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Progesterone clearance rate in lactating dairy cows with two levels of dry matter and metabolisable energy intakes

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Abstract

The aim of these studies was to determine the effect of levels of dry matter (DM) and metabolisable energy (ME) intakes on clearance rate of progesterone (P4) in dairy cows. Thirty-two lactating Holstein–Friesian cows were selected for the study and were fed indoors in individual stalls for a period of 5 weeks. They were individually offered a diet of combinations of pasture, hay and pelleted cereal grain to achieve two different levels of DM and ME. In the first trial, 16 cows were allocated to two groups: (i) high DM (HDM), and (ii) low DM (LDM) intakes, while the amount of ME intake was constant. In the second trial, 16 cows were allocated to two groups: (i) high ME, and (ii) low ME intakes with similar amount of DM intake. A GnRH-agonist (deslorelin) was initially implanted in the ear of each cow to block endogenous P4 secretion. Then 3 weeks later, a CIDR device was inserted into the vagina of each cow and left in place for 11 days. Chromic oxide (Cr_2O_3) capsules were administered to allow daily faecal output (FO) to be estimated. Daily blood, faecal and milk samples were taken during the period of the experiment for P4 and faecal P4 metabolites analyses.

Trial 1: The average milk yield was similar among cows in high and LDM intake groups (26.7 versus 25.0 l per day, $P = 0.2$). The average daily FO was 7.8 kg DM in the HDM and 5.7 in the LDM cows ($P < 0.0001$). Average daily DM intakes were 17.3 kg and 15.4 kg in the HDM and LDM groups, respectively ($P < 0.0001$). The average plasma P4 concentrations were similar between the two groups (1.56 versus 1.60 ng/ml, $P = 0.7$) but milk P4 concentrations were higher in LDM cows (4.6 versus 3.6 ng/ml, $P = 0.02$). The average daily excretion rate of P4 into the

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milk was higher in LDM cows (122.3 versus 88.5 μg, $P = 0.002$). The concentrations of faecal P4 metabolites (FP4M) were not influenced by the level of daily DM intake (2.85 versus 2.90 μg/g, $P = 0.6$). The average daily yields of FP4M were higher among cows in the HDM group (23.2 versus 16.3 mg, $P = 0.01$).

Trial 2: The average milk yield was 31.2 l per day in HME cows compared to 25.0 l per day in LME cows ($P < 0.0001$). The average daily FO was 7.8 kg DM in LME and 5.8 kg DM in HME cows ($P < 0.0001$), and the average DM content of faeces was higher in LME cows (15.8 versus 12.7%, $P = 0.01$). The average daily ME intake was 213 MJ per day in HME group compared to 183 MJ per day in LME group ($P < 0.0001$). The average plasma and milk P4 concentrations were similar between the two groups (plasma $P4 = 1.54$ versus 1.56 ng/ml, $P = 0.4$; milk P4: 3.7 versus 3.6 ng/ml, $P = 0.6$). The average daily excretion rate of P4 into the milk was higher in HME cows (114 versus 88.5 μg, $P = 0.03$). Concentrations of FP4M were not influenced by the level of daily ME intake (2.5 versus 2.85 μg/g, $P = 0.08$). However, daily yields of FP4M were greater in the LME group (23.2 versus 14.4 mg, $P = 0.01$).

In conclusion, this study was unable to establish a relationship between the level of DM and ME in the diet with the excretion rates of FP4M metabolites and plasma P4 concentrations.

Keywords: Progesterone; Feed intake; Faecal progesterone metabolites; Cattle-endocrinology

1. Introduction

Nutritional management plays an important role in the postpartum reproductive performance of dairy cattle. The most important nutritional factor is the overall energy balance of the animal. It has been shown that an energy deficit (Villa-Godoy et al., 1988; Butler and Smith, 1989) and reduced bodyweight (McClure, 1970; Heinonen et al., 1988) at the time of insemination can be related to poor fertility. The effect of energy intake on embryo survival in both overfed and underfed ewes (Parr et al., 1987; Rhind et al., 1989) have also been reported.

