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Full Length Article

Effect of incremental levels of crude degummed canola oil on milk progesterone, plasma luteinizing and follicle stimulating hormones of primiparous Holstein–Friesian cows in a pasture-based system

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Abstract Dietary supplementation of lactating cows with fat can alter the profiles of key reproductive hormones and boost postpartum energy balance. However, published data under Australian pasture-based dairy production conditions are scanty and inconsistent. Therefore, the objective of this study was to determine whether dietary inclusion of crude degummed canola oil (CDCO) at incremental levels for eight-weeks will have significant influence on progesterone (P4), luteinizing hormone (LH) and follicle stimulating hormone (FSH) of primiparous Holstein–Friesian cows grazing pastures. We tested the hypothesis that postpartum supplementation of primiparous Holstein–Friesian cows with dietary CDCO in a pasture-based system will alter the concentrations of P4, LH and FSH reproductive hormones. A random allocation of twenty primiparous Holstein–Friesian cows into four treatment groups that consisted of a wheat-based pelleted basal diet with no supplemental CDCO (control), or a wheat-based pelleted basal diet with CDCO added at 25 ml/kg (low),

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35 ml/kg (medium) and 50 ml/kg (high) was employed in an eight-week feeding trial after two weeks of adjustment. Supplementation levels of CDCO and week of data collection were significant sources of variation ($P < 0.05$) that influenced FSH and P4 concentrations. However, there was no significant effect of supplementation on LH concentration ($P > 0.05$). It was apparent that cows in the high (0.459 ng/ml), medium (0.367 ng/ml) and low (0.251 ng/ml) levels of oil treatments had higher mean plasma FSH concentrations compared to the control (0.172 ng/ml) cows. It was concluded that the current levels of CDCO can be used in pasture-based dairy systems to increase FSH, but not LH and P4.

1. Introduction

Primiparous Holstein–Friesian cows are known to have lower postpartum conception rates than multiparous cows in pasture-based systems [1]. The pressures of milk production accompanied by extreme negative energy balance (NEBAL) at this stage of lactation are the main reasons suggested for poor reproductive performance [2]. The effect of negative energy balance on oestrus cycle is well documented. Energy-challenged cows having cycles of poor reproductive performances have been associated with atypical reproductive hormonal profiles [3]. It has been suggested previously that the secretion of steroids and gonadotropins is inhibited in cows suffering from negative energy balance [3,4]. For instance, cows losing body condition were shown to have lower serum LH compared to cows gaining body condition postpartum [5,6]. A study conducted in the UK revealed that pregnancy rate to first service declined from 55.6% to 39.7% in modern cows [3]. Atypical hormonal pattern of the cows was implicated for the fertility decline [3]. Another study conducted in Tasmania, Australia with over a million records of dairy cows from 428 pasture-based dairy farms, also revealed a decline in fertility [7]. Early resumption of postpartum oestrus cycle is essential for reproductive performance in cows [8]. However, it is dependent on the energy status and availability of adequate circulation of some key reproductive hormones; progesterone (P4), luteinizing hormone (LH) and follicle stimulating hormone (FSH) in plasma [9]. Australian dairy farmers have utilized supplements (mainly wheat and barley) partially in seasons when rainfall is below average to boost energy intake of individual cows to increase milk production. Fat supplementation is not popular in Tasmania because of its unknown effects on performances of cows. However, fat supplementation to dairy cows can provide two benefits; alteration of reproductive hormones and increasing the energy density of the rations consumed by lactating cows [3,10,11]. Studies conducted on the effect of fat supplementation on reproductive hormones in dairy cows have been conflicting and inconsistent [11]. For instance, some studies reported increased P4 concentrations in cows [12,13], while others either found no change [8] or a decrease in P4 concentrations [14]. Some studies have also shown that LH and FSH were influenced by fat supplementation, while in others, they were unaltered or decreased [10,11]. In addition, previous studies have mostly focused on grain-fed stall-barn dairy systems. There is limited published data on fat supplementation in pasture-based systems under Australian conditions. This suggests that further studies in different production systems are required to enable informed choices and tailored decisions when feeding lactating cows with specific dietary fat supplements, hence the justification for our study in a typical Australian pasture-based dairy production system. Therefore, we hypothesized that feeding crude degummed canola oil (CDCO) to primiparous Holstein–Friesian cows for eight weeks in a pasture-based dairy system postpartum, could alter the concentrations of P4, LH and FSH reproductive hormones in milk and plasma. The objective of this study was to determine whether dietary inclusion of CDCO at incremental levels for eight weeks will have significant influence on the concentrations of P4, LH and FSH in primiparous Holstein–Friesian cows grazing pastures.

