Effect of grass dry matter intake and fat supplementation on progesterone metabolism in lactating dairy cows

I.A. Hutchinson,[a,c] **R.J. Dewhurst**,[b] **A.C.O. Evans,**[c] **P. Lonergan,**[c] **and S.T. Butler**[a,1]

[a]Teagasc, Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland

[b]Teagasc, Animal & Grassland Research and Innovation Centre, Grange, Dunsany, Co. Meath, Ireland

[c]School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland

[1]Corresponding author. Tel.: +353 2542252

Email address: stephen.butler@teagasc.ie

ABSTRACT

Progesterone (P4) metabolism in dairy cattle can be manipulated by alterations in dry matter intake and diet composition. Our objectives were to determine the effects of grazing allowance and fat supplementation on P4 metabolism in lactating dairy cows. Forty mid- to late-lactation Holstein-Friesian dairy cows were used in a completely randomised block design, with a 2 x 2 factorial arrangement of treatments. Cows were assigned to receive 1 of 2 pasture allowances [ad libitum allowance (AL – 9.5 kg DM/day) or restricted allowance (R – 7 kg DM/day)] and 1 of 2 fat supplementation treatments [750 g/day saturated fat (F) or no fat supplement (NF)]. All cows received an additional 4 kg/d of concentrate. Grass dry matter intake (GDMI) was measured 5 wk after the initiation of dietary treatment. Cows were treated with prostaglandin $F_{2\alpha}$

($PGF_{2\alpha}$) to eliminate the endogenous source of P4, and 2 intravaginal progesterone releasing devices (CIDR) were inserted into each cow for a period of 8 days. Regular blood samples were taken prior to and following the removal of the CIDRs, and analysed for P4 concentrations. The half-life (t½) and metabolic clearance rate (MCR) of P4 was calculated for each cow. There was no effect of GDMI or fat supplementation on the t½ or MCR of P4. There was a tendency for an interaction between GDMI and fat supplementation on the t½ of P4; cows on the R-F diet tended to have a longer P4 t½ than cows on the AL-F diet. It was concluded that greater alterations in GDMI than achieved in the current study are required to change P4 metabolism. A combination of fat supplementation and restricted feeding slows P4 clearance, which may have beneficial implications for fertility.

KEYWORDS: Progesterone metabolism, dairy cattle, fat supplementation, dry matter intake

1. INTRODUCTION

Genetic selection programmes during the last two decades have resulted in a modern dairy cow capable of producing large volumes of milk on high intake diets. The improvement has come at the expense of fertility and longevity, however, and the reproductive performance of dairy cows has been declining over the last fifty years [1]. Embryo loss is the greatest factor contributing to reproductive inefficiency in dairy cows, with combined embryonic and foetal loss rates in high producing dairy cows averaging approximately 60% [2].

There is substantial evidence of a link between embryo survival and systemic concentrations of P4 in both the cycle prior to ovulation and during the early luteal phase of the cycle following insemination [2]. Positive linear and quadratic

relationships have been identified between milk P4 concentrations on days 4, 5, 6 and 7 following insemination, and also between the rate of change in P4 concentrations between days 4 and 7 inclusive, and embryo survival [3,4]. Low circulating P4 concentrations around the time the developing blastocyst arrives in the uterine horn may affect the volume and/or composition of uterine secretions essential for embryo survival, rate of conceptus development and the ability of the embryo to produce bovine interferon-tau (bIFN-τ) [5].

Progesterone in blood is almost completely metabolised in a single pass through the liver [6]. Liver blood flow (LBF) and metabolic clearance rate (MCR) of P4 are elevated by increasing dry matter intake (DMI) in pigs [7], sheep [6], and dairy cattle [8,9]. Increased MCR of P4 reduces peripheral plasma P4 concentrations due to the inability of the corpus luteum to sufficiently increase its rate of P4 secretion to maintain homeostasis [6].

Fat supplementation has been shown to increase plasma P4 concentrations [10-12]. This has been hypothesised to be due to increased plasma cholesterol concentrations, the precursor essential for steroid synthesis [13]. This hypothesis has been questioned by Hawkins et al. [12], however, who observed that fat supplementation resulted in greater P4 half life in circulation compared with control cows receiving no fat supplement. This indicates that reduced MCR is a major contributing factor to the increased plasma P4 concentrations in cows fed a high lipid diet.

Our objective was to determine the effects of herbage allowance and dietary fat supplementation on the half life and clearance rate of plasma P4 in lactating dairy cows. We hypothesised that substituting a proportion of GDMI with supplementary fat would reduce the MCR of P4.

