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Effects of level of feeding and progesterone dose on plasma and faecal progesterone in ovariectomised cows

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Abstract

The effects of two levels of feeding and two doses of progesterone (P4) on plasma and faecal progesterone metabolites (FP4M) were studied using a total of 24 ovariectomised (OVX), non-lactating, Holstein–Friesian cows. Cows were grazed on improved ryegrass/white clover pastures and allowed ad libitum access to pasture or were restricted to grazing for a total of 4 h per day in two 2 h periods. Progesterone (P4) was administered as one or two, simultaneous, intravaginal progesterone devices (CIDR). The cows were adapted to their pasture supply for 2 weeks before the start of the progesterone treatments. The progesterone devices were administered for 11 days and the cows were dosed with slow release chromic oxide capsules during the P4 treatment to allow faecal output (FO) to be estimated. Daily blood samples for P4 assay and weekly samples for blood metabolite assay were collected. Faecal samples were collected per rectum daily and assayed for pregnanes containing a 20-oxo-, 20α- or a 20β-OH group by enzyme immunoassay (EIA).

Daily FO was higher ($P < 0.001$) for ad libitum than pasture restricted cows (6.3 vs 4.1 kg DM) but was similar for both doses of P4. The average mass of P4 released from a CIDR device over a 11-day period was higher for cows allowed ad libitum pasture compared with those on restricted pasture (0.64 vs 0.60 g; $P = 0.04$). Plasma P4 concentrations, however, were higher in restricted than ad libitum fed cows (1× CIDR: 1.81 vs 1.41 ng/ml; 2× CIDR: 4.10 vs 3.46 ng/ml). Increasing the progesterone dose significantly ($P < 0.001$) increased both the concentrations and daily totals of the faecal pregnanes assayed and total FP4M. Restricted pasture cows had higher ($P < 0.001$) pregnanes and FP4M concentrations than cows fed ad libitum. Daily total faecal pregnane and FP4M did not differ between feeding levels except for faecal 20α-pregnane which was highest for ad libitum fed cows ($P < 0.05$).

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These results showed that the plasma concentrations of P4 in CIDR-treated OVX cows were negatively associated with the level of feeding. Level of feeding and dose of P4 affected the concentrations of FP4M, but the daily excretion rate of FP4M was not positively influenced by the level of feeding.

Keywords: Progesterone; Faecal progesterone metabolites; Cattle-endocrinology; Feeding; Nutrition

1. Introduction

Several management factors may contribute to the efficiency of reproductive performance in the postpartum dairy cow. The most important nutritional factor is the overall energy balance of the animal (Beam and Butler, 1999). Both feed intake and body tissue reserves are used as an energy source for body function and milk production. The gut and liver of a dairy cow can be considered to operate together as a functional unit. This unit supplies nutrients to the body but metabolises other components, such as steroid hormones, to be voided into the bile. Alterations in liver metabolism by changing the amount of feed offered could arise as a result of variation in metabolic events associated with milk production. It has been shown that the nutritional flushing response observed in sheep may be associated with an increased concentration of mixed function oxidase (MFO) enzymes, which could increase the metabolism of steroids (Thomas et al., 1987).

Progesterone supplementation and high level of blood P4 concentration during dioestrus can enhance the conception rates in dairy cattle (Meisterling and Dailey, 1987; Lamming et al., 1989; Macmillan and Peterson, 1993). It has also been shown that low P4 concentrations in early dioestrus may initiate premature luteolysis (Erb et al., 1976) and increase the incidence of developmentally delayed conceptuses (Robinson et al., 1989; Kleemann et al., 1994). High P4 concentrations have been suggested to enhance embryo development (Bulman and Lamming, 1978) while low progesterone post-breeding can reduce fertility (Larson et al., 1997). Factors which may alter the dynamics of P4 production and metabolism could also influence pregnancy rates in dairy cows.