The link between negative energy balance (NEB) and fertility may be related to blood progesterone (P4) concentrations (Butler, 2000). Peripheral P4 concentrations during the postpartum ovulatory cycle is reduced or moderated by NEB (Villa-Godoy et al., 1988; Spicer et al., 1990; Staples et al., 1990). Cows with the most negative energy status during the postpartum period had lower serum P4 levels during their third oestrous cycles postpartum (Villa-Godoy et al., 1988). NEB during early stages of follicular growth and development could also lead to lower P4 secretion (Britt, 1992). The level of P4 secretion may partially explain the pattern of serum P4 concentrations in lactating cows.

The effects of dietary intake on P4 clearance also must be considered. Nolan et al. (1998) reported that plasma P4 concentrations were about 25% lower in heifers fed a high energy diet as compared to those fed a low-energy diet, presumably as a result of increased clearance rate of P4. During the breeding period, when metabolic demands or other physiological processes are greatest, any increase in P4 clearance due to high dietary intake may result in lower plasma P4 concentrations and reduce fertility (Butler, 2000).

The splanchnic tissues play a major role in supplying precursors for milk synthesis. They metabolise other components, such as steroid hormones, otherwise voided into the bile. Therefore, alterations in liver metabolism by changing the amount of feed offered could

contribute to variation in metabolic events associated with milk production. In the cows, flow rate of portal vein blood in cows was directly related to daily digestible DM and energy intakes (Huntington et al., 1981; Wieghart et al., 1986). This may alter splanchnic clearance of P4 (Symonds and Prime, 1989).

These studies were conducted to investigate the effect of levels of dry matter (DM) and metabolisable energy (ME) intake on the clearance rate of P4 in lactating dairy cows.

2. Materials and methods

2.1. Animals and experimental procedures

Thirty-two lactating Holstein–Friesian cows, 4–5 weeks postpartum, were included in the study. Cows were carefully matched on age, parity, milk production, days open and reproductive performance history, and fed indoors for a period of 5 weeks in individual stanchions, which allowed enough head movement for the cows to get up and lie down. Rubber matting (∼7 cm thickness) was installed on the concrete floor to prevent lameness, to make cows comfortable and to prevent slipping. They were milked at 06:00 and 15:00 daily and then individually offered their diets (after each milking) for 5 h. They were then held as a single group on a bare paddock. Animals were fed twice daily, a diet of pasture, hay and pelleted cereal grain to achieve two different levels of DM and ME, at 09:00 and 16:00. Refusals of each feed offered were collected and recorded separately.

2.1.1. Trial 1

Sixteen lactating dairy cows were allocated to two groups and offered either a (i) high DM (HDM), or (ii) low DM (LDM) diet with a similar ME (Table 1). Cows in the HDM intake group were offered pasture, hay and low-energy pellets (11.3 MJ/kg), and cows in the LDM intake group were offered with pasture and high energy-pellets (13.3 MJ/kg). They were offered the pellets first, and then followed by hay and pasture in the HDM intake group, and pasture in the LDM intake group.

2.1.2. Trial 2

Sixteen lactating dairy cows were allocated to two groups and offered either a (i) high ME, or (ii) low ME diet with a similar DM (Table 2). Cows in the high ME intake group (HME) were offered pasture and high energy pellets (13.3 MJ/kg), and cows in the low ME intake group (LME) offered pasture, hay and low energy pellets (11.3 MJ/kg). They were

Table 1
The values of the various feed types in the ration

	Digestibility (%)	DM (%)	CP (%)	ME (MJ)
Low energy pellet	75.3	96.8	16.7	11.3
High energy pellet	86.4	96.5	12.9	13.3
Pasture (Ryegrass and Clover)	77.5	15.4	23.8	11.2
Hay	64.6	82.4	14.1	9.0

