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# Plasma, milk and faecal progesterone concentrations during the oestrous cycle of lactating dairy cows with different milk yields

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**Abstract**

The hypotheses tested in this study were that neither average progesterone (P4) concentrations in plasma and milk nor average progesterone metabolites concentrations in faeces would differ during an oestrous cycle in two groups of cows with differing daily milk yields. High producing (HP = 8) and low producing (LP = 8) dairy cows were selected randomly for the study. Their oestrous cycles were initially synchronised using P4 and prostaglandin F2α. Chromic oxide capsules were administered twice daily to measure total faecal output. Samples of blood, faeces and milk were taken daily throughout one oestrous cycle, plasma and milk P4, and faecal progesterone metabolites (FP4M) assayed. The average daily milk yields in the two groups were 30.8 and 21.9 l per day, respectively ($P < 0.0001$), although daily faecal output was similar in both the groups (HP, 7.7 versus LP, 6.9 kg DM; $P = 0.24$). Mean plasma and milk P4 concentrations were similar in both the groups (plasma P4, 4.12 versus 4.05 ng/ml; $P = 0.3$; milk P4, 8.2 versus 8.3; $P = 0.9$) during dioestrus. Average daily excretion of P4 to the milk was greater in HP than LP cows (252 versus 185 μg, $P = 0.04$). Neither concentration nor the daily yield of FP4Ms was affected by level of milk yield (concentration: 12.2 versus 11.5 μg/g; daily yield: 89.1 versus 82.9 mg per day; $P > 0.05$). These data showed that the concentrations of P4 in plasma and milk, and the concentrations and daily yields of FP4M were not affected by the level of daily milk yields which differed by about 41% of the LP average of 21.9 l.


*Keywords:* Progesterone; Faecal progesterone metabolites; Dairy cows; Milk yield; Oestrous cycle

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## 1. Introduction

High producing (HP) cows have greater nutritional demands to support their milk production. These cows may also exhibit poorer conception rates than lower-yielding cows (Stevenson et al., 1983; Lean et al., 1989). They have a delicate balance between normality and metabolic disturbances, which can adversely affect reproductive efficiency. There has been considerable interest in factors that influence the reproductive performance of these cows. Several workers (Stevenson et al., 1983; Nebel and McGilliard, 1993; Laben et al., 1982) have implicated an antagonism between high production and fertility.

The link between negative energy balance and fertility in HP cows may be related to blood progesterone (P4) concentrations (Butler, 2000). Regulatory mechanism controlling luteal function and blood P4 concentrations may have an important role on the fertility of dairy cows as variations in plasma P4 can affect fertilization, embryo transport and embryo survival (Garrett et al., 1988; Mee et al., 1991). Plasma P4 concentration has also been correlated with the incidence of delayed conception and reduced fertility in dairy cows (Lukaszewska and Hansel, 1980; Macmillan and Peterson, 1993; Garverick and Bierschwal, 1978).

Cows with higher milk production in early lactation have a higher rate of metabolism than low producing cows (Huntington, 1990; Butler, 2000). The concentration of P4 in blood is related to its production and clearance rates. Therefore, cows with higher milk production and with an elevated rate of metabolism may metabolise a greater proportion of the P4 in their blood stream leading to lower circulating concentrations. Neither luteal function nor ovarian venous P4 levels are modified by level of nutrition (Abecia et al., 1995, 1997). The liver possesses an intrinsic enzymatic capability to metabolise high concentrations of P4 involving cytochrome enzyme complexes (mixed function oxidase) in liver microsomes (Payne et al., 1991; Kaddouri et al., 1992). Differences in peripheral blood P4 concentrations are more likely due to metabolism and/or the clearance rate of P4 than to production rate by the ovary.