2. Materials and methods

All experimental procedures were in accordance with the University of Tasmania Animal Ethics Committee guidelines, the 1993 Tasmania Animal Welfare Act and the 2004 Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

2.1. Location

The experiment was carried out at the University of Tasmania's Dairy Research Centre, Tasmanian Institute of Agriculture (TIA) Elliot Dairy Research Farm in Somerset, North-Western Tasmania, Australia, from September to November 2012. Tasmania is Australia's smallest state with a land size of 68,000 sq km and located within the cool, temperate, climatic zone at latitude 42° South and longitude 147° East. It is characterized by four distinct seasons – winter, autumn, spring and summer. The experiment was carried out in spring when the annual rainfall was 2500 mm and humidity was approximately 60%.

2.2. Animals and treatments

The condition and energy status of the experimental cows was visually assessed based on body condition score (BCS) on a scale of 1–8 [15,16]. Twenty 20 primiparous, spring-calving, purebred, Holstein–Friesian cows (average liveweight of 400 ± 40 kg, BCS 4 ± 1 and 40 ± 8 days in milk (DIM), were randomly allocated into 1 of 4 treatments of CDCO (25 ml/kgDM, 35 ml/kgDM and 50 ml/kgDM) and the control (no CDCO – 0 ml/kgDM). This replicated herd of cows ($n = 5$ per treatment group) receiving CDCO supplements was placed under the same management and rotated in electric fenced paddocks with the Control cows offered wheat-based pellets without CDCO. Together, the animals had access to

3000 kgDM of forages, a mixture of ryegrass (*Lolium perenne*), cocksfoot (*Dactylis glomerata*), and white clover (*Trifolium repens*) pasture grazed at the two-leaf stage. Water was offered *ad libitum*. The treated cows grazed the same pasture allotment as the Control cows but were offered CDCO plus wheat-based pellet at the rate of 50 ml/kgDM (for the high level of supplementation group), 35 ml/kgDM (medium level of supplementation group) and 25 ml/kgDM (low level of supplementation group). The current level of CDCO was calculated based on 7% total fat allowed in the diet of grazing cows [17]. Each cow received 6 kg of the pelleted supplements daily for eight weeks, after two weeks of adjustment. Supplements were offered to cows in two splits; morning (3 kg) and evening (3 kg) milking sessions at 0500 h and 1500 h. There was no feed residual left over from any of the groups. The exact pasture intake was difficult to estimate as the case is under grazing conditions. The chemical compositions of the treatment, control and basal feeds are presented in Table 1.

2.3. Feed chemical composition and analysis

Dry matter (DM) content of the basal and experimental diets was determined by drying samples to a constant temperature at 65 °C in a fan forced oven, finely ground to pass through a 2 mm sieve using Laboratory Mill (Thomas Model 4 Wiley® Mill; Thomas Scientific), and further drying at 105 °C for 24 h. The DM was computed as the difference between the initial and final weights of samples. Ash content was determined by combusting samples in a furnace at 600 °C for 8 h. Neutral detergent (NDF) and acid detergent fiber (ADF) contents were measured using an Ankom fiber analyzer (ANKOM220; ANKOM Technology, USA). The analysis for total nitrogen was determined using a Thermo Finnigan EA 1112 Series Flash Elemental Analyzer [18] and the values multiplied by 6.25 to give the crude protein (CP) percentage. Ether extract (EE) was determined using an Ankom fat/oil extractor (ANKOMXT15; ANKOM Technology, USA). Metabolisable energy (ME) was calculated as per Noblet and Perez [19].