2. MATERIALS AND METHODS

2.1. Animals and Treatments

All experimental procedures involving animals were licensed by the Department of Health and Children, Ireland, in accordance with the Cruelty to Animals Act (Ireland 1876) and the European Community Directive 86/609/EC. Forty mid- to late-lactation (178 DIM ± 12 days SD) Holstein-Friesian cows were blocked on the basis of parity, calving date, body weight (BW) and body condition score (BCS), and randomly assigned to 1 of 2 pasture allowances [ad libitum allowance (AL – 9.5 kg DM/day) or restricted allowance (R – 7 kg DM/day)] and 1 of 2 fat supplementation treatments (750 g/day saturated fat [Palmit 80, Trouw Nutrition, Belfast (F)] or no fat supplement (NF)). The experiment was a completely randomized block design with a 2 × 2 factorial arrangement of treatments. The fat supplements were mixed with 1.5 kg of a dairy concentrate and fed in individual feed troughs prior to morning milking. In addition to the 1.5 kg concentrate added to the fat supplement, fat-supplemented cows received an additional 2.5 kg/day of the same concentrate, offered in the parlour during milking. Non fat-supplemented cows received 4 kg/day of the same concentrate, offered in the parlour during milking. A 3-wk period of acclimatisation to the treatments was allowed before any measurements were taken. The nutrient composition of the F and NF concentrates offered are presented in Table 1.

In order to manage the workload, animals were treated in groups of 4 cows per day, one cow from each treatment. Trans-rectal ultrasonagraphy was carried out on all cows on day 28 of dietary treatment in order determine the stage of the oestrous cycle for each cow. On day 34 (+/-5 days) of dietary treatment, cows received an intramuscular injection of 5 mL $PGF_{2\alpha}$ (Lutalyse, Pfizer Animal Health, Dublin, Ireland), followed by two similar injections at am and pm milkings 11 days later to

regress any corpus luteum present and eliminate endogenous P4 synthesis. Two intravaginal P4 releasing devices (Eazi-breed CIDR containing 1.38 g P4, Pfizer Animal Health) were inserted into each cow the morning after the third $PGF_{2\alpha}$ injection, and removed 8 days later. On the day prior to CIDR removal a jugular catheter was inserted into each cow. Frequent blood samples were taken prior to and after CIDR removal to determine the t½ and MCR of P4. Blood samples were collected into lithium heparin vacutainers (Becton Dickinson, Plymouth, UK) at -60, -45, -30, -15, 0, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420, 540 and 660 minutes relative to CIDR removal. Catheter patency was maintained by flushing 1 mL of heparinised saline during frequent sampling periods. Cows were moved to individual tie-stalls for the period of frequent blood samples, and fed grass silage instead of fresh grass. Feed was removed immediately prior to the initiation of frequent blood sampling, and fresh feed not offered until 3 h after CIDR removal to eliminate the acute effects of feed intake on P4 metabolism. Water was offered ad libitum during the frequent blood sampling period. Cows were milked once a day at 12.00 noon on the day they were in the individual stalls. Trans-rectal ultrasonography was carried out on all cows the day after the frequent bleeding period to check for the presence of a CL. In total, 6 cows (2 AL-NF, 2 R-F, 1 AL-F and 1 R-NF) had a functioning CL during the period of frequent bleeding, and were excluded from subsequent analyses.

2.2. Blood sampling and hormone and metabolite analysis

Blood samples from the coccygeal vessels were collected into lithium heparin vacutainers from each cow before morning milking on the day of initiation of dietary treatment, on day 21 of dietary treatment, and immediately prior to CIDR insertion (day 42 ± 5 days). A blood sample was also taken before the evening milking on the

day of CIDR insertion, and daily thereafter during the P4 supplementation period before the morning milking. Following collection of blood samples from either coccygeal vessels or jugular catheters, all blood samples were centrifuged at 2000 x *g* for 15 min at 5 °C. The plasma was harvested and decanted into 1.5 mL tubes, sealed with an airtight cap and stored at -20 °C until further analysis.

All samples were analysed for plasma P4 concentrations. After removal of CIDR's, progesterone was deemed to have reached baseline concentrations at < 0.5 ng/mL. Data from the first 60 minutes after CIDR removal were used to calculate MCR and t½ data. Blood samples collected on days 1, 21, and mid-way through the P4 supplementation period (day 46 ± 5 days) were analysed for IGF-1 and insulin concentrations. Plasma P4 and insulin concentrations were determined using solid-phase fluoro-immunoassays (AutoDELFIA, PerkinElmer Life and Analytical Sciences, Turku, Finland), with appropriate kits (Unitech BD Ltd., Dublin, Ireland). Plasma IGF-I concentrations were quantified by radioimmunoassay, following ethanol:acetone:acetic acid extraction as described by Butler et al. [14]. The inter and intra-assay coefficients of variation for insulin, IGF-1 and P4 were 11.1 % and 14.2 %, 7.7 % and 8.0 %, and 11.7 % and 10.7 %, respectively.