Table 2
DM and ME intakes, FO and milk yield by the cows in each group

Groups	DM intake	ME intake (MJ)	FO (kg DM)	Milk yield (l per day)
HDM	17.3 ± 0.17	183.1 ± 1.50	7.8 ± 0.2	24.9 ± 0.57
LDM	15.4 ± 0.13	181.4 ± 1.60	5.7 ± 0.3	26.7 ± 1.23
High ME	17.9 ± 0.40	213.3 ± 4.60	5.8 ± 0.4	31.2 ± 1.24
Low ME	17.3 ± 0.17	183.1 ± 1.50	7.8 ± 0.2	24.9 ± 0.57

fed with pellets first, and then followed by pasture in the high ME intake group, and hay and pasture in the LME intake group.

Water was available to the cows during the period between feeding and milking time when the cows were held on a bare paddock. Pasture was cut to approximately 40 mm, twice daily, and harvested using a loader wagon. A quick DM content of pasture was measured using a microwave (high for 9 min) with the amount of pasture offered based on estimates of the DM content of pasture. A representative sample of each of the feeds offered and refused was dried at 105 °C to constant weight to determine the actual DM content. An additional sub-sample of all feed offered were bulked on a weekly basis and dried at 65 °C for 72 h, ground and analysed for in vitro DM digestibility (DMD) and nitrogen (N). DMD was determined by the method of Clarke et al. (1982). ME was calculated from DMD (ME = (DMD × 0.17)−2). The nitrogen content was determined by the Kjeldahl method. Crude protein (CP) was calculated from nitrogen (CP = N × 6.25). The values of the various feed types (pasture, hay and pellets) are given in Table 1. DM intake of pasture, hay and pellets and average DM, DMD, ME and CP of the experimental diets are presented in Table 3.

Our previous observations (Rabiee et al., 2001d) showed that ovarian P4 production could be prevented by strategic use of a deslorelin implant to create P4-free animals similar to ovariectomised cows. In order to block endogenous P4 release each cow received an implant of a 6 mg GnRH-agonist (Deslorelin; 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 10 days later. Weekly and daily blood samplings were made before and after prostaglandin F2α injection.

Table 3
DM intake of pasture, hay and pellets and average DM, DMD, ME and CP content of the experimental diets

Intake (kg DM per cow per day)	HDM	LDM	HME	LME
Pasture	6.3	10.4	11.0	6.3
Hay	4.9	–	–	4.9
Low energy pellet	6.2	–	–	6.2
High energy pellet	–	4.9	6.8	–
Diet composition				
DM (%)	32.5	21.2	22.8	32.5
DMD (g/kg DM)	73.5	79.8	80.4	73.5
ME (MJME/kg DM)	10.7	11.8	11.9	10.7
CP (g/kg DM)	18.6	20.2	19.5	18.6

Cr_2O_3 capsules (controlled release device, Captec, NZ) with a mean release rate 1.43 g Cr_2O_3 per day were administrated to allow daily faecal output (FO) to be estimated over the last 17 days. Daily faecal samples were taken for a period of 10 days, following a 7-day equilibration period.

A CIDR device (InterAg, NZ) containing 1.9 g P4 was inserted into the vagina of each cow 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, milk and faecal sampling procedures and assays

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, ^{125}I kit (Spectriat®, Kit, Orion Dianostica, Espoo, Finland). The inter-assay CVs were 16, 5.6 and 5.4% for low, medium and high concentrations, respectively. The sensitivity of the assay was 0.1 ng/ml. Weekly blood samples were also taken into a tube without anti-coagulant to measure blood metabolites including glucose, non-esterified fatty acids (NEFAs), β-hydroxybutyrate (BHB) and urea. Blood glucose concentrations were 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); blood urea using an enzymatic reaction (Trace Scientific, Australia) (Talke and Schubert, 1965).

Weekly milk samples were collected from each cow at a p.m. and a.m. milking using a standard herd recording devices for 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 p.m. and a.m. milking.