2.4. Milk and blood sample collection

Milk samples (n = 480) were collected 3 times a week during morning milking (0500 h) throughout the 8 weeks experimental period to capture the ephemeral progesterone in milk. The milk sample collection was initiated before breeding (day −32) and completed after breeding (day 32). Aliquots of fresh milk samples were collected using milking point controller (MPC; 680) fitted onto the De Laval herringbone milking machine. The milk aliquots were stored in plastic vials at −20 °C until analysed. Blood samples were collected from each experimental cow after the morning milking (0500 h) on the day before and after the initiation of the CDCO treatment, and weekly before and after commencement of oestrous cycle, this collection strategy was largely set to target the pulsatile nature of gonadotropins. The collection of blood samples was restricted by the guidelines of University of Tasmania Animal Ethics Committee. All samples were withdrawn from the coccygeal vein into lithium heparin vacutainers. Approximately thirty minutes thereafter, all collected blood samples were centrifuged using Eppendorf (5810 R) at 1125×g for 10 min at 4 °C. The plasma were harvested and then decanted into 2 ml vials, sealed with an airtight cap and stored at −20 °C until analysed. Milk samples were analysed for milk P4 concentrations, while the plasma samples were analysed for LH and FSH concentrations.

2.5. Synchronization of the oestrus cycle

A double injection protocol over 14 days to synchronize oestrus was initiated in week three of dietary treatment with Ovuprost (2 ml), with artificial insemination on the morning of day 15. Ovuprost has a GnRH-PGF2a format. The Ovuprost was purchased from a local veterinary clinic (Wynyard, Tasmania, Australia) and injected by a trained technician. All cows had heat detector (ESTROTECT™) patches mounted on their caudal region after Ovuprost injection.

2.6. Progesterone assay

Milk P4 concentration was determined by competitive immunoassay, using appropriate P4 ELISA kit (ENZO® Life Science, Lause, Switzerland), as described by Tijssen [20]. Milk P4 assay included a high, medium and low control with equal representation of each treatment in an assay. Three quality control samples were prepared from known concentrations

Table 1 Chemical composition of feed components.

[a,b]Chemical composition (%DM)	[c]Feed components		
	Treatment (canola oil)	Control (No canola oil)	Basal diet (Pasture)
MC	8.2	9.1	5.5
DM	91.8	90.9	94.5
ADF	8.0	9.0	27.7
OM	90.3	91.1	90.7
NDF	20.0	21.1	45.9
EE	6.2	2.1	3.0
Ash	9.7	8.9	9.3
NFC	52.8	59.0	23.9
CP	12.7	10.4	21.0
ME (kJ/100 g DM)	4083.3	4065.7	3999.2

[a] All feeds were analysed based on a dry weight basis.
[b] Moisture content (MC), Dry matter (DM), organic matter (OM), neutral detergent fibre (NDF), acid detergent fibre (ADF), non-fibrous carbohydrate (NFC), ether extract (EE), crude protein (CP) and metabolisable energy (ME).
[c] Treatment = feed with added canola oil. Control = feed without canola oil, Basal diet = feed containing mainly pasture.

of P4 (1 pg/ml) provided in ELISA kit for determination of extraction efficiency of P4 concentration in milk. The milk samples were randomly selected and 1 ml of each sample was extracted once with diethyl ether (1 ml each time). The extraction was dried, re-suspended in 250 μl of assay buffer, vortexed twice and run directly in ELISA assay for P4 analysis. Once the concentration of P4 was confirmed to be sufficient in the milk samples, the remaining samples were then evaluated by non-extraction method directly in ELISA. All the milk samples had high, medium and low control. Each treatment was equally represented in each assay. Samples for a cow on each treatment were completed in single assay. The inter-assay coefficient of variation (CV) for P4 were 6.8% (low), 8.3% (medium) and 2.7% (high) and the intra-assay CV for P4 were 7.6% (low), 5.4% (medium) and 4.9% (high).