The P4 clearance rate from the body may have an influence on the circulating concentration which in turn may influence the conception rate of cows. The concentration of P4 in blood is related to production and clearance rate (Williams and Cumming, 1982). The metabolic clearance rate of progesterone also determines the relationship between progesterone production rate and plasma concentration (Parr, 1992). The inverse relationship between feeding level and concentration of plasma P4 has been attributed to differences in clearance rate of progesterone rather than changes in hormone production (Parr et al., 1987). Cumming et al. (1971) were the first to recognise that modifying the level of feed intake could alter plasma P4 concentration. The cause of this response is thought to be an increase in hepatic blood flow with increased feed intake and consequently an increase in the metabolism of P4 by the liver. Progesterone is rapidly metabolised by the liver and metabolites are excreted into the bile and voided in the faeces (Palme et al., 1996). The most recent data have demonstrated that faecal P4 changes reflect plasma and milk profiles and therefore luteal and/or placental function (Schwarzenberger et al.,

1996b, 1997; Palme et al., 1996). Therefore, the analysis of faecal P4 metabolites has emerged as an appropriate method for monitoring progesterone clearance rate in cattle. Our previous findings (Rabiee et al., 2001a,b) and studies in sheep (Parr, 1992; Parr et al., 1993) showed that the clearance rate of P4 in dairy cows can alter plasma P4 concentration. The present study was conducted to examine the effects of level of feeding and the dose of P4 on plasma and faecal progesterone metabolites (FP4M) in dairy cows.

2. Materials and methods

2.1. Animals and experimental procedures

A total of 24 non-lactating Holstein–Friesian cows were used in a 2 × 2 factorial study. They were non-lactating multiparous Holstein–Friesian cows, aged 4–7 years which were selected from a herd of normally cycling cattle and then ovariectomised (OVX) using an Ecraseur instrument. This procedure was followed with selected antibiotic for 3–4 days post-operatively. Four weeks following the ovariectomy, the cows were allocated at random to four treatments: (i) 1× CIDR and restricted access to pasture; (ii) 1× CIDR and ad libitum access to pasture; (iii) 2× CIDRs and restricted access to pasture; (iv) 2× CIDRs and ad libitum access to pasture. Each cow had one or two intravaginal devices (CIDR, InterAg, NZ) containing 1.9 g P4 inserted into the vagina according to group allocation and these were left in place for 11 days. Cows in the ad libitum groups had unrestricted access to improved pastures of ryegrass and white clover, whereas the restricted group had access to similar pastures for only 4 h per day in two periods between 08.00 and 10.00, and 15.00 and 17.00 h. Following each grazing period, restricted pasture cows then were held as a single group on a bare paddock. Cows were adapted to their pasture for 2 weeks before dosing with slow release chromic oxide capsules and insertion of the CIDR devices. Slow release chromic oxide (Cr_2O_3) capsules (Controlled release device, Captec, NZ, mean release rate 1.43 g active per day) were administered once to allow daily faecal output (FO) to be estimated for a period of 10 days following a 7-day adjustment period. Faecal samples for chromic oxide assay were collected twice daily at 08.00 and 15.00 h. Pasture samples taken during the trial were analysed for dry matter, crude protein and digestibility. Blood and faecal samples were also collected daily for plasma P4 assay faecal P4 metabolites assay. Body weights of the cows were recorded weekly for a period of 5 weeks. The body weight data were analysed only for the last 3 weeks of trial during the P4 treatment.

2.2. Blood 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 of collection (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). The inter-assay CVs were 7.5, 8.0 and 9.0% for low, medium and high concentrations respectively. The

sensitivity of the assay was 0.05 ng/ml. Weekly blood samples were also taken using tubes without anti-coagulant to measure blood metabolites including glucose, non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB) and urea. Blood glucose concentrations were determined using the hexokinase enzymatic system (Trace Scientific, Australia); blood NEFA 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).

Faecal samples were collected daily at 08.00 h 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., 1996a) and the extracts analysed by enzyme immunoassays (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., 1996a,b), 20α-OH-pregnanes (5β-pregnane-3α,20α-diol 3HS:BSA (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., 1996b, 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 assays was 7 ng/g.

