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The effect of level of feed intake on progesterone clearance rate by measuring faecal progesterone metabolites in grazing dairy cows

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Abstract

The objective of the present study was to determine the effect of level of feed intake of pasture on P4 clearance rates in dairy cows. Twelve non-lactating Holstein–Friesian cows aged 4–9 years were randomly allocated to a restricted or ad libitum group. The ad libitum group had unrestricted access to irrigated pasture, whereas the restricted group had access for only 2 h per day. Each animal was drenched orally twice daily with a chromic oxide capsule to allow daily feed intake to be estimated from faecal output (FO). Endogenous progesterone (P4) production was eliminated by subcutanously implanting a capsule containing 6 mg of a potent GnRH-agonist (deslorelin) into the ear of each animal 3 weeks before inserting a CIDR device containing 1.9 g P4 into the vagina. Two luteolytic PGF2α were given 10 days later. Each device was removed after 11 days and residual P4 measured. Daily plasma samples were assayed for P4. Faecal samples were also taken daily and assayed for pregnanes (FP4M) containing a 20-oxo-, a 20α- or a 20β-OH group with EIAs.

The average daily dry matter (DM) intake of pasture was higher for cows in the ad libitum group (15.9 versus 6.3 kg DM, $P = 0.001$). Their plasma P4 concentrations were lower (1.08 versus 1.71 ng/ml, $P = 0.05$), even though the average residual P4 content of the used CIDR devices was not affected by feed intake (1.20 versus 1.25 g, $P > 0.05$). The concentrations of FP4M were not affected by level of feed intake (20-oxo-: 3.3 versus 1.7, 20α-: 3.5 versus 3.7, 20β-: 2.1 versus 3.2 μg/g DM). Daily excretion rates of 20-oxo- and 20α- were higher in ad libitum cows (20-oxo-: 17.8 versus 4.3 mg per day, $P = 0.05$; 20α-: 18.2 versus 8.9 mg per day, $P = 0.001$), but daily yield of faecal 20β- was not affected by feed intake (11.9 versus 8.6 mg per day, $P = 0.5$). These results show that there was a negative relationship between feed intake and plasma P4 concentrations

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in these CIDR-treated GnRH-downregulated Holstein cows. Concentrations of FP4M were not affected by level of feed intake or FO, but daily excretion rate of FP4M was associated with the volume of faeces.

Keywords: Faecal progesterone metabolites; Feed intake; Progesterone; Faecal output

1. Introduction

Nutritional management plays an important role in the postpartum reproductive performance of dairy cattle, especially the overall energy balance of the animal. Both feed intake (FI) and body tissue reserves are used as energy source for body function and milk production. Progesterone (P4) has been recognised as the hormone that maintains pregnancy (Mee et al., 1991; Taylor and Rajamahendran, 1994). Attention has been focused on the dynamics of P4 production and metabolism, and how nutrition influences these parameters (Butler, 1998). Most of the research that has addressed the influence of nutrition on the concentrations of P4 and oestradiol has focused on production. However, little is known about the production rate of P4; or whether the changes in plasma concentration are related either to differences in production rate, or to alternations in the rate of its catabolism.

The concentration of P4 in blood is related to production and clearance rate. Aspects of nutrition have been shown to affect both rates. Most of the research that has focused on P4 production has suggested that subnormal P4 levels in energy-deficient cows are sometimes a result of decreased CL function (Carstairs et al., 1980; Ducker et al., 1985; Villa-Godoy et al., 1988). Cows in negative energy balance and low levels of P4 may be at a greater risk for premature luteolysis than cows with normal P4 levels and a positive energy balance. Low level of energy in the diet also can affect P4 concentrations as shown by Smith (1988) that a low serum glucose concentration was found to decrease LH pulse frequency and amplitude and result in diminished P4 production by the CL.

The P4 clearance rate from the body is considered to have an influence on the circulating concentration and may in turn influence the conception rate of cows. The splanchnic extraction rate of P4 was found to be about 90% in sheep to account for approximately 27% of whole body metabolic clearance (Bedford et al., 1974). The blood flow rate to the liver may be modified by FI (Symonds and Prime, 1989), and thus be expected to substantially alter splanchnic clearance rates. Cumming et al. (1971) were the first to recognise that modifying the level of FI also altered the plasma concentration of P4. The cause of this response was thought to be an increase in hepatic blood flow with increased FI and consequently an increase in the metabolism of P4 by the liver. An inverse relationship between feed level and concentrations of peripheral plasma P4 was attributed to differences in the clearance rate of P4 rather than to changes in its entry rate into systemic blood circulation (Williams and Cumming, 1982; Parr et al., 1993). There is limited information regarding the effect of level of feeding on P4 metabolism in dairy cattle. It has been suggested (Vasconcelos et al., 1998; Sangsritavong et al., 2000) that high feed consumption could alter the clearance rate of P4 in the dairy cows.