2.3. Milk production

Milking took place at 07 00 h and 16 00 h daily. Individual milk yields (kg) were recorded at each milking (Dairymaster, Causeway, Co. Kerry, Ireland). Milk fat, protein and lactose concentrations were determined weekly in successive p.m. and a.m. milk samples. The concentrations of these constituents were determined using Milkoscan 203 (Foss Electric, Hillerød, Denmark). Solids-corrected milk (SCM) yield was calculated using the equation of Tyrrell and Reid [15]. All cows were weighed

175 weekly, and body condition score (BCS) was recorded weekly by a single experienced
176 technician on a 1 to 5 scale (1 = emaciated, 5 = extremely fat) with 0.25 increments
177 [16].
178
179 ***2.4. Herbage mass determination and sampling***
180 Paddock herbage mass (HM; > 35 mm) was determined twice weekly by harvesting 2
181 strips (1.2 m × 10 m) per treatment with a motor Agria (Etesia UK Ltd., Warwick,
182 UK). Ten grass height measurements were recorded before and after cutting on each
183 cut strip using a folding pasture plate meter with a steel plate (diameter 355 mm and
184 3.2 kg/m; Jenquip, Fielding, New Zealand). All mown herbage from each strip was
185 collected, weighed and sampled (0.3 kg). A bulk sub-sample of approximately 0.1 kg
186 was taken from each mown strip within each paddock and oven dried for 48 h at
187 40 °C in preparation for chemical analysis. Dry Matter content, ash, ADF, NDF and
188 OMD were measured as described by Wims et al. [17]. A further sub sample of 0.1 kg
189 (fresh weight) of the herbage sample from each mown strip was dried for 16 h at 90
190 °C for DM determination. The chemical composition of the grass offered is presented
191 in Table 1. Cows were managed in four groups of 10 according to treatment (AL-NF,
192 AL-F, R-NF, R-F), and grazing areas were allocated in 12-h blocks.
193 Pre-grazing sward height was determined daily throughout the experiment by taking
194 30 measurements across the two diagonals of the paddock for each treatment, using
195 the plate meter described above. Pre-grazing herbage mass was recorded for each of
196 the four treatments. Post-grazing sward heights were measured immediately after
197 daily grazing.
198
199 ***2.5. Grass dry matter intake***

Individual animal grass DMI (GDMI) was estimated during wk 5 of the experimental period using the n-alkane technique [18] as modified by Dillon and Stakelum [19]. All cows were dosed twice daily, before milking, for twelve consecutive days with a paper bung (Carl Roth, GmbH and Co. KG, Karlesruhe, Germany) containing 500 mg of dotriacontane (C32). From day 7 to 12 of dosing, faecal grab samples were collected from each cow twice daily before morning and evening milking and stored at -20 °C. The faeces samples were then thawed and bulked (12 g of each collected sample) and oven dried for 48 h at 40 °C in preparation for chemical analysis. In conjunction with the faecal collection, the diet of the animals was also sampled. Herbage representative of that grazed was cut to ground level using hand shears in each paddock before a.m. grazing on days 6 to 11 (inclusive) of the GDMI measurement period. Two samples of approximately 25 individual grass snips were taken from each paddock and stored at -20 °C following collection. Herbage samples were then bowl-chopped, freeze-dried, and milled through a 1 mm screen before chemical analysis. The ratio of herbage C33 (tritriacontane) to dosed C32 was used to estimate GDMI. The n-alkane concentration was determined as described by Dillon [20].

2.6. Statistical analysis

All statistical analyses were carried out using SAS (SAS System Inc., Cary NC, USA). Daily measurements of milk yield were collapsed into weekly means. A test for normality was performed on all the blood analyte data using the UNIVARIATE procedure of SAS. Insulin and IGF-1 variables had a non-normal distribution and were log-transformed prior to analysis to generate a normal distribution. Milk production, milk composition, cow body weight, BCS, plasma P4 concentrations

during the CIDR supplementation period, and plasma IGF-1 and insulin data were analysed using mixed models with repeated measures, using the satterthwaite adjustment to calculate denominator degrees of freedom. Grazing allowance, fat treatment, time and their interactions were included in all models. Parity and calving date were included as adjustment variables in all repeated measures models; if non-significant, these variables were removed and the models were re-run.

The t½ and MCR of P4 were calculated using the NLIN procedure in SAS. Data from the first 60 min following CIDR removal were fitted to the following equation:

$f(t) = b * e^{(c*t)}$

where

t = time,

b = parameter for starting concentration of P4,

c = parameter for rate of decay.

