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## Adaptation of some energetic parameters during transition period in dairy cows

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### ABSTRACT

The aim of this study was to evaluate the effect of peripartum period and lactation class on serum  $\beta$ -hydroxybutyrate, non-esterified fatty acids (NEFA), insulin and glucose concentration in dairy cows. Thirty-five multiparous Holstein cows were selected from a high-producing dairy farm. The animals were divided according to their lactation class: second lactation group (L2,  $n=16$ ), third lactation group (L3,  $n=10$ ) and fourth lactation group (L4,  $n=9$ ). Blood sampling were performed  $7 \pm 5$  days before calving (Pre/C) and  $7 \pm 5$  days after calving (Post/C). Two-way analysis of variance was applied to determine significant effects of lactation class (L2, L3 and L4) and period (Pre/C and Post/C) on studied parameters. Increased NEFA values and lower insulin levels were found in Post/C respect to Pre/C in L2 and L4. These changes highlight the difficulty of dairy cows to cope with the energy demand characterizing the transition period. Improving in knowledge of energetic metabolism changes may help to supply a new strategy of farm management and reproductive performance in dairy cows during the transition period.

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Dairy cows; biochemical parameters; transition period; energetic metabolism

## 1. Introduction

The transition period is an important and vulnerable life stage in dairy cows (Grummer 1995; Drackley 1999) characterized by greatly increased risk of disease (Van Saun 2006). It has been demonstrated that nutritional management in the early dry period is important for maintaining the health and productivity of transition cows (i.e. after cessation of milking) (Dann et al. 2006). Within four days postpartum, the demands for glucose, amino acids and fatty acids due to milk production, are two to five times higher than prepartum requirements (Bell 1995). For these reasons, the transition from late gestation to early lactation is a metabolic changing period for dairy cows, during which most infectious and metabolic diseases are likely to occur (Mallard et al. 1998; Ingvarsen et al. 2003). Cows unable to adapt to this challenging time are more prone to negative subsequent events, and the associations between excessive negative energy balance (NEB) in dairy cows and these detrimental health effects have been reported by several authors (Hammon et al. 2006; McArt et al. 2013). The NEB status is characterized by alterations in blood metabolite and hormone profile (Piccione et al. 2012). During the period of NEB, key hormone expression and tissue responsiveness alter to increase lipolysis and decrease lipogenesis, causing high levels of non-esterified fatty acids (NEFAs) and  $\beta$ -hydroxybutyrate (BHB) concentration which are indicative of lipid mobilization and fatty acid oxidation (Sakha et al. 2006; Wathes et al. 2009).

Glucose metabolism takes on increased significance in the ruminants during pregnancy. (Morgante et al. 2012). Increased

glucose requirements of the gravid uterus during late pregnancy and even greater requirements of the lactating mammary glands necessitate major adjustments in glucose production and utilization in maternal liver, adipose tissue and other tissues (Fiore et al. 2015). NEB occurs as the energy demands for milk production cannot be met by feed intake alone (Herdt 2000). While NEB is a physiologically normal process, excessive NEB reflects poor adaptation and results in adverse health and production effects after calving. The severe NEB during the peripartum might predispose the cow to several pathological conditions like retained placenta, milk fever, metritis, mastitis, clinical ketosis and displaced abomasum (LeBlanc et al. 2005; Duffield et al. 2009). The severity and duration of NEB is reflected by the increase in circulating NEFA and BHB and the degree of decrease in glucose concentrations (Drackley 1999).

The case definition of subclinical ketosis (SCK) is characterized by serum BHB levels  $>1.0 \leq 1.4$  mmol/L in the absence of clinical signs of ketosis (Iwersen et al. 2009; Rollin et al. 2010). Chapinal et al. (2012) demonstrated that serum BHB levels of 1.4 mmol/L and 1.2 mmol/L during the first and second week after calving, respectively, were associated with considerable milk losses (1.5–2.4 kg/day). In ruminants, ingested carbohydrates are fermented to short-chain fatty acids by rumen microbes, and thus most glucose must be synthesized by the liver (Reynolds et al. 1988). As lactose is a major component in milk, gluconeogenesis is closely linked to lactogenesis as the amount of available glucose will determine the quantity of milk produced (Mepham 1993). After parturition, there is a

