

Original article

Excretion rate of progesterone in milk and faeces in lactating dairy cows with two levels of milk yield

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Abstract — This study was conducted to measure the effect of the level of daily milk yield on the excretion rate of progesterone (P4) in milk and faeces in high-producing (HP) and low-producing (LP) lactating dairy cows. A GnRH-agonist was implanted to block endogenous production of P4. A CIDR device was inserted into the vagina and left in place for 11 days. The average and peak milk yields were greater in HP cows ($P < 0.0001$). Mean plasma concentrations of P4 were also similar in both groups ($P = 0.44$), even though the average mass of P4 delivered from a CIDR device was higher with HP cows ($P = 0.02$). Average milk P4 concentration was similar in both groups ($P = 0.81$), so that average daily excretion of P4 in the milk was greater with HP cows ($P = 0.05$). The concentrations ($P = 0.83$) and daily yields ($P = 0.4$) of total faecal progesterone metabolites were not affected by level of milk yield. These data show that the concentrations of plasma and milk P4, and the concentration and yield of P4 metabolites are not affected by the levels of daily milk yield.

progesterone / faecal progesterone metabolite / dairy cow

Résumé — Taux d'élimination de la progestérone dans le lait et les fèces des vaches laitières selon leur production de lait. Cette étude a pour but de mesurer le taux d'élimination de la progestérone (P4) dans le lait et les fèces chez des vaches à haute (HP) ou basse (LP) production laitière quotidienne. Un agoniste de GnRH a été implanté pour bloquer la production endogène de P4. Un dispositif CIDR a été inséré dans le vagin et laissé en place pendant 11 jours. Les productions laitières moyennes ou maximum ont été plus élevées ($P < 0,0001$) chez les vaches HP que chez les LP. Dans le plasma, les concentrations moyennes de P4 ont été similaires dans les deux lots ($P = 0,44$) bien que la quantité moyenne de P4 libérée par le CIDR ait été plus élevée chez les vaches HP ($P = 0,02$). Dans le lait, la concentration moyenne de P4 a été équivalente dans les deux lots ($P = 0,81$). Ceci signifie que l'excrétion moyenne de P4 dans le lait a été supérieure chez les vaches HP par comparaison aux LP ($P = 0,05$). Les concentrations ($P = 0,83$) et les excréctions journalières ($P = 0,40$) des métabolites fécaux

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totaux de P4 n'ont pas été affectées par le niveau de la production laitière. Ces résultats montrent que la concentration de P4 dans le plasma et dans le lait et que la concentration et l'excrétion des métabolites de P4 dans les fèces ne sont pas affectées par la production journalière de lait.

progesterone / métabolite fécal de progesterone / vache laitière

1. INTRODUCTION

The reproductive performance of cows influences production and also the profits of the entire dairy enterprise. High-producing (HP) dairy cows exhibit poorer conception rates than lower-yielding ones [11]. Many workers [10, 19, 39] implicated an antagonism between high production and fertility. The end result of lowered conception rates is increased days open and increased culling from failure to breed with reduced genetic gain through decreased selection pressure.

High-producing cows have a delicate balance between normality and some metabolic imbalances which could affect reproductive efficiency adversely. Progesterone (P4) has an important role in facilitating normal fertilization, embryo transport, embryo survival, follicular development, ovulation and incidence of oestrus [6, 18, 41]. Hepatic P4 clearance rate is perceived to have an influence on circulating plasma concentrations, which in turn may influence the P4 production rate [22]. Around 95% of all P4 produced is metabolised in the liver, with the breakdown products being excreted in the faeces [22]. Plasma P4 concentrations were related to the level of feed intake and rate of blood flow to the liver, rather than the entry rate of P4 to the system [23, 24, 42]. Our previous observations [28, 29] showed that P4 clearance rate in dairy cows can be estimated by measuring faecal P4 metabolites. The objective of the present study was to measure the concentration of plasma P4 and the concentrations and daily yield of faecal P4 metabolites (FP4M) in cows with different levels of milk production.

