Effect of sward condition on metabolic endocrinology during the early postpartum period in primiparous grazing dairy cows and its association with productive and reproductive performance

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ABSTRACT

The effect of different sward herbage allowances and a total mixed ration (TMR) management on milk production, body condition, first postpartum ovulation and endocrine/metabolic parameters were investigated. Primiparous Holstein dairy cows ($n = 44$) were randomly assigned to one of the following grazing treatments ($n = 11$ each): high (HA, 30 kg), medium (MA, 15 kg) and low (LA, 7.5 kg) estimated grass DM available/cow/d and a TMR group fed ad-lib from calving to 56 days after calving. Body condition score (BCS) was registered every 15 days from one month before to two months after calving. Non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), cholesterol, plasma protein, albumin, urea, insulin, insulin like growth factor-I (IGF-I) and leptin were determined in plasma every 15 days from 15 days before to 56 days after calving. Progesterone was determined 2 times per week after parturition to determine first ovulation. TMR group had higher milk production in the first 56 days in milk than the HA and MA groups (P<0.05) which did not differ, and were in turn greater than LA cows (P<0.01). Overall, the TMR and HA groups had a greater BCS, protein and albumin concentrations than the other groups, suggesting a better energy balance. While HA cows presented a better metabolic status (smaller BCS losses, lower plasma NEFA and greater urea concentrations) than MA cows during the early postpartum period (15–30 days postpartum, dpp, P<0.05), HA cows differed (greater plasma cholesterol, albumin and urea concentrations) from LA cows later on (45–60 dpp, P<0.05). Greater plasma insulin and IGF-I concentrations were found in the TMR group (P<0.05), which is consistent with the higher nutrient density offered to this group. The reinitiation of ovarian cyclicity was delayed in MA cows one month after calving when compared to TMR and HA cows (P<0.05), which is consistent with the greater NEFA and lower urea concentrations in this period. The lowest probability of first ovulation throughout the study was observed in LA cows (P<0.05), which was associated with their endocrine and metabolic profile. In conclusion, sward allowance affects metabolic signals which in turn are associated with a different productive and reproductive performance.

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1. Introduction

Appropriate nutrition and management strategies during the early postpartum period are crucial to maximize productivity, fertility, and to prevent metabolic diseases in dairy cows (Grummer, 1995; Drackley, 1999). While under indoor feeding conditions the manipulation of the quantity and quality of nutrients for dairy cows can be controlled, nutrient intake cannot be accurately predicted when pasture is the main component of the diet. Moreover, to achieve the best profitability in pasture based dairy production systems the optimum relation among pasture conditions, stocking rate, and animal performance must be determined. Thus, in periods of the productive cycle, dairy cows may be limited in their access to nutrients. Besides, it has been reported that grazing dairy cows do not get sufficient dry matter (DM) intake to sustain the high milk production that could be achieved with their actual genetic potential (Kolver and Muller, 1998).

It is well accepted that the critical period for dairy cows resides in the metabolic adaptation to the negative energy balance (NEB) due to lactation requirements during the first weeks of lactation. The magnitude of the NEB that occurs during the peripartum period can be monitored by metabolic and endocrine profiles in blood (*e.g.*, non-esterified fatty acids (NEFA), insulin, insulin like growth factor I (IGF-I)). Changes in these metabolites and hormones are associated with productive and reproductive performances. It has been reported that NEB is more frequently severe in primiparous than in multiparous dairy cows under grazing conditions, which has been also associated with differential endocrine patterns and longer anovulatory intervals (Meikle et al., 2004; McEvoy et al., 2009; Adrien et al., 2012). Moreover, primiparous cows graze a very low proportion of the allowed grazing time (<35%) and at a very low rate (<25 bites/min, Chilibroste et al., 2012), when compared to multiparous cows from the same herd under different grazing scenarios (Chilibroste et al., 2007), which suggest a more selective grazing process.