A faecal sample was taken from each cow (blank) before chromic oxide administration. 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 (Leaver, 1982) was used to measure the excretion rate of P4 metabolites through the faeces.

2.3. Residual drug content of CIDR

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

2.4. Statistical analysis

The data derived from the last eight consecutive days of the 11-day treatment period were analysed. The effects of time (days), diet and dose of exogenous progesterone (number of CIDRs) on plasma P4, FP4M, blood metabolites, body weight and interactions between diet and time were analysed using GLM with repeated measures analysis of variance included in the model in SPSS v. 9.0 (SPSS, 1999). Levels of feeding and number of CIDR devices (dose of P4) were included in the statistical model. A two-sample *t*-test was used to compare the means of FO between restricted and ad libitum fed cows.

3. Results

Daily FO was greater in ad libitum fed cows (6.3 vs 4.1 kg DM for ad libitum and restricted cows, respectively $P < 0.001$), but similar within each group for cows treated with one or two CIDR devices ($P > 0.05$). The dry matter content of faeces was also similar between groups (11 vs 12%). The pasture grazed by the cows averaged 27% DM, 22% crude protein and 10.2% ME with a DM digestibility of 72%.

The average plasma P4 concentration in cows receiving 2× CIDR devices was significantly higher ($P < 0.001$) than those with a single device for both restricted (1.81 vs 1.44 ng/ml) and ad libitum cows (4.1 vs 3.36 ng/ml). There was no significant interaction between the level of feeding and dose of P4.

The average residual content of the CIDR devices used with cows in the pasture restricted group was 1.30 g compared to 1.26 g in the ad libitum group ($P = 0.04$). Consequently, the estimated amount of P4 released over the 11-day insertion period was lower among cows in the restricted group than those in the ad libitum group (0.60 vs 0.64 g per device).

The faecal concentrations of 20-oxo-, 20α- and 20β- and total FP4M, generally, were significantly higher for restricted than ad libitum pasture fed cows and for cows dosed with two intravaginal P4 devices (Table 1). There were no significant interactions between feeding level and dose of P4, except for faecal 20-oxo-pregnane ($P = 0.001$; Table 1).

Table 1
The concentration and daily total (mean ± S.E.) of faecal 20-oxo-, 20α-OH and 20β-OH pregnanes in OVX non-lactating dairy cows treated with intravaginal progesterone devices

	20-Oxo-pregnane	20α-Pregnane	20β-Pregnane	Total FP4M
1× CIDR				
Restricted				
Concentration (μg/g)	1.1 ± 0.1	3.4 ± 0.6	0.7 ± 0.1	5.2 ± 0.7
Total (mg per day)	4.6 ± 0.8	14.5 ± 3.4	2.8 ± 0.5	22.0 ± 4.0
Ad libitum				
Concentration (μg/g)	0.8 ± 0.06	1.4 ± 0.2	0.4 ± 0.05	2.7 ± 0.3
Total (mg per day)	5.3 ± 0.7	9.5 ± 2.2	2.8 ± 0.6	17.6 ± 3.4
2× CIDR				
Restricted				
Concentration (μg/g)	2.2 ± 0.2	4.5 ± 0.5	1.6 ± 0.3	8.3 ± 0.8
Total (mg per day)	9.2 ± 0.8	18.6 ± 2.0	6.2 ± 0.8	34.1 ± 3.2
Ad libitum				
Concentration (μg/g)	1.4 ± 0.1	2.2 ± 0.2	0.7 ± 0.1	4.4 ± 0.4
Total (mg per day)	8.8 ± 1.2	14.1 ± 1.8	4.7 ± 0.9	27.6 ± 3.7
Levels of significance				
Restricted vs ad libitum				
Concentration	0.001	0.001	0.006	0.001
Total	NS	0.05	NS	NS
1× CIDR vs 2× CIDR				
Concentration	0.001	0.03	0.002	0.002
Total	0.001	0.09	0.001	0.006