Half life and MCR of P4 were calculated using the parameter for rate of decay, using the equations:

t½ (min) = ln(2)/c.

MCR (%/min) = c*100

Half life data were not normally distributed and were square root-transformed prior to analysis to generate a normal distribution. Half life and MCR data were analysed using mixed models (PROC MIXED) in SAS. Grazing allowance, fat treatment and their interaction were included as fixed effects, and block was included as a random effect. Calving date, parity and starting concentration of P4 (parameter b) were included as adjustment variables, but were not significant so were removed from the model for MCR. In the t½ model, there was a tendency for an effect of starting concentration ($P = 0.08$), so this was included in the model. Regression analysis

(PROC REG) was used to determine the relationship between P4 t½ and GDMI, and between mean circulating concentrations of P4 during the CIDR supplementation period and GDMI. In all statistical analyses, data were considered significant when $P < 0.05$ and a trend declared when $P < 0.1$.

3. RESULTS

3.1. Milk production and composition, body weight and body condition score

Milk production and composition, body weight and BCS data are presented in Table 2. Milk yield was 1.48 kg/d less for cows receiving the R grazing allowance compared with cows receiving the AL grazing allowance ($P = 0.002$). Cows receiving the R grazing allowance produced 0.06 kg/d less milk fat ($P = 0.001$), 0.05 kg/d less milk protein ($P = 0.006$) and 0.07 kg/d less milk lactose ($P = 0.02$) compared with cows receiving the AL grazing allowance. Collectively, this resulted in a 1.50 kg/d increase ($P = 0.001$) in SCM yield for cows receiving the AL grazing allowance compared with cows receiving the R grazing allowance. There was no effect of grazing allowance on milk fat, protein or lactose concentration. There was no overall effect of fat supplementation on milk yield or composition. There was, however, an interaction between grazing allowance and fat supplementation on milk fat production ($P = 0.04$). Fat supplementation increased milk fat production for cows receiving the AL grazing allowance by 0.04 kg/d ($P = 0.03$), but did not affect milk fat production for cows receiving the R grazing allowance. There was a tendency towards an interaction between grazing allowance and fat supplementation on SCM yield ($P = 0.06$), as fat supplementation tended to increase SCM for cows receiving the AL grazing allowance ($P = 0.09$), but had no effect on SCM for cows receiving the R grazing

allowance. There was no effect of grazing allowance, fat supplementation or their interaction on body weight or BCS.

3.2. Dry matter intake, pre and post grazing sward heights

Grass dry matter intake and pre and post grazing sward heights are presented in Table 3. Grass dry matter intake was 1.20 kg/d less for cows receiving the R grazing allowance compared with those receiving the AL grazing allowance ($P = 0.008$). Pre-grazing sward heights were not affected by grazing treatments, but post-grazing heights were 0.27 cm lower in paddocks grazed by cows receiving the R grazing allowance compared with paddocks grazed by cows receiving the AL grazing allowance ($P < 0.001$). There was no effect of fat supplementation and no interaction between fat supplementation and grazing allowance on GDMI, pre- or post-grazing heights.

3.3. Plasma progesterone half-life and clearance rate

Progesterone t½, MCR, and plasma P4 concentrations during the CIDR supplementation period are summarised in Table 4. Progesterone clearance profiles after CIDR removal, P4 t½ and MCR data are illustrated in Figure 1. Mean plasma P4 concentrations in all treatment groups had declined to < 0.5 ng/mL by 240 minutes after CIDR removal. There was no effect of fat supplementation or grazing allowance on P4 MCR, and no interaction between these two parameters on P4 MCR. There was no effect of fat supplementation or grazing allowance on P4 t½; however, there was a tendency towards an interaction ($P = 0.095$). Progesterone t½ tended to be 7.1 min longer ($P = 0.095$) for cows receiving the R-F treatment compared with cows

