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Effect of Prepartum Energetic Supplementation on Productive and Reproductive Characteristics, and Metabolic and Hormonal Profiles in Dairy Cows under Grazing Conditions

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Contents

The effect of cracked corn grain supplementation (3.5 kg/day) during 3 weeks before the expected calving date on milk production and composition, body condition score (BCS), metabolic and hormonal profiles and length of postpartum anoestrus was evaluated in multiparous Holstein dairy cows under grazing conditions (Energy supplemented group, n = 10; Control group, n = 10). Body condition score was weekly recorded during the peripartum period, from days −21 to +35 (parturition = day 0). Non-esterified fatty acids, β-hydroxybutyrate, cholesterol, urea, insulin, insulin-like growth factor I (IGF-I), leptin, thyroxine (T_4) and 3,3'' 5-triiodothyroinine (T_3) were weekly determined in plasma from days −21 to +35. The reinitiation of ovarian cyclicity was twice weekly determined by ovarian ultrasonography and confirmed by plasma progesterone concentrations. Cows fed energy concentrate prepartum had higher BCS during the prepartum and postpartum and produced more milk. Non-esterified fatty acids plasma concentrations were significantly higher in the energy group, while cholesterol was higher in the control group. Treated cows had higher levels of plasma insulin, IGF-I and leptin pre-calving. IGF-I, leptin and T_4 were diminished during the early postpartum period in both groups. Insulin levels were also diminished in the control group, but levels remained high in the energy-supplemented group. Treated cows ovulated sooner after parturition than controls. We conclude that Energetic supplementation prepartum in cows under grazing conditions increased milk production and reduced the reinitiation of ovarian activity, consistent with a better EB (BCS), higher prepartum levels of IGF-I, leptin and insulin, and higher insulin levels during early postpartum.

Introduction

During the transition period (3 weeks before to 3 weeks after calving), cows must adapt their metabolism to the high demands of lactation and to a different diet to meet the requirements of milk production (Drackley 1999), and the energy required for harvesting their feed in a pasture-based dairy system (Acosta 1997). As the amount of energy required for maintenance and milk production exceeds the amount of energy obtained from dietary sources, the cow is in a negative energy balance (NEB; Chilliard 1999). Thus, to achieve high levels of milk production in the early postpartum period, the cow must mobilize body reserves that in turn, are related to the energy intake in the dry period. Fat stores are primarily used for lactation and maintenance, with reproductive processes receiving a lower priority (Butler 2003). In previous studies, we found that dairy cows under a grazing system lost between 0.5 and 1 point of body condition score (BCS) in the month before calving (Cavestany et al. 2005), and BCS at calving was related to the first ovulation postpartum (Meikle et al. 2004).

The main endocrine signals that have been suggested to inform the reproductive axis of the energy balance status are insulin, insulin-like growth factor I (IGF-I) and leptin, both in indoor and grazing production systems (Butler 2003; Meikle et al. 2004). Insulin-like growth factor I and insulin act as promoters of follicular growth and thus have an effect on the resumption of ovarian cyclicity (Spicer and Echternkamp 1995). Leptin also may act as a major modulator of reproduction by regulating feed intake and through a local effect in the ovary to regulate follicle size, and possibly oocyte quality (O'Callaghan and Boland' 1999). Nutritional and metabolic status in the early postpartum period can be predicted by IGF-I levels (Zulu et al. 2002) and by insulin that is important in the homeostatic control of energy metabolism. Insulin is positively correlated with energy intake (Chilliard 1999), although different feedstuffs may affect plasma levels of this hormone differently (Rabelo et al. 2001; Robinson et al. 2002). Leptin is involved in the regulation of energy metabolism; it signals the amount of white adipose tissue deposits within the body (O'Callaghan and Boland 1999'; Chilliard et al. 2005). These metabolic hormones (IGF-I, leptin and insulin) are higher in cows in positive energy balance (EB; Lucy 2003). Lactation hypoleptinaemia is probably not due only to negative EB and could play a role to increase the efficiency of energy utilization during both early and late lactation phases (Chilliard et al. 2005). Thyroid hormones are major modulators of metabolism. In dairy cows, plasma T_3 and T_4 concentrations were negatively correlated with daily milk yield (Tiirats 1997). Besides, thyroid hormones have been reported to stimulate ovarian function acting directly on granulosa and thecal cells by increasing progesterone production and aromatase activity (Spicer et al. 2001).

Feeding a higher quantity of fermentable carbohydrates during the prepartum transition period prepares the microbial population to lactation diets, promotes development of ruminal papillae, increases absorptive capacity of the rumen epithelium and reduces lipolysis by delivering more glycogenic precursor to the liver (Grummer 1995; Rabelo et al. 2005). Metabolic response to cows fed high-energy density diets depends

on the form on which energy is given. While some reported that these diets resulted in increased leptin, IGF-I and insulin concentrations during the prepartum period but not during postpartum period, but had no effect on milk production (Holtenius et al. 2003), others did not find this response (Robinson et al. 2002). Similarly, under grazing conditions, Roche et al. (2005) showed that the level of feeding – not referring to energy density and/or concentrate supplementation – during the last month before calving increased prepartum leptin, IGF-I and glucose, but not during postpartum period and had no effect on milk production.