We have recently demonstrated in primiparous cows during early lactation, that when daily herbage allowance (DHA) is increased from low (7.5 kg DM/cow/d) to medium (15 kg DM/cow/d) a high response in milk production is obtained (0.43 L/kg extra DHA), while a high DHA (30 kg DM/cow/d) did not differ in milk production from medium DHA (Chilibroste et al., 2012). Interestingly, while the slopes for probability of grazing and bites rates were not different between high and medium DHA, they were greater than of cows on low DHA. Besides, a higher BCS loss was found in medium and low DHA when compared to high DHA (Chilibroste et al., 2012). This led us to hypothesize that the medium DHA achieved a similar milk production than high DHA by maximizing ingestive behaviour processes and energy mobilization, which should be reflected in the endocrine and metabolic parameters. Thus, in order to explain productive and reproductive outcomes after different planes of offered pasture, the present study aimed to contribute to the understanding of the adaptive and integrative responses at productive, ingestive, metabolic, and endocrine level of the primiparous dairy cow in the critical period of early postpartum.

The objective of the present study was to determine the effect of different sward herbage allowances during the first 56 days postpartum in primiparous dairy cows on endocrine and metabolic aspects and its relation with BCS evolution, milk production, and re-initiation of ovarian cyclicity.

2. Materials and methods

2.1. Animals and treatments

Animal experimentation was in compliance with regulations set by the Ethical Committee of the University of Uruguay (Montevideo, Uruguay). The experiment was completed at the EEMAC Research Station, Agronomy Faculty, Uruguay (30° S, 53 W).

Primiparous Holstein dairy cows ($n = 44$, body weight without fasting one month before calving (BW) 595 ± 41 kg, age at calving 2.96 ± 0.11 years and BCS 3.7 ± 0.3) calving between March 25th and April 15th (*i.e.*, during autumn) were selected from the herd of the experimental farm. Cows were blocked by BW, age and BCS, and randomly assigned to one of the following grazing treatments ($n = 11$): high (HA, 30 kg estimated grass DM available/cow/d), medium (MA, 15 kg estimated grass DM available/cow/d) and low herbage allowance (LA, 7.5 kg estimated grass DM available/cow/d), and a control group offered a totally mixed ration in a paddock (TMR group) fed *ad libitum* from calving to 56 days in milk. Cows grazed in a 7-day rotational system wherein the three treatments were moved weekly to a new set of plot adjacent independent grazing paddocks separated by electric fences with the same sward condition. To achieve the targeted sward allowances, the 11 cows per treatment grazed plots of 1, 0.5 and 0.25 ha for HA, MA and LA, respectively. The experiment was designed so that herbage height and mass at the end of the paddock occupation differed among treatments covering a range from restricted condition for grazing (herbage height below 5–7 cm, LA) to non restricted conditions for grazing (HA, herbage height above 10–12 cm). No GP was re-grazed during the experiment. Pre and postgrazing sward mass was estimated with a rising plate metre as described in Chilibroste et al. (2012). Actual DHA were 36.5, 17.2 and 9.1 kg DM/cow/day for HA, MA and LA respectively, values which were within the target range. Cows were milked at 5:00 and 16:00 h and allowed to graze between 8:00 and 15:00 h daily on a 2nd year pasture of 24% tall fescue (*Festuca arundinacea*), 30% birdsfoot trefoil (*Lotus corniculatus*) and 38% white clover (*Trifolium repens*). Chemical composition of pasture samples taken during the experiment varied between 135–172 g CP/kg DM, 440–482 g NDF/kg DM and 292–305 g ADF/kg DM. Mean sward mass availability before grazing was 2750 ± 275 kg/ha DM without significant differences between grazing paddocks Chilibroste et al. (2012). In the 8 weeks of the experiment, the temperature ranged from 13 to 20 °C (mean 16.8), relative humidity ranged from 67 to 95% (mean 74.3%) and precipitation from 0 to 145 mm (mean 72.8 mm), Chilibroste et al. (2012). All cows were individually supplemented at

18:00 h with a mixture of corn silage (10 kg) compound feed (4.8 kg) and grass hay (0.4 kg) on a fresh weight basis. The mixture was formulated according to requirements (NRC, 2001) and with roughage to concentrate ratio close to 55:45. The mixture was designed to meet maintenance metabolizable energy needs plus 8–10 L/d of milk (NRC, 2001), leaving any difference in performance to the effect of treatments. Besides, the 18:00 h meal, the TMR was also offered at 6:30, 10:30 and 14:30 h to the TMR group. Each feeding session lasted for 2 h. Milk production was measured daily. BCS was registered weekly (scale 1 = emaciated, 5 = fat) by one trained observed using the scale of Edmonson et al. (1989).