We have previously investigated P4 clearance rate in lactating dairy cows which were intentionally rendered anoestrus before being administered P4 per vaginum (Rabiee et al., 2001b). In these studies, the excretion rates of P4 into the milk and faeces were similar between two groups of cows (non-cycling) with different levels of milk yield. The hypotheses tested in this study were that neither average progesterone (P4) concentrations in plasma and milk nor average progesterone metabolites concentrations in faeces would differ during an oestrous cycle in two groups of cows with differing daily milk yields. The objective of the present study was to monitor plasma and milk P4 concentrations and also the concentrations and daily yields of P4 metabolites in the faeces during an oestrous cycle in two groups of cycling cows with differing daily milk yields. All procedures involving the experimental protocol and use of animals were approved by Ellinbank Research Centre's Animal Ethics Committee (Department of Natural Resources and Environment, Vic., Australia).

## 2. Materials and methods

### *2.1. Animals and experimental protocol*

Sixteen Holstein–Friesian cows, 4–9 years old and 4 weeks postpartum were randomly selected from a group of cycling cows, carefully matched for calving dates and lactation

number and then ranked according to their milk yields and randomly allocated to two groups: (i) high producing (HP = 8, average: 30 l per day or more); and (ii) low producing (LP = 8, average: 20 l per day or less) groups. The group allocations were conducted prior to the oestrous synchronization program. Average milk production records during the post-calving period were used as a base for the group allocation. The difference in milk production between the two groups was 41%. Crushed barely (2.0 kg per cow per day) was equally fed to the cows in both groups twice daily during a.m. and p.m. milking. The duration of access to pasture, type of pasture and amount of barely were similar for all the cows in both groups. They were as one herd and had unrestricted access to improved pastures of ryegrass and white clover. Pasture samples were taken twice weekly during the period of study and analysed for dry matter (DM), crude protein (CP), metabolisable energy (ME) and digestibility. Representative pasture samples were collected and dried at 105 °C to constant weight to determine DM content. Samples of all feeds were bulked on a weekly basis and dried at 65 °C for 72 h, ground and analysed for in vitro dry matter digestibility (DMD) and nitrogen (N). DMD was determined by the method of Clarke et al. (1982), metabolisable energy (ME) was calculated from DMD (ME = DMD × 0.17). The nitrogen content was determined by the Kjeldahl method. Crude protein was calculated from nitrogen (CP = N × 6.25).

Milking times were at 06:15 and 15:00 h and individual milk yields were recorded routinely at each milking (ALPRO TM System, Alfa Laval Agri, Sweden). Body weights of the cows were recorded weekly following the a.m. milking for a period of 5 weeks.

Oestrus was synchronised by insertion of an intravaginal device (CIDR device) containing 1.9 g progesterone (InterAg, NZ) into the vagina of each animal for 7 days, with an injection of prostaglandin F2α (2 ml Prosolvin, Intervet, Australia) at 09:00 h on the day of device removal.

### *2.2. Blood, milk and faecal sampling procedures and assays*

Blood, milk and faecal sampling were conducted daily; it commenced a day following the day of CIDR removal and continued for a period of one oestrous cycle (21–22 days). The average interval from calving to the start of sampling for the two groups was 5 weeks. The day of first oestrus was designated as day 0, and subsequent samples were collected relative to day 0. Blood samples were taken from a coccygeal vessel into vacutainer tubes (lithium heparin) at 07:30 h and completed in an hour. Each sample was centrifuged within 10 min (1000 × g for 15 min at 4 °C) and plasma stored at −20 °C until assayed for P4 by direct RIA using a commercial, solid phase, $^{125}$I kit (Spectriat® Kit, Orion Dianostica, Espoo, Finland). This assay has been validated for cow plasma (Rabiee et al., 1997b) by determining known amounts of P4 which have been added to charcoal-stripped cow plasma. The inter-assay CVs were 16, 5.6, and 5.4% for low, medium and high concentrations, respectively. The assay sensitivity was 0.03 ng/ml. Weekly blood samples were also taken into the tubes without anit-coagulant to measure blood glucose, non-esterified fatty acids (NEFAs), β-hydroxybutyrate (BHB) and urea. Blood glucose concentration was measured using the hexokinase enzymatic system (Trace Scientific, Australia); blood NEFAs using the acyl CoA synthetase coupled enzymatic system (Randox, Australia) with Randox reagents (Matsubara et al., 1983); blood BHB using 3HBDeOH enzymatic system (McMurray et al.,

1984); and blood urea using an enzymatic reaction (Trace Scientific, Australia; Talke and Schubert, 1965).