2. MATERIALS AND METHODS

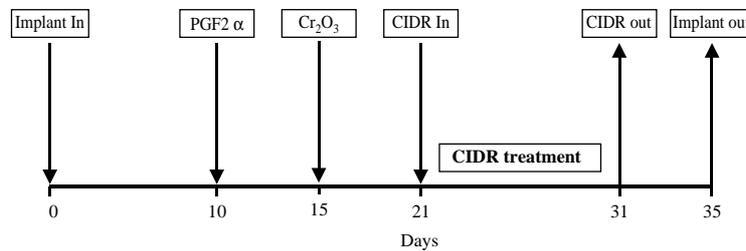
2.1. Animals and experimental protocol

Sixteen lactating Holstein-Friesian cows, 4–9 years old and 4 weeks post-partum were randomly selected, from a herd of 250 cows, and ranked according to their milk yields and allocated to two groups; (i) high (HP = 8) or (ii) low (LP = 8) producing groups. They were as one herd and had unrestricted access to improved pastures of rye-grass and white clover. Milking times were at 0615 and 1500 h and individual milk yields were recorded routinely at each milking (ALPRO TM System, Alfa Laval Agri, Sweden). Body weight and body condition score (1–5 scale) were recorded weekly.

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).

Our previous observations and other studies [7, 30] showed that ovarian P4 production could be prevented by strategic use of a GnRH-agonist to create progesterone-free animals similar to ovariectomised cows. In order to block endogenous production of P4, each cow initially received a 6 mg Deslorelin (GnRH-agonist; D-Trp⁶-D-Trp⁶-Pro⁹-des-Gly¹⁰-GnRH ethylamide) ear implant (Peptech Animal Health, Sydney, Australia) initially, followed by two injections of prostaglandin F_{2α} (2 mL Prosolvin, Intervet, Melbourne) at 0800 and 1600 h, 10 days later (Fig. 1). The implants were left in place for 5 weeks. Weekly and daily

Figure 1. Description of the timing of deslorelin implantation, PGF2 α injection, chromic oxide administration and the insertion of CIDR device in this experiment.



blood samplings were made before and after prostaglandin F2 α injection.

Chromic oxide (Cr₂O₃) capsules (gelatine capsules containing 10.3 g Cr₂O₃) were administered at 0800 and 1600 h each day for 19 days to allow daily faecal output to be estimated over the last 12 days of this period (Fig. 1).

Each cow had an intra-vaginal device (CIDR, InterAg, NZ) containing 1.87 \pm 0.04 g P4 inserted into the vagina 3 weeks after deslorelin implantation and left in place for 11 days (Fig. 1). Blood and faecal samples were taken to monitor P4 profile during the CIDR treatment period.

2.2. Blood, milk and faecal sampling procedures and assays

Blood samples were taken daily from a coccygeal vessel into Vacutainer tubes (lithium heparin). Each sample was centrifuged within 10 min (3000 rpm for 15 min at 4 °C) and plasma stored at -20 °C until assayed for P4 by direct RIA using a commercial, solid phase, ¹²⁵I kit (Coat-A-Count[®], Kit, Los Angeles, California, USA). 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⁻¹. Weekly blood samples were also taken into the tubes without anti-coagulant to measure blood glucose, non-esterified fatty acids (NEFAs), beta-hydroxybutyrate (BHB) and urea. Blood glucose concentrations were measured using the hexokinase enzymatic system (Trace Scientific,

Australia); blood NEFAs using the Acyl CoA synthetase coupled enzymatic system (Randox, Australia) with Randox reagents [15]; blood BHB using 3HBDeOH enzymatic system [17]; and blood urea was measured using an enzymatic reaction (Trace Scientific, Australia) [40].

Weekly milk samples were collected using standard herd recording meters during a period of 6 weeks. A sample of whole milk was preserved with 0.5% bronopol and refrigerated at 4 °C. Milk fat, protein and lactose were determined by Milkoscan (Foss Electric, Denmark) from aliquot samples of milk taken at each pm and am milking.