Milk samples were 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 (Spectriat®, Kit, Orion Dianostica, Espoo, Finland) kit. The inter-assay CVs were 10.8, 6.6 and 6.1% for low, medium and high concentrations, respectively. The sensitivity of the assay was 0.47 ng/ml.

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. (1996b). 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; Schwarzenberger et al., 1996b), 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 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 (Palme et al., 1996, 1997; Schwarzenberger et al., 1996a, 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 assay was 7 ng/g.

Faecal samples for Cr_2O_3: A faecal sample was taken from each cow (blank) before Cr_2O_3 administration commenced. Morning and afternoon samples were weighed, bulked 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 FO 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. Data analysis

The results were analysed after excluding the first 3 days of observations following the CIDR insertion. Values over the last 8 days of CIDR exposure were pooled and mean ± S.E. are presented. The effects of time (day) and diet on plasma and milk P4, FP4M, blood metabolites, body weight and interactions between diet and time were analyzed using GLM with repeated measures analysis included in the model in SPSS v. 9.0 (SPSS, 1999). A two-sample *t*-test was used to compare the means of FO between restricted and ad libitum cows. The recovery rate of P4, the percentage of P4 that was excreted into the milk or faeces relative to the total P4 administrated, was calculated using the following formula:

$$\text{P4 recovery rate} = \frac{\text{amount of P4 recovered in milk or faeces}}{\text{total P4 administered}} \times 100$$

3. Results

3.1. Trial 1

3.1.1. Milk production, faecal output and nutrient intake

Average daily milk yield was not affected by DM intake ($P = 0.2$, Table 4). The average daily milk yield ranged from 21 to 31 l per day in LDM and 22 to 28 l per day in HDM groups.

The average daily FOs were 7.8 kg DM in HDM and 5.7 kg DM in LDM cows ($P < 0.0001$, Table 2), with the average DM content of faeces during the 8-day period higher among cows in the HDM group (15.8 versus 13.8% $P = 0.04$). Their average daily DM intake was 17.3 kg compared to 15.4 kg in the LDM group ($P < 0.0001$, Table 2), although ME intake was similar in the two groups (average = 182 MJ per day). The average dietary CP concentration (%) was similar for the two groups (HDM = 18.9 versus LDM = 20.3%, $P > 0.05$).

3.1.2. Plasma and milk progesterone

Average plasma P4 concentrations were similar for the two groups ($P = 0.7$, Table 3), but the concentrations of milk P4 were higher in the LDM group ($P = 0.02$, Table 4).

Table 4
Concentrations, yield (mean ± S.E.) 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 P4 device

	Plasma P4 (ng/ml)		Milk P4 concentration (ng/ml)		Daily milk yield (l per day)		Daily yield of milk P4 (ug per day)		P4 released from CIDR (g)	
	HDM	LDM	HDM	LDM	HDM	LDM	HDM	LDM	HDM	LDM
Mean ± S.E.	1.56 ± 0.09	1.60 ± 0.06	3.6 ± 0.2	4.6 ± 0.3	25.0 ± 0.6	26.7 ± 1.2	88.5 ± 4.6	122.3 ± 9.8	0.85 ± 0.04	0.84 ± 0.02
Time (day)	<0.0001		<0.0001		0.05		<0.0001		–	
Group	0.7		0.02		0.2		0.002		0.7	
Group × day	0.3		0.6		0.9		0.2		–	
	HME	LME	HME	LME	HME	LME	HME	LME	HME	LME
Mean ± S.E.	1.54 ± 0.06	1.56 ± 0.09	3.7 ± 0.3	3.6 ± 0.2	31.2 ± 1.2	25.0 ± 0.6	114.0 ± 9.8	88.5 ± 4.6	0.84 ± 0.02	0.85 ± 0.04
Time (day)	<0.0001		<0.0001		0.2		<0.0001		–	
Group	0.4		0.6		<0.0001		0.03		0.8	
Group × day	0.8		0.7		0.4		0.3		–	

Plasma and milk P4 concentrations varied through time ($P < 0.0001$), but interactions of day and DM intake were not significant ($P > 0.05$). The average daily excretion rate of P4 into the milk (daily yield of milk $P4$ = daily milk yield × milk P4 concentration) was higher in LDM cows ($P = 0.002$). The mass of P4 lost from the CIDR devices was similar in both groups ($P = 0.7$, Table 4). The recovery rates of P4 in the milk were similar between the two groups (average = 0.15%), and ranged from 0.1 to 0.15% in HDM, and from 0.13 to 0.20% in LDM cows.