Table 2
Body weight during progesterone treatment and concentrations (mean ± S.E.) of blood glucose, NEFA, BHB and urea, and significance of the effect of time in OVX non-lactating dairy cows treated with intravaginal progesterone devices[a]

No. CIDR	Diet	Body weight (kg)	Glucose (mM/l)	NEFA (mM/l)	BHB (mM/l)	Urea (mM/l)
1× CIDR	Restricted	574.8 ± 19	3.5 ± 0.08	0.45 ± 0.04	0.26 ± 0.04	5.2 ± 0.13
	Ad libitum	614.4 ± 18	3.7 ± 0.05	0.28 ± 0.03	0.27 ± 0.02	5.6 ± 0.20
2× CIDR	Restricted	556.0 ± 37	3.7 ± 0.06	0.57 ± 0.12	0.29 ± 0.02	4.7 ± 0.33
	Ad libitum	595.0 ± 7	3.7 ± 0.02	0.27 ± 0.10	0.27 ± 0.02	5.6 ± 0.21
Levels of significance	Restricted vs ad libitum	NS	NS	0.008	NS	0.007
	1× CIDR vs 2× CIDR	NS	NS	NS	NS	NS
	Time (weeks)	NS	0.001	0.001	0.004	0.002

[a] NEFA: non-esterified fatty acids; BHB: β-hydroxybutyrate.

The average faecal concentrations of 20-oxo-, 20α- and 20β-pregnanes and total FP4M decreased significantly through time ($P = 0.005$). The level of feeding did not influence the average daily total of faecal 20-oxo-, 20β-pregnanes and total FP4M, but influenced the daily total of faecal 20α-pregnanes which was higher for restricted than ad libitum pasture cows ($P = 0.05$, Table 1). The average daily total of faecal 20-oxo-, 20β-pregnanes and total FP4M were significantly higher (Table 1) for cows treated with two P4 devices and there were no interaction between level of feeding and dose of P4. Recovery rates of P4 metabolites in the faeces were 38% (26–37%) and 30% (17–57%) in cows with 1× CIDR device compared to 32% (23–43%) and 24% (17–36%) for those two groups with 2× CIDR devices ($P = 0.02$). The average recovery rate of P4 metabolites in the restricted pasture groups (33%) did not differ significantly from those in the ad libitum groups (27%).

The average body weight of cows in the restricted pasture groups did not differ significantly from those in the ad libitum groups during the period of P4 treatment (Table 2). The dose of P4 had no significant effect on the concentration of blood metabolites. There were no differences in the concentrations of blood glucose and BHB between restricted and ad libitum cows (Table 2). The average concentration of NEFA was higher in restricted cows (Table 2, $P = 0.008$), while the average blood urea was higher in ad libitum group (Table 2, $P = 0.007$). The concentrations of blood metabolites fluctuated through time in both restricted and ad libitum groups ($P < 0.001$), but these concentrations were not affected by interactions involving either level of feeding and dose of P4.

4. Discussion

The effects of levels of feeding and amount of P4 delivered using CIDR devices on progesterone metabolism in dairy cows were investigated by measuring plasma P4 and FP4M in OVX cows. Plasma P4 concentrations were influenced negatively by the level of

feeding. However, this study failed to establish a quantitative association between plasma P4 concentration and daily total of FP4M.

