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

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

Keywords : Banteng, *Bos javanicus*, ovarian function, progesterone

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. The Banteng is that found in Thailand is a subspecies of *Bos javanicus birmanicus* which also found in other countries on the Asian mainland. The 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 for the reduction in population.

An increase in the number of Banteng is urgently required. A possible way is to breed more animals in zoological areas and then release them in protected natural forests. Studies of the Banteng reproductive function is important for breeding management and progesterone determination in females could be a criteria to monitor the ovarian function. However, taking blood sample from wild animals seem to be impossible, therefore, the non-invasive method where the concentration of progesterone is determined in faeces was tested as an alternative, since faecal sample easy can 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 Chiangmai Zoo, were used for this study with 2 males (bull no. 1-2) as the controls. They were divided into 2 groups and kept in two separate restricted 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 the hot (April-May), rainy (August-September) and cold (December -January 96) seasons in 1995 and during the 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). Progesterone analysis from extracted faecal samples was performed 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. The faecal progesterone concentration was expressed in nanogram per gram dry faeces (ng/g dry faeces).

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 íC 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.

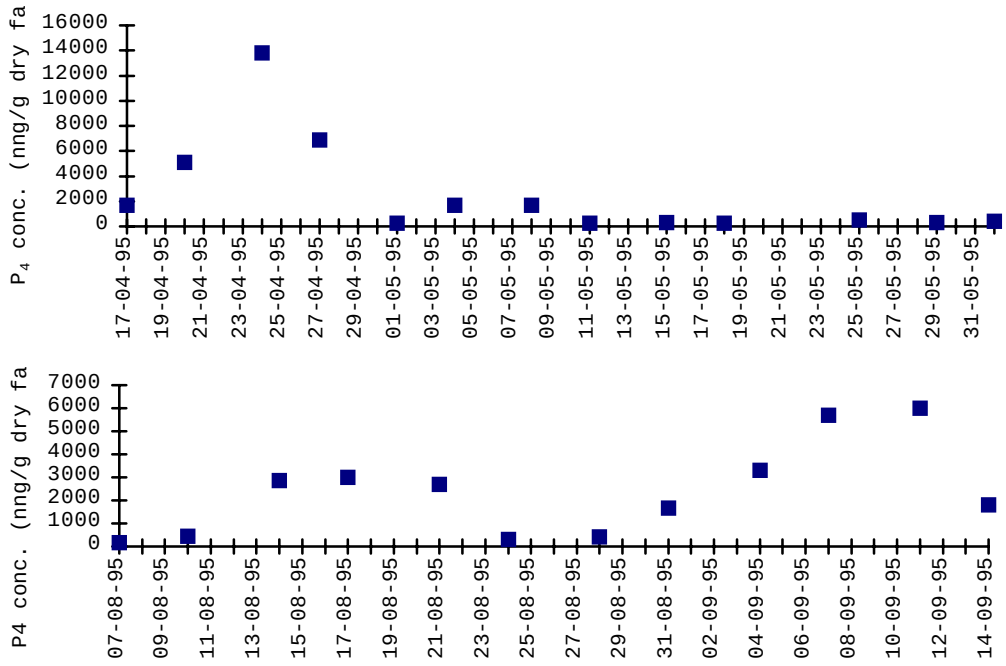
Progesterone analysis from extracted faecal samples by RIA, based on the method described by Pongpiachan and Apichartsrungkoon (1990). Antisera was raised in rabbit against progesterone linked with molecule of bovine serum albumin. Labeled progesterone used in the assay was [1,2,6,7 - ^{3}H] - P_4 . 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

Oestrous cycles presented by progesterone profiles were found in cyclic non-pregnant cows (cow no. 1,5,6 and 7; some part of 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 ; some part of 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 in the samples from 1995 and 1997, respectively. Maximum levels of progesterone concentrations were 8,195. 5 ± 2,908 and 4,540 ± 1,761 ng/g dry faeces in the samples from 1995 and 1997, respectively. Basal levels of progesterone concentrations were not significantly different between the samples collected from 1995 and 1997, whereas maximum levels of progesterone concentrations of the samples collected from 1995 were significantly ($P<0.01$) higher than those from 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 oestrous cycle length of cyclic cows was 20.94 ± 4.3 days.



