Animal Science Papers and Reports vol. 33 (2015) no. 2, 129-136
Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

Glucose infusion response to some biochemical parameters in dairy cows during the transition period*

Enrico Fiore[1], Antonio Barberio[2], Massimo Morgante[1], Maria Rizzo[3], Elisabetta Giudice[4], Giuseppe Piccione[3], Marcello Lora[1], Matteo Gianesella[1]**

[1] Department of Animal Medicine, Productions and Health (MAPS), University of Padua, Viale dell'Universita 16, 35020 Legnaro (Padua), Italy

[2] Experimental Zooprophylactic Institute of Venice, Viale dell'Universita 12, 35020 Legnaro (Padua), Italy

[3] Department of Veterinary Sciences, University of Messina, Polo Universitario dell'Annunziata, 98168, Messina, Italy

[4] Department of Biological and Environmental Sciences, University of Messina, Viale Ferdinando Stagno d'Alcontres 31, 98166, S. Agata-Messina, Italy

(Accepted March 24, 2015)

The aim of this study was to evaluate the effect of pre-partum versus post-partum periods on the biochemical profile in dairy cows. The effect of glucose tolerance test (GTT) on glucose ($Glu_{on\text{-}farm}$) and β-Hydroxybutyrate ($BHB_{on\text{-}farm}$) levels was also assessed. 42 Holstein cows from two different farms (farm A and farm B) were enrolled in the study. Body Condition Score (BCS) was determined for both groups. The GTT was carried out 7±5 days pre-partum and 7±5 days post-partum. In both periods blood samples were taken at T0 (pre-glucose administration), at T10 and T80 (10 and 80 minutes post-glucose administration). All samples were analysed for total protein, albumin, globulin, glucose, urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), calcium (Ca), phosphorus (P), sodium (Na), chloride (Cl), potassium (K), magnesium (Mg) and $BHB_{on\text{-}farm.}$ Two-way ANOVA was applied to determine significant effects of the transition period (pre-partum vs. post-partum) and farm (A vs. B) on the studied parameters as well as the effect of time before and after the GTT on $Glu_{on\text{-}farm}$. The average values of BCS, glucose, urea, Ca, Cl and K are significantly lower in the post-partum when compared to the pre-partum period (P<0.05). The increase in AST concentrations during the post-partum period (P<0.05) is considered a suitable indicator of hepatic

*This work was financially supported by the IZSVe (research project No. RC 17/10)
**Corresponding author: giuseppe.piccione@unime.it

steatosis. The glucose tended to increase significantly at T10 (P<0.05) and returned to pre-infusion concentrations at T80. These results confirm that the transition period is an important metabolic challenge to high-yielding dairy cows. Therefore, this study provides useful information in order to prevent the outset of nutritional imbalance that typically occurs in high production dairy cows.

KEY WORDS: biochemical parameters / dairy cows / Glucose Tolerance Test (GTT)

High-yielding dairy cows undergo a tremendous set of metabolic adaptations as they go from late pregnancy to early lactation. In fact, it is well known that during pregnancy all the metabolic pathways are involved in sustaining the foetus growth [Bell 2000]. The transition period in dairy cattle usually refers to the interval between 3 weeks pre-partum and 3 weeks post-partum and it is characterized by dramatic changes in metabolism and host defense mechanisms that are associated with an increased risk of disease [Contreras and Sordillo 2011, Jóźwik *et al*. 2012a]. During the peripartum period, the highest incidence of pathological conditions is observed in dairy cows. Late in pregnancy the feed intake in ruminants is reduced despite increased nutritional requirements for foetal development and mammogenesis/lactogenesis [Ingvartsen 2006], while nutrient demand to support conceptus growth and initiate milk synthesis is increasing. The nutritional status of dairy cattle has a significant influence on the onset of the transition cow production diseases that result in financial losses for farmers and reduced animal welfare [Mulligan *et al.* 2006].

Hayirli *et al.* [2002] demonstrated that dairy cattle that were over-conditioned (BCS > 4.0; using a scale of 1-5) in the last 3 weeks of gestation had a much greater depression in feed intake during the immediate pre-partum period when compared to cows with lower BCS.