Weekly milk samples were collected with standard herd recording meters for six consecutive weeks during the sampling period. A sample of whole milk was preserved with 0.5% bronopol and refrigerated at 4 °C. Milk fat, protein and lactose concentrations were determined by Milkoscan (Foss Electric, Denmark) from aliquot samples of milk taken at each a.m. and p.m. milking. Whole milk samples were also taken daily into 10 ml vials coated with 0.5% bronopol and stored at −20 °C until assayed for P4 by direct RIA using a commercial, solid phase, $^{125}$I kit (Spectriat® Kit, Orion Dianostica, Espoo, Finland). The inter-assay CVs were 10.8, 6.6 and 6.1 for low, medium and high concentrations, respectively. The assay sensitivity was 0.47 ng/ml.

Faecal samples were collected directly from the rectum into 25 ml scintillation vials and immediately stored at −20 °C until assayed. A 0.5 g sample was extracted with methanol as described by Schwarzenberger et al. (1996). Faecal extracts were analysed by EIAs for immunoreactive progesterone metabolites. Briefly, the group-specific antibodies used in the EIAs were raised in rabbits. The assays included 20-oxo-pregnanes (antibody: 5α-pregnane-3β-ol-20-one 3HS:BSA; Schwarzenberger et al., 1996), 20α-OH-pregnanes (5β-pregnane-3α,20α-diol 3HS:BSA; trivial name pregnanediol; Schwarzenberger et al., 1993), and 20β-OH-pregnanes (antibody: 4-pregnene-20β-ol-3-one 3CMO:BSA; Schwarzenberger et al., 1993, 1996). Significant cross-reactivities in these assays were those with 5-reduced progesterone metabolites. Results were designated as measurements of pregnanes. Several previous publications have shown that these are the principal metabolites of progesterone excreted into the faeces of cattle (Palme et al., 1996, 1997; Schwarzenberger et al., 1996, 1997). Three assays were used in this study in order to measure the entire range of faecal pregnanes. The intra- and inter-assay coefficients of variation for these assays were similar to those described previously and ranged between 10 and 15%, respectively. The sensitivity of the assays was 7 ng/g.

Chromic oxide ($Cr_2O_3$) capsules (gelatine capsules containing 10.3 g $Cr_2O_3$) were administered at 08:00 and 16:00 h each day for 19 days to allow daily faecal output per cow to be estimated during the last 10 days of this period. A faecal sample was taken from each cow (blank) before routine chromic oxide administration. Gelatine capsules were administrated to each cow orally using an applicator. Faecal samples were taken in aluminium containers at the same time over the study period. Morning and afternoon samples were bulked and analysed for chromic oxide. Faecal samples were weighed and oven-dried at 100 °C for 3 days and then ground. Concentrations of chromium in the faeces were determined using a modification of the method of Williams et al. (1962). Estimated faecal output (Leaver, 1982) was used to measure the excretion rate of progesterone metabolites through the faeces.

### *2.3. Data analysis*

The results were analysed after excluding the first 10 days following CIDR removal for synchrony to minimise the daily variations. The data for the duration of luteal phase (days 7–16 of dioestrus) were included in the statistical analysis when plasma P4 concentrations were at the steady state and with a minimum fluctuation. The effect of time (day) and level of milk yield on plasma and milk P4 concentrations and interactions between level of milk

yield and time were analysed using GLM with repeated measures analysis included in the model in SPSS version 9.0 (SPSS, 1999). A two-sample *t*-test was used to compare the means of faecal output between HP and LP cows. Pearson correlation was also used to analyse the correlation between milk yield and P4 concentrations; and milk yield and faecal output. Four cows were excluded from the study (two from each group), because of their failure to respond to the synchrony program.