Blood was collected from the jugular vein in heparinized vials once a week from one month before up to calving at 7:00 h. After calving, bleeding was performed twice a week immediately after milking and before grazing or TMR feeding (TMR group) until two months after calving. Blood samples after the extraction were stored at 4 °C and centrifuged within an hour after collection, and the plasma was stored at −20 °C until analysis.

2.2. Metabolite determination

The metabolic profiles were determined in one assay in the Laboratory DILAVE of Uruguay. Blood biochemistry was analyzed according to the following colorimetric methodologies: cholesterol: CHOD-PAP (Wiener Lab 861231904), total protein: Biuret reaction (Wiener Lab 864102502), albumin: Bromcresol green (Wiener Lab 861250000), and urea: Urease UV (Wiener Lab 861237004). For these determinations commercial kits from Weiner Laboratory (Rosario, Argentina) were used and calibrated with control calibrator serum (Wiener Lab 861244507) on a Vitalab Selectra 2 autoanalyzer (Vital Scientific, Dieren, The Netherlands). For quality controls, Lyotrol N (Ref. 62373), and P (Ref. 62 373), and internal controls of the Laboratory DILAVE were used. Concentrations of NEFA were determined by the method ACS-ACOD (kit NEFA-C, Wako Chemicals, USA) and BHB by the d-3-hydroxybutyrate kit (Randox Laboratories Ltd., Ardmore, UK), control calibrators include in the kits and internal controls of the Laboratory DILAVE were used. The intra-assay CV for all determinations was less or equal than 11%.

2.3. Hormone determination

Concentrations of insulin, IGF-I, leptin and progesterone (P4) were determined in the Laboratory of Nuclear Techniques, Veterinary Faculty, Montevideo, Uruguay. The metabolic hormones were determined every 15 days (periods), while progesterone was determined twice a week from calving until the first two samples considered of luteal concentration. Insulin concentrations were determined by a ^{125}I-Insulin radioimmunoassay (RIA) kit (Diagnostic Products Co., Los Angeles, CA, USA) as previously determined (Pereira et al., 2010). The sensitivity of the assay was 1.1 μIU/mL and the intra-assay CV was 8.2 and 9.4% for low concentration (2.5 μIU/mL) and medium (12.6 μIU/mL) controls, respectively. Concentrations of IGF-I were determined using a immunoradiometric assay with a commercial kit (IGF-I-RIACT Cis Bio International, Gif-sur-Yvette, France) as previously described (Adrien et al., 2012). The sensitivity of the assay was 0.8 ng/mL and the intra-assay CV for control 1 (74 ng/mL) and control 2 (535 ng/mL) were 5 and 8% respectively. Leptin concentrations were determined by a liquid-phase RIA using a commercial Multi-Species Leptin kit (RIA kit, Millipore, Cat XL-85K) previously reported in bovines (Pinotti and Rosi, 2006). The RIA had a sensitivity of 1.4 ng/mL. All samples were determined in the same assay and the intra-assay coefficients of variation for control 1 (4.2 ng/mL) and control 2 (18.8 ng/mL) were 11.6 and 9.7% respectively. Progesterone was determined using a commercial kit (Diagnostic Products Co Los Angeles, CA, USA). The sensitivity of the assay was 0.1 ng/mL and the intra-assay CV for the low (0.5 ng/mL), medium (2 ng/mL) and high (10 ng/mL) controls were 15.6, 8.1 and 5% respectively. The inter-assays CV for the same controls were 16.1, 9.4 and 7% respectively.