298 receiving the AL-F treatment. There was no relationship between GDMI and P4 t½ (r^2
299 = 0.02, *P* = 0.4).
300 Mean plasma P4 concentrations during the CIDR supplementation period were not
301 affected by grazing allowance. Mean plasma P4 concentrations were 0.79 ng/mL
302 greater (*P* = 0.005) for cows receiving the NF treatment compared with cows
303 receiving the F treatment. Mean plasma P4 concentrations for cows receiving the AL-
304 NF treatment were 1.09 ng/mL greater (*P* = 0.01) compared with cows receiving the
305 AL-F treatment. There was a significant relationship between mean plasma P4
306 concentrations during the CIDR supplementation period and GDMI (r^2 = 0.27; *P* =
307 0.002). The regression equation was:
308 Mean P4 = -0.31 × GDMI + 7.28 + ε
309
310 ***3.4. Plasma insulin and IGF-1 concentrations***
311 There was no effect of grazing allowance (*P* = 0.4), and no interaction between
312 grazing allowance and fat supplementation (*P* = 0.2) on mean plasma insulin
313 concentrations. Mean plasma insulin concentrations were 1.79 µIU/mL (*P* < 0.001)
314 greater for cows receiving the fat supplement compared with those not receiving the
315 fat supplement.
316 Mean plasma IGF-1 concentrations were 20.7 ng/mL greater (*P* = 0.03) for cows
317 receiving the AL grazing allowance compared with cows receiving the R grazing
318 allowance. There was no effect (*P* = 0.6) of fat supplementation on mean plasma IGF-
319 1 concentrations, but there was a tendency for an interaction between grazing
320 allowance and fat supplementation on mean plasma IGF-1 concentrations (*P* = 0.06).
321 Fat supplementation reduced mean plasma IGF-1 concentrations for cows receiving
322 the AL grazing allowance, but increased mean plasma IGF-1 concentrations for cows

receiving the R grazing allowance (P = 0.06). Mean plasma IGF-1 concentrations were 37.7 ng/mL greater for cows receiving the AL-NF treatment compared with cows receiving the R-NF treatment.

4. DISCUSSION

The objective of the current study was to determine the effects of different levels of GDMI and fat supplementation on the t½ and MCR of P4 in lactating dairy cows. The dietary treatments utilised in the current study had little effect on either the t½ or MCR of plasma P4, although there was a tendency for an increased t½ of plasma P4 in fat-supplemented cows on the R grazing treatment.

A number of studies have established a positive association between DMI and the MCR of P4 [9,21]. The lack of an effect of GDMI on MCR or t½ of P4 in the current study is likely due to the relatively small difference (1.20 kg, or 11.9 %) in GDMI between the AL and R grazing treatments. By comparison, the treatments used by Sangsritavong et al. [9] maintained a DMI difference of 7.08 kg DM/d between treatments. Studies in sheep that identified increased MCR of P4 with increasing DMI also utilised much greater increments in DMI than the current study [6].

It seems likely that in the current study the 12% difference in GDMI was insufficient to significantly alter LBF and hence P4 metabolism. This is supported by the work of Rabiee et al. [22], who failed to detect a relationship between DMI and indicators of P4 metabolism at 12 % differences in DMI. The GDMI treatments implemented in the current study were designed to reflect practical differences in DMI that may occur during periods of feed restriction arising from a pasture growth deficit. This is in contrast to Sangsritavong et al. [9], who compared cows on ad libitum intake with cows fed a diet restricted to half the level required for maintenance.

348 The current experiment was initially designed to maintain a 2.5 kg DM/d difference in
349 GDMI between treatments, at lower levels of GDMI than were achieved during the
350 study. It is evident from the grazing measurements data (Table 3) that all cows grazed
351 to a very low post-grazing height, which accounts for the additional intake above the
352 pre-determined levels. In addition to this, cows on the R grazing allowance grazed to
353 a lower post-grazing height compared with cows on the AL grazing allowance,
354 resulting in smaller than expected differences in GDMI between cows on the AL and
355 R grazing treatments.
356 There was no effect of fat supplementation on the MCR or t½ of P4 in the current
357 study. This is in contrast to the findings of Hawkins et al. [12], who demonstrated an
358 increased t½ of P4 when feeding Ca salts of palm oil (Megalac) to beef heifers. The
359 level of inclusion of supplemental fat in the diet in the current study (5 % of DMI)
360 was the maximum rate allowable, while still avoiding potential negative effects on
361 rumen function, and was similar to the inclusion rates used by Hawkins et al. [12] (6-
362 7% of DMI). It is important to note the considerable disparity between the values for
363 P4 t½ in the study of Hawkins et al. [12] (170 and 113 min) compared with the values
364 observed in the current study (29 – 37 min). This variation is most likely due to the
365 vastly different metabolic status of lactating dairy cows compared with the beef
366 heifers. The P4 t½ values observed in the current study are consistent with the work of
367 Miller et al. [23] who estimated a P4 t½ of 33.8 min in cows that were infused with
368 radiolabeled P4. The absence of an effect of fat supplementation on P4 metabolism in
369 the current study is supported by Piccinato et al. [24], who failed to detect any
370 differences in *in vivo* P4 metabolism in cows infused with linseed oil (rich in C18:3),
371 despite observing an inhibitory effect on P4 metabolism *in vitro* when liver slices
372 were incubated with C18:3.