There are many reports in the literature on the relationships of energy balance and fertility, almost all of them related to confined dairy systems with TMR feeding; we did not find in the literature reports that link energy balance with hormonal and metabolic profiles in Holstein cows under a pasture-based production system. In accordance with this, in this study, we aimed to modify the signals that link prepartum EB with postpartum milk production and reproductive performance in grazing Holstein cows, by achieving a better EB through a prepartum energy supplementation concentrate. Our working hypothesis was to determine whether a 3-week prepartum 3.5 kg cracked corn grain supplementation could influence the periparturient metabolic and endocrine changes, milk production and length of postpartum anoestrus in pasture-fed dairy cows with low BCS before calving.

Materials and Methods

Experimental design

From the experimental herd of the dairy farm research station of INIA La Estanzuela (Colonia, Uruguay), 20 multiparous Holstein cows with an average body weight of 578 ± 14 kg and BCS of 2.53.0 ± 0.1 (scale 1–5, Edmonson et al. 1989) were used. The cows were expected to calve at the beginning of the austral autumn (March) and 28 days before the individual expected calving date, cows were separated from the herd and allocated in a different paddock of natural pastures. At day 21 before the expected calving date, cows were assigned to two prepartum treatment groups, considering BCS and days dry. Treatments were: Energy group (n = 10) that received (at 8:00 AM) 3.5 kg/day of cracked corn grain [82% organic dry matter digestibility (ODMD), 9% neutral detergent fibre (NDF) and 7.7% acid detergent fibre (ADF); 13.5 MJ] each cow in individual feeders, and Control group (n = 10) with no concentrate. Cows were offered also bales of hay of improved pasture [legumes (white clover, *Trifolium repens*), and Gramineae (rye grass, *Lolium multiflorum*; 54.5% ODMD, 12.4% crude protein (CP), 60.1% NDF and 47.2% ADF] *ad libitum*, and both groups were kept in separate paddocks of improved natural pastures (11.8% CP, 58.2% NDF and 34.7% ADF; 9.9 MJ). After parturition, both groups were group fed and received the same diet, that consisted in 4.0 kg/day of a commercial concentrate (19.3% CP, 28.3% NDF and 14.1% ADF; 7.1 MJ) administered twice a day individually during milking time. This was complemented with 12 kg/day (fresh base) of corn silage (73.9% ODMD, 6.2% CP, 41.1% NDF and 28.3% ADF; 10.6 MJ) and the animals had access to a daily strip of improved pastures consisting in a mixture of alfalfa (*Medicago sativa*), white clover and tall fescue (*Festuca arundinacea*) (62.5% ODMD, 17.7% CP, 52.4% NDF and 38.3% ADF; 9.2 MJ). Nutrient composition was calculated on a dry matter basis. Under this pasture-based system, grazing is done in different paddocks, which are managed under a rotational system. The estimated distance walked per day was 2 km during the prepartum and 2–2.5 km during the postpartum period, depending on the location of the pasture strip (this distance does not include the one corresponding to grazing). Body condition score was determined weekly from the beginning of the experiment until week 5 postpartum by the same operator. Milk production was recorded daily and then averaged for each week. For determinations of milk composition (fat and protein content), a composite sample of four consecutive milking of each week was taken during the first 5 weeks postpartum. Milk fat production was analysed by the Mojonnier method and milk protein production was analysed by the Kjeldahl method (with a Bentley 2000 equipment; Bentley Instruments Inc., Chaska, MN, USA).

Determination of ovarian cyclicity

The reinitiation of ovarian cyclicity was monitored twice a week by transrectal ovarian ultrasonography using a 5.0 MHz linear probe (Aloka 500; Aloka, Tokyo, Japan). Ovulation was determined by disappearance of the largest follicle followed by the formation of a corpus luteum, which was confirmed by plasma progesterone concentrations, from samples taken twice a week starting at the second week postpartum. The reinitiation of ovarian cyclicity was defined as the day when progesterone increased from basal concentrations in two consecutive samples of >1.6 nM or one sample of >3.2 nM (Meikle et al. 2004).

Blood biochemistry

Blood samples were obtained weekly in the morning before the administration of concentrate by jugular venipuncture in heparinized vacuum tubes, from day −28 to day 35 (parturition: day 0), centrifuged and then stored at −20°C until analysis. Samples analysed were those corresponding to days −21, −7, +7, +21 and +35. Urea was analysed by the Urease UV method with a Weiner (Weiner Laboratories, Rosario, Argentina) kit # 861237004; total cholesterol with the CHOD-PAP method with a Weiner kit # 861231904; non-esterified fatty acids (NEFA) were analysed by the ACS-ACOD (acil-CoA sinthetase & acil-CoA oxidase) method with a NEFA-C kit # 994-75409 of Wako Chemicals (Richmond, VA, USA); β-hydroxybutyrate (BHB) with 3-HBDH-NAD + 3-hydroxybutyrate dehydrogenase-NAD + method (Ranbut, Randox Laboratories Ltd, Crumlin, Co., Antrim, UK). For quality controls, it was used Lyotrol N and P, as well as internal controls of the veterinary laboratory "'Miguel Rubino"' (DILAVE, Uruguay). The intra-assay coefficient of variation was ≤3.7% for all the parameters, and the interassay CV was ≤9.6%.