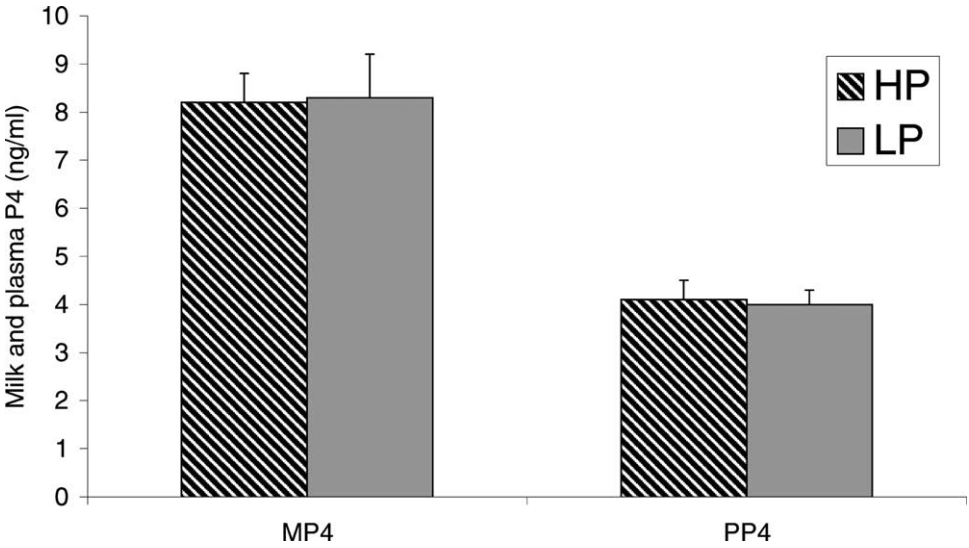


Fig. 1. Mean (±S.E.) plasma and milk progesterone concentrations in high producing (HP = 6) and low producing (LP = 6) lacatating dairy cows during dieoestrus.

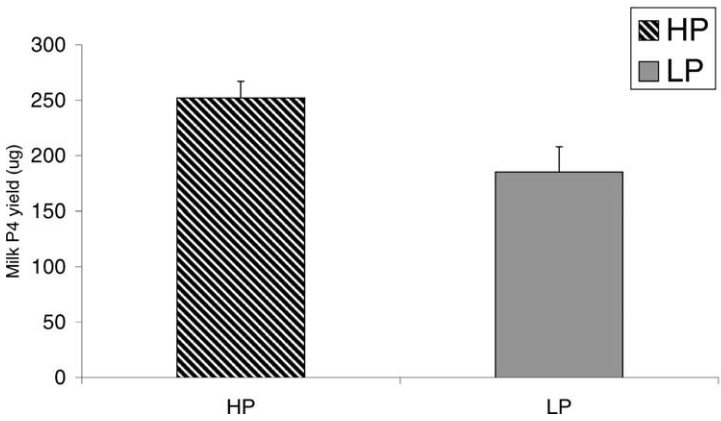


Fig. 2. Mean (±S.E.) of daily excretion of milk progesterone in high producing (HP = 6) and low producing (LP = 6) lacatating dairy cows during dieoestrus.