373 It is possible that the duration of the fat supplementation period may provide some
374 explanation for the contrasting results between the current study and those reported by
375 Hawkins et al. [12]. Hawkins et al. [12] fed supplemental fat for at least 150 d prior to
376 measurement of P4 metabolism, at 6-7 % of DMI. In the current study, although
377 levels of fat supplementation were similar (5 % of DMI), fat was fed for
378 approximately 50 d prior to measurement of P4 metabolism.
379 Rabiee et al. [25] reported that CIDR (1 or 2) supplementation increased circulating
380 P4 concentrations in feed-restricted cows compared with ad libitum fed cows on a
381 grass-based diet. Conversely, in the current study we found no differences in plasma
382 P4 concentrations during the CIDR supplementation period between cows on the R
383 and AL grazing allowances. This may be due to the difference in DMI between
384 restricted and ad libitum treatments; in the current study we achieved a difference of
385 1.2 kg DM/d, whereas Rabiee et al. [25] reported a treatment difference of 2.2 kg
386 DM/d.
387 The reduction in plasma P4 concentrations observed in the current study during the
388 CIDR supplementation period in fat-supplemented cows compared with cows
389 receiving no fat indicates either increased P4 metabolism, or possibly impaired
390 delivery of P4 from the CIDR in fat-supplemented cows. It is possible that dietary fat
391 supplementation affects vaginal absorption of P4 from the CIDR, P4 binding proteins
392 in plasma, or both. Further work is required to investigate potential effects of diet on
393 P4 delivery from intravaginal devices.
394 The decrease in plasma IGF-1 concentrations with reduced GDMI in the current study
395 is consistent with previous observations of reduced plasma IGF-1 concentrations with
396 feed restriction [26]. The increase in plasma insulin concentrations with fat
397 supplementation observed in the current study is consistent with Lammoglia et al.

398 [11]. Lemley et al. [27] reported reduced hepatic abundance of the P4 catabolic
399 enzymes *CYP2C* and *CYP3A* in dairy cows infused with insulin. In a subsequent
400 study, Lemley et al. [28] fed dairy cows isoenergetic and isonitrogenous diets
401 designed to cause divergent insulin secretion, and measured P4 metabolism and
402 hepatic *CYP2C* and *CYP3A* abundance. Dietary treatment increased plasma insulin
403 concentrations by 22%, and decreased *CYP2C* and *CYP3A* activity by 50%, resulting
404 in increased P4 t½ [28]. In the current study, plasma insulin concentrations were
405 increased by 48.6% in fat supplemented cows compared to those not receiving fat, but
406 this was not reflected in any changes in P4 metabolism. It is unclear why the
407 relationship between plasma insulin and P4 metabolism observed by Lemley et al.
408 [28] was not replicated in the current study. The elevated plasma insulin
409 concentrations observed in R-F-treated cows may, however, explain the tendency
410 towards an increased P4 t½ in these animals.
411
412 **5. CONCLUSIONS**
413 The dietary treatments utilised in the current study had little effect on the t½ or MCR
414 of plasma P4. The tendency towards a longer t½ in fat-supplemented cows on the R
415 grazing allowance would appear to support our initial hypothesis. The absence of an
416 effect of either GDMI or fat supplementation on plasma P4 metabolism does not
417 support the use of either restricted feeding or fat supplementation as a means to
418 increase plasma P4 concentrations. Previous reports of reduced MCR of P4 in feed-
419 restricted cows required reductions in DMI that would be impractical on commercial
420 dairy farms. The current study does provide evidence, however, that a combination of
421 fat supplementation and restricted feeding slows P4 clearance in lactating dairy cows.
422 This may have practical implications, particularly in pasture-based systems where

423 pasture supply can be limited in spring. Supplementing fat to cows on a restricted
424 pasture diet could increase circulating P4 concentrations, with beneficial implications
425 for embryo survival and fertility.
426
427
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529 **Table 1:** Nutrient composition of the concentrated offered to fat-supplemented (F)
530 and non fat-supplemented (NF) cows, and chemical composition of the grass offered.
531 Values are the means of samples collected throughout the study, followed by the
532 standard deviation of the mean in parenthesis.