2.7. Luteinising and follicle stimulating hormone assay

Plasma LH and FSH concentrations were measured by a double-antibody radioimmunoassay, as previously described for ovine gonadotropins [21,22]. Hormone concentrations and assay quality control data were calculated using the computer programme of [23].

The LH assay used a primary antiserum raised in rabbit (NIH, AFP-240580) against bovine LH. Bovine LH (NIH, AFP-11118B) was used as the assay standard and for iodination.

Briefly, 100 μl assay buffer (0.5% BSA/0.03 M sodium phosphate monobasic/0.12 M sodium phosphate dibasic/ 0.1% sodium azide/0.1% triton-X), first antibody (1:140,000) diluted in 1:2000 normal rabbit serum (NRS) and 100 μl of iodinated bovine LH were added to duplicate plastic tubes containing either standard (0.5–50 ng/ml) or 300 μl bovine plasma. After incubation at 32 °C for 24 h the antibody-bound hormone was separated from the free hormone by the addition of goat-anti-rabbit (GAR) (1:500). The tubes were incubated with second antibody overnight at 32 °C before centrifugation (3200 rpm; 30 min; 4 °C), after which the supernatant was aspirated and the precipitate counted. All samples were assayed in a single assay. The sensitivity of the LH assay was 0.1 ng/ml and the intra-assay coefficient of variation was 7.3%.

The FSH assay used a rabbit anti-bovine FSH antiserum (NIH, AFP-7722291) and bovine FSH (NIH, AFP-9294C) was used as the assay standard and for iodination.

Assay buffer (100 μl), first antibody (1:15,000) diluted in 1:2000 NRS and 100 μl of iodinated bovine FSH were added to duplicate plastic tubes containing either standard (2.5–320 ng/ml) or 300 μl bovine plasma. After incubation at 32 °C for 24 h, second antibody (GAR 1:400) was added. The tubes were incubated at 32 °C before the addition of 100 μl of 10% polyethyleneglycol then incubated for 3 h at 4 °C. This was followed by centrifugation (3200 rpm; 30 min; 4 °C), after which the supernatant was aspirated and the precipitate counted. All samples were assayed in a single assay. The sensitivity of the FSH assay was 0.1 ng/ml and the intra-assay coefficient of variation was 7.7%.

2.8. Statistical analysis

Initially, summary statistics by level and week of CDCO supplementation were computed to give means, standard deviations standard error, variance, minimum and maximum values that were scrutinised for any data entry errors. Testing for linear, cubic and quadratic orthogonal contrasts by regressing the dependent on explanatory variables was carried out using PROC REG (SAS, 2009). However, linear, quadratic and cubic orthogonal contrasts were tested for and found to be inconsequential. Therefore, repeated measures analysis of variance was employed fitting fixed effects and second-order interactions. Subsequently, P4, LH and FSH were analysed by repeated measures analysis of variance using PROC MIXED (SAS, 2009) utilising compound symmetry covariance structure and week of supplementation as the repeated effects. The model included treatment, week of lactation and interaction between treatment and week of lactation as fixed effects, while base line hormone values and cows were fitted as random effects and the degrees of freedom were estimated by the Satterthwaite method (SAS, 2009). Variables of interest having significant treatment and or week of lactation effects are presented in Tables and Figures as pooled LSM ± SEM and differences between means were considered significant at the $P < 0.05$ threshold unless otherwise stated. Significant differences and mean separations were carried out using Tukey's probability pairwise comparison tests (SAS, 2009).

3. Results

3.1. Milk progesterone profile

The reported data focused on the observed temporal changes of mean milk P4 (pg/ml) over the experimental period. It was observed that feeding CDCO to primiparous Holstein–Friesian had no significant effect ($P > 0.05$) on P4 in milk (Table 2). However, as the week of supplementation progressed, CDCO diet supplement appeared to significantly affect ($P < 0.05$) P4. The treatment by period interaction yielded no significant effect ($P > 0.05$) on P4. Weekly trend for mean P4 concentration in milk was very similar across the groups (Fig. 1). The observed temporal changes of milk P4 concentration from day −32 to day 32 indicated that regardless of the group, the secretion of P4 in milk was consistently similar throughout (Fig. 2).

