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Body condition score loss, hepatic lipidosis and selected blood metabolites in Holstein cows during transition period*

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The aim of this study was to investigate the relationship between the loss in body condition score (BCS) and loss (ΔBCS), energy balance (EB), hepatic lipidosis and blood serum concentration of non esterified fatty acids (NEFA), glucose, triacylglycerol (TAG) and total bilirubin (tBIL) in healthy dairy cows during transition period. Twenty healthy Holstein cows were included and categorized into groups based on BCS loss (ΔBCS) between dry period and early lactation (ΔBCS <0.75 and ≥0.75). Significant differences between groups (p<0.05) were observed for blood serum NEFA, glucose and tBIL. Cows with high ΔBCS (≥0.75) between dry period and early lactation showed increased blood serum NEFA, TAG and tBIL concentrations and lower blood serum glucose concentration

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during transition period, compared to the low ΔBCS cows (<0.75). Metabolic profiles of cows during transition period indicate high level of lipid mobilization from adipose tissue, possible decreased liver cells TAG export and gluconeogenic ability and impaired bilirubin metabolism if ΔBCS is increased by more than 0.75 points. ΔBCS was in relation with energy balance (EB) in transition period.

KEY WORDS: body condition score loss / cows / metabolic profile / transition period

Transition period in dairy cows is defined as 3 weeks before and after parturition. It is the most critical time in dairy cows life cycle, with highest incidence of metabolic diseases and infections occuring during that period. [Goff and Horst 1997, Drackey 1999, Jóźwik *et al.* 2012]. Homeorhesis is favored since mammary gland utilizes most of the nutrients and metabolites for the milk synthesis, almost regardless of the other body needs [Bauman and Currie 1980, Cincović *et al*. 2012]. This situation is characterized by negative energy balance (NEB), which is the major driving force for substantional endocrine, metabolic and body condition changes. Dairy cows in NEB are forced to mobilize their body fat reserves, with large quantities of NEFA being released from adipose tissue in order to meet energy demands [Markusfeld *et al.* 1997, Rukkwamsuk *et al.*1999, Djokovic *et al.* 2011, 2013]. If the amount of ingested nutrients and mobilized endogenous energy is enough to cover the increased energy demands, lactation is maintained and dairy cows health is uncompromised, but they experience a dramatic body condition loss during the first weeks of lactation [Rukkwamsuk *et al*. 1999, Gerloff 2000]. The body condition scoring system represents useful management tool to asses nutritional status in dairy cows [Edmonson *et al.* 1989]. Most of the dairy cows gain weight during late gestation and dry period, because of the *ad libitum* nutrition. Unrestricted feed consumption with the increased energy intake in dairy cows during dry period could leed to syndrome called "fat cow syndrome" [Morrow 1976]. Those dairy cows become obese, and the risk for their health disorders during transition period and subsequent lactation dramatically increases. However, not all obese cows develop metabolic diseases after parturition, indicating there are other factors contributing to their pathogenesis. The absolute amount of body condition score loss (ΔBCS) could be good indicator of nutritive status and correlates well with the high incidence of reproductive and metabolic diseases [Kalaitzakis *et al*. 2007, Šamanc *et al*. 2010]. First blood serum metabolic profiles included packed cell volume and hemoglobin along with glucose, proteins and minerals [Payne *et al.* 1970]. Blood serum metabolic profiles of NEFA, TAG, glucose and tBIL have been used to monitor dairy cows nutritional and liver health status during transition period [Drackley *et al*.1991, Kim and Suh 2003, Fratrić *et al.*2009, Jóźwik *et al.* 2012]. Such profiles have also been used to monitor herd health and to find subclinical disease, to predict risk of ketosis or abomasal displacement as well as to investigate herd problems with metabolic disorders [Oetzel 2004, Le Blanc *et al* .2007, van Dorland *et al*.2009, Le Blanc 2010]. There is a valid opinion that blood serum metabolic profiles can be used to identify nutritional dysbalances before they could have negative impact on animals health and milk production [Whitaker 2004, Le Blanc 2010, Jóźwik *et al.* 2012].

The aim of this study was to evaluate the relationship between body condition score loss (ΔBCS), EB, hepatic lipidosis and blood serum concentration of NEFA, glucose, TAG and tBIL in healthy dairy cows during transition period.