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decrease in insulin production by the pancreas (Drackley et al. 2001) which results in decreased glucose utilization by insulin-sensitive organs (e.g. adipose tissue and muscle). In response to a decrease in serum glucose concentration, an increase in lipolysis releases NEFA (Bertics et al. 1992; Herdt 2000). The liver converts 15–20% of NEFA (Drackley & Andersen 2006), in ketone bodies (acetone, acetoacetic acid and BHB), in triacylglycerols (TAGs) and packaged into very low-density lipoproteins for transport back to the adipose tissue or stored as TAGs. Excessive fat accumulation in the liver impairs normal liver function (Murondoti et al. 2004), which may lead to hyperketonemia (Herdt 2000). The identification of potential differences in energetic metabolism from late pregnancy to early lactation, may give new and useful information about the guidelines for the management strategies during the transition period (Fiore et al. 2015). In view of such considerations, the aim of this study was to evaluate the changes in some serum metabolic parameters, including BHB, NEFA, insulin and glucose, in dairy cows during the transition period.

## 2. Materials and methods

Thirty-five multiparous Holstein cows were selected from a high-producing dairy farm in the Northeast Italy (45° 24' N, 12° 52' E, 12 m above sea level): 16 in second lactation (L2), 10 in third lactation (L3) and 9 in fourth lactation (L4). The study was performed from June 2013 to October 2013. Farm makes a dry period of 60 days and a period of steaming-up of 15 days before calving. Farm was selected with a milk production (about 10,000 kg for year); milk yield quality was not different for all cows: an average of 3.7% of milk-fat and 3.4% of milk-protein. All the animals were clinically healthy and free from internal and external parasites. Their health status was evaluated based on rectal temperature, heart rate, respiratory profile, appetite, faecal consistency and hematologic profile. The ratio and the chemical composition of diets administered during steaming-up and subsequent early lactation is reported in Table 1.

Blood was collected by jugular venipuncture into vacuum tubes without anticoagulant agent (BD Vacutainer Systems, Pre-analytical Solutions, Plymouth, UK) at  $7 \pm 5$  days pre-calving (Pre/C) and  $7 \pm 5$  days post-calving (Post/C). Blood samples were refrigerated and later processed in the laboratory within one hour. Following standing at room temperature for 20 min, the tubes were centrifuged (Labfuge 400, Heraeus) at 1750 g for 10 min and the obtained serum stored at  $-18^{\circ}\text{C}$  until analysed. Sera have been tested for glucose by means of an automated analyzer (BM Hitachi 911, ROCHE, Basel, Switzerland). On Cobas C 501 (Roche Diagnostics, Mannheim,

Germany) were performed NEFA and BHB serum concentration analysis: NEFA concentrations were determined by using a colorimetric method, NEFA RX Monza test (kit no. FA 115, Randox, Crumlin, UK), while BHB concentrations were determined by RANBUT RX Monza test (kit no. RB 1007, Randox, Crumlin, UK). Insulin concentration was quantified with a commercial 125I-IRMA kit developed for human samples (BI-Ins IRMA kit; CIS Bio International Ltd.) and previously validated for bovine plasma samples (Kerestes et al. 2009). Two-way analysis of variance was used to determine significant effects of lactation class (L2, L3 and L4) and period (Pre/C and Post/C) on the considered parameters (BHB, NEFA, insulin and glucose). Significant differences were considered at  $P$  value  $<.05$ . Bonferroni's multiple comparison test was applied for *post hoc* comparison. All statistical analyses were performed using Statistica 7.0 (StatSoft Inc., Tulsa, OK, USA). All the results were expressed as mean values  $\pm$  standard deviation.