Hormone determination

Progesterone was determined by radioimmunoassay using a commercial kit (Coat-a-Count; DPC, Diagnostic Products Co., Los Angeles, CA, USA). The intra- and inter-coefficients of variation were 6% and 11%. The sensitivity was 0.1 nM.

Thyroxine (T_4) and 3,3′,5-tri-iodothyronine (T_3) were determined by ^{125}I-Spec RIA-coated tube kits (Institute of Isotopes Co., Ltd, Budapest, Hungary). The sensitivity was 0.5 nM (T_4) and 0.19 nM (T_3). The intra-assays CV were 6.4–8.1% for T_4 and 6.0–8.3% for T_3. The interassays CV were ≤5.8% and ≤6.5%, respectively.

The plasma insulin content was quantified as free insulin with a commercial ^{125}I-labelled radioimmunometric sandwich assay kit (BI-Insulin IRMA kit; CIS Bio International Ltd, Subsidiary of Schering S.A., Gif-Sur-Yvette, France; sensitivity: 0.86 pM; intra-assay and interassay CV: from 1.3% to 5.6% and ≤8.5%, respectively).

The plasma IGF-I concentrations were determined with a ^{125}I-labelled two-site immunoradiometric method, which includes a preceding extraction of IGF-I with an ethanolic HCl solution, and a before-assay neutralization of extracts (DSL-5600 Active IGF-I-coated Tube IRMA Kit; Diagnostic Systems Laboratories Inc., Webster, TX, USA; sensitivity: 0.11 nM; intra-assay and interassay CV: from 3.4% to 6.6% and ≤7.0%, respectively). The assay was run in accordance with the manufacturer's instruction, with the exception of an overnight incubation of the neutralized extract on +4°C (rather than its 3 h incubation on room temperature). Both the insulin and IGF-I assays were validated for bovine plasma and serum samples. In both assay systems, the binding pattern of serially diluted bovine plasma samples was parallel to that of the standard curves. The recovery rates of added known quantity of insulin (35.9, 89.8 and 179.5 pmol per sample) and IGF-I (0.78, 2.60 and 7.80 nmol per sample, given either before or after the extraction) to standard bovine plasma samples (n = 3; all with predetermined low insulin and IGF-I content) were the following: 94–106% (insulin), 78–88% (IGF-I given before extraction) and 89–107% (IGF-I given after extraction).

Plasma leptin concentration was quantified with a ruminant-specific ^{125}I-RIA (Delavaud et al. 2002) adapted and modified by Kulcsár et al. (2006). This version of assay was validated for bovine plasma as defined previously (Meikle et al. 2004; sensitivity: 0.032 nM; interassay and intraassay CV: 12.2%, 5.6%, 6.1%, and 10.1%, 4.6% and 5.3% in ranges of quality control samples with known "'low"', "'medium"' and "'high"' leptin content, respectively).

Statistical analysis

Milk production, BCS, metabolites and hormones concentrations were analysed by the Proc Mixed (Statistical Analysis System, SAS Institute, Cary, NC, USA) and the model included treatment, weekly observations and their interactions. The covariance structure was autoregressive order 1 and cow within treatment was set as random effect. Least-square means were compared with LSD with a significance level of 5%. Previous lactation milk production, BCS at the beginning of the experiment and number of days dry were tested as covariables but the effect was not significant, therefore, they were not included in the analysis. Ovarian cyclicity was analysed by Proc GLM (SAS) with the prepartum treatment as the fixed effect. Proportion of cows ovulating in the early postpartum was analysed by chi-square procedure. Results are presented as least-square means ± pooled standard error. Pearson's correlation coefficients were calculated to study relationships between variables.

Results

Body condition score

Body condition score was similar in both groups at the beginning of the trial, but from 7 days prepartum through remaining the experimental period, cows in the Energy group had higher BCS than cows in the Control group (Table 1, $p < 0.01$), except at calving and at the first week postpartum, where BCS was similar in both groups. In the Energy group, cows increased their BCS during the first 2 weeks of treatment and there was a decrease from the week prior to parturition to 1 week postpartum ($p < 0.01$; Fig. 1). Thereafter, it remained steady during the remaining of the trial, but from weeks 3 to 5 postpartum, it was higher than in the

Table 1. Fixed effects included in the model for measured parameters in cows under grazing conditions (fixed effects are treatment, experimental week and their interaction)

Variable	Treatment	Week	Treatment*week
Body condition score	**	***	*
Milk production	*	***	NS
Non-esterified fatty acids	*	***	0.15
β-Hydroxybutyrate	NS	**	***
Cholesterol	*	***	NS
Urea	0.15	*	**
Insulin	**	*	***
IGF-I	0.09	***	*
Leptin	0.08	***	NS
3,3′5-Triiodothyroinine	0.12	***	NS
Thyroxine	NS	***	NS

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; NS = $p > 0.2$.