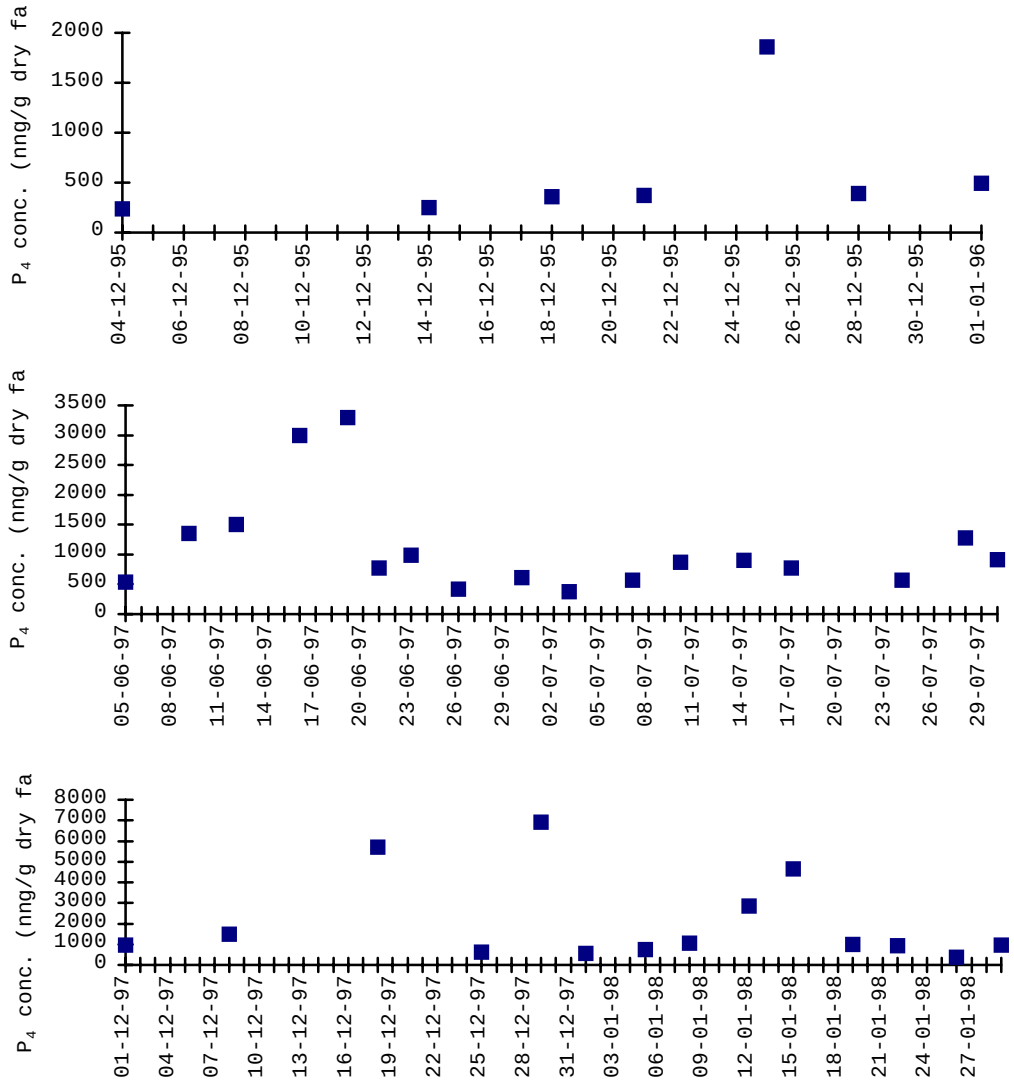


Figure 1: Faecal progesterone concentrations of cow no. 1 during the hot, rainy and cold seasons in 1995 and during the rainy and cold seasons in 1997 respectively.