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, ¹²⁵I (Spectriat[®], Kit, Orion Diagnostica, Espoo, Finland) kit. There is little information regarding P4 metabolites in the milk, however, the antibodies used in this assay mainly cross-reacted with pregnenolone (3.9%, Orion Diagnostica, Espoo, Finland). The inter-assay CVs were 10.8, 6.6 and 6.1% for low, medium and high concentrations, respectively. The sensitivity of assay was 0.47 ng·mL⁻¹. Metabolic clearance rate (MCR) of P4 by the mammary gland was calculated for the whole period of CIDR treatment using the following formula:

$$\text{MCR} = \frac{\text{Average daily P4 release} / \text{Average daily milk P4 concentration}}{\text{Average body weight}} .$$

Daily 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. [35]. Faecal extracts were analysed by EIAs for immunoreactive P4 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; [35]), 20α -OH-pregnanes (5β -pregnane- $3\alpha,20\alpha$ -diol 3HS:BSA; trivial name pregnanediol; [34]), and 20β -OH-pregnanes (antibody: 4-pregnene- 20β -ol-3-one 3CMO:BSA; [33]). Significant cross-reactivities in these assays were those with 5-reduced P4 metabolites. Results were designated as measurements of pregnanes. Several previous publications have shown that these are the principal metabolites of P4 excreted into the faeces of cattle [20, 21, 35, 37]. 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 assay sensitivity was $7\text{ ng}\cdot\text{g}^{-1}$. The cross-reactivity of these antibodies has been reported by Schwarzenberger et al. [36].

A faecal sample was taken from each cow (blank) before routine chromic oxide administration. Gelatine capsules were administered to each cow by means of 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. [43]. Estimated faecal output [12] was used to measure the excretion rate of P4 metabolites through the faeces.

A Soxhlet extraction technique was used to determine the residual content of P4 in used CIDR devices [31].

2.3. Data analysis

The results were analysed after excluding the first 3 days of observations after CIDR insertion. Three cows were excluded from the statistical analyses (one from HP and two from LP group) because the endogenous production of P4 was not blocked completely after deslorelin implantation. The effect of time (day) and level of milk production on plasma P4, FP4M, blood metabolites and milk composition and interactions between diet and time were analysed using GLM with repeated measures analysis included in the model in SPSS v. 9.0 [38]. The body weight of cows was used as a covariant in the model. A non-parametric test was used to analyse the MCR between the two groups.

3. RESULTS

3.1. Pasture analysis and faecal output

The pasture grazed by the cows averaged 17% DM, 23.2% crude protein and 12.2% ME with a digestibility of 84%. The crushed barley contained 89% DM, 12.3% crude protein and 11.5% ME with a 79.3% digestibility. The average daily faecal outputs (FO) were similar for the two groups (6.4 vs. 5.04 kg DM, $P = 0.34$) with average dry matter content of faeces being 9.0% in HP cows compared to 10% in LP cows ($P = 0.5$).

3.2. Body weight, blood metabolites

The average body weight was greater in HP cows compared to those in the LP group (508 vs. 458 kg, $P = 0.01$) and HP cows did significantly lose body weight through time ($P = 0.01$). The average blood glucose concentration was higher for cows in the LP group (3.5 vs. 3.3 mM, $P = 0.02$), but blood NEFAs concentration was higher in HP cows (0.64 vs. 0.52 mM, $P = 0.1$). The average concentration of blood BHB

tended to be higher in HP cows (1.04 vs. 0.8 mM, $P = 0.09$). There were no significant differences in the concentrations of blood urea (7.8 vs. 6.9 mM, $P = 0.3$) between two groups.

3.3. Milk production and composition

Daily milk yield was recorded for a period of 100 days. The average and peak milk yields were higher in HP cows ($P < 0.0001$, Tab. I). Average daily milk production ranged from 27 to 29 L·day⁻¹ in HP and from 17 to 22 L·day⁻¹ in LP cows. There was no interaction between the level of milk yield and body weight of the cows. The average milk fat contents (HP: 4.1% vs. LP: 4.6%) and protein contents (HP: 3.0% vs. LP: 3.2%) were higher in LP cows ($P = 0.01$), but milk lactose content were similar in both groups (4.9% vs. 5.0%, $P = 0.1$).