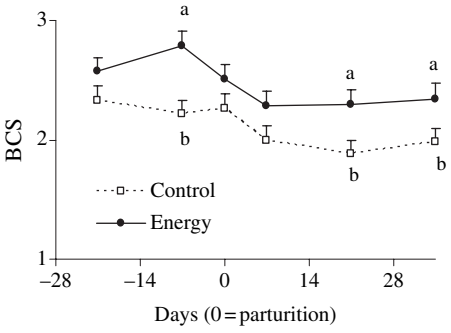

Fig. 1. Evolution of body condition score in Control and Energy (cracked corn prepartum supplementation) groups in dairy cows under grazing conditions (a, b: different letters between treatments at the same point differ, $p < 0.05$)

Control group. BCS in the Control group decreased only in the first week postpartum and did not recover to their initial level during the remaining of the experimental period. In the Control group, BCS score was positively correlated with IGF-I ($r = 0.41$, $p < 0.001$, $n = 43$), leptin ($r = 0.49$, $p < 0.001$, $n = 43$) and T_4 ($r = 0.35$, $p < 0.05$, $n = 43$) and negatively correlated with BHB and urea ($r = -0.25$, $p = 0.08$ and $r = -0.26$, $p = 0.07$, respectively, $n = 43$). BCS was positively correlated with IGF-I, and leptin ($r = 0.37$ and 0.30, $p < 0.05$, $n = 41$) and T_4 ($r = 0.45$, $p < 0.005$, $n = 41$) in the Energy group.

Milk production and composition

Milk production was higher for the Energy group at weeks 2 and 4 postpartum ($p < 0.05$; Table 1, Fig. 2a). In the Control group, milk was negatively correlated with milk protein per cent, IGF-I and leptin ($r = -0.47$, -0.42 and -0.49, respectively, $p < 0.05$, $n = 26$). In the Control group, milk was positively correlated with T_4 ($r = 0.37$, $p < 0.05$, $n = 30$), and to cholesterol ($r = 0.49$, $p < 0.01$, $n = 29$). Milk protein and fat percentage showed a decreasing pattern as the lactation progressed, and there were no treatment differences ($p > 0.5$; Fig. 2b,c). In the Control group, fat was negatively correlated with cholesterol ($r = 0.49$, $p < 0.04$, $n = 20$). In the Energy group was positively correlated to IGF-I and urea ($r = 0.48$ and 0.56, $p < 0.05$, $n = 19$) and negatively correlated with insulin ($r = -0.43$, $p = 0.06$, $n = 19$).

Metabolites and hormones

There was a treatment effect on plasma NEFA levels ($p < 0.05$; Table 1), and while prepartum concentrations were similar in both groups, postpartum levels were higher during the first 2 weeks postpartum in the Energy group. Cows in this group had a marked increase in NEFA after calving and presented a decrease from the first week postpartum. Cows in the Control group did not have a postpartum increase, but also showed a decrease during the postpartum period (Fig. 3a). In the Energy group, NEFA was negatively

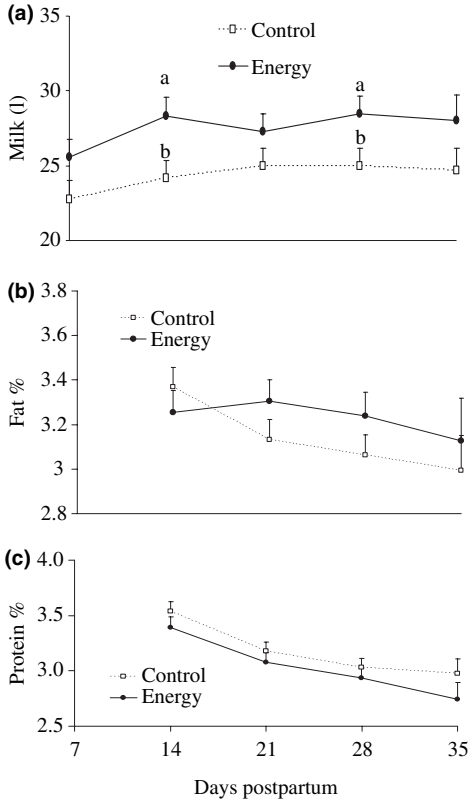

Fig. 2. Milk production (a), milk fat percentage (b) and milk protein percentage (c) in Control and Energy (cracked corn prepartum supplementation) groups in dairy cows under grazing conditions (a, b: different letters between treatments at the same point differ, $p < 0.05$)