2.4. Statistics analyses

The experiment was run as a completed randomized block design. Milk, BCS, hormone and metabolite concentrations were analyzed as repeated measurements in time using MIXED procedure (Statistical Analysis System, SAS Institute, 2002), with a statistical model that included the effect of block, treatment, periods related to calving and interactions. The covariance structure was heterogeneous first order autoregressive and degrees of freedom were adjusted by the Kenward–Rogers method. Tukey–Kramer tests were conducted to analyze differences between groups in the same period and between periods within the same group. For metabolite and hormone concentrations, postpartum days were categorized in intervals of 15 days during the experimental period, and data are presented in graphs as least square means ± pooled standard error (the mean of the interval is labelled in the figure). The reinitiation of ovarian cyclicity was defined as the day when progesterone (P4) concentration in plasma sample was above 1 ng/mL and was followed by another consecutive sample of luteal concentrations. Days to first ovulation was defined as the number of days from calving to reinitiation of ovarian cyclicity. The probability for first ovulation was estimated by the proportion of cows with first ovulation confirmed every 5 days from day 15 to 56 postpartum. The probability of reinitiation of ovarian cyclicity was performed using Genmod. Data are presented in figures as least square means and pooled standard errors. Means were considered to differ when P<0.05, unless stated otherwise.
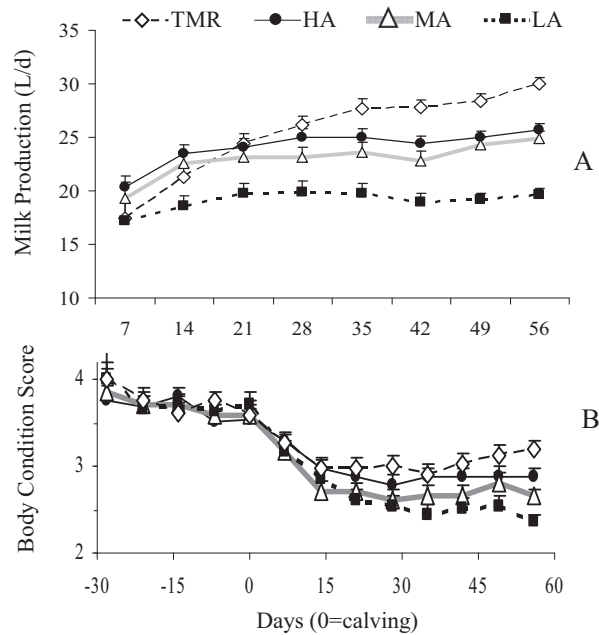
Fig. 1. Milk daily production (A) and body condition score (B, BCS) throughout the study for cows in TMR, high (HA), medium (MA) and low (LA) pasture allowances groups. Milk production and BCS were affected by treatment (P<0.0001 and P=0.01, respectively), period (P<0.0001 for both) and by the interaction treatment and period (P<0.01 and P=0.02, respectively).

3. Results

3.1. Milk production and body condition score

Milk yield was greater (P<0.05) in TMR group (25.4 ± 0.4 L/d) than the HA and MA groups (24.1 ± 0.3 and 23.0 ± 0.40 L/d) which did not differ, and these were in turn greater (P<0.05) than LA cows (19.1 ± 0.4 L/d). While milk production of all groups were different from the LA group already from 14 days postpartum (dpp, P<0.05) until the end of the study, differences among the TMR *vs.* HA/MA groups were found at 35 dpp and thereafter (P<0.05, Fig. 1A). No other differences were detected.

Cows of all the treatments mobilized reserves during the first 3 weeks after calving but while HA cows lost approximately 0.5 point of BCS, LA cows lost 1 point of BCS (Fig. 1B). While differences among the TMR and MA and LA groups were evident throughout the experiment (P<0.05), TMR cows presented greater BCS than HA cows at 49 and 56 dpp. High herbage allowance (HA) cows tended (P=0.06) to present greater BCS than LA cows on 35 dpp and than MA cows on 42 dpp.

3.2. Metabolite concentrations

NEFA concentrations increased on day 15 after calving for all pasture-based groups, and decreased in the first month after calving (P<0.05), while no fluctuations in NEFA concentrations were found in the TMR group (Fig. 2A). On 15 dpp, NEFA concentrations were greater in MA group than the rest of the groups (P<0.05). β-Hydroxylbutyrate concentrations were greater on 15 and/or 30 dpp than at calving in the grazing groups (P<0.05), but no increases were found in the TMR group (Fig. 2B). TMR cows had lower BHB concentrations than the pasture-based groups from day 15 after calving onwards (P<0.05). Cholesterol concentrations were low at calving and increased thereafter in all groups (P<0.05). Cholesterol concentrations in the TMR group were lower than the rest of the groups from 15 dpp until the end of the experiment (P<0.05, Fig. 2C). Cholesterol concentrations in HA cows were greater than LA cows on days 30 and 45 postpartum (P<0.05). Similarly, cholesterol concentration at 45 dpp was greater in MA than LA cows (P<0.05).