3.2. Plasma luteinizing hormone

There was no significant effect ($P > 0.05$) of feeding CDCO to Holstein–Friesian cows on plasma LH (Table 2). In addition, week of supplementation had no significant effect ($P > 0.05$) on plasma LH. The interaction between treatment and week was also not a significant ($P > 0.05$) source of variation (Table 2). Weekly plasma LH trend was similar regardless of the group (Fig. 3).

3.3. Plasma follicle stimulating hormone

Result of the multivariate analysis of variance (*P*-values) for the effects of treatment and week of supplementation on plasma FSH is presented in Table 2. Treatment had significant ($P < 0.05$) influence on plasma FSH, however, week of supplementation ($P > 0.05$) and interaction between treatment and week of supplementation ($P > 0.05$) did not significantly

Table 2 Multi-trait analysis of variance (*P*-values) for fixed and interaction effects of treatment and week of supplementation on progesterone (P4), luteinising (LH) and follicle stimulating hormones (FSH) in Holstein–Friesian cows.

[a]Effect	[b]Milk and plasma hormones		
	P4	LH	FSH
[c]TRT	0.0832	0.4829	**0.0002**
[d]Week	**0.0293**	0.1364	0.1972
[e]Week * TRT	0.4456	0.4984	0.9999

[a] All *p*-values in bold were significant ($P < 0.05$).
[b] Progesterone hormone (P4, mmol/mL), luteinising hormone (LH, mmol/mL), follicle stimulating hormone (FSH, mmol/mL).
[c] TRT, imposed treatment.
[d] Week, week of supplementation.
[e] Week * TRT, two way interaction of week of supplementation by imposed treatment. Each group had five cows.

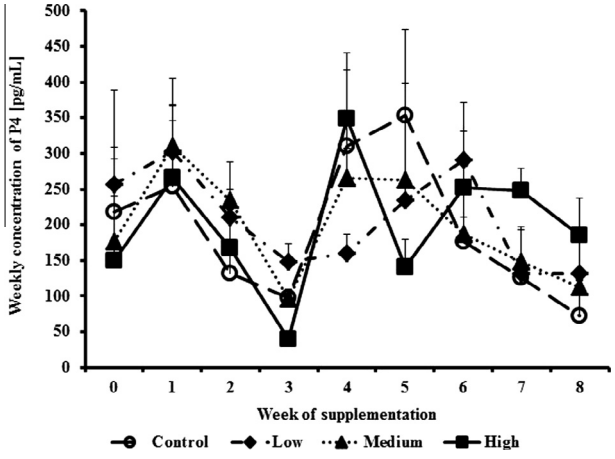

Figure 1 Weekly progesterone profile. Interaction between incremental level of CDCO supplement and week of supplementation on weekly progesterone (P4) concentration in milk of primiparous Holstein–Friesian cows grazing pasture for eight weeks. (control (○), low (♦), medium (▲) and high (■) levels of CDCO). Each group had five cows. *Note:* week 0, week fat before supplementation; week 1, week when fat supplementation started.

affect mean plasma FSH concentration. Cows in the high treatment group consistently produced greater plasma FSH throughout the weeks of supplementation. The trend was followed closely by the medium group cows; whereas the mean FSH concentrations of the low and control groups were lower (Fig. 4). The cows in the high group (0.459 ± 0.05 ng/ml) recorded the greatest total plasma concentration of FSH, followed by the medium group (0.367 ± 0.07), then the low group (0.251 ± 0.02) in comparison to the control (0.172 ± 0.02; Fig. 5).