## 3. Results

The average and peak milk yields (maximum daily milk production) for a period of one cycle were higher in HP cows (average: 30.8 versus 21.9 l per day; peak: 33.5 versus 24.7 l per day; $P < 0.0001$). Average daily milk production for individual cows ranged from 28 to 34 l per day in HP cows and 18 to 25 l per day in LP cows. The average and peak milk

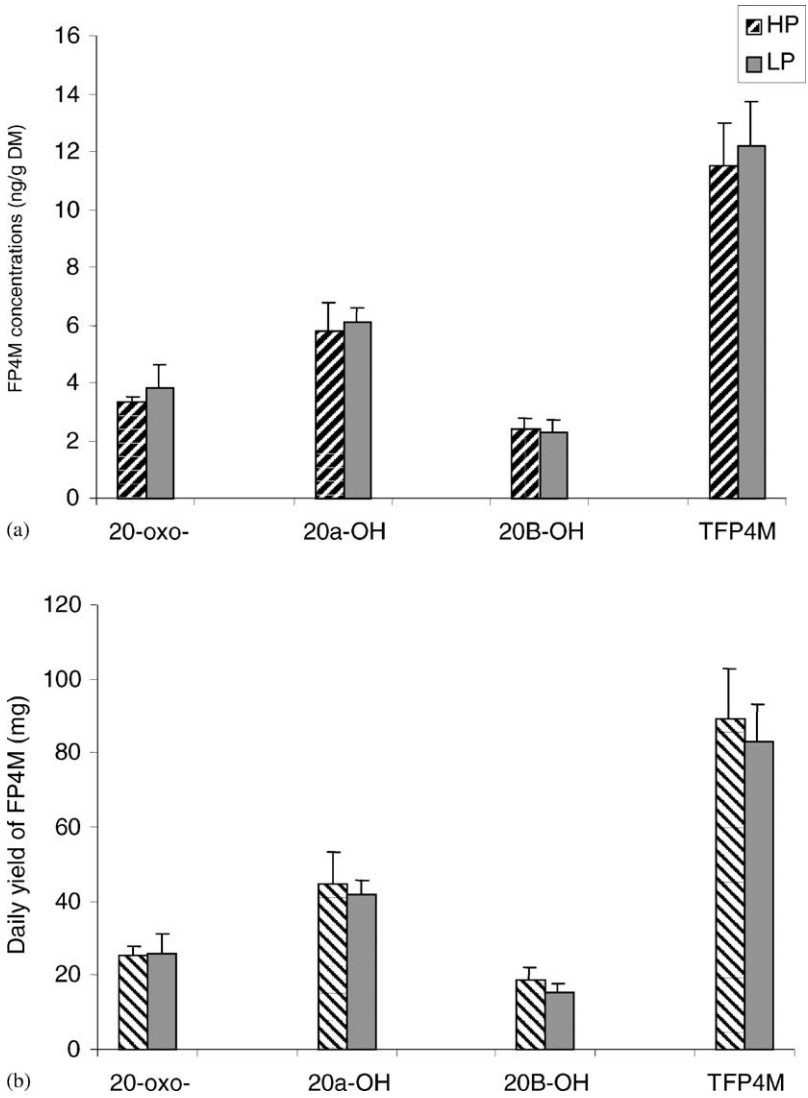


Fig. 3. (a) Mean (±S.E.) concentrations and (b) daily yields of faecal progesterone metabolites (20-oxo-, 20α-OH and 20β-OH-pregnanes and total P4 metabolites (PF4M)) in high producing (HP = 6) and low producing (LP) lactating dairy cows during dioestrus.

yields differed significantly among HP cows ($P = 0.03$), and also tended to be different among LP cows ($P = 0.06$).

The average daily faecal outputs (per cow) were similar in both the groups (7.7 versus 6.9 kg DM per cow; $P = 0.24$), and of similar dry matter content during the 8-day sampling period (0.7 versus 0.8% per cow, $P > 0.05$). The correlation between daily faecal output and milk yield in both the groups was not significant ($R^2 = 0.21$; $P = 0.14$). The pasture grazed by the cows averaged 15% DM, 22.5% crude protein and 11.8% metabolisable energy (ME) with a digestibility of 81%. The crushed barley contained 95% DM, 12.4% crude protein and 11.5% ME with a digestibility of 79.3%.