3.4. Plasma and milk progesterone

The daily plasma concentrations of P4 were consistently higher in LP cows (Fig. 2), but the average plasma P4 concentrations were not statistically different ($P = 0.44$, Tab. I). The concentrations of milk P4 were similar between the two groups ($P = 0.81$, Tab. I) and plasma and milk P4 concentrations did not vary with day ($P = 0.2$). The daily excretion rate of P4 into the milk (average [daily yield of milk P4 = daily milk yield × daily milk P4 concentration]) was higher in HP cows (65.3 vs. 57.5 µg·day⁻¹, $P = 0.05$), possibly because the mass of P4 lost from the CIDR devices used with the HP cows also was higher (0.73 vs. 0.68 g, $P = 0.02$). Recovery rates of P4 in the milk were similar in both groups (0.1%), and ranged from 0.08 to 0.14% in HP, and from 0.07 to 0.10% in LP cows ($P = 0.5$). The MCR of P4 by the mammary gland tended to be higher in HP cows (58.9 vs. 48.9 L·day⁻¹·kg⁻¹, $P = 0.086$), and ranged from 42 to 75 L·day⁻¹·kg⁻¹ in HP cows, and from 41 to 65 L·day⁻¹·kg⁻¹ in LP cows.

3.5. Concentrations and daily yield of FP4M

The average concentrations of faecal 20-oxo-pregnanes (20-oxo-), 20 α -OH (20 α -) and 20 β -OH (20 β -) were similar in both groups (Tab. II) and daily concentrations of FP4M did not vary (Fig. 3, $P = 0.83$). Neither were they affected by the level of milk yield ($P = 0.83$). Interactions of day and milk production group also were not significant ($P = 0.5$, Tab. II). Daily yield of faecal 20 α -, 20-oxo-, 20 β - and total FP4M were not different significantly between the HP and LP cows (Tab. II). The average daily yield of total FP4M among cows ranged from 20 to 56 mg in the HP group ($P = 0.01$) and from 18 to 32 mg in the LP group ($P = 0.05$). Recovery rates of P4 metabolites in the faeces were 51% (36 to 87%) and 37% (30 to 53%) in HP and LP cows, respectively ($P = 0.6$). There was more variation in the recovery rate of total FP4M among cows in the HP group compared to those in the LP group.

4. DISCUSSION

Concentrations of plasma and milk P4 and FP4M in lactating dairy cows with two different levels of milk production were monitored to explore the relationship between milk yield and excretion rate of P4 in the milk and faeces. This study showed that plasma P4 concentrations did not differ among cows with two levels of milk yield. Whereas the entry rate of P4 into the systemic circulation and excretion rate of P4 to the milk were greater in HP cows, the excretion rate of P4 in faeces was similar for the two groups.

Both HP and LP cows had unrestricted access to pasture (ad libitum). Average daily milk production for the HP cows was about 9 L (40%) higher (Tab. I) and their average body weight was also greater ($P = 0.01$). There is generally a positive association between the level of feed intake and milk

Table I. Concentrations, yield and significance of plasma and milk P4, milk yield and P4 release from a CIDR device in lactating dairy cows implanted with a subcutaneous GnRH-agonist and treated with an intra-vaginal progesterone device.

	PP4 (ng·mL ⁻¹)		Milk P4 (ng·mL ⁻¹)		Daily milk yield (L·day ⁻¹)		Daily yield of milk P4 (µg·day ⁻¹)		P4 release from CIDR (g)	
	HP	LP	HP	LP	HP	LP	HP	LP	HP	LP
Mean (M)	1.2 ± 0.1	1.3 ± 0.2	2.3 ± 0.1	2.9 ± 0.3	28.5 ± 0.2	20.1 ± 0.9	65.3 ± 3.5	57.5 ± 5.0	0.73 ± 0.02	0.68 ± 0.01
<i>P</i> value for M (mean difference ± SE)	0.34 (-0.16 ± 0.17)		0.07 (-0.60 ± 0.30)		< 0.0001 (8.0 ± 0.93)		0.13 (9.0 ± 5.6)		0.07 (0.06 ± 0.03)	
<i>P</i> value for M & mean difference ± E (adjusted for BW*)	0.44 (0.13 ± 0.16)		0.81 (-0.08 ± 0.30)		< 0.0001 (7.0 ± 1.1)		0.05 (15.3 ± 6.8)		0.02 (0.09 ± 0.03)	
<i>P</i> value for Covariant	0.015		0.02		0.08		0.17		0.12	

* Body weight (Covariant).