4. Discussion

4.1. Milk progesterone profile

Progesterone plays a pivotal role in dairy cow reproduction by regulating gonadotropic hormones, oestrus cycle and maintaining

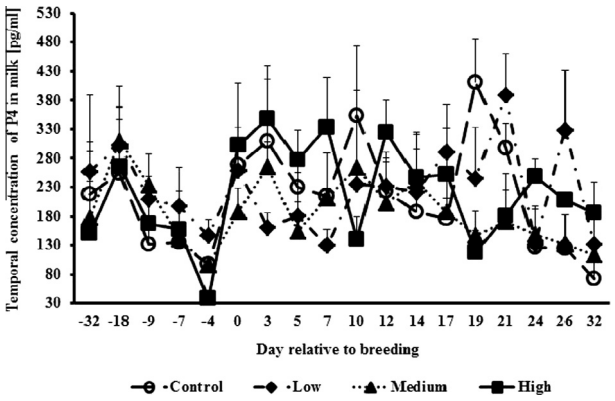

Figure 2 Temporal changes to progesterone profile. Temporal changes in milk P4 concentrations during the treatment period for primiparous Holstein–Friesian. The treatment lasted eight weeks and the cows were subjected to control (○), low (♦), medium (▲) and high (■) levels of CDCO and control (○) diets. Each group had five cows. All values were least square means (±SEM). *Note:* −4 d, synchronization initiated; 0 d, heat detection began; 3 d, breeding initiated.

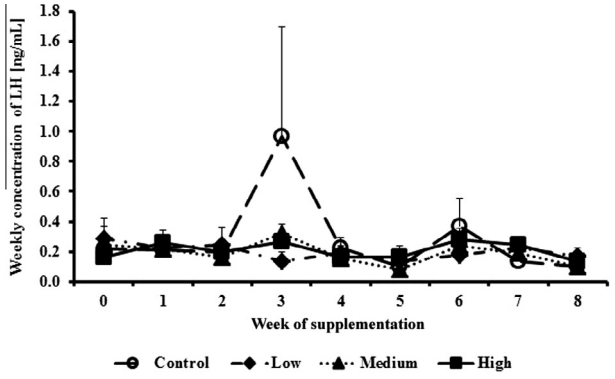

Figure 3 Weekly luteinising hormone profile. Interaction between incremental level of CDCO supplement and week of supplementation on weekly plasma LH concentration of primiparous Holstein–Friesian cows grazing pasture for eight weeks. (control (○), low (♦), medium (▲) and high (■) levels of CDCO). Each group had five cows. *Note:* week 0, week before fat supplementation; week 1, week when fat supplementation started.

pregnancy to term [9]. However, the secretion of progesterone is dependent on the availability of cholesterol *in vivo*. Cholesterol contains high and low density lipoproteins [24], which are precursors for progesterone synthesis [11]. Fat supplements have been used efficiently to manipulate progesterone synthesis by altering the concentration of plasma cholesterol in dairy cows [25]. For instance, feeding dietary fat increased [26] or decreased [14] progesterone synthesis, while in other studies no change was found [8]. However, in the current study feeding a dietary fat source did not influence plasma P4 concentration. Canola is known to contain phytosterol and triglycerol [27]. Plants with high contents of phytosterol are comprised of low cholesterol [27]. A previous study reported that phytosterol can significantly reduce cholesterol in humans with hypercholesterolemic conditions [28]. Therefore, the lack of significant effect observed in the present study could be that CDCO was hypocholesterolemic

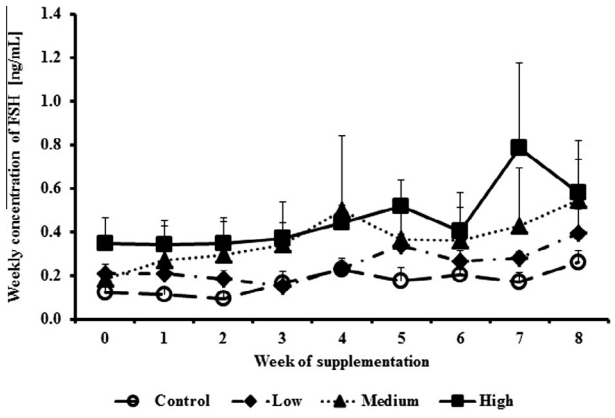

Figure 4 Weekly follicle stimulating hormone profile. Interaction between incremental level of CDCO supplement and week of supplementation on weekly plasma FSH concentration of primiparous Holstein–Friesian cows grazing pasture for eight weeks. (control (○), low (♦), medium (▲) and high (■)). Each group had five cows. *Note:* week 0, week before fat supplementation; week 1, week when fat supplementation started.