The plasma concentrations of P4 were similar in both HP and LP cows ($P = 0.3$; Fig. 1). The concentrations of milk P4 were also similar between the two groups ($P = 0.9$; Fig. 2) but both, plasma and milk P4, varied with day ($P = 0.001$), without creating significant interactions involving day and production group ($P = 0.4$). The average daily excretion rate of P4 in milk (average daily yield of milk P4 = average daily milk yield × average milk P4 concentration) in HP cows was 252 μg per day compared to 165 μg per day in LP cows ($P = 0.03$; Fig. 1). The correlation between milk P4 concentrations and daily milk yield was not significant ($R^2 = 0.008$, $P = 0.70$).

The concentrations of FP4M (20-oxo-, 20α-OH (20α-) and 20β-OH (20β-)-pregnanes) varied with day ($P = 0.001$), but not with milk production group. Interactions of day and milk production group were not significant ($P = 0.9$). There were no differences in the daily yield of faecal 20-oxo-, 20α- and 20β-pregnanes between HP and LP cows ($P > 0.05$; Fig. 3). Average daily yields of total FP4M among cows ranged from 63 to 154 mg in the HP group ($P < 0.01$) and 66 to 132 mg in the LP group ($P < 0.05$).

Average body weights were not different for the two groups (HP: 572 versus LP: 515 kg, $P = 0.2$). There were no differences in the concentrations of blood glucose (3.6 versus 3.6 mM, $P = 0.8$), BHB (0.45 versus 0.50 mM, $P = 0.6$), NEFAs (0.23 versus 0.20 mM, $P = 0.3$) or urea (6.2 versus 6.5 mM, $P = 0.5$) between the two groups.

The average milk fat (HP: 4.2% versus LP: 3.9%), protein (HP: 3.0% versus LP: 3.2%) and lactose (HP: 4.9% versus LP: 5.0%) contents were similar for both the groups ($P > 0.05$).

## 4. Discussion

Measuring P4 concentrations in plasma and milk and also the concentrations and daily yields of FP4M throughout an oestrous cycle in two groups of cows with differing daily milk yields, demonstrated that neither average P4 concentrations in plasma and milk nor the concentrations and daily yields of FP4M were affected by production group.

The difference in average daily milk production between the two groups was 9.00 l, 41% of the LP average of 21.9 l. However, daily faecal output was similar for the two groups, indicating the amount of daily feed intake did not differ greatly. Daily feed intake is also highly correlated with other factors that may influence portal or hepatic blood flow (Huntington and Reynolds, 1989; Parr, 1992), including liveweight and energy density of the diet. Blood metabolites concentrations also indicated that the cows were at similar metabolic status during the experimental period. Therefore, greater milk production in the HP cows in this study may have been due to differences in potential genetic capability for

milk production involving possibly differences in feed conversion efficiency. These factors and other possible elements that may have contributed to a greater milk production in HP cows need to be investigated further.

Similarities in plasma P4 concentrations and also in milk P4 concentrations between the two groups may have been due to the similarities in the daily faecal output and excretion rate of FP4M. There is generally a positive association between the level of feed intake and milk yield in dairy cows. The portal and hepatic blood flows have been higher in lactating dairy cows compared to those in beef heifer and beef steer (Huntington and Reynolds, 1989). Our previous observations in lactating dairy cows (Rabiee et al., 2001c) and non-lactating cows (Rabiee et al., 2001a,b) demonstrated that P4 clearance rate was similar among non-cycling high and low producing lactating cows, and higher in non-lactating cows which were placed on a restricted diet. This could be due to the amount of faecal output and daily excretion rate of FP4M which were similar for both HP and LP lactating cows but higher in restricted non-lactating cows (Rabiee et al., 2001a,b). These observations and others in cattle (Vasconcelos et al., 1998; Sangsritavong et al., 2000) and sheep (Parr et al., 1987, 1993) indicated that plasma P4 concentration may be influenced by the level of feed intake and blood flow to the liver and gut. Therefore, similarities in plasma and milk P4 concentrations between the two groups may have been due to the lack of differences in daily feed intake or faecal output and excretion rate of FP4M than differences in milk production.