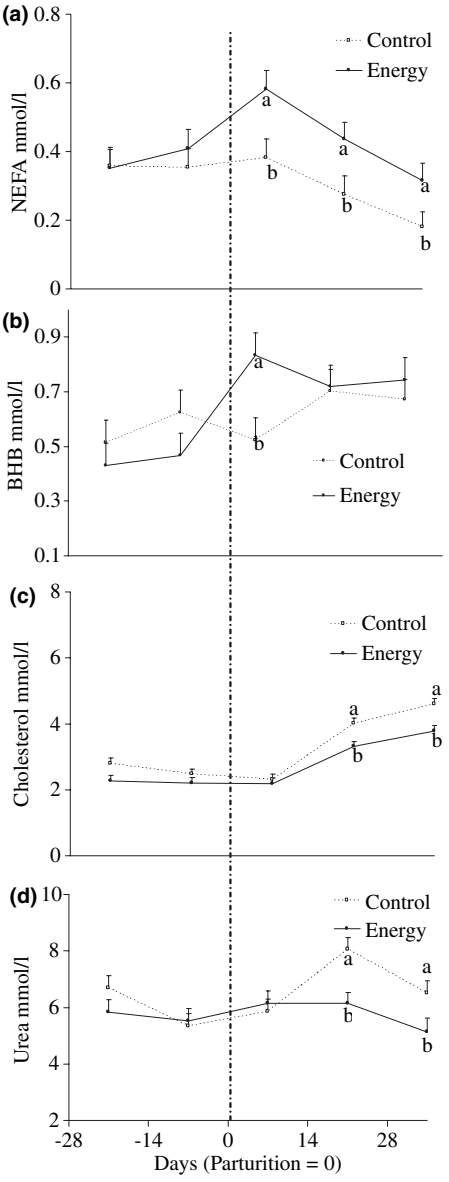

Fig. 3. Levels of (a) non-esterified fatty acids, (b) β-hydroxybutyrate, (c) cholesterol and (d) urea in Control and Energy (cracked corn prepartum supplementation) groups in dairy cows under grazing conditions (a, b: different letters between treatments at the same point differ, $p < 0.05$)

correlated to, T_3 and T_4 ($r = -0.30$ and -0.58, $p < 0.05$ and $p < 0.001$, $n = 49$) and positively correlated to BHB ($r = 0.58$, $p < 0.001$, $n = 50$).

The BHB concentrations were affected by the interaction treatment*week ($p < 0.001$; Table 1). While Energy group had a significant increase after calving and maintained this level for the remaining of the trial, the Control group maintained lower BHB levels until the first week postpartum and increased by the second week postpartum. BHB levels were different in both groups only at the first week postpartum ($p < 0.01$; Fig. 3b). In the Control group, BHB was positively correlated to T_3 and T_4 ($r = 0.56$ and $r = 0.57$, $p < 0.001$, $n = 50$). In the Energy group, BHB was negatively correlated with T_4, IGF-I and leptin ($r = -0.60$, $r = -0.54$ and $r = -0.46$, $p < 0.001$, $n = 49$).

The plasma cholesterol levels were different between groups ($p < 0.05$). There was a significant effect of week, cholesterol levels in the Energy group were higher in weeks 3 and 5 postpartum ($p < 0.05$), but no significant interaction among them (Table 1). Both groups had constant and similar levels during the prepartum, and rose after calving. Treatment differences were found after the third week postpartum, where the Control group had higher levels ($p < 0.05$), and so remained up to the end of the trial (Fig. 3c). In the Control group, cholesterol was positively correlated to BHB, urea, T_3 and T_4 ($r = 0.56$, $p < 0.001$, $r = 0.37$, $p < 0.01$, $r = 0.49$, $p < 0.001$ and $r = 0.29$, $p = 0.06$, $n = 44$) and negatively correlated to leptin ($r = -0.32$, $p < 0.05$, $n = 44$). In the Energy group, BHB was positively correlated to milk, and T_3 ($r = 0.49$, $p < 0.01$, $n = 29$ and $r = 0.38$, $p < 0.01$, $n = 48$), and negatively correlated to IGF-I and leptin ($r = -0.33$ and $r = -0.36$, $p < 0.05$, $n = 48$).

There was a significant effect of week and week*treatment interactions ($p < 0.01$; Table 1) in plasma urea that increased markedly in the Control group during the postpartum period while in the Energy group there was an increase up to the first week postpartum ($p < 0.05$), to return to initial levels at the end of the trial. Urea was higher in the Control group at weeks 3 and 5 postpartum ($p < 0.05$), and decreased in both groups from the third week postpartum to the end of the trial (Fig. 3d). In the Control group, urea was positively correlated to BHB and cholesterol ($r = 0.54$ and $r = 0.37$, $p < 0.01$, $n = 50$). In the Energy group, urea was positively correlated to milk fat per cent ($r = 0.56$, $p < 0.05$, $n = 29$), and negatively correlated to leptin ($r = -0.28$, $p < 0.05$, $n = 48$).

While similar at the beginning of the trial, plasma insulin levels were consistently higher in Energy group ($p < 0.05$), and also showed a treatment*week interaction (Table 1). This treatment difference was due to a marked decrease before parturition in the Control group compared with the Energy group that maintained high levels during the postpartum period (Fig. 4a).