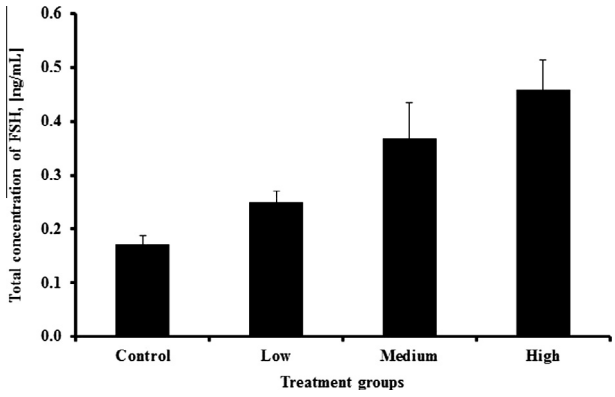

Figure 5 Total follicle stimulating hormone concentration. Total mean concentrations of FSH in plasma of primiparous Holstein–Friesian cows receiving 0 ml kg^{-1}DM (control), 25 ml kg^{-1}DM (low), 35 ml kg^{-1}DM (medium) and 50 ml kg^{-1}DM (high) levels of CDCO supplementation for eight weeks. Each group had five cows.

(low cholesterol). In addition, no significant differences were observed between the unsupplemented and supplemented cows on the concentration of P4 in milk (Table 2). This indicates that the levels of fat used in the current study were inadequate, and that higher levels of CDCO than the current levels used in this study, are probably required. The current result also indicates that the effect of fat supplementation on P4 secretion might be dependent on the dosage of chemical composition (i.e. lipoproteins) and specific fatty acids of the experiment dietary fat. This argument is supported by Staples et al. [11] who stated that the effect of fat on reproduction is independent of the cow's energy status, but rather depends on the specific fatty acid composition of the supplemented fat. Boken et al. [12] also found that enhancement of plasma P4 concentration through fat supplementation was also accompanied by greater body weight loss, which further support that the effect of dietary fat supplement might fatty acids specific. The fat source used in this study was unprotected and may have been vulnerable to rumen biohydrogenation and affected its chemical composition post-ruminal and hence reducing its impact on P4 synthesis [11].

4.2. Plasma luteinizing hormone and follicle stimulating hormone

The lack of changes in the LH concentration during the fat supplementation found in the present study is in agreement with that reported previously [4]. However, contrasting this study is that of (Lucy, 1991 #50) Sklan [29] which showed that feeding primiparous cows with an inert fat source increases their plasma LH concentration during folliculogenesis. However, in the same study, they found that LH concentration was increased at the luteal stage in primiparous cows but not in multiparous cows. This seems to suggest that the effect of supplemented fats on LH synthesis may be elicited at different stages of oestrus cycle in cows at different parity and lactation stages. The non-significant effect of fat observed in the present study, however, could be due to the levels, form and duration of the supplementated fat. Boland and Lonergan [30] have also argued that a pulsatile LH secretion is not affected by short period dietary changes in ruminant. Most of the previous studies had a longer period of fat supplementation [29]. In the present study, feeding only took place for eight weeks. It seems that longer feeding (>8 weeks) might be required for grazing primiparous Holstein Friesian cows. The levels of CDCO used in the current study did not exceed 50 ml kg^{-1}DM. This is because based on calculations, levels above 50 ml kg^{-1}DM would have exceeded the 7% critical level of total fat allowed in the diet of grazing cows. However, greater levels of CDCO supplementation than utilized in this study might be required. The energy level of supplemented fat is usually essential for gonadotropins synthesis [6]. The fat source, CDCO, used in this study had similar metabolisable energy to the control feed (4083.3 vs 4065.7 kJ/100 g DM). Randel [6] argued that a diet low in energy leads to low pulsatile release of LH. Therefore, greater level of CDCO with greater metabolisable energy than currently applied needs to be considered. However, in the present study the concentration of plasma LH was determined from weekly samples. Plasma LH is secreted in a pulsatile manner and the current plasma LH concentration result from weekly samples may not be that informative, because samples may have been collected from between or during a pulse.

