Transition period in healthy and diseased dairy cows: evaluation of metabolic modifications

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SUMMARY

Introduction - The time from late pregnancy to early lactation is known as transition period and is characterized by a metabolic stress that leads to a high incidence of metabolic, infectious, and reproductive disorders associated with a severe negative energy balance (NEB). During the period of NEB there is an increase of lipolysis and a decrease of lipogenesis causing a striking increase of blood levels of βhydroxybutyrate (BHB) and non-esterified fatty acids (NEFA). Moreover, an alteration of several blood metabolites including urea, calcium, magnesium, glucose and insulin concentrations is typical during NEB in association with increased hepatic gluconeogenesis. Inflammatory conditions during the transition period may cause a shift in liver protein synthesis, including a marked reduction in albumin and cholesterol serum concentrations.

Aim - The aim of this study was to evaluate the changes of Body Condition Score (BCS) and some haematochemical parameters in healthy and diseased high yielding dairy cows during the transition period. Moreover, the relationship between glucose, βhydroxybutyrate (BHB) and non-esterified fatty acids (NEFA) in healthy and diseased high yielding dairy cows was evaluated during the transition period.

Materials and methods - Blood sampling and BCS values were collected from 24 dairy cows 15 ± 5 days prepartum (T0), 10 ± 2 days postpartum (T1), 30 ± 2 days postpartum (T2) and 50 ± 2 days postpartum (T3). On blood samples, serum glucose, BHB, NEFA, total proteins (TP), albumin, globulins and urea concentration was evaluated. Two way repeated measure analysis of variance (ANOVA) and Person's correlation test were applied on obtained data.

Results and discussion - A significant BCS loss during the postpartum in both groups was found. The decrease of glucose values and the increase of NEFA and BHB values were found suggesting the difficulty of dairy cows to cope with the energy demand. Serum TP, albumin and globulins values were higher during the lactation than prepartum. Serum urea values showed significant differences during the different physiological phases.

Conclusions - Our data contributed to improve the knowledge on changes of some blood metabolite concentration during the transition period for a deeper interpretation of clinical data and an easier diseases diagnosis in high yielding dairy cows.

KEY WORDS

Haematochemical parameters, dairy cow, transition period, animal welfare, body condition score.

INTRODUCTION

High yielding dairy cows have been selected to support high milk production through their ability to mobilize fat and muscle[1]. The time from late pregnancy to early lactation is known as transition period and it spans from 3 weeks before to 3 weeks after the parturition[2]. A metabolic stress is likely to occur during this period[3] leading to a high incidence of metabolic, infectious, and reproductive disorders[3,4]. Some of these alterations are related to increases in energy requirements driven by both foetal needs and lactogenesis[5]. At the onset of lactation, the dairy cows must cope with an increase in energy demand by the mammary gland for milk production[4]. In addition, high rates of body condition score (BCS) losses along with a gradual decline in dry matter intake (DMI) and alterations in blood metabolite and hormone profiles occur during this period and are known to be associated with a severe negative energy balance (NEB)[3]. Thus, during the period of NEB key hormone expression and tissue responsiveness alter to increase lipolysis and decrease lipogenesis causing a striking increase of blood levels of βhydroxybutyrate (BHB) and non-esterified fatty acids (NEFA) in blood concentration[3,5]. Hepatic uptake of fatty acids is positively related to the plasma concentrations of NEFA. It is well known that an apparent reduction in liver function due to accumulated triglycerides in the hepatocytes occurs[6]. Development of these hepatic lesions have been associated with an increased disease incidence in the postpartum period such as retained foetal membranes, metritis, ketosis, mastitis, displacement of the abomasum, hypocalcemia, immunosup-

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pression and lower milk production in dairy cow[4,7]. Moreover, an alteration of several blood metabolites including urea, calcium, magnesium, glucose and insulin concentrations is typical during NEB in association with increased hepatic gluconeogenesis[7,8]. Inflammatory conditions during the transition period may cause a shift in liver protein synthesis, including a marked reduction in albumin and cholesterol serum concentrations[7]. BCS is still an object of research for the prevention of excessive negative metabolic changes in dairy cows, as it shows good correlation with increased risk of fatty liver or ketosis[6]. In view of this, the aim of the present study was to evaluate the changes of BCS and some haematochemical parameters, and the correlation between glucose, BHB and NEFA in healthy and diseased high yielding dairy cows during the transition period.

MATERIALS AND METHODS

In our study, protocols of animal husbandry and experimentation were reviewed and approved in accordance with the standards recommended by the *Guide for the Care and Use of Laboratory Animals* and Directive 2010/63/EU for animal experiments.