Table II. Concentration, daily yield and significance of FP4M in lactating dairy cows implanted with a subcutaneous GnRH-agonist and treated with an intra-vaginal progesterone device.

	Faecal 20-oxo-pregnanes		Faecal 20α-OH		Faecal 20β-OH		Total FP4M	
	HP	LP	HP	LP	HP	LP	HP	LP
Concentration (µg·g ⁻¹ DM)	1.3 ± 0.2	1.3 ± 0.1	3.7 ± 0.6	2.8 ± 0.3	0.3 ± 0.04	0.1 ± 0.1	5.3 ± 0.6	4.6 ± 0.2
<i>P</i> value for Con. & mean difference ± SE (adjusted for BW*)	0.75 (10.0 ± 30.1)		0.94 (7.2 ± 86.0)		0.80 (4.0 ± 15.5)		0.83 (21.2 ± 95.1)	
Daily yield of FP4M (mg)	8.2 ± 1.2	6.6 ± 0.6	24 ± 4.8	14.2 ± 2.0	2.1 ± 0.2	2.2 ± 0.6	34.3 ± 5.0	23.0 ± 2.0
<i>P</i> value for yield & mean difference ± SE (adjusted for BW*)	0.30 (2.1 ± 1.9)		0.53 (4.4 ± 6.8)		0.51 (0.5 ± 0.7)		0.36 (7.0 ± 7.3)	

* Body weight (Covariant).

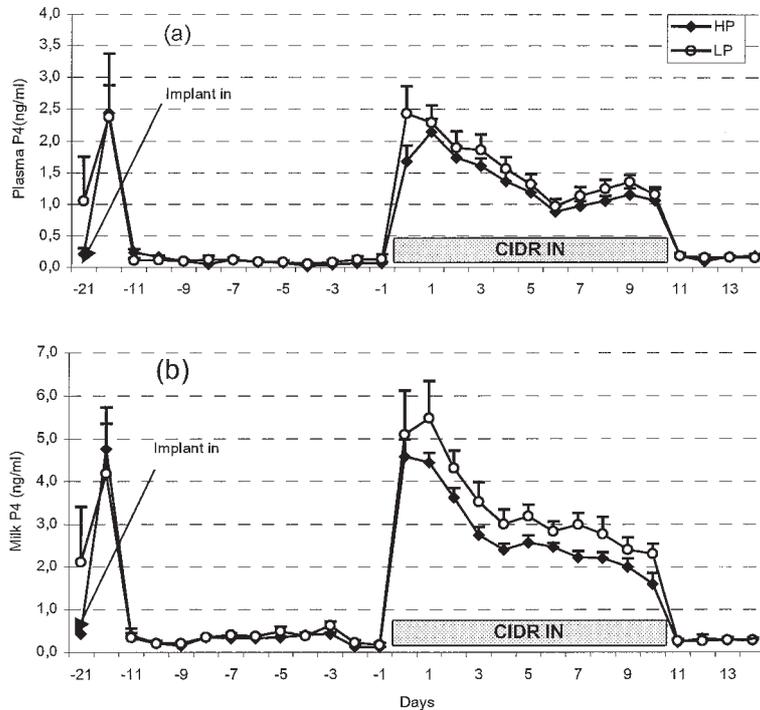


Figure 2. Daily concentrations of plasma (a) and milk (b) P4 in lactating dairy cows before and during treatment with a CIDR device.

yield in dairy cows. While feed intake was not measured in this study, daily faecal output data did not differ between HP and LP cows ($P = 0.34$), suggesting that differences in milk production were due to differences in efficiency of digestion associated with greater mobilisation of body mass.

Cows in the HP group had greater weight losses compared to those in LP group (18.0 vs. 4.0 kg). Progesterone has been identified as the major steroid in extracts of bovine muscle and fat tissues [13]. It has been suggested that cows may be able to release P4 into the systemic circulation by mobilising body fat or possibly muscle tissue [16]. Fat may act as a depot in dairy cattle that can accumulate a store of P4 to levels 5 to 10 times that of the concentration in blood. The role of P4 content of body fat on plasma P4 levels during early lactation is not fully understood but deserves further study.

