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Effect of organic trace minerals supplementation during early postpartum on milk composition, and metabolic and hormonal profiles in grazing dairy heifers

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Abstract

The effect of complexed trace minerals supplementation on productive, reproductive and metabolic variables during early postpartum of grazing primiparous dairy cows =) was investigated. Pasture-fed Holstein heifers were offered (supplemented group; SG, n = 60) or not (control group; CG, n = 60) a commercial trace mineral supplement during the first 90 days of lactation. Milk production and composition were determined monthly. Reproductive function was evaluated by calving-first service interval, number of services per conception, and pregnancy rates. Non-esterified fatty acids, β-hydroxybutyrate, insulin, and insulin-like growth factor-1 were determined on blood samples collected on days postpartum –7 ± 4, 30 and 60. Supplementation did not affect milk production or its composition, except for fat percentage that tended to be higher in SG than CG. Calving-first service and calving-conception intervals were shorter in SG than CG but number of services per conception and pregnancy rates did not differ between treatments. No lameness was observed during the period evaluated and although a beneficial effect of SG was found in locomotion score, it was not clinically relevant (scores 1-2). Metabolites and hormones concentrations did not differ between treatments. Organic trace mineral supplementation had a transient beneficial effect on milk composition on the intervals from calving to first service and from calving to conception, with no changes in metabolic and endocrine profiles during the transition period primiparous dairy cows on grazing conditions.

Additional key words: dairy cows; Holstein heifers; metabolic-endocrine profile microminerals; transition.

Resumen

Efecto de la suplementación con minerales orgánicos traza durante el postparto temprano sobre la composición de la leche y los perfiles metabólicos y hormonales en novillas de leche

Se investigó el efecto de la suplementación con complejos de minerales traza sobre variables productivas, reproductivas y metabólicas durante el postparto temprano en vacas lecheras primíparas en pastoreo. Novillas Holstein (n = 120) fueron suplementadas (SG) o no (grupo control; CG) con un preparado comercial de minerales traza durante los primeros 90 días de lactación. La producción de leche y su composición se determinaron mensualmente. La actividad reproductiva fue evaluada por el intervalo parto-primera inseminación artificial (IA), el número de servicios por concepción y la tasa de gestación. Se determinaron los ácidos grasos no esterificados, el β-hidroxibutirato, la insulina y el factor de crecimiento similar a la insulina-1 en muestras de sangre tomadas los días postparto –7 ± 4, 30 y 60. La suplementación no afectó la producción de leche ni su composición, excepto por una tendencia del porcentaje de grasa a ser mayor en el SG que en el CG. Los intervalos parto-primera IA y parto-concepción fueron menor en SG que en CG, pero el número de IA por concepción y las tasas de gestación no fueron diferentes entre los dos grupos. No

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Abbreviations used: BCS (body condition score); BHB (β-hydroxybutyrate); CG (control group); IGF-1 (insulin like growth factor I); NEFA (non-esterified fatty acids); SG (supplemented group).

se observaron cojeras durante el periodo evaluado y a pesar de que se encontró un efecto beneficioso sobre el índice de locomoción en SG, no fue relevante clínicamente (índices 1-2). Las concentraciones de metabolitos y hormonas no fueron diferentes entre grupos. En conclusión, la suplementación con minerales orgánicos traza tuvo un efecto beneficioso transitorio sobre la composición de la leche y los intervalos parto-primera IA y parto-concepción, sin afectar los perfiles endocrino-metabólicos de novillas en pastoreo.

Palabras clave adicionales: microminerales; novillas Holstein; perfil metabólico-endocrino; transición; vacas lecheras.

Introduction

During the transition period (from three weeks before to three weeks after parturition) dairy cows *(Bos taurus)* undergo critical metabolic changes as they shift from the pregnant, non-lactating state to the non-pregnant, lactating state, with the concomitant rapid increase in the energy requirements of the cow to meet the demands for milk production (Drackley, 1999). In the past decades, milk production per cow has increased notably —because of either improved nutritional management and/or genetic selection strategies—, and it has been accompanied by a higher incidence of diseases and decreased reproductive efficiency (Roche *et al.*, 2000). Indeed, the periparturient period often represents a dramatic experience for dairy cows since most of the pathologies and metabolic disorders observed in dairy herds occur during this period (Goff & Horst, 1997). In pasture-based milk production systems, primiparous cows seem to be the category most affected by this transition: they present greater prevalence of foot lesions than multiparous cows (Ramos & Freire, 2006); longer anestrus and worse reproductive performance (Meikle *et al.*, 2004) and more pronounced metabolic disorders (Cavestany *et al.*, 2005).