3.1.3. Body weight, blood metabolites and milk composition

The average body weight in HDM cows was 492 kg compared to 480 kg in LDM cows ($P = 0.7$, Table 6). There were some changes in the body weight of cows during the period of study in both groups ($P = 0.01$), but the weight loss was not significant by the completion of study. The concentrations of blood glucose tended to be higher for cows in the LDM group ($P = 0.08$, Table 6), but their blood concentrations of BHB were lower ($P = 0.01$, Table 6). The concentration of blood urea tended to be higher in HDM cows ($P = 0.07$, Table 6). There were no differences in the concentrations of blood NEFAs between the two groups ($P = 0.6$, Table 6). The average milk fat content tended to be higher for cows in the HDM group ($P = 0.1$), but the average milk protein content was lower ($P = 0.001$). The average milk lactose content was similar in both groups ($P = 0.7$ Table 6).

3.1.4. Concentrations and daily yields of FP4M

The concentrations of faecal 20α-OH and 20β-OH and total FP4M were not affected by the level of DM intake (Table 5), except for faecal 20-oxo-pregnanes ($P = 0.02$, Table 5). The concentrations of 20-oxo, 20α-, 20β- and total FP4M changed significantly through time ($P < 0.001$), but interactions of day and level of daily DM intake were not significant ($P > 0.05$). Daily DM intake did not influence the daily yields of faecal 20-oxo-, 20β- ($P > 0.05$, Table 5), but altered the daily yield of faecal 20α- and total FP4M ($P < 0.05$, Table 5). Differences in average daily yield of total FP4M among cows ranged from 12 to 34 mg in the HDM group ($P = 0.05$) and from 9 to 27 mg in the LDM group ($P = 0.05$). Recovery rates of P4 metabolites in the faeces were 30% (from 15 to 45%) and 21% (from 12 to 35%) among cows in the HDM and LDM groups, respectively ($P = 0.05$).

3.2. Trial 2

3.2.1. Milk production, faecal output and nutrient intake

The average milk yields for the LME and HME cows were 25.0 and 31.2 l/cow per day, respectively ($P < 0.0001$, Table 4), ranging from 22 to 28 l per day in LME group and from 25 to 36 l per day in HME group.

The HME cows had higher daily FO (7.8 versus 5.8 kg DM, respectively, $P < 0.0001$). Faecal DM content of faeces during the 8-day period was higher in LME cows (15.8 versus12.7%, $P = 0.01$) but daily ME intake was only 183 MJ per day in the LME group compared to 213 MJ per day in the HME group ($P < 0.0001$, Table 2). The average DM intakes were 17.9 and 17.4 kg per day in HME and LME cows, respectively ($P = 0.24$). The average dietary CP concentration (%) was similar between the two groups (HDM = 18.9 versus LDM = 19.7%, $P > 0.05$).