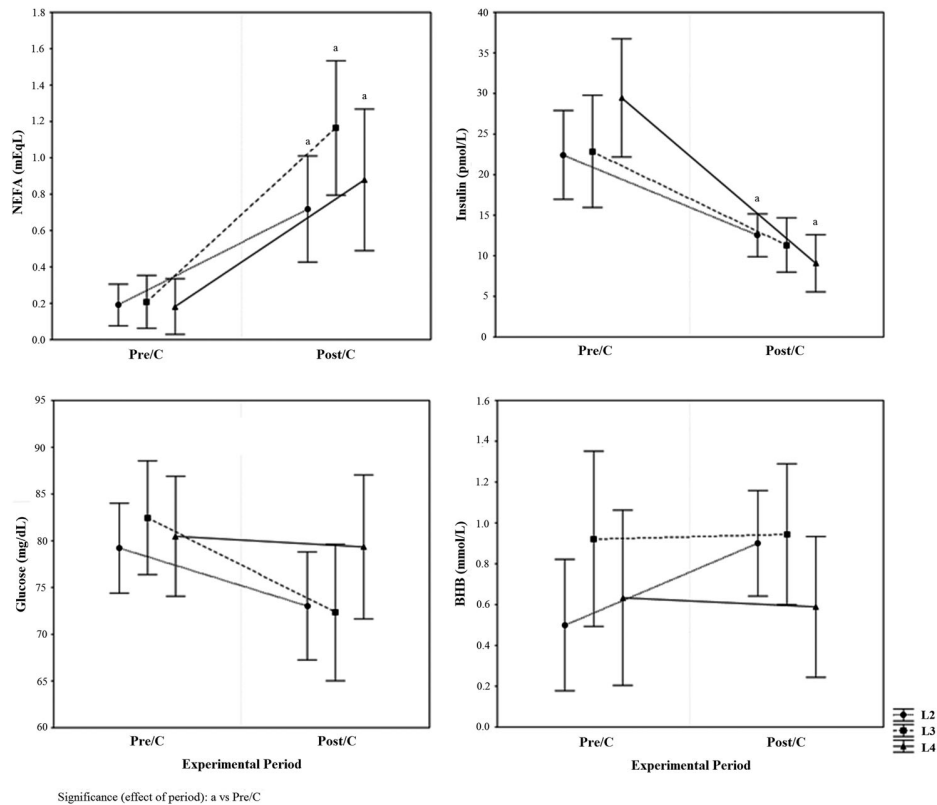
## 3. Results and discussions

The results obtained in the present study showed a significant effect of periods (Pre/C and Post/C) on NEFA and insulin concentrations ( $P <.05$ ) (Figure 1). In particular, the application of Bonferroni's *post hoc* comparison showed increased NEFA values during Post/C respect to Pre/C in L2 and L4 and decreased insulin values in Post/C compared with Pre/C in L2 and L4. No effect of lactation class was found on serum BHB, NEFA, insulin, glucose levels ( $P >.05$ ).

Insulin and NEFA trends obtained in the present study reflect the metabolic adaptation to postpartum phase (Zhao & Keating 2007). The liver plays a key role in the metabolic adaptation, through coordination and interconversion of nutrients to support pregnancy and lactation. During this physiologic phase insulin resistance inhibits glucose use in insulin-dependent tissues, such as muscle and adipose tissues: glucose can be deviated mainly to non-insulin-dependent tissues such as the mammary gland for milk production (Contreras & Sordillo 2011). Lower insulin levels found during postpartum period respect to prepartum period may be due to a decreased responsiveness of pancreatic  $\beta$ -cells to a state of hyperglycemia, caused by factors which inhibit the release of insulin, such as the increase of NEFA. Higher NEFA values during postpartum period indicated the activation of lipid mobilization that represented another metabolic mechanism of adaptation to postpartum period (Piccione et al. 2012). Effectively, it is well-known that low insulin concentration and reduced insulin sensitivity of the tissues around parturition increase lipid mobilization and induces further rises in serum NEFA concentrations (Hayirli 2006). It has been stated that the increase of serum NEFA concentration led to the increase of ketogenesis by hepatocytes (Grummer et al. 2004). The values of BHB obtained from peripartum cows enrolled in this study remained unchanged throughout the monitoring period and not exceeded the 1.00 mmol/L indicating no SCK condition (Geishauser et al. 2000). This finding is discordant compared to the results obtained from van Dorland et al. (2009), who showed higher BHB values during postpartum period than prepartum time suggesting that the cows enrolled in their study suffered from a larger energy deficit.

**Table 1.** Analytical composition of diets administered during pre-calving and post-calving.

Chemical composition (%)	Pre-calving	Post-calving
Crude protein	13.37	16.59
Ethereal extract	4.30	6.01
Neutral detergent fibre	40.77	30.17
Acid detergent fibre	24.82	20.37
Nonfibre carbohydrates	34.17	38.81
Starch	14.74	28.46



**Figure 1.** Trends of serum BHB, NEFA, insulin, glucose values measured in dairy cows during prepartum (Pre/C) and postpartum (Post/C) periods. Vertical bars denote the 95% confidence intervals.

#### 4. Conclusions

The decrease of insulin and the increase of NEFA found in this study showed the difficulty of dairy cows to cope with the energy demand characterizing the transition period.

Interactions between time period and health status suggest peripartum blood metabolite levels may provide some indication to postpartum disease risk and can be useful as a herd monitoring tool that may help to supply a new strategy for the improvement of farm management.

#### Disclosure statement

No potential conflict of interest was reported by the authors.

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