The negative energy balance characteristic of the transition period is evidenced by the increase of non-esterified fatty acids (NEFA) along with increased ketonic bodies such as β-hydroxybutyrate (BHB) (Whitaker *et al.*, 1999) in stabulation and in grazing conditions, the mainly anabolic hormones insulin and insulin like growth factor 1 (IGF-1), decrease before calving and remain low during early postpartum as reflect of peripheral catabolism (Lucy, 2001; Meikle *et al.*, 2004). These hormones play key roles in the reproductive processes and the recovery of its concentrations after calving has been associated to reproductive success (Butler, 2000). On the other hand, deficiency of certain metabolites can cause several of the pathologies that accumulate in this period (Grummer, 1993) and it has been reported that there can be a dramatic disbalance between the output (mainly in the form of milk) and the input of trace minerals involved in diverse metabolic processes, which ultimately impact productive and reproductive variables (Wilde, 2006).

Supplementation with complexed trace minerals is a frequent practice in dairy cattle nutrition programs. Feeding of zinc methionine complex to dairy cows reduced the somatic cell count, increases milk yield and improved integrity of hoof tissue (Tomlinson *et al.*, 2004; Bicalho *et al.*, 2007). Moreover, if this complex is complemented with Mn-, and Cu-specific aminoacids complexes and cobalt glucoheptonate, further improvement of milk production, reproductive performance and claw integrity is observed (Nocek *et al.*, 2000; Bicalho *et al.*, 2007). Several studies have also reported improvements in reproductive performance, immune response and hoof health in ruminants supplemented with these minerals (Hutcheson, 1990; Chirase *et al.*, 1991; Graham, 1991; Campbell *et al.*, 1999; Nocek *et al.*, 2000; Lucy, 2001; Margerison *et al.*, 2002) but we have not found reports on how this supplementation may affect the plasma concentrations of metabolites and metabolic hormones. Moreover, the experimental designs have been performed under stabulation conditions with controlled nutritional schemes and less is known about its repercussions under grazing conditions.

The aim of this study was to determine the impact of a short supplementation with complexed trace minerals on productive, reproductive and metabolic characters during early postpartum of grazing primiparous dairy cows.

Material and methods

Experimental design

Animal experimentation was performed according to the commercial farm practices and the Animal Experimentation Committee of the University of Montevideo, Uruguay. Primiparous Holstein heifers (2.8 ± 0.2 units of body condition score (BCS), 23 to 25 months of age at calving, calving period: April 24th to May 28th, 2008) from a commercial dairy herd of Colonia (34°25'S, Uruguay) were assigned randomly to two groups: one group supplemented with complexed trace minerals (supplemented group; SG; n = 60) and a control group (CG; n = 60), without supplementation. Both groups grazed in weekly plots of rye grass (*Lolium multiflorum*; estimated maximal herbage mass of 1500 kg dry matter (DM) ha^{-1} and forage allowance of 7.5 kg DM cow^{-1} day^{-1}) in the morning and of alfalfa (*Medicago sativa;* estimated maximal herbage mass of 2,000 kg DM ha^{-1} and forage allowance of 8 kg DM cow^{-1} day^{-1} in the afternoon. In addition cows were supplemented 12 kg day^{-1} (as-fed basis) of corn silage, and a concentrate of 5 kg day^{-1} of high moisture corn grain, 2 kg day^{-1} of sunflower expeller and 4.5 g day^{-1} of a premix of vitamins and minerals (Zoodry, Roche Ltd., Basel, Switzerland) fed individually. The diet was formulated according the NRC requirements and supplied 17.4% of crude protein and 1.64 Mcal net energy of lactation kg^{-1} DM). During the first 90 days in milk, the SG group received daily a complex (Availa® 4, Zinpro Corp., Eden Prairie, MN, USA; 7 g cow^{-1}) containing 360 mg of zinc methionine, 200 mg of manganese methionine, 125 mg of copper lysine, and 25 mg of cobalt glucoheptonate, mixed in the concentrate. Food refusals were about 5-10% of the amount offered in both groups. Body condition score was determined by the same observer at the beginning of the experiment (7 ± 4 days before anticipated calving) and on days of lactation 30 and 60, using a 5-point scale (1 = emaciated, 5 = fat; Ramos & Freire, 2006). Cows were milked twice a day and milk production and quality were determined monthly (15 to 90 days in milk). Milk samples (10 mL) at each milking were individually collected and analysed for content of fat, protein and somatic cell count. Milk fat was analysed by the Mojonnier method and milk protein was analysed by the Kjeldahl method (with a Bentley 2000 equipment; Bentley Instruments Inc., Chaska, MN, USA).