Glucose metabolism in ruminants proves to be particularly significant during pregnancy and it appears to be a primary energy source for the foetus [Aldoretta and Hay 1999]. To go successfully through the transition period to lactation, dairy cows must develop metabolic adaptations that enable increased gluconeogenesis to face the heavy demands for lactose, mobilization of sufficient body fat reserves to face the energetic demands of lactation, and calcium mobilization to face the increased demands for calcium. Furthermore, this negative energy balance (NEB) is aggravated by the nutrient prioritization towards the mammary gland. Intense selection for milk production has resulted in an immense priority for the high-producing dairy cow to allocate energy to milk production, to the detriment of body reserves [Leroy *et al.* 2008]. As catalytic components of enzymes or to regulate several mechanisms involved in pregnancy and lactation [Goff 2006, Liesegang *et al.* 2007, Tanritanir *et al.* 2009] all animals require minerals (calcium (Ca), phosphorus (P), sodium (Na), chloride (Cl), potassium (K), and magnesium (Mg) for growth, reproduction and lactation. Inadequate amounts of these macrominerals can greatly influence the incidence of many disease [Goff *et al.* 1991, Wilde 2006, Gelfert and Staufenbiel 2008, Goff 2008].

The aim of this study was to describe how some biochemical parameters change in dairy cows during the transition period, by evaluating cows' response following a glucose tolerance test (GTT). The identification of differences in energetic metabolism between late pregnancy and early lactation might provide useful information to improve animal management strategies during the transition period.

Material and methods

Farm condition and Animals

Forty-two multiparous Holstein cows were selected from two high production dairy farms in the northern Italy. The data were collected from October 2011 to February 2012. The cows were divided into two equal groups on the basis of the farm of origin: farm A was in the Vicenza region, and farm B was in the Padua region, (Italy). Both farms apply a dry period of 60 days and a period of steaming-up of 15 days before calving. Farm A and farm B had a similar milk production (about 10000 (kg) a year) and quality (3.7% milk-fat, 3.4% 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, fecal consistency, and hematological profile. No animals showed any sign of diseases during the study. The animals were kept under the natural photoperiod and ambient temperature. Body Condition Score (BCS, 0 to 5 scale) was evaluated for each subject before the trial. All cows were fed a balanced diet in accordance with the nutritional requirements of the transition period. Table 1 shows the chemical composition of diets used during steaming-up and subsequent early lactation. Water was available *ad libitum*.

Table 1. Mean chemical composition (%) of diets of dairy cows during the final part of dry period (steaming-up) and early lactation

Period	Farm A		Farm B	
	steaming-up	early lactation	steaming-up	early lactation
Crude protein	12.63	16.59	12.87	16.66
Ethreal extract	4.18	6.01	4.55	5.32
Ash	7.55	7.42	7.83	7.34
Ndf	43.25	33.17	43.99	33.41
Nfc	33.39	38.81	33.56	38.27
Dry metter degradable	58.58	68.48	59.68	68.22
Adf	24.66	20.37	24.46	20.12
Starch	16.71	25.46	16.49	25.94
Calcium	0.39	1.06	0.41	0.98
Phosphorus	0.24	0.45	0.24	0.43
Magnesium	0.30	0.33	0.29	0.31
Sodium	0.10	0.58	0.11	0.62
Potassium	0.43	1.45	0.51	1.39
Chlorine	0.20	0.32	0.19	0.28
Sulfur	0.16	0.22	0.16	0.21

Blood sampling and analysis

The glucose tolerance test was carried out 7±5 days before (pre-partum) and 7±5 days after calving (post-partum). This easy technique assesses an animal's ability to maintain glucose homeostasis. All animals received sterile 50% glucose solution (0.25 g/kg body weight). Polyethylene catheters were aseptically inserted into both external jugular veins (one for the injection and the second for blood sampling) on the day prior to the experiment. Infusions were injected and blood samples were collected through the catheters without noticeable stress to the animals. From the opposite vein, blood samples were collected into vacuum tubes containing EDTA (Terumo Co., Tokyo, Japan) at T0 (pre-administration), and at T10 and T80 (10 and 80 min post-glucose administration). From the collection we developed on-farm ketone and glucose monitoring with a small hand-held meter that measures either whole blood BHB or whole blood glucose (OPTIUM XCEED). The samples obtained at T0 were refrigerated and were later processed in the laboratory for analyses. The tubes were centrifuged at 3.500 rpm for 10 min and the obtained plasma was stored at -18°C until analysed. Plasma was assessed by means of a BT1500 automated photometer analyzer (Biotecnica Instruments S.p.A., Roma, Italy) for the following biochemical parameters: Albumin, Globulin, Total Proteins, Urea, AST, ALT, Calcium, Phosphorus, Sodium, Chloride, Potassium and Magnesium. Globulins were determined by the difference between Total Proteins and Albumin concentrations.