The average plasma P4 concentrations were consistently lower among ad libitum cows, despite higher delivery rate of P4. Lack of interaction between level of P4 delivered by a CIDR device and level of feeding indicated that both level of feeding and the entry rate of P4 into the systemic circulation could alter plasma P4 concentrations independently. Our previous observations (Rabiee et al., 2001a,b) and other studies in the cow (Sangsritavong et al., 2000; Vasconcelos et al., 1998) and the sheep (Parr, 1992; Parr et al., 1993; Abecia et al., 1995, 1997) support these observations. However, it has been reported that the effect of ad libitum feeding on plasma P4 in cattle is more variable than in sheep. Ad libitum 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. Lomax and Baird (1983) showed that the blood flow rate through the liver was 52% higher in normally fed, lactating cows as compared to non-lactating cows, and was decreased by fasting in both groups of cows. The gut and liver are also able to metabolise steroid hormones to be voided into the bile and then faeces. This may indicate that greater feed intake is associated with higher blood flow to the liver and greater P4 clearance and lower plasma concentrations of P4. However, our study failed to show an apparent association between plasma P4 and the concentration and daily total of FP4M in restricted and ad libitum fed cows. This suggests that other factors rather than the volume of faeces could also affect the excretion rate of P4 and plasma P4 concentrations.

The level of feeding significantly altered the concentrations of FP4M in restricted pasture cows ($P < 0.05$) which contrasts our previous observations (Rabiee et al., 2001b). These showed that FP4M were not affected significantly by level of feeding. The severity of feed restriction (4 vs 2 h), DM content (27 vs 20%) and the digestibility of the pasture (73 vs 65%) differed between our two studies, and may partly explain some of the differences observed in the FP4M concentrations.

Daily total of faecal 20-oxo- and 20β-pregnane were not influenced by the level of feeding (Table 1). Higher daily FO in ad libitum cows (6.3 kg DM) than restricted cows (4.1 kg DM) may indicate that daily excretion rate of FP4M may have been influenced by the concentrations of P4 metabolites in the faeces rather than daily FO. Other factors such as diet composition, passage rate of faeces and re-absorption rate of faecal P4 may also have altered the excretion rate of P4 metabolites. The recovery rates of P4 in the faeces were similar between groups, even though the daily FO was higher in the ad libitum group and entry rates of P4 into the systemic circulation were greater among cows with 2× CIDR devices. These findings differ from our previous observations (Rabiee et al., 2001a,b) which reported that these rates were positively associated with the daily FO. Investigations of faecal P4 changes associated with the control of P4 production from the CL, or progesterone release from the CIDR device in this study, are complicated by the factors that can influence the concentration of FP4M (such as diet composition, frequency of feeding), and by the complex control mechanisms that regulate liver metabolism. There is limited information regarding the effect of feeding on the concentration and daily yield of FP4M in dairy cattle. However, lack of understanding of those parameters involved in progesterone metabolism, makes it difficult to establish a biological explanation for a quantitative relationship between plasma P4 and daily yield of FP4M.

Greater residual content of used CIDR devices in the restricted group (1.30 g) than those in the ad libitum pasture group (1.26 g) are supported with our previous observations with OVX cows (Rabiee et al., 1999) and lactating cows (Rabiee et al., 2001c). These studies also showed that cows which were grazing had higher P4 release rates from a CIDR device compared to those measured from cows in confined conditions. These differences may have been associated with housing or diet as well as other factors which may affect the rate of blood flow to the anterior part of vagina and daily physical activity of cows. Walking may enhance absorption rate of P4 from vaginal fluid, and also alter the frequency or pattern of contact of the device with the vaginal mucosa to consequently increase the amount of P4 released from a device.

Plasma P4 concentrations were higher in cows which received 2× CIDR devices, regardless of the level of feeding. Other studies in dairy cows (Macmillan et al., 1991; Munro, 1987) also showed that simultaneous insertion of three devices into the vagina of cows resulted in a tripling of the plasma P4 concentrations (8.4 vs 2.8 ng/ml). Adams et al. (1992) used different doses of P4 (30, 150 and 300 mg) in heifers and found that the mean plasma P4 concentrations were 2.1, 7.5 and 12.2 ng/ml, respectively. These data suggest that higher plasma P4 level can be maintained by enhancing the entry rate of P4 delivery into the systemic circulation irrespective of level of feeding. Therefore, administration of P4 could have a potential benefit in improving fertility in dairy cows as shown by other studies (Lukaszewska and Hansel, 1980; Garrett et al., 1988; Lamming et al., 1989; Pope et al., 1995).