Table 5
Concentration, daily yield (mean ± S.E.) and significance of FP4M in lactating dairy cows implanted with a subcutaneous GnRH-agonist and treated with an intra-vaginal P4 device

	Faecal 20-oxo-pregnanes		Faecal 20α-OH		Faecal 20β-OH		Total FP4M	
	HDM	LDM	HDM	LDM	HDM	LDM	HDM	LDM
Concentration (μg/g DM)	0.53 ± 0.04	0.87 ± 0.08	2.0 ± 0.4	1.6 ± 0.3	0.32 ± 0.02	0.51±0.06	2.85 ± 0.33	2.9 ± 0.33
P-value	0.02		0.20		0.11		0.60	
Daily yield of FP4M (mg)	4.3 ± 0.37	4.87 ± 0.58	16.3 ± 2.8	8.6 ± 1.6	2.6 ± 0.20	2.8 ± 0.40	23.2 ± 2.6	16.3 ± 2.2
P-value	0.40		0.03		0.60		0.05	
	HME	LME	HME	LME	HME	LME	HME	LME
Concentration (μg/g DM)	0.70 ± 0.05	0.53 ± 0.04	1.4 ± 0.10	2.0 ± 0.40	0.40 ± 0.04	0.32 ± 0.02	2.5 ± 0.2	2.85 ± 0.33
P-value	0.50		0.05		0.73		0.08	
Daily yield of FP4M (mg)	4.04 ± 0.50	4.32 ± 0.37	8.1 ± 0.94	16.3 ± 2.8	2.2 ± 0.23	2.6 ± 0.2	14.4 ± 1.4	23.2 ± 2.6
P-value	0.90		0.05		0.73		0.08	

Table 6
Mean (±S.E.) and significance of analysis of variance of body weight, blood metabolites and milk composition in HP and LP cows treated with a subcutaneous GnRH-agonist (deslorelin) and intra-vaginal P4 device

	Body weight (kg)	Glucose (mM)	NEFAs (mM)	BHB (mM)	Urea (mM)	Milk fat (%)	Milk protein (%)	Milk lactose (%)
HDM	492 ± 20	3.5 ± 0.06	0.36 ± 0.07	0.65 ± 0.06	7.5 ± 0.3	4.1 ± 0.2	2.9 ± 0.03	4.9 ± 0.07
LDM	480 ± 18	3.7 ± 0.07	0.41 ± 0.07	0.41 ± 0.05	6.7 ± 0.2	3.7 ± 0.2	3.1 ± 0.05	4.9 ± 0.1
Time (week)	0.001	<0.0001	<0.0001	0.02	0.001	0.03	0.2	0.5
Group	0.7	0.08	0.6	0.01	0.07	0.1	0.001	0.7
Time × group	0.4	0.04	0.4	0.08	0.01	0.2	0.007	0.05
HME	492 ± 15	3.8 ± 0.05	0.33 ± 0.03	0.34 ± 0.02	5.8 ± 0.2	3.5 ± 0.3	3.1 ± 0.09	5.0 ± 0.05
LME	492 ± 20	3.5 ± 0.06	0.36 ± 0.07	0.65 ± 0.06	7.5 ± 0.3	4.1 ± 0.2	2.9 ± 0.03	4.9 ± 0.07
Time (week)	0.004	<0.0001	<0.0001	0.05	<0.0001	0.08	0.02	0.13
Group	0.9	0.002	0.7	<0.0001	0.001	0.07	0.01	0.3
Time × group	0.7	0.14	0.5	0.1	0.01	0.05	0.04	0.01

NEFAs: non-esterified fatty acids; BHB: β-hydroxybutyrate.

3.2.2. Plasma and milk progesterone

Average plasma and milk P4 concentrations were similar in the two groups (plasma P4: $P = 0.4$; milk P4: $P = 0.6$, Table 4). Plasma and milk P4 concentrations varied through time ($P < 0.001$), but interactions of day and DM were not significant ($P > 0.05$). The average daily excretion rate of P4 into the milk (daily yield of milk $P4$ = daily milk yield × milk P4 concentration) was higher in HME cows ($P = 0.03$) although the mass of P4 lost from the CIDR devices was similar in both groups ($P = 0.8$) (Table 4). Recovery rates of P4 in the milk were similar between the two groups (average = 0.13%), and ranged from 0.10 to 0.18% in HME, and from 0.10 to 0.15% in LME cows.