The main route of excretion of P4 is through the liver and then as bile to the faeces. Therefore, the analysis of faecal P4 metabolites (FP4M) has emerged as an appropriate

method for monitoring ovarian function, as already shown in cattle (Desaulniers et al., 1989; Larter et al., 1994; Schwarzenberger et al., 1996). Our previous study (Rabiee et al., 2001a) showed that by measuring faecal P4 metabolites, it was possible to investigate P4 clearance rate in dairy cows. The objective of the present study was to determine the effect of level of FI of pasture on P4 clearance rates in dairy cows. In this study, an external source of P4 was administered to cows implanted with a potent GnRH-agonist, deslorelin, to investigate the effect of level of feeding on plasma P4 and the concentrations and daily yield of FP4M.

2. Materials and methods

2.1. Animals and experimental protocol

Twelve non-lactating multiparous Holstein–Friesian cows aged 4–9 years were randomly allocated to similar 1ive-weight groupings: (I) restricted; and (II) ad libitum. They were located in two separate paddocks for a period of 5 weeks. Cows were adjusted to their pasture for 3 weeks before dosing with chromic oxide (Cr_2O_3) capsules and treatment with P4. Body weight and body condition score (one to five scale) were recorded weekly during the trial. Cows in the ad libitum group had unrestricted access to graze pasture during the day, whereas the restricted group had access for only 2 h per day in two periods, first between 08.00 and 09.00 h and then from 15.00 to 16.00 h. Both groups were handled twice daily for blood and faecal sampling and chromic oxide administration. The pasture grazed by both groups was mainly improved ryegrass and white clover. Pasture samples taken during the trial were analysed for dry matter (DM), crude protein and digestibility.

Our previous observations (Rabiee et al., 2001b) and other studies (D'Occhio et al., 1996) have shown that ovarian P4 production could be prevented by strategic use of a deslorelin implant to create progesterone-free animals similar to ovariectomised cows. Briefly, the GnRH-agonist, deslorelin, suppresses pulsatile secretion of LH (Gong et al., 1996) and blocks corpus luteum function and P4 production (D'Occhio et al., 1996). In this study each cow received a 6 mg deslorelin ear implant (Peptech® Animal Health, Sydney, Australia) initially, followed by two injections of prostaglandin F2α (2 ml Prosolvin, Intervet, Melbourne) at 08.00 and 16.00 h for 10 days later. Weekly and daily blood samplings were made before and after prostaglandin F2α injection.

Chromic oxide (Cr_2O_3) capsules (gelatine capsules containing 10.3 g Cr_2O_3) were administrated at 08.00 and 16.00 h each day for 19 days to facilitate estimating daily FO over the last 12 days.

A CIDR device (InterAg, NZ) containing 1.9 g P4 was inserted into the vagina 3 weeks after deslorelin implantation and left in place for 11 days. Blood and faecal samples were taken to monitor P4 profile during the CIDR treatment period.

2.2. Blood and faecal sampling procedures and assays

Blood samples were taken daily from a coccygeal blood 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 commer cial, solid phase, ^{125}I kit (Coat-A-Count®, Kit, Los Angeles, CA, USA). The inter-assay CVs were 7.5, 8.0 and 9.0% for low, medium and high concentrations, respectively. Weekly blood samples were also taken into tubes without anti-coagulant to measure blood metabolites including glucose, non-esterified fatty acids (NEFAs), beta-hydroxybutyrate (BHB) and urea. Blood glucose concentration was determined using the hexokinase enzymatic system (Trace Scientific, Australia); blood NEFAs using the Acyl CoA synthetase coupled enzymatic system (Randox, Australia) using 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).

Faecal samples were collected directly from the rectum into 25 ml polycarbonate containers and immediately stored at −20°C until assayed. A 0.5 g sub-sample was extracted with methanol (Schwarzenberger et al., 1996) and the extracts 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; 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., 1991). Significant cross-reactivities in these assays were those with five-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 (Palme et al., 1996, 1997; Schwarzenberger et al., 1996, 1997). Three assays were used in this study in order to measure the 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 assay was 7 ng/g.