Plasma total protein concentrations were low at calving in all groups and increased thereafter. TMR cows had greater protein concentrations than LA cows at 30 and 45 dpp, P<0.05 (Fig. 3A). HA cows had lower protein concentrations than TMR cows at 60 dpp, P<0.05. Albumin concentrations were low at calving and were maintained or decreased (LA group) throughout the study. Greater albumin concentrations were found in TMR and HA cows than LA cows on 30 or 45 dpp, 0.05 (Fig. 3B). Urea concentrations increased along the study (Fig. 3C). MA group presented (P<0.05) or tended (P=0.11) to present lower urea concentrations than HA and TMR groups respectively at days 15 and 30 dpp, respectively. On 60 dpp, urea concentrations in TMR and HA cows were greater than LA cows (P<0.05), and in turn TMR cows had greater urea concentrations than MA cows (P<0.05).
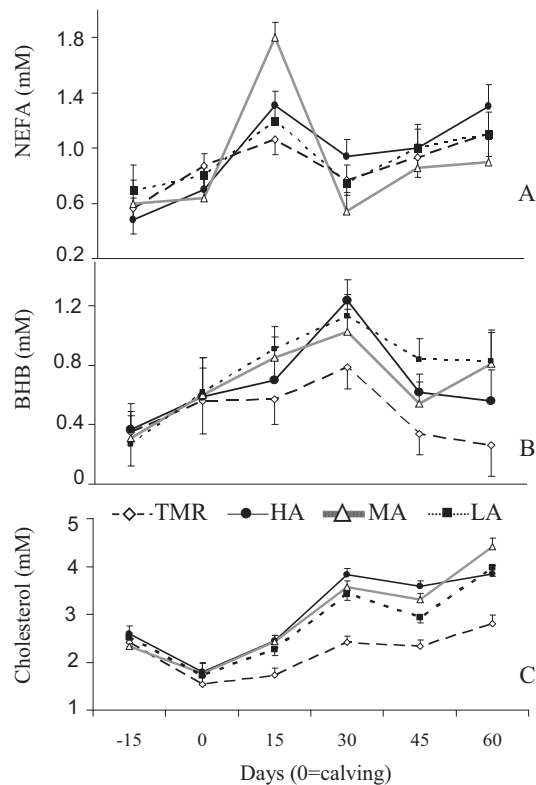
Fig. 2. Plasma non-esterified fatty acids (A), β-hydroxybutirate (B) and cholesterol (C) plasma concentrations throughout the study for cows in TMR, high (HA), medium (MA) and low (LA) pasture allowances groups. Non-esterified fatty acids, β-hydroxybutirate and cholesterol were affected by treatment (P=0.1, P<0.0001 and P<0.0001 respectively), period (P<0.0001 for all) and by the interaction treatment and period (P=0.11, P<0.001 and P<0.0001, respectively).

3.3. Hormone concentrations

A sharp decrease in IGF-I concentrations was observed around calving in all groups and remained low in the pastured-based groups (Fig. 4A). While IGF-I concentrations increased during early postpartum in the TMR group, increases in the HA and MA groups were observed at 60 dpp, and no significant increase could be detected in LA group. The TMR cows presented greater IGF-I concentrations than MA cows at 15 dpp (P<0.05), and tended to differ from all pastured-based groups from 30 dpp (P≤0.15), which become significant thereafter. At the end of the experiment IGF-I concentrations of the TMR group did not differ from HA group, but were greater (P<0.05) or tended (P=0.07) to be greater than LA or MA respectively. No differences among pastured-based groups were found.

Although no main effects were found for insulin concentrations, when means were compared, insulin concentrations in the TMR group increased on 30 dpp and remained high until the end of the experiment, but no increases could be detected in the pasture-based groups. Insulin concentration in the TMR cows was greater than the grazing cows (P<0.05).

Leptin concentrations increased from calving to 15 dpp in the TMR and HA cows and remained constant thereafter (Fig. 4C), but were unchanged in MA and LA cows during the experiment.

3.4. Reinitiation of ovarian cyclicity

The probability of the reinitiation of ovarian cyclicity after calving was affected by the treatment at 30, 35, and 55 dpp (P<0.03) and tended to be affected at 40, 45 and 50 dpp (P<0.08). One month after calving, MA cows had lower probability to reinitiate ovarian cyclicity than TMR and HA cows (Fig. 5). HA cows tended to present a greater probability of cyclicity than LA cows (P=0.15), which in turn tended to be higher than MA cows on day 30 postpartum (P=0.13). On days 40–50 postpartum, TMR cows presented greater probabillity than MA and LA cows, and all groups differed from LA cows at day 55 postpartum.