3.2.3. Body weight, blood metabolites and milk composition

Average body weight was the same in both groups (492 versus 492 kg, $P = 0.9$), but there were some changes in the body weight of cows during the period of study in both groups ($P = 0.04$). Although they did not lose weight significantly by the completion of study. The concentration of blood glucose was higher in HME group ($P = 0.002$, Table 6), whereas blood concentrations of BHB ($P < 0.0001$, Table 6) and urea ($P = 0.01$, Table 6) were higher in LME cows. There were no differences in the concentrations of blood NEFAs between the two groups ($P = 0.7$). The average milk fat content tended to be higher in LME group ($P = 0.1$), but the average milk protein content was higher in HME cows ($P = 0.01$). The average milk lactose content was similar in both groups ($P = 0.3$ Table 6).

3.2.4. Concentrations and daily yields of FP4M

The concentrations of faecal 20-oxo-, 20β- and total FP4M were similar between HME and LME groups (Table 5, $P > 0.05$), except for faecal 20α- concentrations (Table 5, $P = 0.05$). The concentrations of FP4M changed through time ($P < 0.0001$), but interactions of day and daily ME intake were not significant ($P > 0.05$). Daily yields of faecal 20α- and total FP4M were affected by daily ME intake (Table 5, $P = 0.01$), but ME intake did not influence the average daily yields of faecal 20-oxo-, 20β- (Table 5, $P > 0.05$). Differences in average daily yield of total FP4M among cows ranged from 9 to 22 mg in the HME group ($P = 0.05$) and from 12 to 33 mg in the LME group ($P = 0.05$). Recovery rates of P4 metabolites in the faeces were 19% (from 11 to 29%) and 30% (15 to 45%) in HME and LME cows ($P = 0.02$), respectively.

4. Discussion

These studies were conducted to determine the effect of levels of DM and ME intake on P4 clearance rate in lactating dairy cows. There were no significant associations between plasma P4 concentrations and daily intakes of DM and ME. While the current studies were unable to demonstrate a relationship between daily yields of FP4M and plasma P4 concentrations, both ME and DM intake influenced the excretion rates of FP4M.

Average daily milk yields were similar among cows in HDM and LDM groups, but the HME cows produced significantly more milk than the LME cows. The average body weight was similar between the groups and did not change over the 6 weeks experimental period. The results indicate that differences in milk yield were associated with daily ME intake

rather than DM intake or mobilisation of body tissue. The lack of tissue mobilisation was probably a reflection of the low body condition score of the animals at the commencement of the experiment. The absence of tissue mobilisation eliminates the release of stored-P4 into the systemic circulation as a contributing factor to treatment differences.

The average plasma P4 concentrations and mass of P4 delivered from a CIDR device were similar between the two groups in each trial (Table 4). Huntington et al. (1981) and Wieghart et al. (1986) demonstrated that there is a significant positive relationship between portal blood flow rate and level of DM and ME intake. Therefore, changes in metabolic flux across the splanchnic tissues would have a major affect on the metabolic status of the animal. The liver and gut are the functional units that supply nutrients to the body and metabolise other components, such as steroid hormones, to be voided into the bile and then the faeces. It was expected that changes in the level of DM or ME intake could alter the rate of blood flow to the liver, and consequently the clearance rate of P4, as suggested by other studies in cattle (Sangsritavong et al., 2000; Vasconcelos et al., 1998; Rabiee et al., 2001a,b) and sheep (Williams and Cumming, 1982; Parr, 1992; Parr et al., 1993; Rhind et al., 1989). Recent observations (Nolan et al., 1998) have shown that plasma P4 concentrations in cattle were proportionately 0.25 higher in animals on low diet (40 MJ ME per day) compared with heifers on a high diet (120 MJ ME per day). They suggested that this difference was likely to be caused by changes in liver size and consequently steroid metabolism, as occurs in sheep. It is possible that the 12 and 16% increases in DM and ME intake, respectively, were insufficient to significantly alter blood flow and therefore excretion of P4 by the liver.