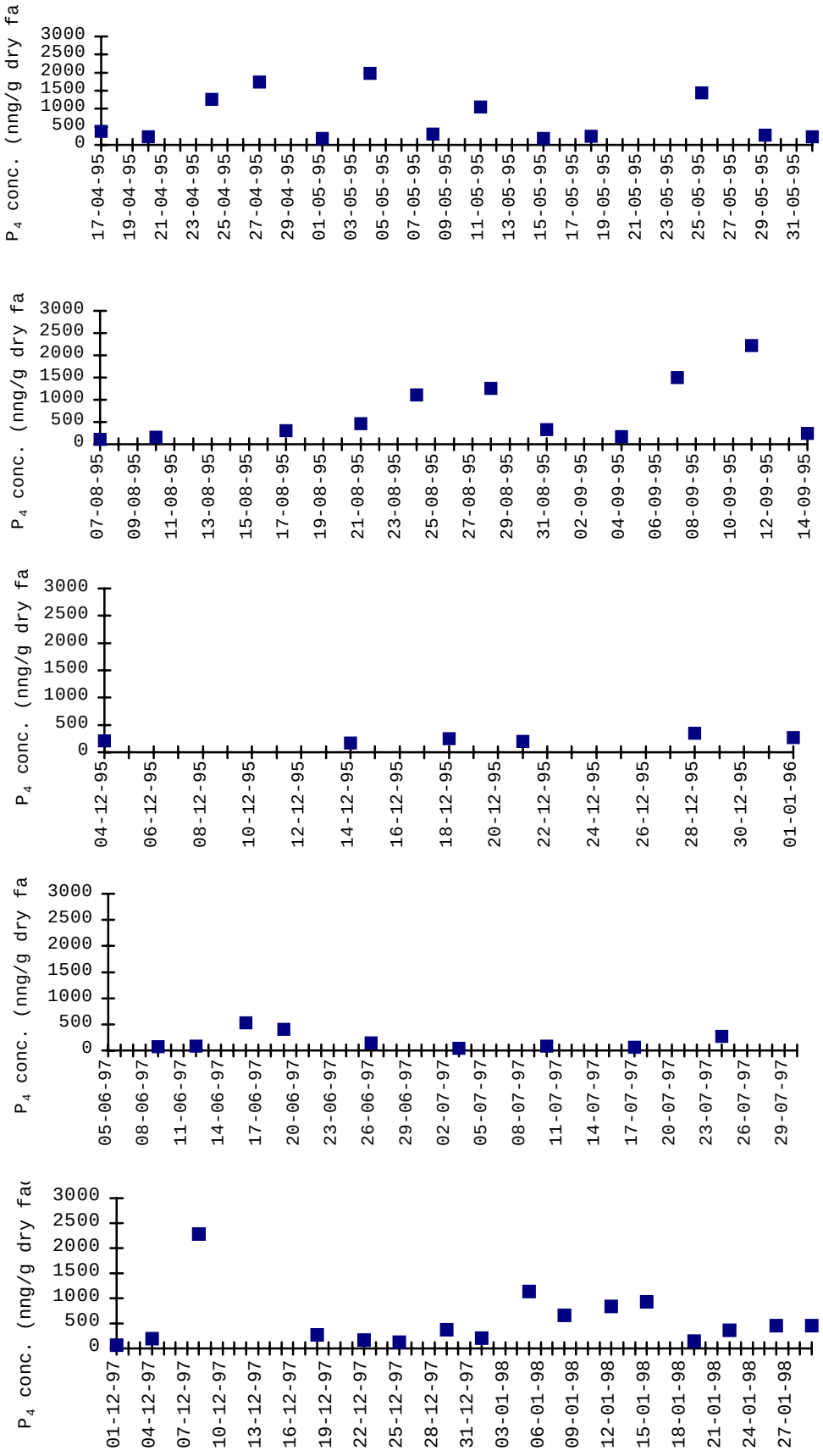

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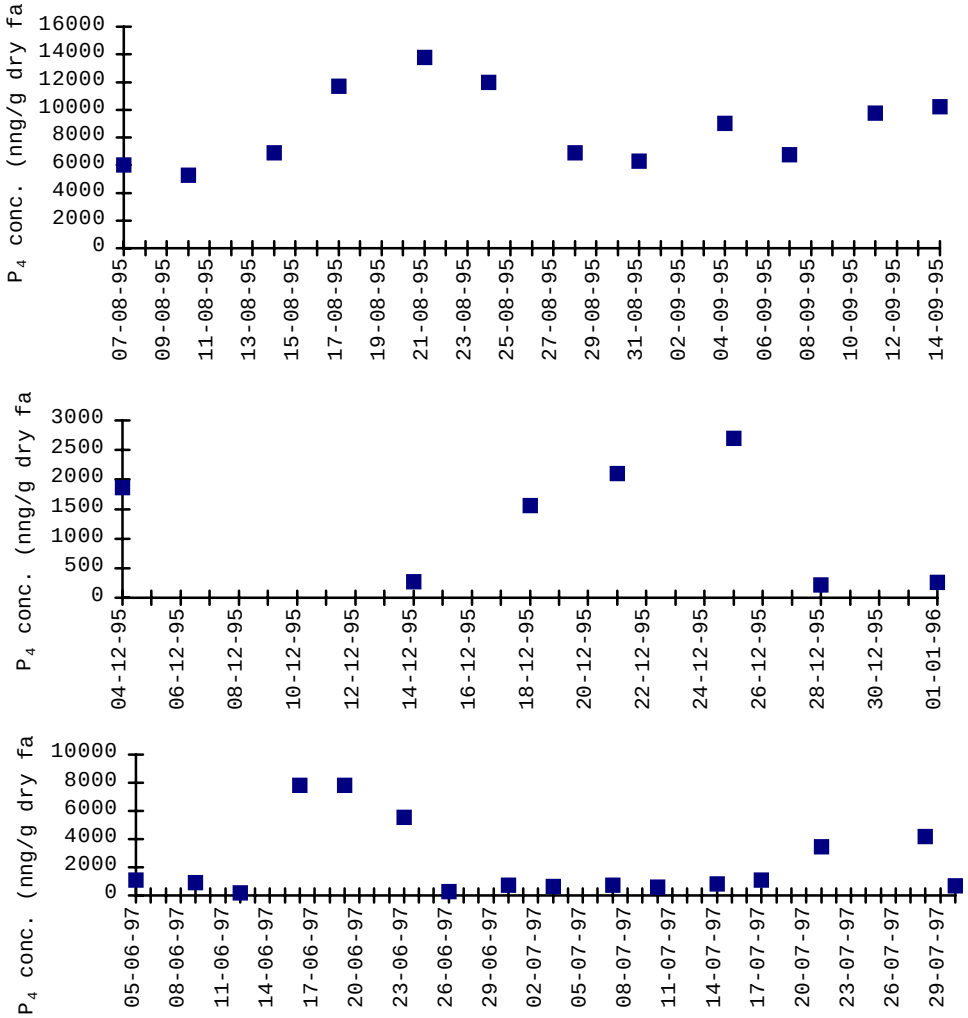
Figure 2: Faecal progesterone concentrations of bull no. 2 during the hot, rainy and cold seasons in 1995 and during the rainy and cold seasons in 1997 respectively.

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 dates means that the cows started pregnancy in March 1995, June 1995 and July 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, 693 ± 4,202 and 4,342.5 ± 1,109 ng/g dry faeces in the samples from 1995 and 1997, respectively. The level of progesterone at peaks of the samples collected from 1995 was significantly ($P<0.01$) higher than those from 1997.