Mean IGF-I levels throughout the experimental period differed, there was also an effect of week ($p < 0.001$) and a significant treatment*week interaction ($p < 0.05$) (Table 1). Treatment difference was because of the higher levels found during the last week prepartum in the Energy group ($p < 0.01$, Fig. 4b). Both groups decreased after calving, and the levels remained low throughout the experimental period. In

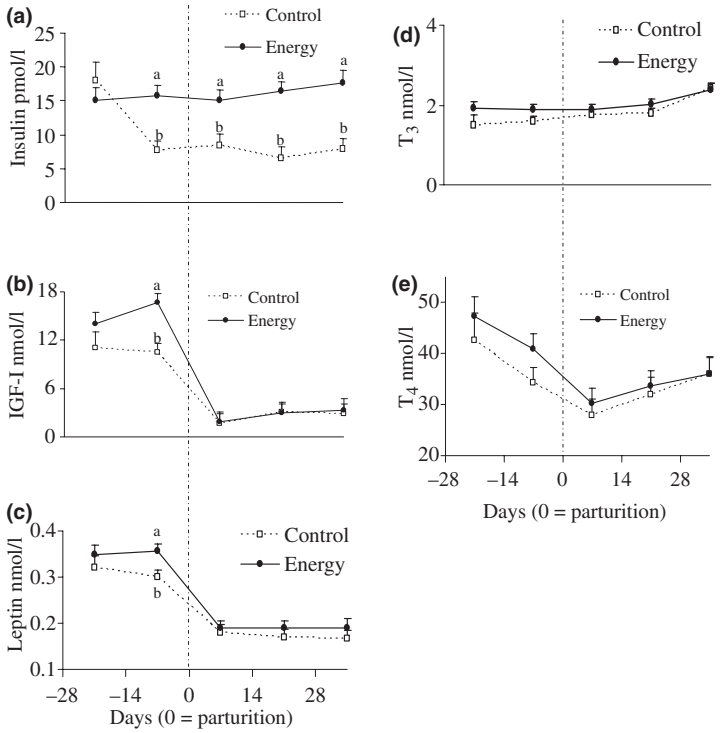

Fig. 4. Concentrations of (a) insulin, (b) IGF-I, (c) leptin, (d) T_3 and (e) T_4 in Control and Energy (cracked corn prepartum supplementation) groups in dairy cows under grazing conditions (a, b: different letters between treatments at the same point differ, $p < 0.05$ for insulin and leptin, and $p < 0.01$ for IGF-I)

the Control group, IGF-I was positively correlated to BCS, leptin and T_4 ($r = 0.41$, $p < 0.01$, $r = 0.95$, and $r = 0.56$, and $p < 0.001$, $n = 44$) and negatively correlated to milk ($r = -0.42$, $p < 0.05$, $n = 29$). In the Energy group, IGF-I was positively correlated to milk fat per cent, BCS, T_4 and leptin ($r = 0.48$, $p < 0.05$, $n = 19$, $r = 0.36$, $p < 0.05$, $n = 41$, $r = 0.60$, $p < 0.01$, $n = 49$, $r = 0.94$, $p < 0.001$, $n = 49$), and negatively correlated to BHB, NEFA and cholesterol ($r = -0.54$, $p < 0.001$, $r = -0.26$, $p = 0.06$, $r = -0.33$, $p < 0.05$, $n = 49$).

Leptin was high during the prepartum period, dropped drastically after calving in both groups, and remained low for the rest of the trial, although it was a not higher in the Energy group than in the Control group (Table 1). Leptin was higher in treated cows during the first week prepartum ($p < 0.05$), but levels were similar in both groups during the postpartum period (Fig. 4c). In the Control group, leptin was positively correlated to BCS, T_4 and IGF-I ($r = 0.49$, $r = 0.60$ and $r = 0.94$, $p < 0.001$, $n = 44$), and negatively correlated to milk production and cholesterol ($r = -0.49$, $p < 0.05$, $n = 26$ and $r = -0.32$, $p < 005$, $n = 44$). In the Energy group, leptin was positively correlated to BCS, T_4 and IGF-I ($r = 0.29$, $p = 0.05$, $n = 41$; $r = 0.59$, $p < 0.001$, $n = 49$ and $r = 0.94$, $p < 0.001$, $n = 49$) and negatively correlated to BHB, and urea ($r = -0.46$, $p < 0.001$, $n = 49$ and $r = -0.28$, $p = 0.05$, $n = 48$).

The T_3 values were not influenced by treatment (Table 1), and increased throughout the experimental period (Fig. 4d). There were no treatment differences in the T_4 levels (Table 1), but both groups decreased until the first week postpartum, and then increased until the end of the trial. The Energy group tend to present higher T_4 levels during the prepartum ($p = 0.12$), but not during the postpartum period (Fig. 4e). T_3 was positively correlated to T_4 (0.34, $p < 0.05$, $n = 44$).