Feeding CDCO to primiparous Holstein–Friesian increased the mean plasma concentration of FSH in the present study. A previous study that fed three levels of total dietary fat to dairy cows found no significant differences in plasma FSH regardless of the diet [10]. Availability of FSH in the ovary is associated with the growth and development of ovarian follicles through a cycle of positive and negative feedback mechanisms between the anterior pituitary, hypothalamus and ovary axis [31]. In another study, feeding soybean to beef cows increased the number of medium size follicles compared to fish oil and saturated fat treatments [32]. Vegetable oil contains more linoleic acid (an ω6 fatty acid) as compared with eicosapentaenoic and docosahexaenoic acids (ω3 fatty acids) in fish oil, which suggests that specific fatty acids within the supplemented fat do have specific physiological functions on the secretion of FSH [33]. It is suggested that linoleic acid assists the synthesis

of PGF2α [33]. Increased concentration of PGF2α at uterine level causes the regression of formed corpus luteum, thus stimulating the return to oestrus cycle [33]. As a consequence, the secretion of FSH is initiated in the anterior pituitary gland to facilitate recruitment of ovary follicles [4]. On the other hand, glucose is also spared at the mammary gland as a result of milk fat depression following fat supplementation [34]. Consequently, excess glucose arising from the mammary gland is suggested to be channelled to the hypothalamus–pituitary axis to boost the energy levels and to enable secretion of more gonadotropin hormones [11].

It has been suggested that fat supplementation might have a direct effect on the hypothalamo-pituitary-ovary axis, which would affect the availability of gonadotropins [4]. However, the lack of significant effect of treatment on LH suggests that fat supplements may have a counter effect on gonadotropin hormones, where having a significant effect on one might not be true for the other. This statement is supported by the work of Kane and Hawkins [35] who found that feeding undegradable intact protein at different levels to cycling beef heifers enhanced secretion of FSH, but not LH.

5. Conclusions

Fat supplementation has been reported to improve reproductive performance in dairy cattle by altering the concentrations of key reproductive hormones. In the current study, feeding CDCO to primiparous Holstein Friesian cows for eight weeks increased the plasma FSH as the supplement levels increased. However, no changes were observed in the concentrations of P4 and LH between treatments, which suggests that greater levels of CDCO than were currently used might be required to alter the profiles of P4 and LH in cows. However, the present study does provide evidence that CDCO supplementation to primiparous Holstein–Friesian cows at 25 ml/kg^{-1}DM, 35 ml/kg^{-1}DM and 50 ml/kg^{-1}DM in a pasture-based system could enhance the circulating plasma FSH without affecting the concentrations of LH and P4 in plasma and milk under Australian pasture-based conditions. This could have practical beneficial implications for reproductive success in pasture-based dairy systems, considering that FSH is essential for growth, development and maturation of ovarian follicles. However, due to non-significant differences between supplemented and unsupplemented cows in P4 and LH, we propose that higher levels of CDCO than the current levels used in this study, are probably required. Furthermore, poor reproductive performance experienced by primiparous Holstein–Friesian cows grazing pasture might not be due to atypical hormonal profiles, because other factors may be involved. The full extent of how lipid supplementation alters the dynamics of steroids and gonadotropic hormones in dairy cows still eludes us and warrants further investigation into other molecular genetic factors such as gene expression and mRNA profiles of supplemented cows to provide a better understanding of CDCO's role in future applications as a dietary fat supplement for lactating cows.

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