The level of feeding did not significantly influence the mean body weight groups. Steroids are selectively stored in fat, so that any dietary regime that results in fat mobilisation is likely to result in the release of stored P4. O'Callaghan and Boland (1999) suggested that this may account for some of the increased P4 evident in animals on low dietary intakes. However, the duration of this study was not long enough to measure the body weight response to the level of feeding or estimate any changes in energy status of cows.

5. Conclusions

The higher concentrations of plasma P4 and FP4M in the restricted groups and cows with 2× CIDR suggest that both level of feeding and dose of exogenous P4 could influence the concentrations of P4 in plasma and faeces. The absence of an apparent positive association between level of feeding and daily total of FP4M may indicate that faecal excretion rates of P4 metabolites may be controlled by other factors such as diet composition and digestibility which deserve further studies.

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References

Abecia, J.A., Rhind, S.M., Bramley, T.A., Mcmillen, S.R., 1995. Steroid production and LH receptor concentrations of ovarian follicles and corpora lutea and associated rates of ova wastage in ewes given high and low levels of food intake before and after mating. Anim. Prod. 61, 57–62.

Abecia, J.A., Lozano, J.M., Forcada, F., Zarazaga, L., 1997. Effect of level of dietary energy and protein on embryo survival and progesterone production on day eight of pregnancy in Rasa Aragonesa ewes. Anim. Reprod. Sci. 48, 209–218.

Adams, G.P., Matteri, R.L., Ginther, O.J., 1992. Effect of progesterone on ovarian follicular waves and circulating follicle-stimulating hormone in heifers. J. Reprod. Fertil. 95, 627–640.

Beam, S.W., Butler, W.R., 1999. Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. J. Reprod. Fertil. 54 (Suppl.), 411–424.

Bulman, D.C., Lamming, G.E., 1978. Milk progesterone levels in relation to conception, repeat breeding and factors affecting acyclicity in dairy cows. J. Reprod. Fertil. 54, 447–458.

Cumming, I.A., Mole, B.J., Obst, J., Blockey, M.A., Winfield, C.G., Goding, J.R., 1971. Increase in plasma progesterone caused by undernutrition during early pregnancy in the ewe. J. Reprod. Fertil. 24, 146–147.

Erb, R.E., Garverick, H.A., Randel, R.D., Brown, B.L., Callahan, C.J., 1976. Profiles of reproductive hormones associated with fertile and nonfertile inseminations of dairy cows. Theriogenology 5, 227–242.

Garrett, J.E., Geisert, R.D., Zavy, M.T., Morgan, G.L., 1988. Evidence of maternal regulation of early conceptus growth and development in beef cattle. J. Reprod. Fertil. 84, 437–446.

Kleemann, D.O., Walker, S.K., Seamark, R.F., 1994. Enhanced fetal growth in sheep administered progesterone during the first three days of pregnancy. J. Reprod. Fertil. 102, 411–417.

Lamming, G.E., Darwash, A.O., Back, H.L., 1989. Corpus luteum function in dairy cows and embryo mortality. J. Reprod. Fertil. 37 (Suppl.), 24–252.

Larson, S.F., Butler, W.R., Currie, W.B., 1997. Reduced fertility associated with low progesterone postbreeding and increased milk urea nitrogen in lactating cows. J. Dairy Sci. 80, 1288–1295.

Leaver, J.D., 1982. In: Le Du, Y.L.P., Penning, P.D. (Eds.), Herbage Intake Handbook: Animal Based Techniques for Estimating Herbage Intake. British Grassland Publication, pp. 37–73.

Lomax, M.A., Baird, G.D., 1983. Blood flow and nutrient exchange across the liver and gut of the dairy cow. Br. J. Nutr. 49, 481–489.

Lukaszewska, J., Hansel, W., 1980. Corpus luteum maintenance during early pregnancy in the cow. J. Reprod. Fertil. 59, 485–493.