Nutrient Composition (DM basis)	Concentrate		Grass
	NF	F	
DM (g/kg)	927.3 (0.81)	927.5 (2.43)	237.6 (28.59)
DM composition (g/kg of DM)			
CP (g/kg of DM)	133.0 (16.60)	104.3 (6.32)	235.4 (39.13)
CF (g/kg of DM)	80.7 (6.06)	53.3 (2.07)	-
OMD	-	-	795.3 (39.81)
NDF	-	-	413.2 (41.72)
ADF	-	-	247.5 (33.52)
Ash (g/kg of DM)	93.8 (2.56)	60.2 (3.60)	160.5 (41.27)
Oil (acid hydrolysis; %)	2.65 (0.37)	31.6 (2.74)	-

533 CP, Crude protein; CF, Crude fibre; OMD, Digestibility of organic matter; NDF, Neutral-detergent
534 fibre; ADF, Acid-detergent fibre.
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Table 2: Milk production and compostion, body weight and body condition score of cows on an ad libitum (AL) or restricted (R) grazing allowance, with (F) or without (NF) fat supplementation

	AL[1]		R[1]			P-value		
	NF[2]	F[2]	NF[2]	F[2]	SEM	G[3]	F[3]	G*F[3]
Milk yield (kg/d)	19.63^{ab}	20.20^{a}	18.63^{bc}	18.24^{c}	0.461	0.002	0.8	0.3
Fat								
kg/d	0.76^{a}	0.80^{b}	0.73^{a}	0.72^{a}	0.027	0.001	0.3	0.04
%	4.06	4.22	4.19	4.15	0.092	0.7	0.4	0.17
Protein								
kg/d	0.66^{ac}	0.69^{c}	0.62^{a}	0.62^{a}	0.022	0.006	0.6	0.3
%	3.39	3.41	3.39	3.36	0.024	0.3	0.9	0.3
Lactose								
kg/d	0.89^{a}	0.92^{a}	0.85^{ab}	0.82^{b}	0.034	0.020	0.9	0.4
%	4.82^{ab}	4.82^{ab}	4.83^{a}	4.78^{b}	0.034	0.5	0.11	0.13
SCM (kg/d)	18.49^{ac}	19.46^{a}	17.76^{bc}	17.19^{b}	0.418	0.001	0.6	0.064
BCS	2.93	2.94	2.97	2.93	0.028	0.3	0.5	0.3
BW (kg)	512	509	507	511	2.9	0.7	0.9	0.2

[abc]Within row means not sharing the same superscript differ significantly (P<0.05)
[1]AL = 9.5 kg DM/d grazing allowance; R = 7 kg DM/d grazing allowance.
[2]NF = 0 g/d fat supplementation; F = 750 g/d fat supplementation.
[3]Effect of grazing allowance (G), fat supplementation (F) and their interaction.

Table 3: Grass dry matter intake (GDMI) of cows on an ad libitum (AL) or restricted (R) grazing allowance, with (F) or without (NF) fat supplementation, and pre- and post- grazing heights in paddocks grazed by cows on an ad libitum (AL) or restricted (R) grazing allowance, with (F) or without (NF) fat supplementation.

	AL[1]		R[1]			P-value		
	NF[2]	F[2]	NF[2]	F[2]	SEM	G[3]	F[3]	G*F[3]
GDMI (kg/d)	11.36[a]	11.25[a]	9.83[b]	10.38[ab]	0.425	0.008	0.6	0.4
Pre-grazing height (cm)	4.92	4.84	4.90	4.87	0.137	0.9	0.7	0.9
Post-grazing height (cm)	3.03[a]	3.04[a]	2.77[b]	2.78[b]	0.043	<.0001	0.8	0.9

[abc]Within row means not sharing the same superscript differ significantly (P<0.05)
[1]AL = 9.5 kg DM/d grazing allowance; R = 7 kg DM/d grazing allowance.
[2]NF = 0 g/d fat supplementation; F = 750 g/d fat supplementation.
[3]Effect of grazing allowance (G), fat supplementation (F) and their interaction.

568 **Table 4:** Half life and clearance rate of plasma P4, and plasma P4 concentrations during the CIDR supplementation period of cows on an ad-
569 libitum (AL) or restricted (R) grazing allowance, with (F) or without (NF) fat supplementation.

	AL[1]		R[1]			P-value		
	NF[2]	F[2]	NF[2]	F[2]	SEM	G[3]	F[3]	G*F[3]
Clearance rate (%/minute)	2.26	2.32	2.32	1.94	0.191	0.4	0.4	0.3
Half life (minutes)[4]	34.2 (27.7 - 41.3)	29.9 (24.4 - 35.8)	31.2 (25.9 - 37.1)	37.0 (30.9 - 43.6)	-	0.5	0.9	0.095
Mean plasma P4 during CIDR supplementation period (ng/mL)	4.96[a]	3.87[b]	4.51[ab]	4.01[b]	0.334	0.6	0.005	0.3