General management was similar for all the animals, and oestrus detection, pregnancy assessment and artificial insemination were performed by the same person. Conception was assessed by rectal palpation on days 45 and 60 after insemination. The reproductive function was evaluated by calving-to-first service interval, number of services per conception and conception rates.

The detection of lame cows during de supplementation time was performed daily by the stockman and workers of the farm. The personnel was already trained, but received a specific course for monitoring the lame cow previous to the experiment by the first author of this study. An observation of the locomotion score was performed using the 1 to 5 scale (1 = no lameness, 5 = extremely lame) (Sprecher *et al.*, 1997) approximately one month postpartum (range: 20 to 50 days) in 49 cows of each group.

Blood sampling and determination of NEFA, BHB, insulin and IGF-1

Blood samples were collected in the morning by coccygeal venipuncture into heparinized evacuated tubes (Vacutainer, Becton Dickinson, NJ, USA) on days postpartum –7 ± 4, 30 and 60. Samples were centrifuged for 15 min at 3000 rpm and plasma was stored at –20 °C until analysis.

Non-esterified fatty acids were determined in all samples by the ACS-ACOD (acil-CoA synthetase & acil-CoA oxidase) method with the NEFA-C kit of Wako Chemicals (Richmond, VA, USA) and β-hydroxybutyrate (BHB) with 3-HBDH-NAD+3-hydroxybutyrate dehydrogenase-NAD+ method (Ranbut, Randox Laboratories Ltd, Antrim, UK). Both metabolites were determined in a single assay and the intra-assay coefficient of variation (CV) was below 10%.

Hormone determinations were performed at the Laboratory of Nuclear Techniques, Veterinary Faculty, University of Uruguay. Insulin concentrations were determined in all samples by ^{125}I-insulin radioimmunoanalysis kit (Coat-A-Count Insulin, Diagnostic Products Corp., Los Angeles, CA, USA). The sensitivity of the assay was 1.4 µIU mL^{-1}. The intra-assay CV for the control (4.2 µIU mL^{-1}) was 13.3%.

The concentration of IGF-1 was determined in a subset of 20 animals selected randomly from each treatment group by an immunoradiometric method using a commercial kit (IGF1-RIACT Cis Bio Int, GIF-Sur-Yvette Cedex, France). The assay sensitivity was 0.68 ng mL^{-1} and the the intra-assay CV for control 1 (51 ng mL^{-1}) and control 2 (709.3 ng mL^{-1}) were 3.5 and 16.2 %, respectively.

Statistical analysis

Milk production, BCS, metabolites and hormones concentrations were analysed by the mixed procedure (Statistical Analysis System, SAS Institute, Cary, NC, USA) with the following model using cow as the experimental unit:

$$Y_{ijk} = \mu + \tau_i + d_j + (\tau d)_{ij} + c_k + \beta X_l + e_{ijkl}$$

where μ = effect of the mean, τ_i = effect of the i^{th} treatment, d_j = effect of j^{th} time point, c_k = k^{th} cow random effect, βX= effect of the l^{th} prepartum BCS covariate, and e_{ijkl} = effect of the random residual error associated with the measurement on the k^{th} cow at the j^{th} time point on the i^{th} treatment with the l^{th} prepartum BCS. The covariance structure was autoregressive order 1. Impacts of postpartum alterations were assessed from a comparison of confidence intervals (α = 0.05) between covariate values and postpartum samples. Data are presented as least square means ± pooled standard errors. Reproductive parameters were analysed using a generalised lineal model with the Genmod procedure (Statistical Analysis System, SAS Institute, Cary, NC, USA) using the following model $Y_{ijk} = \mu + \tau_i + \beta X_k + e_{ijk}$; where μ = effect of the mean, τ_i = effect of the i^{th} treatment, βX_l = effect of the l^{th} prepartum BCS covariate, and e_{ikl} = effect of the random residual error associated the k^{th} cow on the i^{th} treatment with the l^{th} prepartum BCS. Data are presented as least square means ± pooled standard errors. Means were considered different when $p < 0.05$.