The average mass of P4 delivered from a CIDR device was greater in HP cows, but mean plasma P4 concentrations were similar between the two groups (Tab. I). Although liver metabolism was likely greater in HP cows, higher P4 delivery from the CIDR device may have been able to maintain the plasma P4 level in these cows. Splanchnic tissues play a major role in supplying precursors for milk synthesis. Therefore, changes in metabolic flux across the splanchnic tissues will be expected to have significant effects on milk synthesis and on the metabolic clearance of P4. Other studies [29] reported that plasma P4 was higher in cows on a restricted diet compared to cows fed ad libitum, while loss of P4 from a CIDR device was similar for both groups of cows. Studies by Rabiee et al. [26] showed that the rate of P4 release from a CIDR device may have been affected by the housing and

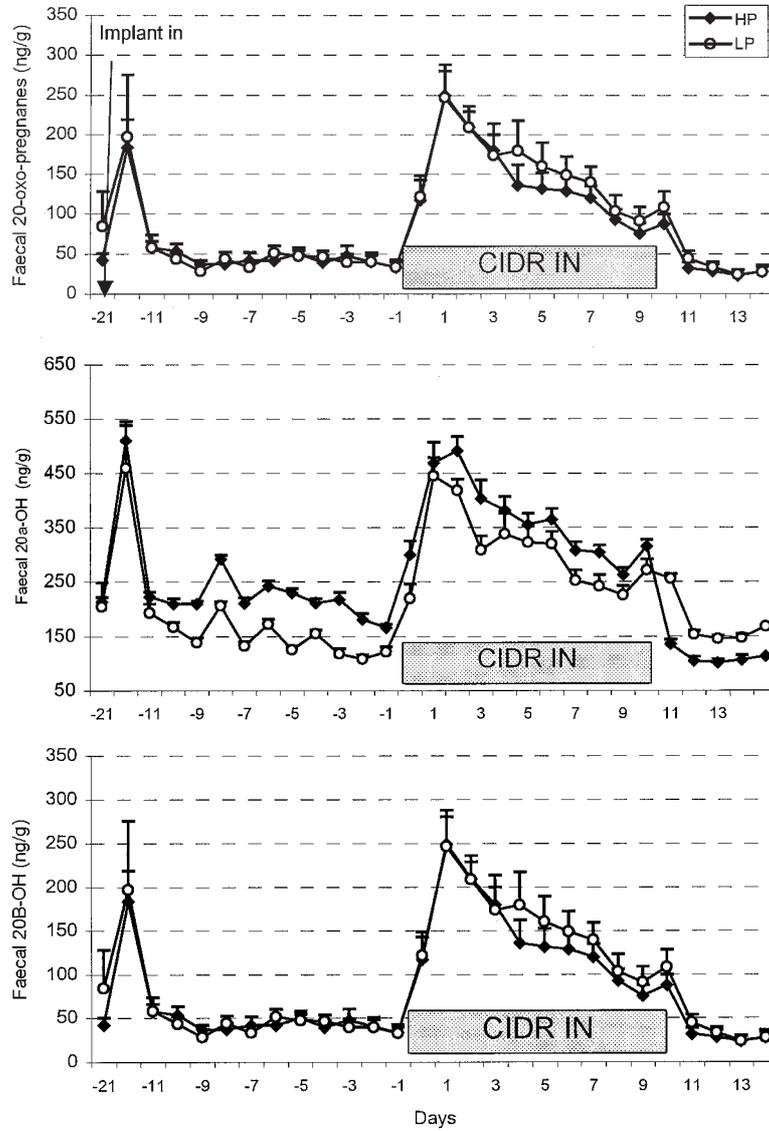


Figure 3. Daily concentrations of faecal 20-oxo-, 20 α - and 20 β -pregnanes in lactating dairy cows before and during treatment with a CIDR device.

physical activity. In the present study differences in the faecal output between HP and LP cows were not as great as that found in a previous study [25, 27, 28]. Studies in cattle [28, 29, 32] and sheep [23, 24] showed that plasma P4 concentration is influenced

by the level of feed intake and blood flow to the liver and gut. Therefore, similarities in plasma P4 concentrations between the HP and LP groups may have been due to the lack of difference in faecal output between the two groups, with greater body size

resulting in greater dilution of circulating P4, higher P4 delivery and also higher excretion rate of P4 in the milk in HP cows (Tab. I).