Animals

Twenty-four pregnant multiparous Holstein Friesian cows were enrolled in this study. The artificial insemination was performed for each dairy cows. Each animal was kept under natural photoperiod and ambient temperature. All the animals were clinically healthy and free from internal and external parasites at the beginning of the experimental period. The health status of the dairy cows was evaluated based on parity, dystocia and milk yield. No dystocia was found. Milk yield quality was optimal with an average of 3.7% of milk fat, and an average of 3.4% of milk protein. Rectal temperature, heart rate, respiratory profile, appetite, faecal consistency, and hematologic profile were assessed in all cows in order to evaluate their health status. After the partum, 13 animals presented one or more periparturient pathologies, including metritis, mastitis, or retained placenta. The average age, expressed in months, was 55.31 ± 12.45 in healthy group (n. 11) and 56.85 ± 16.40 in diseased group (n. 13). All the animals were multiparous and the mean of the parity was 3.18 ± 0.94 for healthy group and 3.31 ± 1.32 for diseased group.

Sample collection

BCS and blood samples were collected from each animal at 4 time points: 15 ± 5 days prepartum (T0), 10 ± 2 days postpartum (T1), 30 ± 2 days postpartum (T2) and 50 ± 2 days postpartum (T3). BCS (0 to 5 scale) was evaluated in each subject[9]. Blood samples were collected by jugular venipuncture with 22G needles into vacuum tubes (BD Vacutainer Systems®) with no anticoagulant additive.

Haematochemical parameters

Blood samples were centrifuged at 3500 rpm for 10 minutes and the obtained sera were transferred into plastic tube and stored at -20°C until assay. Glucose, BHB and NEFA were assessed by a UV spectrophotometer, Cobas C 501 analyzer (Roche Diagnostics, Mannheim, Germany) using commercial available kit. Total proteins (TP), albumin, globulins and urea were determined by an automated biochemistry analyzer BT1500 (Biotecnica Instruments S.p.A., Roma, Italy) using commercial available kit.

Statistical analysis

All results were expressed as mean ± standard error of the mean (SEM). Data were tested for normality using the Shapiro Wilk analysis. Two way repeated measure analysis of variance (ANOVA) was used to determine the differences between healthy and diseased animals, the statistically significant effect of sampling time (T0, T1, T2 and T3) on BCS and clinical status on biochemical analytes. P values <0.05 were considered statistically significant. Tukey-Kramer multiple comparisons test was used for post hoc comparisons. The influence of glucose concentration on NEFA and BHB values was evaluated using Person's correlation test. Statistical analysis was performed using the STATISTICA 7 software package (Stat Software Inc., Tulsa, Oklahoma).

RESULTS

The application of ANOVA showed a statistically significant difference between the healthy and diseased groups (P<0.001) in serum urea concentrations and a statistically significant effect of sampling times (P<0.05) on BCS, glucose, BHB, NEFA, TP, albumin, globulins and urea (Fig. 1). Particularly, BCS statistically decreased at T2 vs T0 in healthy group and at T1, T2 and T3 vs T0 in diseased group. Moreover, glucose values increased in both groups at T0 vs T1, T2 and T3, and at T1 vs T2 and T3. BHB concentration increased at T1 vs T0, T2 and T3 in both groups, whereas in diseased group BHB concentrations increased at T3 vs T0 and T2. NEFA concentration increased at T1 vs T0, T2 and T3 in healthy group, whereas in diseased group decreased at T0 vs T1, T2 and T3, and at T2 and T3 vs T1. TP values decreased at T1 vs T0 and T3 in healthy group, whereas in diseased group decreased at T1 compared to T0, T2 and T3. Albumin concentration decreased at T1, T2 and T3 vs T0 in both groups. Globulin levels showed higher values at T2 and T3 vs T1 in healthy group, and higher values at T3 vs T1 in diseased group. Urea levels showed higher values at T1, T2 and T3 vs T0 in both groups, whereas in healthy group the values were higher at T1 vs T2.

The application of Pearson's correlation coefficient showed a negative correlation between glucose and BHB at T2 and T3 in both groups (Table 1). Whereas, a negative correlation between glucose and NEFA was found at T2 and T3 in healthy group and at T3 in diseased group (Table 2).

DISCUSSION

In literature, there is a lack of information on BCS values and haematochemical parameters in healthy and diseased periparturient high yielding dairy cows at different time points. This study demonstrated a significant BCS loss during the dry to early lactation (30 days postpartum) periods in both groups. A BCS gain in healthy group was found, whereas in diseased group the animals continued to have a constant BCS loss. Marked BCS loss from the dry to near calving pe-