Results

Lameness and locomotion score

No lameness was observed during the first 3 months of the supplementation period. The locomotion score was different between groups ($p = 0.02$); a higher locomotion score was found in CG group. The CG presented 31 cows out of 49 (63.3%) with a normal locomotion score (score 1) and 18 cows (36.7%) with a subnormal locomotion score (score 2), whereas the SG group presented 41 cows out of 49 (83.6%) with score 1 and 8 cows (16.4%) with score 2.

Body condition score

Body condition score tended ($p = 0.06$) to be higher in the SG than in the CG and it was affected ($p < 0.01$) by the day postpartum and by their interaction, since the SG showed an increase in BCS during early lactation while the CG maintained its condition (Fig. 1).

Milk yield and composition

The effects of trace mineral supplementation, days postpartum and their interaction on each variable are listed in Table 1. Trace minerals supplementation did not affect milk production (16.9 *vs.* 16.5 ± 0.4 L day^{-1})

Table 1. Fixed effects included in the model for measured variables in grazing primiparous dairy cows (fixed effects are treatment —supplemented *vs.* control—, day post-partum and their interaction)

Variables	Treatment	Day	Treatment* Day[2]
BCS[1]	0.0605	**	*
Milk variables			
Milk (kg day^{-1})	ns	***	*
Solids (kg day^{-1})	ns	***	*
Fat %	0.0689	***	ns
Fat (kg)	ns	***	ns
Protein %	ns	***	ns
Protein (kg)	ns	***	**
SCC	ns	***	ns
Metabolites and hormones			
NEFA	ns	ns	ns
BHB	ns	ns	ns
Insulin	ns	*	ns
IGF-1	ns	ns	ns

[1] BCS = body condition score; SCC = somatic cell count; NEFA = non-esterified fatty acids; BHB = β-hydroxybutyrate; IGF-1 = insulin-like growth factor 1. [2]* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns = $p > 0.1$.

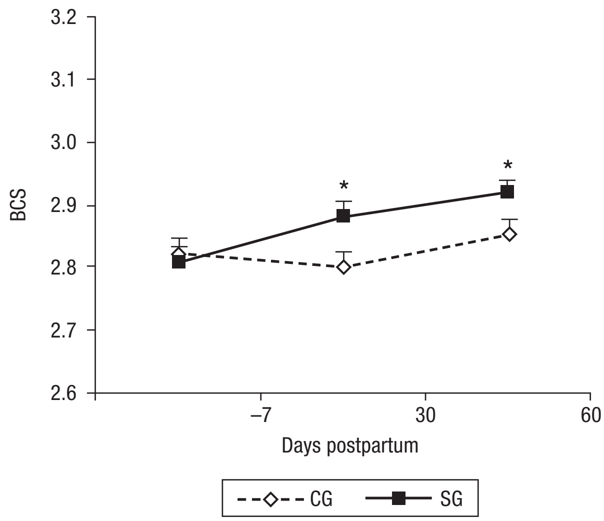

Figure 1. Body condition score (BCS) in Control (CG) and Supplemented (SG) primiparous dairy cows under grazing conditions. Asterisks indicate differences between treatments at the same day, $p < 0.05$.

or any of the parameters measured in milk, except that fat percentage tended ($p = 0.07$) to be higher in the SG than in the CG (Table 1). An interaction between treatment and day postpartum was observed both in milk production ($p < 0.05$), total solids ($p < 0.05$) and protein content ($p < 0.01$) (Fig. 2). On day 30 the SG tended ($p < 0.1$) to have higher milk yield and presented higher ($p < 0.05$) milk fat and protein content, although milk fat and protein percentages were not affected. Although similar levels of solids, and fat and protein contents were observed by day 60, the increase was observed 30 days earlier in the SG than the CG (Fig. 2).