Reproductive parameters

Interval from calving to first ovulation was 25.0 ± 3.7 days for the Energy group and 37.4 ± 3.7 days for the Control group (mean ± SEM, $p < 0.05$). Cows in the Energy group ovulated 12 days sooner than the Control group ($p < 0.05$). While all the cows in the energy group ovulated before 35 days postpartum, only 60% of those in the control group had the first ovulation before this period (Mantel-Haenszel chi-square: 4.75, $p < 0.05$).

Discussion

The administration of an energy supplement to grazing cows increased milk production, BCS during the prepartum and postpartum period, and decreased the postpartum acyclic period. These results are associated with increased prepartum levels of insulin, IGF-I and leptin and postpartum insulin concentrations in the supplemented cows.

The supplementation resulted in an increase in BCS during the first 2 weeks of treatment and until 1 week prior to calving, probably because of the energy content in the corn grain, which probably resulted in a higher estimated energy intake. The better digestion of the nutrients is likely to be produced by a higher activity of the ruminal flora adapted to the high concentrate diet fed in the postpartum period (NRC 2001). The higher BCS of the Energy group during postpartum period could be explained by the better ruminal adaptation to the postpartum diets and a higher dry matter intake (Curtis et al. 1985) as mentioned above.

Energy supplementation increased by 12% the milk production during the experimental period, which could be because of the better adaptation to the postpartum diet. Information regarding the effect of energy supply during prepartum on milk production is conflicting; some studies found an increase (Ingvartsen and Andersen 2000; Overton and Waldron 2004) while others found no effect (Mashek and Beede 2000; Holtenius et al. 2003; Roche et al. 2005) in milk production. These differences might be attributed to the type of supplement, the way of administration and the quantity, as well as the potential production of the cows used in the different experiments. Another factor susceptible to increase milk production in energy group is the higher BCS and the resulting higher body fat mobilization (see plasma NEFA at week 1 in Fig. 3) as reported before (Chilliard 1999; Kokkonen et al. 2005).

The increase in the NEFA levels in the Energy group was associated with a drop in the BCS before and after parturition. This could have been because of an effect of the higher body fatness *per se* (Chilliard et al. 2000), and⁄or to a higher milk production, as also reported by Ingvartsen and Andersen (2000), but opposed to Stockdale and Roche (2002). As the Control group did not show an increase in this metabolite, we suggest that in this group, there was not an NEB that is consistent with the lower milk production and loss of BCS. The decrease in NEFA at the third week postpartum in the Energy group was the consequence of an increase in feed intake, which can be also observed in the BCS improvement. In the energy-supplemented cows, the elevation of NEFA was paralleled with an increased production of BHB. At this moment, the cows in the Energy group were in a deeper NEB, because of a higher milk production and insufficient intake. The increase in the cholesterol levels in the postpartum period could be because of the amount of milk production, to the great energetic demands for lactation that result in an increase of the synthesis of lipoproteins in the liver, but BCS and NEFA profiles suggest that the cholesterol increase is because of a better energy balance as suggested before (Cavestany et al. 2005). The urea increase in Control cows was because of a higher protein catabolism caused by a lower content of glucogenic substrates that forced the cows to use their protein reserves as suggested previously (Jorritsma et al. 2003). These authors found that cows with ruminal flora not adapted to lactation rations might also face higher plasma urea concentrations because of a mismatch between energy and protein at the level of the rumen.

The most interesting finding in our trial was that insulin levels remained consistently higher in the Energy group through all the experimental period. This suggests that feeding more fermentable carbohydrates during the

prepartum transition period may deliver more glucogenic precursor to the liver and enhance insulin synthesis. The higher plasma prepartum insulin concentrations with respect to those in the postpartum period in the control group agree with previous works (Holtenius et al. 2003; Meikle et al. 2004; Kokkonen et al. 2005). Furthermore, increased propionate production as a result of corn-based diet results in an increased secretion of insulin (Brockman and Laarveld 1986). Insulin plays a central role in the homeostatic control of energy metabolism (Chilliard 1999). Dietary changes cause an immediate and rapid stimulus in metabolic hormones and metabolites; the most important of these are glucose and insulin; if the dietary intake is low, insulin concentrations can be reduced (O'Callaghan and Boland' 1999), and this was observed in the Control group that had also lower levels of insulin in the same period. The higher postpartum NEFA together with higher insulinaemia in the Energy group could reflect higher insulin resistance as a result of higher body fatness (Chilliard 1999); although this is true in very obese cows that was not the situation in this study.