Macmillan, K.L., Peterson, A.J., 1993. A new intravaginal progesterone releasing device for cattle (CIDR-B) for oestrous synchronization, increasing pregnancy rates and the treatment of post-partum anoestrus. Anim. Reprod. Sci. 33, 1–25.

Macmillan, K.L., Taufa, V.K., Barnes, D.R., Day, A.M., 1991. Plasma P4 concentrations in heifers and cows treated with a new intravaginal device. Anim. Reprod. Sci. 26, 25–40.

Matsubara, C., Neshikawa, Y., Yoshida, Y., Tateamura, K., 1983. A spectrophotometric method for the determination of free fatty acid in serum using acyl-coenzyme A synthetase and acyl-coenzyme A oxidase. Anal. Biochem. 130, 128–133.

McCann, J.P., Hansel, W., 1986. Relationship between insulin and glucose metabolism and pituitary–ovarian functions in fasted heifers. Biol. Reprod. 34, 630–641.

McMurray, C.H., Blanchflower, W.J., Rice, D.A., 1984. Automated kinetic method for D-3-hydroxybutyrate in plasma and serum. Clin. Chem. 30, 421–425.

Meisterling, E.M., Dailey, R.A., 1987. Use of concentrations of progesterone and estradiol 17-β in milk in monitoring postpartum ovarian function in dairy cows. J. Dairy Sci. 70, 2154–2161.

Munro, R.K., 1987. Concentrations of plasma progesterone in cows after treatment with 3 types of progesterone pessaries. Aust. Vet. J. 64, 385–386.

O'Callaghan, D., Boland, M.P., 1999. Nutritional effects on ovulation, embryo development and the establishment of pregnancy in ruminants. Anim. Sci. 68, 299–314.

Palme, R., Fischer, P., Schildorfer, H., Ismail, M.N., 1996. Excretion of infused 14C-steroid hormones via feces and urine in domestic livestock. Anim. Reprod. Sci. 43, 43–63.

Palme, R., Möstl, E., Brem, G., Shellander, K., Bamberg, E., 1997. Fecal metabolites of infused ^{14}C-progesterone in domestic livestock. Reprod. Domest. Anim. 32, 199–206.

Parr, R.A., Davis, I.F., Fairclough, R.J., Miles, M.A., 1987. Overfeeding during early pregnancy reduces peripheral progesterone concentration and pregnancy rate in sheep. J. Reprod. Fertil. 80, 317–320.

Parr, R.A., 1992. Nutrition–progesterone interactions during early pregnancy in sheep. Reprod. Fertil. Dev. 4, 297–300.

Parr, R.A., Davis, I.F., Miles, M.A., Squires, T.J., 1993. Liver blood flow and metabolic clearance rate of progesterone in sheep. Res. Vet. Sci. 55, 311–316.

Pope, W.F., Cárdenas, H., Wiley, T.M., McClue, K.E., 1995. Dose–response relationships of exogenous progesterone shortly after ovulation on oestrous cycle length, blastocyst development and fertility in sheep. Anim. Reprod. Sci. 30, 109–117.

Rabiee, A.R., Macmillan, K.L., Rathbone, M.J., 1999. Effect of feeding management on progesterone release from CIDR devices in ovariectmised dairy cows. Proc. Aust. Soc. Reprod. Biol. 30, 120.

Rabiee, A.R., Macmillan, K.L., Schwarzenberger, F., 2001a. Progesterone metabolism in ovariectomised non-lactating Holstein–Friesian cows treated with progesterone with two levels of feed intake. Anim. Reprod. Sci. 66, 35–46.

Rabiee, A.R., Macmillan, K.L., Schwarzenberger, F., 2001b. Evaluating the effect of feed intake on progesterone clearance rate by measuring faecal progesterone metabolites in deslorelin-implanted grazing dairy cows. Anim. Reprod. Sci. 67, 205–214.