A faecal sample was taken from each cow (blank) before the routine chromic oxide twice for daily administration. Morning and afternoon samples were weighed, bulked and oven-dried at 100°C for 3 days, and then ground. Chromic oxide concentrations were measured using an X-ray fluorescence spectrometry method (Norrish and Hutton, 1977). Estimated faecal output (FO) (Leaver, 1982) was used to measure the excretion rate of P4 metabolites through the faeces.

A Soxhlet extraction technique was used to determine the residual amount of P4 in used CIDR devices (Rathbone et al., 1998).

2.3. Statistical analysis

The data derived from the last 8 consecutive days of the 11-day treatment period and were analysed. Values over the last 8 days of CIDR exposure were pooled and mean ± S.D. are presented. The effects of time (day) and diet on plasma P4 were analysed using GLM with repeated measures analysis included in the model in SPSS version 9.0 (SPSS, 1988). Two cows were excluded from the study (one from each group), because of their failure to respond completely to deslorelin implantation.

3. Results

3.1. Faecal output and feed analysis

The amount of daily FI and FO were higher in ad libitum cows (FI: 15.9 versus 6.3 kg DM; FO: 5.4 versus 2.5 kg DM; for ad libitum and restricted cows, respectively, $P = 0.001$). The DM content of faeces from restricted cows was higher than ad libitum cows (15 versus 11%, $P = 0.01$). The pasture grazed by cows contained 22.2 versus 17.5% DM, 19.3 versus 26.7% crude protein, and 60 versus 65% digestibility for restricted and ad libitum cows, respectively.

3.2. Plasma progesterone

The plasma concentrations of P4 were higher in restricted than ad libitum cows (1.71 ± 0.04 versus 1.08 ± 0.03; $P = 0.05$), even though the average mass of P4 delivered from a CIDR device during the 11-day period insertion was similar for both groups (0.65 versus 0.70 g, $P = 0.3$). Plasma P4 was not affected by day or interactions of day and the level of FI ($P > 0.05$).

3.3. Faecal progesterone metabolites

The concentrations of FP4M were not affected by day, level of feeding or interactions of day and the level of FI (Fig. 1, $P > 0.05$). The 20-oxo-pregnanes (20-oxo-) concentrations

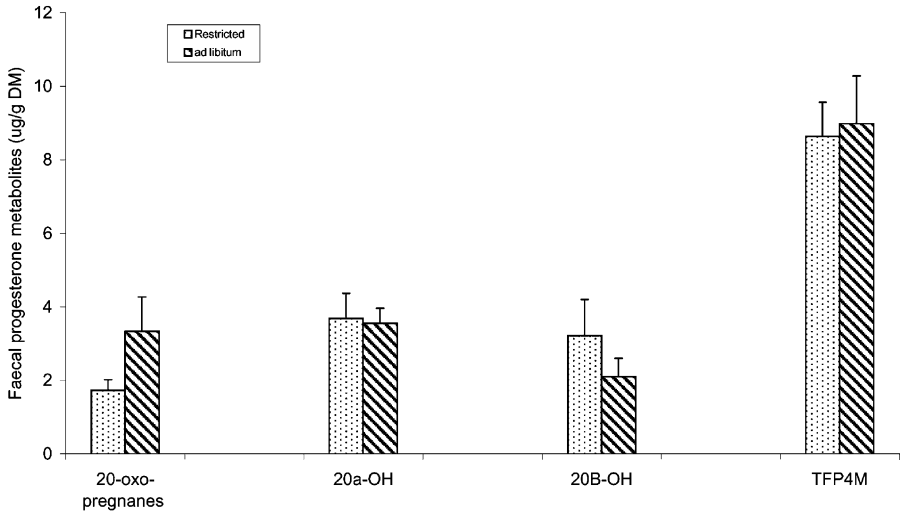

Fig. 1. Concentrations (mean ± S.E.) of fecal 20-oxo-, 20α-, 20β-OH pregnanes and total FP4M in restricted and ad libitum non-lactating cows.