4. Discussion

This study demonstrates that the nutritional treatments affected the endocrine and metabolic profiles, which were also associated with milk production, BCS, and first postpartum ovulation. The data suggest that nutrient availability modifies
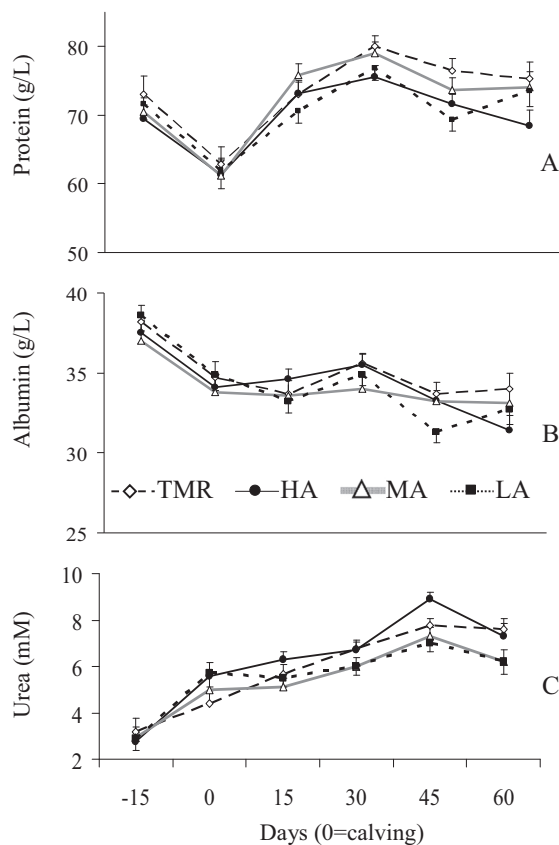
Fig. 3. Plasma total protein (A), albumin (B) and urea plasma concentrations (C) throughout the study for cows in TMR, high (HA), medium (MA) and low (LA) pasture allowances groups. Protein and urea concentrations were affected by treatment (P=0.02 and P=0.01, respectively), protein, albumin and urea concentrations were affected by period (P<0.001 for all) and by the interaction treatment and period (P=0.15, P<0.001 and P=0.1, respectively).

milk production, which is reflected in the metabolic/endocrine status that is related with postpartum reinitiation of ovarian cyclicity.

A higher milk production was found in the TMR group, which is consistent with previous results when concentrates were used compared to grazing groups (Kolver et al., 2002; Horan et al., 2005). It is interesting to note that differences in milk production among the TMR and the higher herbage allowances groups (HA and MA) were found only after 35 dpp, even when TMR cows in an open-sky system had free access to feed. No differences were found between HA and MA cows in milk production, even if herbage allowance of HA cows doubled MA cows, which suggest a stronger metabolic effort for MA cows to maintain milk production. On the contrary, LA cows showed a limited performance in milk production early after calving (14 dpp), suggesting that when nutrient availability is below a threshold (herbage allowance 7.5 kg DM/cow/d, Chilibroste et al., 2012), milk production is set in order to assure cow's homeostasis. Although results from the literature are difficult to compare due to the different treatments used (supplements and concentrates), Kennedy et al. (2008) reported no differences in milk yield until 40 dpp according to herbage allowance, which led the authors to suggest that a low herbage allowance is sufficient during that period. The differences found in milk production between TMR and HA/MA can be related to differences in energy intake and maintenance costs related to walking and grazing activity (Bargo et al., 2002).