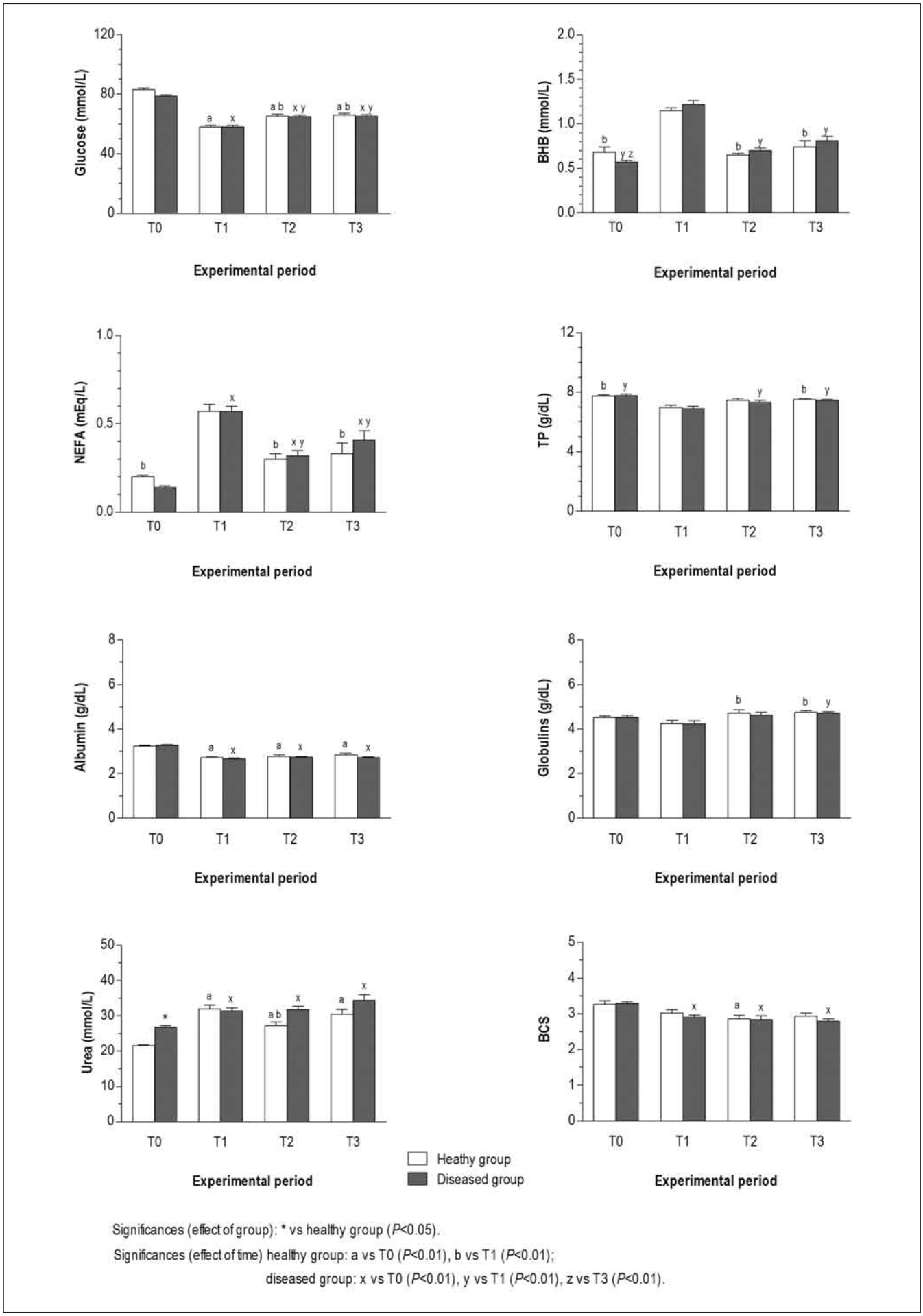

Figure 1 - Mean values (±SEM) of BCS, glucose, BHB, NEFA, TP, albumin, globulins and urea, together with statistical significances, obtained during the experimental period (T0-T3) in healthy animals (white bar) and diseased animals (black bar).

Table 1 - Coefficients of correlation (r) between serum glucose and BHB values obtained from healthy group and diseased group during the experimental period. P values <0.05 were considered statistically significant.

Healthy group	Glucose T0	Glucose T1	Glucose T2	Glucose T3
BHB T0	r=-0.2938 P=0.381			
BHB T1		r=0.0269 P=0.937		
BHB T2			r=-0.6642 P=0.026	
BHB T3				r=-0.6623 P=0.026
Diseased group	Glucose T0	Glucose T1	Glucose T2	Glucose T3
BHB T0	r=-0.0745 P=0.809			
BHB T1		r=0.5198 P=0.069		
BHB T2			r=-0.6071 P=0.028	
BHB T3				r=-0.7190 P=0.006

Table 2 - Coefficients of correlation (r) between serum glucose and NEFA values obtained from healthy group and diseased group during the experimental period. P values <0.05 were considered statistically significant.