The percentage of fat in milk tended ($p = 0.068$) to be higher in the SG than in the CG (3.81 and 3.63 ± 0.67%, respectively). However, fat production was not different between groups (0.62 and 0.61 ± 0.01 kg day^{-1} for SG and CG, respectively). Neither trace minerals supplementation nor its interaction with days postpartum affected somatic cell count in milk (57,000 and 74,000 ± 11,000, for CG and SG, respectively).

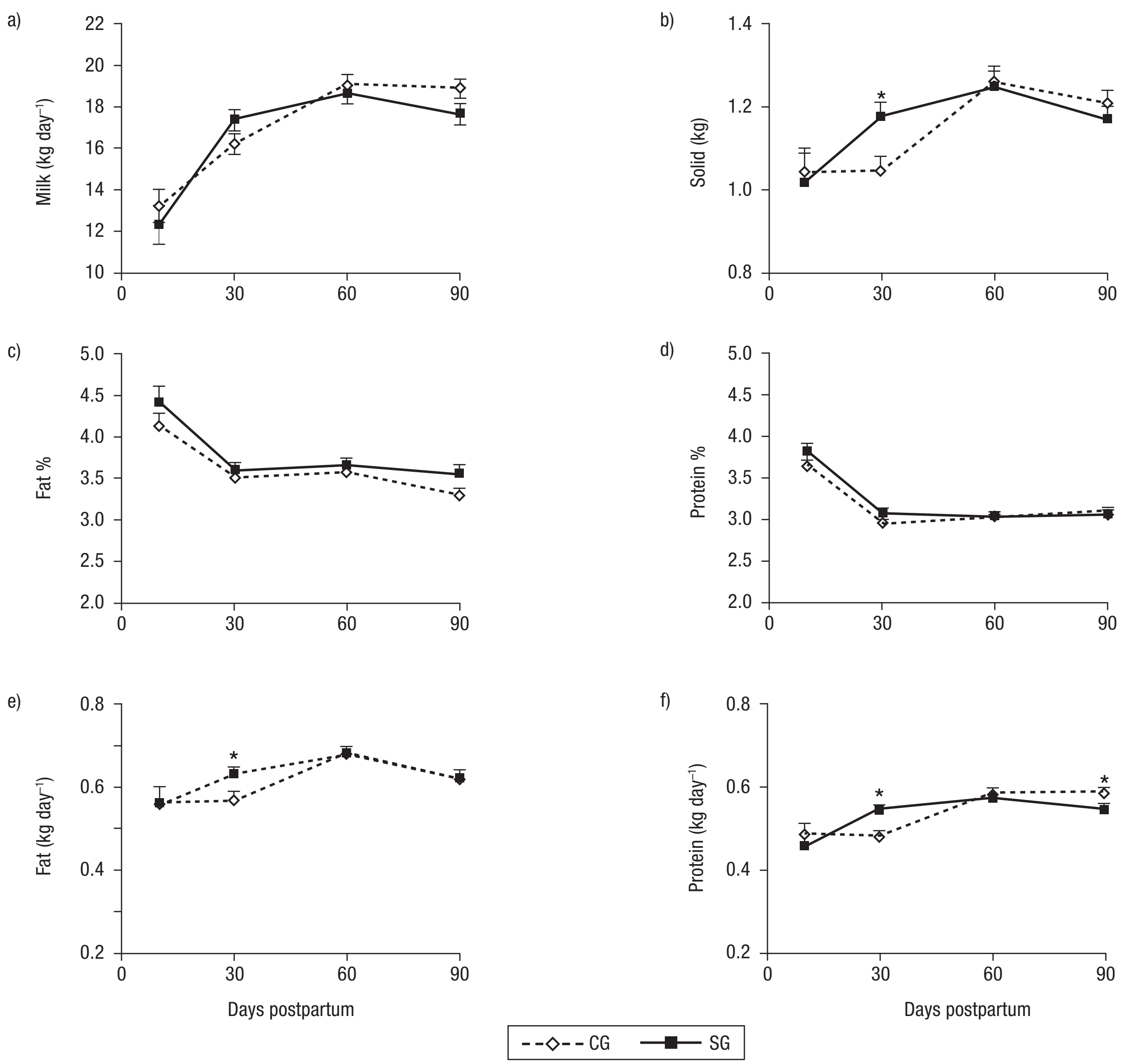

Figure 2. Milk production (a), solids (b), milk fat percentage (c), milk protein percentage (d), kilograms of milk fat (e) and kilograms of milk protein (f) in Control (CG) and Supplemented (SG) primiparous dairy cows under grazing conditions. Asterisks indicate differences between treatments at the same day, $p < 0.05$.