It has been shown that the effect of level of feeding on plasma P4 in cattle is more variable than in sheep. The level of feeding in heifers has increased (McCann and Hansel, 1986), decreased (Villa-Godoy et al., 1990) or had no effect (Spitzer et al., 1978) on P4 concentration when compared with restricted feeding. The absence of a relationship between P4 excretion rates and either DM or ME intake in the current studies suggests that other factors may be involved in influencing portal and hepatic blood flow. It has also been shown (Huntington et al., 1981) that breed, live weight and energy density of the diet may influence portal or hepatic blood flow.

Average milk P4 concentrations were higher among cows in the LDM group than those in HDM group ($P = 0.02$) but did not appear to be related with milk fat concentration, as the daily milk fat yield was similar between the two groups in each study ($P > 0.05$). Since an increase in milk yield in lactating dairy cows is associated with an increase in the mammary gland uptake of P4, it was expected that the higher the milk production, the greater the potential contribution of the mammary gland to metabolic clearance of P4. Despite the higher excretion rate of P4 to the milk in cows with higher ME and lower DM intakes, the recovery rates of P4 were similar (0.13 to 0.15%) among the two groups in each trial. This is supported by other studies in cattle (Heap et al., 1975a,b; Rabiee et al., 2001c). It may indicate that even with greater excretion rate of P4 to the milk among cows in the LDM and HME groups, it is unlikely that the mammary gland and level of milk production could have a significant role in the clearance rate of P4 and also in altering the concentration of plasma P4.

In the current experiments the concentrations of FP4M were similar between the two groups in each trial (Table 5), however daily faecal yield of FP4M were greater with HDM and LME intake cows indicating that excretion rates of P4 metabolites in the faeces were influenced by daily FO. Other studies with lactating and non-lactating dairy cows (Rabiee

et al., 2001a,b,c) and studies in rats (Arts et al., 1992) supported the observations that daily excretion rate of P4 metabolites are more closely related to daily FO than the FP4M concentrations. Studies in sheep (Abecia et al., 1995, 1997; Parr, 1992; Parr et al., 1993) have shown that ovarian P4 production was not influenced by the level of feed intake. Recent studies in cattle (Vasconcelos et al., 1998; Sangsritavong et al., 2000) have also suggested that a reduction in reproductive efficiency in high producing dairy cows may be mediated by steroid metabolism due to high feed consumption. Diet composition and energy density may also be linked with the concentration of mixed function oxidase (MFO) enzymes which could control metabolism of steroid hormones (Thomas et al., 1987).

The recovery rates of P4 in the faeces were higher in cows with higher DM and lower ME intakes; similarly the daily FO was higher in these groups, even though the entry rates of P4 into the systemic circulation were similar among cows in both studies. Since the diet ingredients and DM content of the faeces in this study were different between the groups, diet composition or possibly the volume and DM content of faeces may have altered the quantity of individual metabolites, but did not influence the total concentrations of FP4M. These findings are supported by our previous studies in lactating and non-lactating dairy cows (Rabiee et al., 2001a,b,c) and suggest that factors such as pasture digestibility and the composition of the diet may influence the quantity and quality of individual metabolites. Differences in ME and DM intake in the current experiments were achieved by offering different groups of animals different combinations of pasture, hay and low or high ME pellets. The differences in FO can therefore be explained by differences in total diet digestibility. The HDM and LME treatments consumed a diet with a digestibility of 740 g/kg compared with 810 g/kg for the LDM and HME treatments. Further studies are required to investigate the association between the diet composition and digestibility, the concentrations of MFO in the liver and plasma P4 concentrations.

5. Conclusion

The absence of a relationship between DM or ME intake, plasma and milk concentrations of P4 and FP4M within the ranges investigated in the current experiments suggest that circulating P4 concentrations of lactating dairy cows may be controlled by other factors involved in liver metabolism of steroid hormones. Further studies are required to investigate the association between the diet composition and digestibility, the concentrations of MFO in the liver and plasma P4 concentrations.

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