Healthy group	Glucose T0	Glucose T1	Glucose T2	Glucose T3
NEFA T0	r=0.0342 P=0.920			
NEFA T1		r=0.1708 P=0.616		
NEFA T2			r=-0.6197 P=0.042	
NEFA T3				r=-0.6891 P=0.019
Diseased group	Glucose T0	Glucose T1	Glucose T2	Glucose T3
NEFA T0	r=0.1676 P=0.584			
NEFA T1		r=0.3142 P=0.296		
NEFA T2			r=-0.1773 P=0.562	
NEFA T3				r-=0.7022 P=0.007

riods results in an increased incidence of postpartum metabolic and reproductive diseases[10]. Contrary, other authors, demonstrated that cows that lose more BCS (more loss in body fat reserves) utilize these reserves for milk production without negative effects on reproductive performance or on the incidence of retained placenta[11]. During this period, the high demand of nutrients stimulates the mobilization of the body fat in cows, which may result in the development of metabolic diseases such as milk fever and ketosis fatty liver complex[10]. An association between BCS at calving, reproductive performance and disease was reported[12]. Conflicting results have also been reported on the association between BCS loss during lactation and reproductive performance and disease[13]. Ketosis in dairy cows made a significantly high BCS loss over a prolonged period of time compared with no diseased cows[14].

Our results showed that the general pattern of change in metabolite concentrations was significantly affected by the physiological periods (prepartum and postpartum) and could reflect the health status of dairy cows. According to some authors, our results confirmed that the larger negative energy balances did not influence the plasma glucose concentrations during the first four weeks' postpartum period[15]. Glucose is an important direct controller of metabolic interactions and responses in dairy cows during the transition period[5]. According to some authors, in our study plasma glucose concentrations remained stable or slightly increased during the prepartum transition period, dramatically increased at calving, and then decreased immediately postpartum[8,16]. The glucose concentration increase at calving may result from increased glucagon and glucocorticoid concentrations that promote depletion of hepatic glycogen stores[16]. The outcomes of this study confirm that blood glucose is an insensitive marker of energy status in cattle[6,8]. Our results demonstrated that larger negative energy balances did not influence on plasma glucose concentrations during the first four weeks in postpartum period[2,5,8]. The decrease of glucose concentration and the increase of BHB and NEFA values found at T1 in our study, showed the difficulty of dairy cows to cope with the energy demand characterizing the transition period according to other authors[5]. These results indicated the activation of lipid mobilization that represented another metabolic mechanism of adaptation to postpartum period[2,3]. A negative correlation was found between glucose and both BHB and NEFA in both healthy and diseased group. Effectively, our findings showed that fat mobilization, during the transition period, is characterized by increased serum BHB and NEFA levels and by decreased serum glucose values that could contribute to the suppression of the immune system[2]. BHB levels are more sensitive for detecting subclinical stages of ketosis than NEFA in high yielding dairy cows with a high degree of lipomobilization. Additionally, high lipomobilization negatively impacts liver functions but does not imply that ketosis might be present[4].

In our study, the serum TP and globulins values levels were higher at T0 than T1 in both groups, it can be justified because in cows, the serum TP, β2 and γ1globulins increase at 2 months before term, reaching maximum values at 1 month, and then rapidly decline toward the term in dairy cows[17]. This mechanism reflects the transport of immunoglobulins from serum to the mammary gland, to be transferred to colostrum, that begins several weeks before parturition reaching a peak 1 to 3 days before calving[17]. In agreement with other reports, serum TP concentrations at T1 were lower than concentrations at T0, T2 and T3[17,18]. The decrease of serum TP concentration has been indicated as an effect of a reduction of globulin fraction whereas changes in plasma albumin were not relevant[17,18]. In our study, albumin levels decreased at T1 with a slight increase at T2 and T3, in both groups, as previously reported[19]. A consequence of in-

creased concentrations of NEFA in plasma is the alteration of the physiological functions of albumin. In periparturient dairy cows, plasma concentrations of this protein decreased especially during the first weeks after calving[19]. Since albumin is synthesized by hepatocytes, its concentration is considered a good indicator of liver function[18].

Serum urea concentrations showed significant differences during the different physiological phases. The variation of urea concentration around calving may be due to a variety of factors. Impaired liver function, as commonly occurs after calving, reduces the metabolic clearance of urea. Glucose availability may be supplemented by increased catabolism of amino acids stored in skeletal muscle and other tissue proteins, resulting to an increase of urea production[1,2].

In conclusion, our results confirmed that transition period represents an important metabolic challenge to the high yielding dairy cows. During this period, the interpretation of blood metabolite changes allows to better interpret clinical data and diagnose disease improving the animal welfare in high yielding dairy cows. The present study contributes to improve the knowledge of dairy cow during the transition period, which is a very stressful lifetime and constitutes crucial welfare and economic problem to animals and farmers.

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