Statistical analysis

Two-way analysis of variance was applied to determine significant effects of different periods (pre-partum vs. post-partum) and farms (farm A vs. farm B) on the parameters studied, as well as the effect of time points before and after a glucose tolerance test (T_0, T_{10} and T_{80}) on $Glu_{on\text{-}farm}$. The data collected were statistically analyzed by Prism ver. 4.00 (Graphpad Software, Inc., USA, 2003).

Results and discussion

In our study significantly higher BCS values (P<0.05) were observed in pregnant animals (Fig. 1). During early lactation cows are in negative energy balance and body reserves are an important fuel supply to assist the cow in reaching her genetic potential for milk synthesis [Grummer 1995]. The best BCS at calving is between 3.0 and 3.25 (5 point scale). Below 3.0 BCS cows produce less milk, are less likely to get pregnant, and are more likely to present themselves in an animal welfare-risk category. Over 3.25 BCS cows have a reduced dry matter intake, produce less milk, and are more likely to succumb to periparturient metabolic disorders [Roche *et al.* 2009].

A significant effect (P<0.05) of different periods on glucose, urea, AST, Ca, K and Cl levels was shown. The statistical analysis also confirmed a significant effect of the farm on urea, ALT and P parameters. As given in Table 2, significant differences (P<0.05) between pre-partum and post-partum periods were found in some biochemical

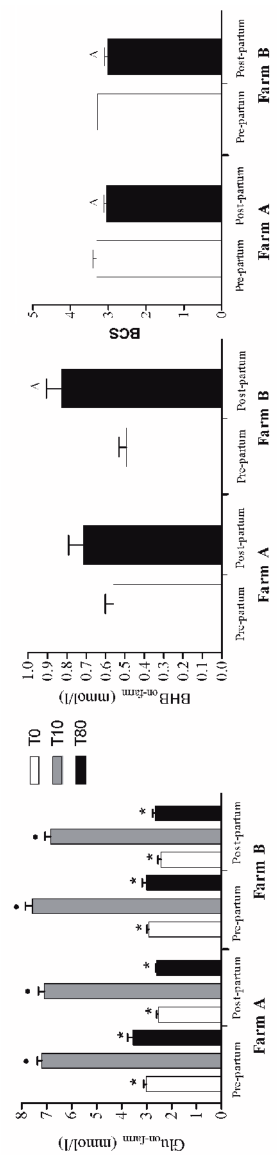

Fig. 1. Averages ±SD and statistical significance of $Glu_{on\text{-}farm}$ at T0 (pre-glucose administration), at T10 and T80 (10 and 80 minutes post-glucose administration), and of $BHB_{on\text{-}farm}$ and BCS obtained during different experimental periods (pre-partum and post-partum) in both farms. Significance: effect of time ($P<0.05$) * vs. T10, • vs. T80; effect of period ($P<0.05$) $^{\Delta}$ vs. Pre-partum.

indexes. Some of these parameters could be used for assessing the health status of the cow.

In this study plasma AST activities during the post-partum period were higher than the accepted reference intervals [Kaneko *et al.* 2008]. A similar trend was previously found by Jóźwik *et al.* [2012b], who investigated some metabolic parameters in lactating high yielding cows. This increase in AST concentration is considered one of the indicators of hepatic steatosis in the post-partum period [Cebra *et al.* 1997]. Kaneko *et al.* [2008] estimated that the AST has a sensitivity of 94% for steatosis and it reveals the presence of liver damage even in the absence of clinical manifestations [Kauppinen 1984, Meyer and Harvey 1998]. Changes in AST concentrations may indicate metabolic disorders involving the liver, including ketosis, even at subclinical levels [Meyer and Harvey 1998]. The concentrations of glucose, urea, calcium, potassium and chloride, although within physiological ranges, were significantly lower in the post-partum period when compared to the pre-partum period ($P<0.05$). Plasma glucose concentrations remain unchanged or slightly increase during the pre-partum transition period, it increased dramatically at calving, and decreased immediately postpartum [Kunz *et al.* 1985, Vazquez-Anon *et al.* 1994].