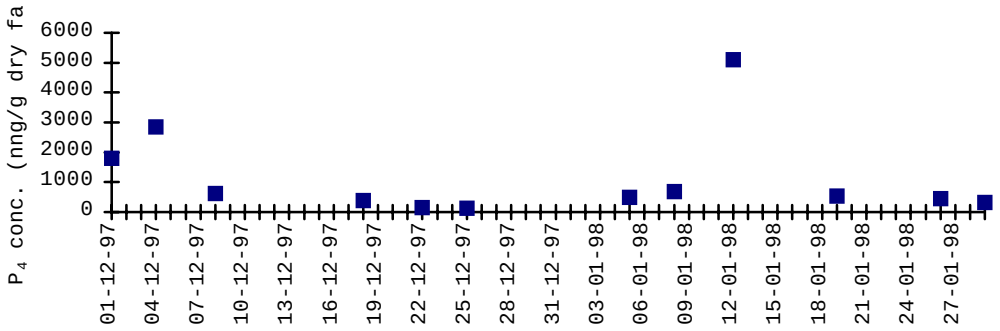
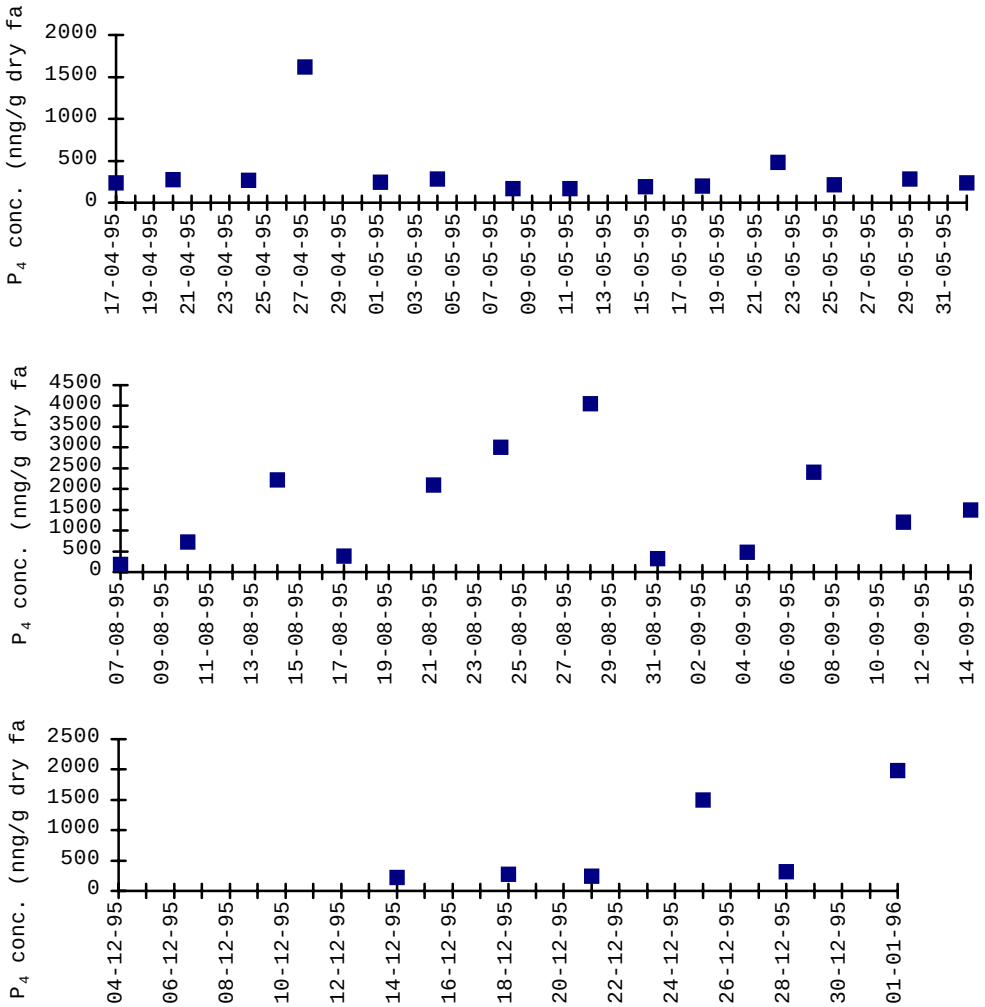


Figure 3: Faecal progesterone concentrations of cow no. 3 during the hot, rainy and cold seasons in 1995 and during the rainy and cold seasons in 1997 respectively.