Similarities in milk fat content and high correlation between milk P4 and fat content of milk (Darling et al., 1974) may partially explain the similarities in the concentrations of P4 in milk between the two groups. Similarities in the concentration of P4 in milk between the two groups also indicated that daily milk yield was not associated with milk P4 concentrations ($R^2 = 0.008$), and greater daily excretion rate of P4 to the milk in HP cows simply could be due to higher milk production ($P = 0.03$; Fig. 2). Despite the higher daily yield of milk P4 in HP cows, the amount of P4 excreted in milk had a limited role (<0.1%) in P4 clearance rate in lactating dairy cows (Heap et al., 1975a,b). In this study, the entry rate of P4 into the systemic circulation was not known. However, even variation in the entry rate of P4 into the circulation was not necessarily reflected at the peripheral level (Rabiee et al., 1997a,b). These data and other findings suggest that the mammary gland and level of milk yield did not have a major role in altering the plasma concentrations of P4.

The concentrations of individual P4 metabolites and total FP4Ms were similar for both production groups, indicating that FP4M concentrations were not affected by level of daily milk yields. Diet composition, digestibility and level of dry matter intake may influence the quantity of different P4 metabolites in the faeces, but the total concentrations of FP4M were similar (11.5 versus 12.2 μg/g) for both the groups.

The average daily yields of faecal 20-oxo-, 20α- and 20β-pregnanes were similar between HP and LP cows ($P > 0.05$), indicating that the daily yields of FP4M were not affected by daily milk yield. Other studies in rats, sheep (Arts et al., 1992; Adams et al., 1994) and cattle (Rabiee et al., 2001a,b) have shown that the excretion rates of steroid metabolites were positively associated with daily faecal output. Thus, similarities in the daily yield of FP4M in this study may have been due to similarities in the amount of daily faecal output. Similarities in the yield of FP4M may have also been reflected in the plasma and milk levels of P4 in both the groups of cows. Vasconcelos et al. (1998) and Sangsritavong et al. (2000) also showed that by feeding cows four times instead of two times per day, a

high level of plasma P4 could be maintained and that a reduction in reproductive efficiency in high producing dairy cows may be mediated by steroid metabolism due to high feed consumption. These findings and other studies in sheep (Parr, 1992; Parr et al., 1987, 1993; Abecia et al., 1995, 1997; Williams and Cumming, 1982) suggest that the peripheral P4 concentrations may have been associated with P4 clearance rate rather than the entry rate of this hormone.

The average body weight did not differ between the two groups ($P = 0.2$). The lack of a significant difference in blood concentrations of glucose, BHB, NEFAs and urea between the two groups also suggest that cows in both the groups had similar body reserve and also were at similar metabolic status. Progesterone has been identified as the major steroid in extracts of bovine muscle and fat tissues (McCracken, 1964) and partitioned readily between plasma and muscles (Lin et al., 1978). Fat tissues may have the potential to release stored P4 into the systemic circulation, however, its role in lactating dairy cows is not known.

## 5. Conclusion

Neither plasma and milk P4 concentrations nor the concentrations and daily yields of FP4M would differ during an oestous cycle in two groups of cows with differing daily milk yield. The greater excretion rate of P4 in the milk of HP cows did not lead to a reduction in the concentration of their peripheral P4. The excretion rate of FP4M to the faeces was related to the daily rate of faecal output. These data may indicate that the concentrations of plasma P4 and excretion rate of P4 to the faeces could be influenced by the level of feeding rather than with the level of milk production. Collectively, similarities in plasma P4 concentration among cows with two levels of milk yield may indicate that the peripheral P4 concentrations were not associated with the level of milk production in lactating dairy cows.

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