Reproductive parameters

Intervals from calving to first service and from calving to conception were shorter ($p < 0.001$) in the SG than in the CG (75.5 ± 6.5 and 84.2 ± 6.5 d and 122 ± 6.7 *vs.* 110 ± 6.7, respectively). However, no differences were found in the number of services per conception (SG: 2.56 ± 0.2; CG: 2.44 ± 0.2) and pregnancy rates (SG: 80%; CG: 79%).

Plasma metabolites and hormones

The effects of trace mineral supplementation, days postpartum and their interaction on each variable are listed in Table 1. No effect of supplementation with trace minerals was observed either in the NEFA or in the BHB concentrations. An effect of the day postpartum was observed ($p < 0.05$): both NEFA and BHB concentrations increased from day –7 to 30 (Fig. 3). Insulin concentrations were not affected by supplementation (2.47 ± 0.17 *vs.* 2.21± 0.17 uUI mL^{-1}, for CG and SG, respectively). Similarly, IGF-1 was not affected by trace minerals supplementation (76.9 ± 9.4 and 77.1 ± 7.6 ng mL^{-1}, for CG and SG, respectively; Fig. 3). However, both insulin and IGF-1 plasma levels decreased ($p < 0.05$) from day –7 to 30 (Fig. 3).

Discussion

The novelty of the present study is that primiparous grazing cows were supplemented with complexed trace minerals during the first trimester of lactation. The vast majority of reports on trace mineral supplementation involve feeding with total mixed rations, and very few have been undertaken in grazing cows. Moreover, in most experiments, supplementation starts in the prepartum. The experiment most comparable to ours is the one by Griffiths *et al.* (2007) where grazing primipa-