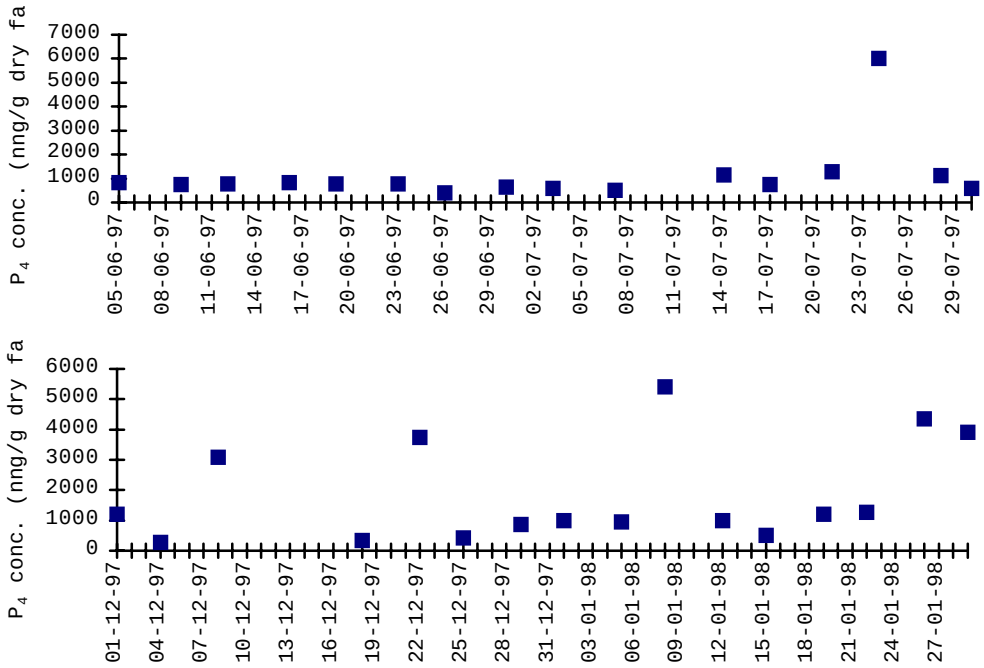


Figure 4: Faecal progesterone concentrations of cow no. 4 during the hot, rainy and cold seasons in 1995 and during the rainy and cold seasons in 1997 respectively.

Oestrous 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

By studies in dairy cattle Mekchay (1994) 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; $Y_{cubic} = -1.157 + 0.0083x - 2.25*10^{-6}x^2 + 2.1*10^{-10}x^3$. 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), by studies in dairy cattle; Schwarzenberger et al. (1991), by studies in mares ; Schwarzenberger et al. (1992), by studies in mares; Schwarzenberger et al. (1993), by studies in okapi and Larter et al. (1994), by studies dairy cattle. Therefore, only faecal samples were used in this study without the collection of blood sample to avoid disturbing the animal.

Progesterone profiles were found for cyclic and pregnant female Bantengs compared to the absence of profiles of the males. However, the progesterone concentrations were not consistent with the ovarian status, particularly in pregnant cows. This may be due to the variation in the diet that the animals received in different seasons. 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 is supported by Fowler (1978) who reported that breeding season of Banteng lasted from June until August. However, Mahannop (1990) reported that Banteng in natural forests may not have a certain breeding season due to the disturbance of man.

Acknowledgements

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