monitor the estrous cycle and pregnancy in the Okapi *(Okapia johstoni)*. Zoo Biol. 12 : 549-559.

Srikosamatara, S. and V. Suteethorn. (1995) Populations of gaur and banteng and their management in Thailand. Nat. Hist. Bul. Siam Soc. (In press).

Wasser, S.K., R. Thomas, P.P. Nair, C. Guidry, J. Southers, J. Lucus, D.E. Wildt and S.L. Monfort. 1993. Effects of dietary fibre on faecal steroid measurements in baboons *(Papio cynocephalus cynocephalus)*. J. Reprod. Fert. 97 : 569-574.