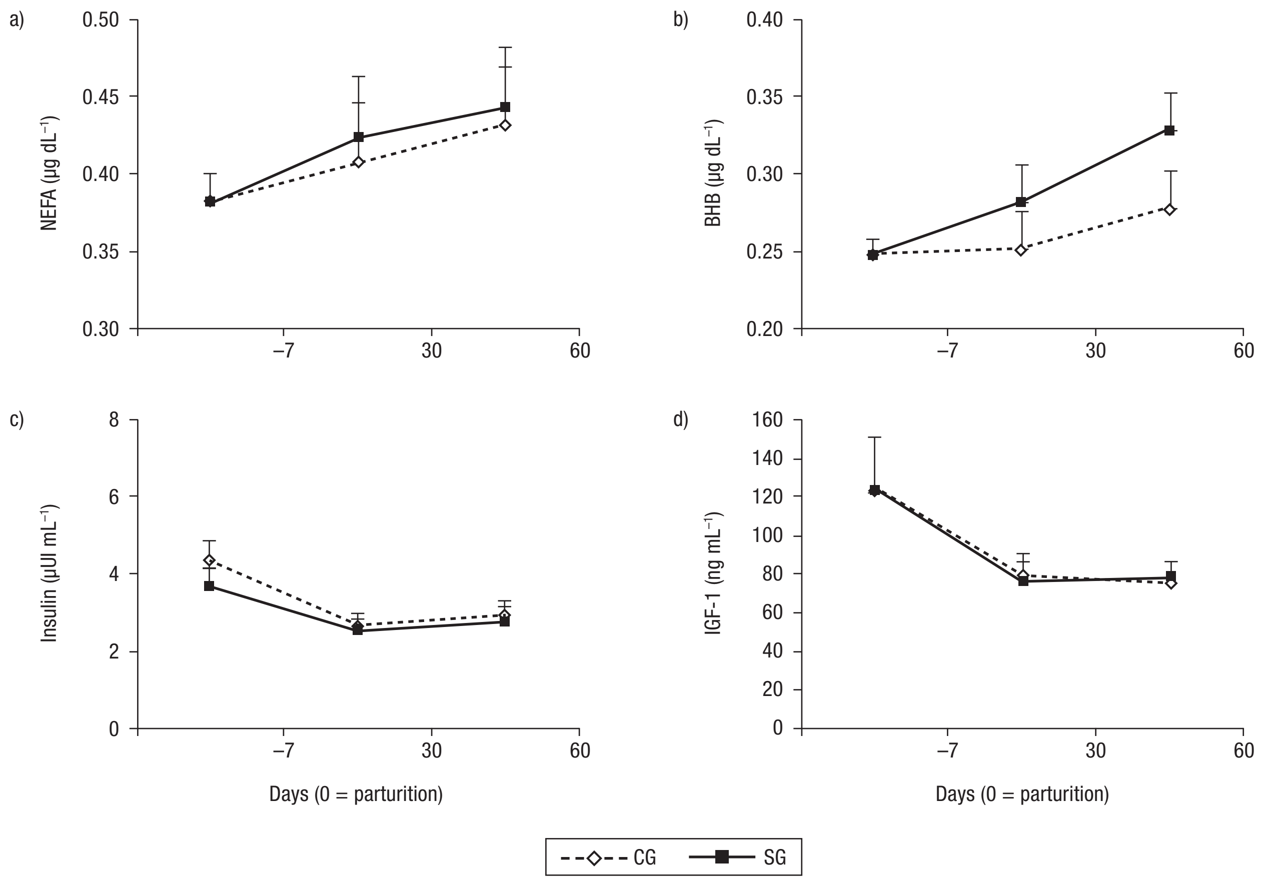

Figure 3. Concentrations of NEFA (a), BHB (b), insulin (c) and IGF-1 (d) in Control (CG) and Supplemented (SG) primiparous dairy cows under grazing conditions.

rous and multiparous cows were used. However, the authors do not provide information about the responses of primiparous *versus* multiparous cows. To our knowledge, this is also the first report on IGF-1 concentrations after trace mineral treatment.

In the present study, trace mineral supplementation did not affect the overall milk production, milk fat and protein content and SCC, although it tended to increase fat percentage on day 90. Data are consistent with the lack of effect of post-partum trace mineral supplementation on productive variables reported by Campbell *et al.* (1999). On the other hand, changes such as increase in milk production and decrease of SCC have been observed, under stabulation conditions and starting supplementation around 45 days pre-partum (Socha & Johnson, 1998; Uchida *et al.*, 2001). The discrepancies may be explained by the differences in experimental models (stabulation *vs.* grazing conditions), different composition of the diets and the composition and amount of the mineral supplementation. Griffiths *et al.* (2007) reported an increase in the production of milk in supplemented grazing cows. However, the supplementation period used in that experiment exceeded by far the treatment period of the present study. Indeed, the meta-regression analysis performed by Rabiee *et al.* (2010) revealed that the type of trace minerals and the duration of treatment before and after calving along with the use of other supplements were the main factor influencing milk yield.

In this study, no lameness was detected during the experimental period, thus, no effect of the supplementation on the disease could be determined. Coincidently with the present results, Griffiths *et al.* (2007) found no effect of supplementation on claw hardness or on the incidence of claw disorders. Even if a beneficial effect of the mineral supplementation was observed in the locomotion score, these scores of locomotion are not clinically relevant. Margerison *et al.* (2002) consider that both score 1 and 2 are the ones corresponding to non-lame cows, score 3 intermediate, while scores 4 and 5 are the indexes for lame cows. Indeed, the effect of lameness on productive and/or reproductive parameters is observed after scores of locomotion score that are more than 2 (Sprecher *et al.*, 1997; Bicalho *et al.*, 2007). Although the control and supplemented animals reached similar levels of milk production and milk fat and protein content, a positive effect of trace mineral supplementation was observed during the first month of lactation, with levels already increasing in the supplemented group and a relative delay in the non-supplemented. These data suggest a beneficial effect in the early post-partum (first month), which is the moment of greater mineral and metabolic imbalance because of the combination of loss of appetite and the steep increase in the metabolic demands of lactation.

Cows receiving trace mineral supplementation showed an increase in BCS during early lactation while BCS was maintained in control cows in discrepancy with previous reports in grazing primiparous dairy cows (Meikle *et al.,* 2004; Adrien *et al*., 2012) in which BCS decreased in early lactation as negative energy balance is established to support milk production. However, the low BCS of cows in this study could explain the difference between this and previous studies, Bewley & Schutz (2008) reviewed that thin cows, as in the present study, have even been shown to gain BCS during early lactation and that thin cows produce more milk directly from feed rather than from energy reserves. In addition, in contrast with our results, Campbell *et al.* (1999) did not report differences in BCS between trace mineral supplemented and control cows.