Rabiee, A.R., Macmillan, K.L., Schwarzenberger, F., 2001c. Progesterone clearance rate in lactating dairy cows treated a CIDR device with two different levels of milk yield. Reprod. Nutr. Dev. 41, 309–319.

Rathbone, M.J., Bunt, C.R., Burggraaf, S., Burke, C.R., Macmillan, K.L., 1998. Optimization of a controlled release intravaginal drug delivery system containing progesterone for the control of the estrus in cattle. In: Proceedings of the International Symposium on Controlled Release of Bioactive Materials, vol. 25, Las Vegas, pp. 249–250.

Robinson, N.A., Lselie, E.K., Walton, J.S., 1989. Effect of treatment with progesterone on pregnancy rate and plasma concentrations of progesterone in Holstein cows. J. Dairy Sci. 72, 202–207.

Sangsritavong, S., Combs, D.K., Sartori, R.F., Wiltbank, M.C., 2000. Liver blood flow and steroid metabolism are increased by both acute feeding and hypertrophy of the digestive tract. J. Dairy Sci. 83 (Suppl. 1) (Abstract 221).

Schwarzenberger, F., Möstl, E., Bamberg, E., Pammer, J., Schmehlik, O., 1991. Concentration of progestagens and oestrogens in the feces of pregnant Lipizzan, Trotter and Thoroughbred mares. J. Reprod. Fertil. 44 (Suppl.), 489–499.

Schwarzenberger, F., Francke, R., Göltenboth, R., 1993. Concentrations of faecal immunoreactive progestagen metabolites during the oestrous cycle and pregnancy in the black rhinoceros (*Diceros bicornis michaeli*). J. Reprod. Fertil. 98, 285–291.

Schwarzenberger, F., Möstl, E., Palme, R., Bamberg, E., 1996a. Faecal steroid analysis for non-invasive monitoring of reproductive status in farm, wild and zoo animals. Anim. Reprod. Sci. 42, 515–526.

Schwarzenberger, F., Son, C.H., Pretting, R., Arbeiter, K., 1996b. Use of group-specific antibodies to detect faecal progesterone metabolites during the oestrous cycle of cows. Theriogenology 46, 23–32.

Schwarzenberger, F., Palme, R., Bamberg, E., Möstl, E., 1997. A review of faecal progesterone metabolite analysis for non-invasive monitoring of reproductive function in mammals. Int. J. Mammal. Biol. 62 (Suppl. II), 214–221.

Spitzer, J.C., Niswender, G.D., Seidel, G.E., Wiltbank, J.N., 1978. Fertilisation and blood levels of progesterone LH in beef heifers on a restricted energy diet. J. Anim. Sci. 46, 1071–1077.

Talke, H., Schubert, G.E., 1965. Enzymatishe hammstoffbestimmung in blut und serum im optishen test nach warburg. Klin Wochachr 43, 174–175.

Thomas, D.L., Thomford, P.J., Crickman, J.G., Cobb, A.R., Dziuk, P.J., 1987. Effects of plane of nutrition and phenobarbital during the pre-mating period on reproduction in ewes fed differentially during the summer and mated in the fall. J. Anim. Sci. 64, 1144–1152.

Vasconcelos, J.L.M., Bungert, K.A., Tsai, S.J., Wechsler, F.S., Wiltbank, M.C., 1998. Acute reduction in serum progesterone concentrations due to feed intake. J. Dairy Sci. 81 (Suppl. 1), 226.

Villa-Godoy, A., Hughes, T.L., Emery, R.S., Enright, W.J., Ealy, A.D., Zinn, S.A., Fogwell, R.L., 1990. Energy balance and body condition influence luteal function in Holstein heifers. Domest. Anim. Endocrinol. 7, 135–148.

Williams, C.H., David, D.J., Iismaa, O., 1962. The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry. J. Agric. Sci. Camb. 59, 381–385.

Williams, A.H., Cumming, I.A., 1982. Inverse relationship between concentration of progesterone and nutrition in ewes. J. Agric. Sci. Camb. 98, 517–522.