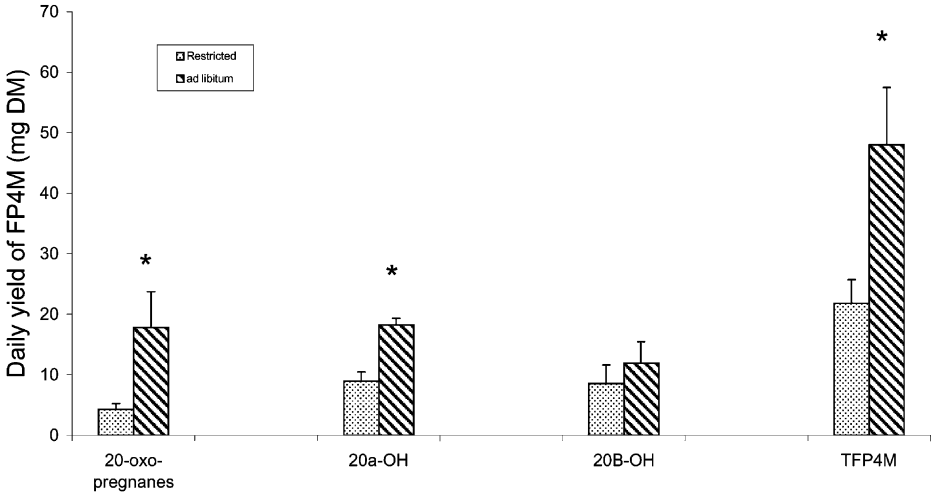

Fig. 2. Daily yields (mean ± S.E.) of fecal 20-oxo-, 20α-, 20β-OH pregnanes and total FP4M in restricted and ad libitum non-lactating dairy cows.

tended to be higher in faecal samples from the ad libitum group, but the concentrations of faecal 20α-OH (20α-) and 20β-OH (20β-) were slightly higher in samples from cows in the restricted group (Fig. 1, $P > 0.05$). The level of FI influenced daily yield of total FP4M (Fig. 2, $P = 0.03$). Daily yields of 20-oxo- ($P = 0.05$) and 20α- ($P = 0.001$) were significantly higher in samples from the ad libitum group, but the difference in daily yield of faecal 20β- between two groups was not significant ($P = 0.5$). Difference in average daily yield of total FP4M among cows ranged from 14 to 35 mg in the restricted group ($P < 0.05$) and from 32 to 85 mg in the ad libitum group ($P < 0.01$). Recovery rates of P4 metabolites in the faeces of ad libitum and restricted groups were 75 (from 0.51 to 1.2) and 36% (from 0.22 to 0.57), respectively ($P < 0.05$). There was more variation in the recovery rate of total FP4M among cows in the ad libitum group compared to those in the restricted group.

3.4. The average body weights and blood metabolites

The body weight of cows in both groups were similar at the beginning of the study (559 versus 547, $P > 0.05$, in restricted and ad libitum cows, respectively). By the completion of study the average body weight was greater in ad libitum cows compared to those in the restricted group (560 versus 528 kg, $P = 0.01$). There were no differences in the concentrations of blood glucose (4.3 versus 4.2 mM, $P = 0.5$) between the two groups. The average concentration of blood NEFAs and BHB were greater in restricted cows (NEFAs: 0.95 versus 0.24 mM, $P < 0.0001$; BHB: 0.35 versus 0.23 mM, $P = 0.03$), while average blood urea concentration was significantly higher in the ad libitum group (7.4 versus 4.2 mM, $P < 0.0001$).

4. Discussion

This study was conducted to investigate the effects of level of feeding on the concentrations of plasma P4 and FP4M, and daily excretion of P4 metabolites into the faeces. Deslorelin-implanted cows were used and a CIDR device was inserted into the vagina for 11 days and the distribution and recovery rate of P4 were studied.