The improvement in body reserves could be the cause of the earlier increase in milk fat and protein content and the better reproductive performance observed in these animals (shorter partum-to-service and partum-to-conception intervals). Although not always associated to increased BCS, beneficial effects of trace mineral supplementation have been reported on time to first estrus post-partum (Campbell *et al.*, 1999; Rabiee *et al.*, 2010), partum-to-service interval, number of services to pregnancy and pregnancy rates (Socha & Johnson, 1998). However, we have not found significant effects on number of services per conception and pregnancy rates, although the number of animals is not the ideal for this kind of calculations. Similarly, Campbell *et al.* (1999) did not observe an effect of treatment on the number of services per conception.

To our knowledge, this is the first time that IGF-1 levels are evaluated during trace mineral supplementation in dairy cows. The IGF-1 plays roles in ovulation and also as a growth promoter during the early embryonic period in ruminants (Butler, 2003) and an increased plasma concentration during trace mineral supplementation could have explained in part the improvements in reproductive function. However, no effect of the treatment was observed on this variable. Organic trace minerals supplementation did not affect insulin concentrations either, in coincidence with Campbell *et al.* (1999), and we have found no effect of trace mineral supplementation on the plasma concentrations of NEFA and BHB.

On the other hand, alterations on endocrine and metabolic profiles were observed during the first 30 days postpartum. Concentrations of insulin and IGF-1 decreased during the postpartum, reflecting the uncoupling of the somatotropic axis of early lactation (Meikle *et al*., 2004; Adrien *et al.*, 2012). This uncoupling has been reported as a metabolic adaptation to cope with the energy demands of lactation and the partition of energy and nutrients towards the mammary gland (Bauman, 2000). Parallel, although BCS did not change or slightly increased during the postpartum, NEFA and BHB concentrations increased in the same period, which is characteristic of a period of lipomobilization such as the start of lactation in dairy cows. Lipolytic response (reflected in increased NEFA and BHB concentrations in this study) could have no significant correlation with BCS, BCS change and/or backfat thickness, as reported in dairy (Theilgaard *et al*., 2002) and beef cows (Hudson, 2011). Cow BCS only assesses subcutaneous fat stores, which represent 25% or less (in thin cows) of total body fat mobilized during early lactation (Bewley & Schutz, 2008). Therefore, mobilization of fat from other deposits as intraabdominal fat, which is more deposited than subcutaneous fat in dairy cows (Bewley & Schutz, 2008), could explain the increase in NEFA and BHB concentrations even when BCS did not decrease.

The organically complexed trace minerals offered to heifers in the present experiment shortened the intervals from calving to first service and from calving to conception and increased milk fat and protein content before it did in the control non-supplemented animals, although it did not alter the overall production. No other parameter was affected. The limited response to the supplement could be due to the high quality of the control diet, which possibly fulfills the requirements of trace elements during this period. On the other hand, most reports on the beneficial effects of trace minerals supplementation have used multiparous cows as experimental unit, whereas we have studied heifers. It is known that primiparous cows normally produce less milk than multiparous cows, and although heifers have energy requirements for growth, their energy balance is challenged to a lesser extent. Moreover, their response to mineral supplementation can differ. For example, primiparous and multiparous cows differed in their milk yield response to dietary cobalt supplementation (Kincaid *et al.*, 2003). Zinc absorption in ruminants declines with increasing parity although the underlying mechanisms are not clear (Wilde, 2006).

Many factors influence the effects of organic trace mineral supplementation on productive and reproductive performance (Spears, 1996; Wilde, 2006; Rabiee *et al.*, 2010), and the different experimental approaches and management conditions (*i.e.,* stabulation *vs.* grazing conditions) make animal response to supplementation variable. The relationship between costs and benefits needs cautious evaluation in each case.

Our results indicate that under the conditions of this experiment, organic trace mineral supplementation offered to grazing primiparous dairy cows during the postpartum, has a transient beneficial effect on milk composition and on the intervals from calving to first service and from calving to conception, with no changes in metabolic and endocrine profiles during the transition period.

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