In contrast to pre-calving IGF-I profiles that were higher in Energy group, this feeding did not maintain postpartum IGF-I levels as has been previously reported by Lucy (2003) and Roche et al. (2005). After parturition IGF-I decreased despite increasing feed intake (Rhoads et al. 2004). Low plasma IGF-I levels in early lactation are generally a consequence of hypoinsulinaemia (McGuire et al. 1995; Butler 2003); however, in the Energy group insulin levels are high in that period. Cows in poor energy status have low circulating concentrations of IGF-I (Pushpakumara et al. 2003). We do not find an explanation regarding to why the Energy group, with increased insulin concentrations during the postpartum period when compared to Controls, did not differ in IGF-I levels. Chilliard (1999) stated that early lactation cows in negative EB are resistant to GH and that this decreased plasma IGF-I postpartum is independent of insulinaemia. More recently, Butler et al. (2003) reported that the liver is refractory to GH during NEB and this uncoupling of the GH-IGF axis results in diminished plasma concentrations of IGF-I. Mashek and Beede (2000) hypothesized that a nutrition-mediated decline in IGF-I during the peripartum period may prevent a full metabolic adaptation to the nutrient demands of lactation, and consequently reduce milk production.

Prepartum higher levels of plasma leptin in the Energy group agree with Holtenius et al. (2003) and Kokkonen et al. (2005) in cows fed confined, and with Roche et al. (2005), who worked with cows under grazing conditions. Leptin acts as an energy reserve signal, secreted by the white adipose tissue (Chilliard et al. 2005) and this is consistent with the BCS increase found in the Energy group. Furthermore, the higher energy intake acts as a short-term stimulus for plasma leptin (Delavaud et al. 2002). The decrease in postpartum leptin levels has also been reported (Chilliard et al. 2005). Leptin levels remained low and constant throughout the rest of the experimental period for both groups, which is in agreement with findings of Holtenius et al. (2003) and Roche et al. (2005), who found that prepartum levels of feeding did not affect blood leptin concentrations postpartum, but not with the observation of Kokkonen et al. (2005) who found that prepartum supplemented cows had higher postpartum plasma leptin. Plasma leptin content had a positive correlation with BCS and was a good indicator of level of body fat in peripartum dairy cows in agreement with Meikle et al. (2004).

The peripheral tissues try to fit their current local energy metabolism to the postpartum catabolic condition, for example, by interfering in the pathway of thyroid hormones, resulting in diminished circulation as observed here, as previously reported (Meikle et al. 2004). Thyroxine and T_3 are the lowest in early lactation when production of milk is the greatest (Meikle et al. 2004). In our results, this decrease near calving was found only in T_4, while T_3 remained constant all over the experimental period. In dairy cattle, peripheral thyroid hormone metabolism plays a major role in regulating the homeostatic and teleophoretic responses involved in the maintenance of high priority functions (Chilliard 1999).

The supplementation on prepartum cows shortened the intervals from parturition to first ovulation. In disagreement with our findings, Keady et al. (2001) under confined conditions, found that energetic supplementation on the dry period delayed the reinitiation of cyclicity. The onset of postpartum ovarian cyclicity was reported to have a negative correlation with the nadir of the NEB (Butler 2003); the faster the cows recover from NEB the sooner they will start cycling. In our study, energetic supplementation increased the hormonal levels, which have been reported to be related with reproduction in the prepartum period (Spicer and Echternkamp 1995), although in the postpartum period, only insulin levels were higher in the Energy group. In agreement with our results, Gong et al. (2002) reported that the diet resulting in a higher insulin concentration reduced the interval from calving to first ovulation. It is likely that increased insulin concentrations promoted the differentiation and maturation of dominant follicles during early lactation, thereby increasing the chance of these dominant follicles ovulating in response to the LH surge (Butler 2003). Insulin stimulates ovarian progesterone and oestradiol production (Spicer and Echternkamp 1995). Britt (1992) hypothesized that ovarian follicles are detrimentally affected by exposure to NEB during their early growth and development. Roth et al. (2001) reported that an acute stress (e.g. NEB) affected follicular steroidogenesis 24 days later. According to this, we assumed that lower insulin and IGF-I levels in the Control group on the week pre-calving affected steroidogenesis and follicle size, and that could be the cause of the longer postpartum acyclic period on that group. Data regarding leptin and reproductive performance are complex and sometimes contradictory. It has been reported that cows with decreased leptin present delayed onset of cyclicity on the postpartum (Kadokawa et al. 2000). On the other hand, leptin may play a permissive role, when increased above a critical threshold (0.250–0.312 nM according to the review by Chilliard et al. 2005), in the activation of the hypothalamus–pituitary axis and consequent reinitiation of ovarian

activity (Meikle et al. 2004). This would allow cows that had higher BCS in the prepartum and postpartum and thus, higher leptin plasma levels in the prepartum and/or the postpartum period (Meikle et al. 2004; Kokkonen et al. 2005) to shorten the interval calving to first ovulation.

Conclusion

Energetic supplementation administered 3 weeks pre-calving in grazing conditions increased milk production and had a positive effect on the reinitiation of ovarian activity, which is consistent with a better EB (BCS), higher prepartum levels of IGF-I, leptin and insulin, and higher insulin levels during early postpartum period. It must be stressed that parameters used to estimate energy balance (i.e. BCS, NEFA, BHB and insulin) are not always univocal, and thus they cannot be used isolated; evaluation of several of these metabolites and/or hormones should give a more clear information on the energy status of the high producing dairy cow.

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