Analyses of residual P4 in CIDR devices inserted for 11 days showed that the small differences in P4 release (0.65 versus 0.70 g, $P > 0.05$) were not statistically significant. Consequently, lower concentrations of peripheral P4 in ad libitum cows compared to those in the restricted group indicated that the inverse relationship between feed level and concentration of plasma P4 could be associated with differences in clearance rate of P4 rather than to changes in the entry rate of the hormone into the blood. Studies in sheep (Parr et al., 1982; Williams and Cumming, 1982; Parr, 1992; Adams et al., 1994) also showed that the level of feeding significantly changed the plasma P4 levels. Several studies (Parr et al., 1979, 1987; Williams and Cumming, 1982; Parr, 1992) highlighted that plasma P4 concentrations were significantly higher in ewes fed a 1/4 maintenance (M) diet compared to an M and 2M diets. An increased FI can cause an elevation in the amount of blood flowing through the gut and liver (Parr et al., 1993). Since P4 is almost completely metabolised after one passage through the liver and gut, an increase in FI may result in a reduction in the concentration of plasma P4. Recent studies in cattle (Vasconcelos et al., 1998; Sangsritavong et al., 2000) have also shown that the frequency of feeding affected peripheral P4 concentration. Peterson and Hnderson (1991) also suggested that individual variations in plasma P4 among cows treated with CIDR devices may have been attributed to variation in the rate of metabolism of P4. In contrast, in a previous study, we showed that when cows were housed in-doors and fed with lucerne and oaten hay, the alteration of the level of feeding did not change the concentrations of plasma P4 (Rabiee et al., 2001a). It may indicate other factors such as housing condition and diet composition could affect the metabolic clearance rate of P4. The entry rate of P4 into the circulation may not be the only factor that can affect peripheral concentration of this hormone.

The concentrations of individual FP4Ms were not affected by level of FI. The total concentrations of FP4M were also similar in both groups (Fig. 1). No comparable studies have been conducted in cattle or other species with P4. However, studies in rats and sheep (Arts et al., 1992; Adams et al., 1994) showed that the reduced estrogen excretion was associated with higher plasma estrogen in rats and sheep on restricted FIs. They concluded that the slower rate of passage of digesta may have provided greater opportunities for re-absorption. Similarity in the concentrations of total FP4M for both groups (8.6 versus 9.0 μg/g) in this study does not support this conclusion. Diet composition, digestibility and level of feeding may influence the quantity of different P4 metabolites in the faeces.

The total daily yield of FP4M was higher among cows in the ad libitum group (Fig. 2, $P = 0.03$). Our previous observations in ovariectomised cows (Rabiee et al., 2001a) and other studies in rats (Arts et al., 1992) also indicated that excretion rates of P4 metabolites were greater in animals fed ad libitum. Studies in sheep (Adams et al., 1994) have also shown that daily peaks of excretion of radioactive estrogen in sheep coincided with enhanced defecation about the time the animals were fed, and the excretion was slower in the restricted sheep. The enhanced estrogen excretion resulted in lower plasma estrogen in rats and sheep

(Arts et al., 1992; Adams et al., 1994). The level of FI and consequently volume of faeces was associated with the differences in the daily excretion rate of FP4M indicating that the excretion rate of P4 metabolites to the faeces was influenced by the level of feeding rather than by the entry rate of P4 into the systemic circulation. The enhanced rate of P4 excretion into the faeces was associated with the lower plasma P4 in ad libitum cows. Since the delivery rate of P4 was similar between the two groups, this suggests that it is the volume of faeces which mainly dictated the total daily excretion rate of P4 metabolites in the faeces. However, the mechanism whereby the level of feeding was able to control concentrations and daily yields of faecal P4 remain to be established.

Studies in ruminants (Palme et al., 1996; Schwarzenberger et al., 1996) showed that the rate of food passage was longer than the passage time of faecal steroids. The lag time was affected by the digestibility of the forage which influenced the rate of passage of digesta (Schwarzenberger et al., 1996). The physical form of faeces and passage rate of faeces also may enhance the re-absorption of FP4M. The significant elevation of plasma P4 in the restricted group may support this explanation. The slower passage rate of faeces in the restricted group, due to gut microbial transformation, may also alter the quantity of different FP4M. Differences in the concentrations of different FP4M (i.e. 20β-OH) in both groups suggest that the re-absorption or further breakdown of metabolites in the gut may be influenced by diet composition.

The recovery rate of FP4M was lower in the restricted group, as it was almost half that of the ad libitum group. The amount of P4 that was delivered by a CIDR device to a cow was very similar in both groups. This suggests that the amount of P4M excreted into the faeces could be related to the amount of daily FI, rather than the amount of P4 administered to the cows.

5. Conclusion

These results showed that a reduction in FI and consequently FO was associated with a reduced excretion rate of P4 metabolites to the faeces. This was linked to an increase in the concentration of peripheral P4 among cows on a restricted pasture diet compared to those fed ad libitum. These data indicate that the concentrations of plasma P4 and excretion rate of P4 to the faeces were influenced by the level of feeding and the resulting changes in metabolism rather than by the entry rate of P4 into circulation.

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