The concentrations of milk P4 were similar in both groups of cows even though the daily milk fat yield was greater in LP cows compared to LP cows (Tab. I), and the average mass of P4 delivered from a CIDR device was higher in HP cows ($P = 0.02$). Previous studies suggest that the concentration of P4 is closely correlated with fat content of milk, and that the mammary metabolism of P4 in dairy cows is low [5]. Given this fact, greater concentration of P4 in the milk of LP cows would be expected. When the concentration of P4 in milk was adjusted for body weight, there was no difference in the concentration of milk P4 between two groups (mean difference = -0.08 , Tab. I). These data and other findings [5, 8, 9] suggest that the metabolic role of the mammary gland is not great and that milk P4 concentration may have been influenced by milk fat content and also the body mass.

Despite the greater excretion rate of P4 to the milk in HP cows, the mammary gland and level of milk yield did not appear to have a significant role in altering the concentration of plasma P4. Greater MCR of P4 by the mammary gland in HP cows (20%) may indicate that they were able to excrete more P4 in the milk. However, the recovery rates of P4 appeared to be similar between the two groups (0.1%) despite the greater excretion of P4 to the milk in HP cows. Other studies [9] also reported that the total amount of the labeled steroid secreted in milk is less than 0.1% of the production rate.

The concentrations of FP4M were not affected by the level of milk production (Tab. II). The concentrations of FP4M also were not influenced by the volume of faeces which is supported by other studies [27–29]. However, these observations contradict other studies in rats and sheep [3, 4] which reported that the level of feed intake and passage rate of faeces could affect the

re-absorption rate of oestrogen from the gut. Small variations in the concentrations of FP4M in both groups of cows compared to other studies [28, 29] suggest that pasture digestibility may influence the quantity and quality of individual metabolites, but not the total concentrations of FP4M.

Average daily yields of total FP4M were similar in both groups (Tab. II, $P = 0.36$). In this study the level of faecal output and concentrations of FP4M were similar in both groups, consequently the daily yield of FP4M did not differ among cows. Other studies with ovariectomised and non-cycling cow [28, 29] and studies in rats [4] also suggested that excretion rates of P4 metabolites were related to the level of faecal output. Similarities in the concentrations of plasma and milk P4 between two groups in this study were more likely to be associated with the level of faecal output rather than level of milk yield. It has been shown [1, 2] that neither luteal function nor ovarian venous P4 levels are modified by level of nutrition in sheep, indicating that P4 production is not affected by the level of feed intake. Blood flow rate through the liver is around 50% higher in normal fed, lactating cows as compared with non-lactating cows, and is decreased by fasting [14]. Accordingly, it is suggested that similarities in the recovery rate of FP4M between the two groups was related to the amount of P4 metabolites excreted to the faeces and daily faecal output rather than delivery rate of P4. This may indicate that the excretion rate of P4 rather than the entry rate of this hormone may influence the peripheral P4 concentrations. Thus, when daily excretion rate of P4 to the faeces did not differ between the two groups, consequently similar peripheral P4 concentrations would be expected.

5. CONCLUSION

These results showed that the level of milk yield did not influence the mean concentration of plasma P4. Similarities in

plasma P4 concentrations between HP and LP cows may have been associated with the similarities in the level of faecal output between the two groups. The greater excretion rate of P4 to the milk in HP cows did not lead to a reduction in the mean of peripheral P4 concentrations among HP cows. The excretion rate of P4 to the milk, MCR and recovery rate of P4 in milk (< 0.1%) did not appear to have a major role in P4 excretion in lactating cows. Collectively, the concentrations of plasma P4 in lactating dairy cows may be influenced by several factors such as the amount feed intake (or volume of faeces) and the excretion rate of P4 metabolites to the faeces.

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