The $Glu_{on\text{-}farm}$ levels were higher pre-partum than in the post-partum period. Statistical differences ($P<0.05$) were also observed at T10 and T80 (Fig. 1). Moreover, a significant effect of sampling times on $Glu_{on\text{-}farm}$, ($P<0.05$) was estimated. The rate of glucose tended to increase after glucose infusion. We may observe an increase of $Glu_{on\text{-}farm}$ after GTT and a return to preinfusion concentrations after 80 min (T80). Pregnant subjects must provide an adequate supply of nutrients for the development of the foetus and produce large amounts of glucose by gluconeogenesis to face the heavy demands for lactose. If cows cannot meet even a slight additional demand for glucose, they become glucose deficient and overproduce ketones. An excessive fat mobilisation may induce an

Table 2. Averages (±SD) of some biochemical parameters and statistical significance obtained in both farms during different experimental periods (pre-partum and post-partum)

Parameter	Experimental conditions			
	farm A		farm B	
	pre-partum	post-partum	pre-partum	post-partum
Total protein (g/dl)	6.81±0.72	7.07±0.70	6.76±0.46	6.60±0.76
Albumin (g/dl)	3.30±0.18	3.38±0.14	3.22±0.16	3.30±0.25
Globulin (g/dl)	3.51±0.70	3.69±0.71	3.54±046	3.30±0.71
Glucose (mmol/l)	80.13±6.75	75.55±8.08$^{\Delta}$	80.84±6.05	75.16±9.28$^{\Delta}$
Urea (mmol/l)	22.65±8.48	15.34±4.51$^{\Delta}$	26.55±5.17*	21.43±5.59*
AST (U/l)	78.40±10.13	118.25±20.89$^{\Delta}$	89.25±16.04	114.26±25.32$^{\Delta}$
ALT (U/l)	21.29±7.03	19.82±5.40	18.21±5.75*	14.43±6.06*
Ca (mmol/l)	10.92±0.50	9.97±0.69$^{\Delta}$	10.92±0.82	9.89±1.43$^{\Delta}$
P (mgl/dl)	5.65±1.24	5.72±1.06	7.12±0.88*	6.14±2.02*
Na (mmol/l)	139.25±3.58	140.75±3.68	139.38±1.27	139.73±3.00
Cl (mmol/l)	105.38±7.49	98.55±3.24	106.65±13.01	95.12±5.88$^{\Delta}$
K (mmol/l)	4.60±0.44	4.25±0.53$^{\Delta}$	4.51±0.42	4.02±0.65$^{\Delta}$
Mg (mg/dl)	2.72±0.49	2.62±0.41	2.57±0.19	2.32±0.65

$^{\Delta}$Differences between periods within farm significant at P<0.05.
*Differences between farms within a period significant at P<0.05.

imbalance in hepatic carbohydrate and fat metabolism, which may result in ketosis [Herdt and Emery 1992, Goff and Horst 1997]. Ketosis is a metabolic disorder that primarily occurs 2-7 weeks after calving [Halse 1978, Gillund *et al.* 2001] with a diagnosis made at a median of 24-28 days post-partum [Řstergaard and Gröhn 1999, Fleischer *et al.* 2001]. The $BHB_{on\text{-}farm}$ values recorded in the present study, although within the physiological range for cows (0.00-0.86 mmol/l, [Radostis *et al.* 2007]), were higher in the post-partum period than pre-partum (Fig. 1). The transition from gestation to lactation is a period of great metabolic stress for dairy cows [Rollin *et al.* 2010]. Metabolic and endocrine adjustments must be made, as dairy cows move from late gestation to early lactation. The importance of a successful transition is obvious [Drackley *et al.* 2001]. A method to diagnose animal disease or to monitor herd health is the basis for developing the metabolic profile, with the main purpose to indicate a herd's susceptibility to production disease.

While confirming that the transition period presents an important metabolic challenge to high-yielding dairy cows, this study could be a useful tool in managing and preventing the onset of nutritional imbalance that typically occurs in high production dairy cows.

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