The effects of nutritional treatments were also evident in BCS evolution which reflect the energy balance: MA and LA cows had a lower BCS than HA and TMR cows, which in turn was higher than HA cows at the end of the study. Higher NEFA levels were detected in the MA group on day 15 pp, which is consistent with the pronounced loss of BCS and high milk yield after calving. There are few studies that have determined metabolic and endocrine profiles in dairy cows under different daily herbage allowance (DHA) treatments. Kennedy et al. (2008) reported lower NEFA concentrations in DHA groups of 16 and 19 kg DM/cow/day when compared to 13 kg DM/cow/day, but besides the considerations regarding milk production, metabolic determinations were performed only once during the specific nutritional treatment (three occasions during postpartum period), which limits the understanding of the evolution of the metabolic milieu. The increase in NEFA concentrations found in all grazing groups on day 15, was followed by increased BHB concentrations reflecting the cow negative energy balance as reported (Ingvartsen and Andersen, 2000; Meikle et al., 2004). Concentrations of both NEFA and BHB remained unchanged in the TMR group, which is consistent with Van Knegsel et al. (2005) who reported low concentration of NEFA and BHB when animals are supplemented with glycogenic nutrients (*e.g.*, foods such as corn as
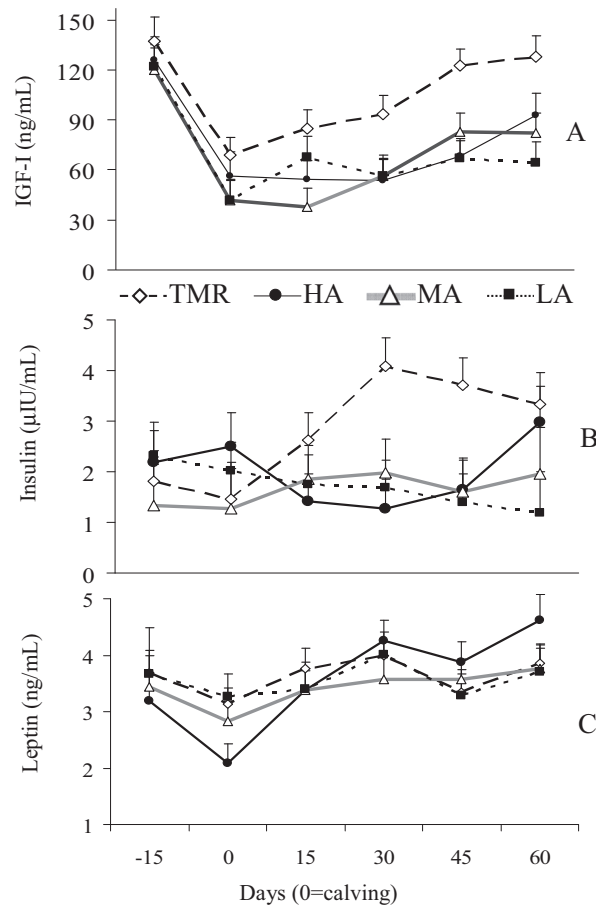
Fig. 4. Plasma insulin like growth factor I (A), insulin (B) and leptin plasma concentrations (C) throughout the study for cows in TMR, high (HA), medium (MA) and low (LA) pasture allowances groups. IGF-I and insulin concentrations tended to be affected by the treatment (P=0.06 for both). IGF-I and leptin were affected by the period (P<0.0001 for both) and IGF-I by the interaction treatment and period (P=0.01).

has been used in the present study). Similar results as ours were reported in grazing dairy cows that were supplemented (Kennedy et al., 2008). Concentrations of BHB in the grazing groups were greater than the TMR group and at 30 dpp were around 1 mmol/L, which is considered to be subclinical ketosis according to Whitaker et al. (1999). This is probably related to increased needs for growth in primiparous cows occurring simultaneously with the demands of lactation and their lower feed intake capacity as described previously (Rémond et al., 1991), which seems to be more acute under grazing conditions.

Cholesterol, total protein, and albumin concentrations decreased around calving which has been associated to the lower intake and negative energy balance during this period (Cavestany et al., 2009; Adrien et al., 2012). Cholesterol levels were higher in HA than LA cows, probably due to increased lipoprotein synthesis as has been previously shown (Bernabucci et al.,
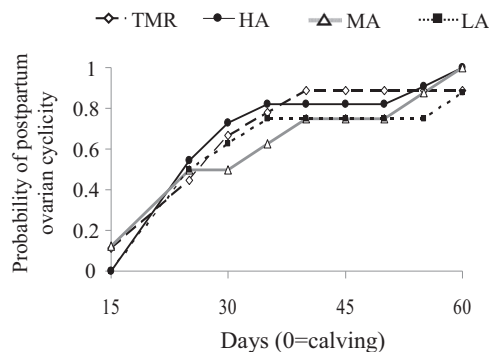

Fig. 5. Probability of days to first ovulation for cows in TMR, high (HA), medium (MA) and low (LA) pasture allowances groups.