570 [abc]Within row means not sharing the same superscript differ significantly (P<0.05)
571 [1]AL = 9.5 kg DM/d grazing allowance; R = 7 kg DM/d grazing allowance.
572 [2]NF = 0 g/d fat supplementation; F = 750 g/d fat supplementation.
573 [3]Effect of grazing allowance (G), fat supplementation (F) and their interaction
574 [4]Half life values are back-transformed least square means, followed by the 95 % confidence limits in parenthesis
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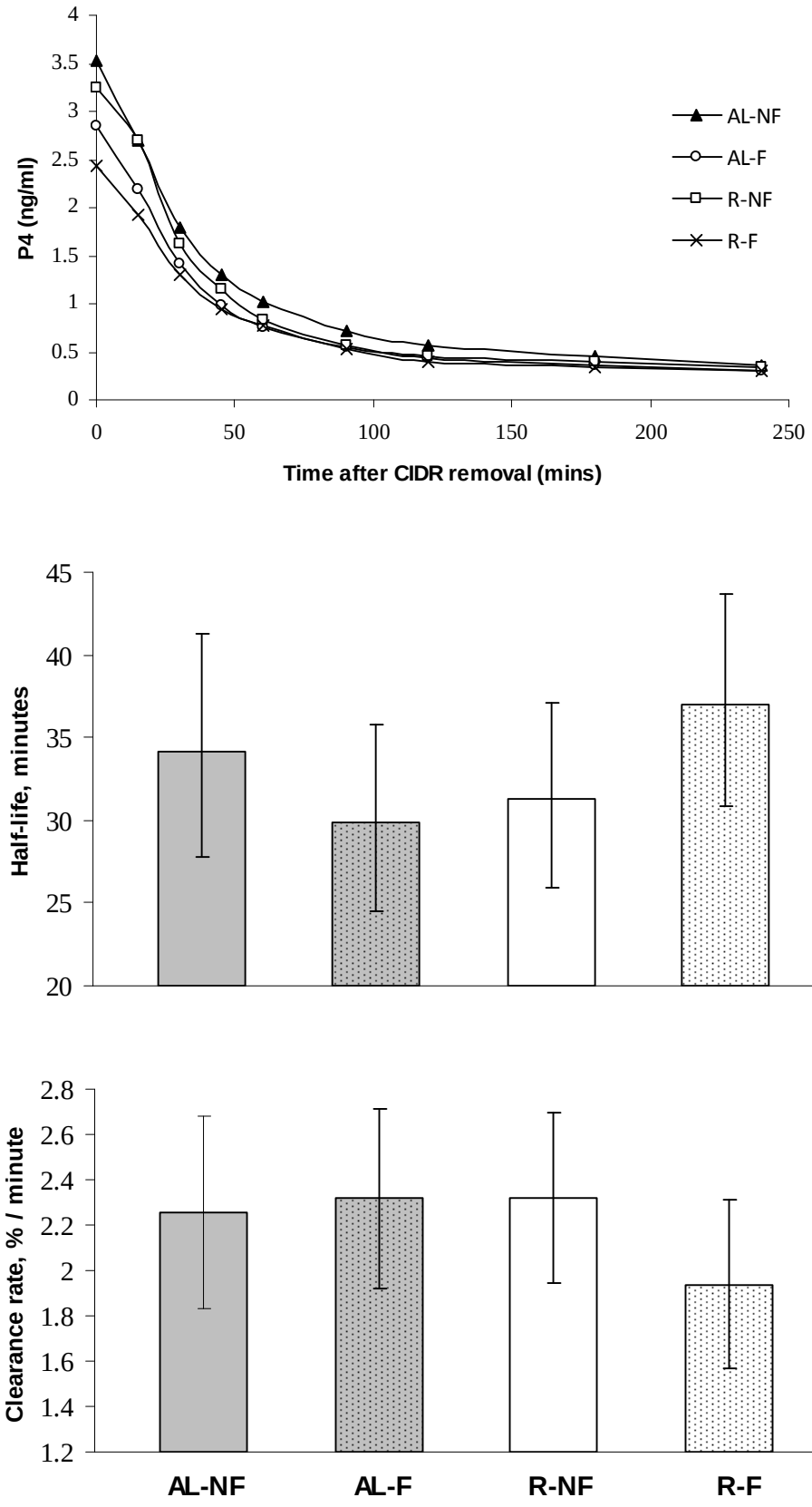

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589 **Figure 1: Hutchinson**

590 **Figure 1:** Progesterone (P4) clearance profiles (upper panel), and half-life (middle
591 panel) and clearance rate (lower panel) of plasma P4 in cows on ad libitum (AL) or
592 restricted (R) grazing allowance, with (F) or without (NF) fat supplementation. Half
593 life and MCR of P4 calculations were performed on data from the first 60 minutes
594 after CIDR removal. Data from 60 – 240 min are included for illustration purposes
595 only. Progesterone clearance profile values are LSM, pooled SEM = 0.215 ng/mL.
596 Half life and clearance rate data are LSM ± 95 % confidence limits.
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