2004). Moreover, cholesterol concentrations have been reported to be positively associated with short interval from calving to conception, which is also associated with better energy balance (Reist et al., 2003). On the other hand, TMR cows presented the lowest cholesterol levels and had the highest BCS, this could indicate that other factors such as specific nutrients have a direct effect on cholesterol concentrations as has been reported previously (Duske et al., 2009). Protein, albumin, and urea profiles can reflect the energy/nitrogen utilization by the ruminant. Lower concentrations of protein and albumin at 30 and 45 dpp were found in LA cows when compared to TMR cows and to TMR and HA cows, respectively, suggesting a worse energy balance in LA cows. This is reinforced by the lower urea concentrations in LA cows compared to TMR and HA cows, which is related to the low herbage allowance offered to LA cows. Similarly, slightly lower urea concentrations were detected at 15 and 30 dpp in MA than in HA or TMR cows, suggesting that MA cows had maximized nitrogen utilization in the rumen. Overall, differences in metabolic profiles were evident in the TMR group throughout the study and especially when compared to LA or MA groups. While metabolic differences were observed among HA cows and MA cows during early postpartum (15–30 dpp), differences among HA and LA cows were observed later on in the postpartum period (45–60 dpp).

Low IGF-I and insulin concentrations were found at calving which is consistent with the NEB that characterizes this period (Bertics et al., 1992), and is in agreement with previous reports (Butler, 2000; Lucy, 2001; Holtenius et al., 2003). This is a metabolic strategy to cope with the negative energy balance, tissues try to fit their current local energy metabolism to this new catabolic state through changes in endocrine signals that shift the metabolism from an anabolic to a catabolic direction (Chilliard, 1999). Greater insulin concentrations were found in the TMR group, which is consistent with the higher nutrient density offered to this group. Besides, this group was under stabulated conditions and no extra requirements to cover grazing activities and the daily trips from the milking parlour to the paddock were needed. The associated IGF-I increase found in the TMR cows is in agreement with the known stimulatory action of insulin and/or nutrient availability on the hepatic IGF-I synthesis (Butler, 2000; Rhoads et al., 2004). Moreover, a reciprocal action has been proposed among pancreas and liver, as a direct IGF-I action on enhanced β-pancreatic cells insulin secretion has been shown (Yoshida et al., 2007).

IGF-I concentrations increased after calving except for LA group, probably due to the energy restriction due to the nutrients offered. This is also associated with leptin concentrations that remained unchanged in this group, while concentrations increased in TMR and HA cows, consistent with a better BCS evolution in these cows. On the other hand, no relevant differences were observed in leptin concentrations among groups. Indeed, in a previous study (Meikle et al., 2004) we found that the association among leptin concentrations and BCS was weaker after calving than before calving; and this association was lost in lean cows. On the other hand, different methods to measure leptin should be considered as the present paper uses a multispecies RIA kit and the previous report used ruminant specific RIA.

The reinitiation of ovarian cyclicity was delayed in MA cows one month after calving when compared to TMR and HA cows, which is consistent with the metabolic profile found in this period. Taking into account the similar milk production among HA and MA cows, BCS evolution and the reinitiation of ovarian cyclicity, we suggest that MA cows presented a greater metabolic stress (greater body lipid mobilization) to maintain milk production with a lower DMI that was reflected in this reproductive parameter. The delayed ovulation in LA cows observed from day 40 until the end of the study, is in agreement with the endocrine/metabolic status of this group provoked by the low herbage allowance. The anestrous duration has been associated with BCS loss (Butler, 2000; Meikle et al., 2004) and it has been demonstrated that the sooner the cows restore their energy balance, the sooner they will start cycling and will become pregnant (Huszenicza et al., 1988). IGF-I concentration is a good indicator of the capacity to resume cyclicity after parturition in agreement with Roberts et al. (1997). Both insulin and IGF-I are known to stimulate follicular growth and ovulation (Spicer et al., 1993). Overall, the endocrine/metabolic data observed in the different treatments is consistent with the results found in the probability of ovulation in each group.

In conclusion, this study shows that when cows were supplemented to meet maintenance metabolizable energy needs plus 8–10 L/d of milk and offered medium DHA (15 kg estimated grass DM available/cow/d, milk production did not differ from high DHA (30 kg DM/cow/d), but this metabolic stress was reflected in the endocrine/metabolic parameters and the delayed reinitiation of ovarian cyclicity found in the first month after calving in the former cows. On the other hand, a low DHA (7.5 kg DM/cow/d) resulted in delayed ovulation, a poor endocrine/metabolic status and BCS evolution throughout the experiment.

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