Material and methods

Animals

Twenty dry cows that had yielded 7858±580 l of milk in the previous lactation were chosen from the commercial dairy herd (PIK Beograd). The cows aged from 4 to 6 years, weighing 661±24 kg in dry period and 576±23 kg in the early lactation. Cows were housed in a tie-stall barn. During the dry period cows were fed a diet consisting of 3 kg lucerne hay, 3 kg wheat straw, 10 kg maize silage (30%DM), 4 kg lucerne haylage, 2 kg maize ear silage (68%DM), 0.5 kg dry sugar beet pulp, 1.5 kg concentrate (12%CP). During the early lactation the diet consisted of 4 kg lucerne hay, 15 kg maize silage (30%DM), 8 kg lucerne haylage, 4 kg maize ear silage (68%DM), 2 kg dry sugerbeet pulp, 2 kg excruded soybean meal, and 4.5 kg concentrate (18%CP). The dietary nutrient content for dairy cows in dry period and early lactation is presented in Table 1. Energy balance was evaluated according to body weight, offered meal and average milk production using NRC [2001] standards.

Table 1. Nutrient content in daily ration for experimental dairy cows in dry period and early lactation

Nutrient	Dry period	Early lactation
Dry matter (DM)	12.10	21.5
Net energy of lactation (NEL), MJ	66.20	153.20
Crude protein (CP), %DM	12.10	18.30
Rumen undegradable protein (RUP), %CP	35.82	39.69
Fat, %DM	3.09	4.92
Fibre, %DM	25.12	17.20
Acid detergent fibre (ADF), %DM	32.33	22.60
Neutral detergent fibre (NDF), %DM	49.08	37.16
Iodine (I), mg/kg (DM)	0.60	0.60

Body condition scoring (using five-point scale with quarter-point divisions) was performed on 20 pregnant Holstein dairy cows by the same investigator using the system provided by Elanco Animal Health. Experimental cows were scored once for body condition during the dry period (2 weeks before calving, 15±2 days), and once during puerperium (2 weeks postpartum day, 15±3 days). Body condition loss was determined as physiologically acceptable if loss was up to 0.7 between two consecutive phases of productive-reproductive cycle (dry period/puerperium) [Kim and Suh 2003, Šamanc *et al*. 2010] and this criterion was used for dividing all experimental cows into two groups: 1 – group of low BCS loss (L group, n=9, ΔBCS <0.75), and 2 – group of cows with high BCS loss (H group, n=11, ΔBCS >0.75). Results for BCS are presented as mean BCS values for two groups of cows and absolute BCS prepartum-postparum difference (ΔBCS).

Biochemical analysis

Blood was sampled twice during the late dry period – in week 1 (7±2 days) and – week 2 (15±2 days) relative to parturition, and twice during early lactation period +1 (7±2 days) and +2 weeks (15±3) days after parturition. Blood samples were collected at 10:00 h a.m. or 4 to 6 hours after milking and feeding, from the jugular vein into sterile disposable test tubes. After clotting for 3 hours at 4°C and centrifugation (1500G, 10 min, 4°C), sera were carefully harvested and stored at -20°C until analysis. Blood samples collected on fluoride, immediately centrifuged in the same way and plasmas were assessed for glucose concentration. The following biochemical blood components were measured at Biochemical Laboratory (INEP, Zemun, Serbia) by different colorimetric techniques using spectrophotometer (COBAS MIRA): NEFA levels were measured by RANDOX (United Kingdom) kit, glucose and tBIL by HUMAN kit (Germany) and TAG by ELITECH kit (France).

Histopathological analysis

Shortly after blood collection, the liver was sampled through percutaneous biopsy at day 15 after calving (15±3 days). Liver specimens were fixed in neutral 10% formaldehyde solution and histopathologically analysed for lipid contents at the Pathological Department of the Faculty of Veterinary Medicine in Belgrade. Sections obtained using a freezing microtome (LEICA 1850, Jung Tissue Freezing Medium) were specifically stained with Sudan III. The liver lipid contents were semi-quantified through computer image analysis (Software Q Win) made using the appliance (LEICA Q 500 MC). Lipid content in the hepatocytes was evaluated using stereological method [Gaal *et al.*1983], and presented as percentage (%).

Statistical

The significance of differences in BCS between groups were determined using Student t-test at the level of significance 5% and 1%. Blood serum metabolic profiles of NEFA, glucose, TAG and tBIL were analysed using XLSTAT (Repeated measures ANOVA with Tukey test) and the model included group, time of blood sampling and their interactions as fixed effects. Results of metabolic profiles are presented as least-square means ± pooled standard error of the mean (LS mean±SEM). Least-square means were compared with Tukey test at a significance level of 5%. Results of the liver fat content are presented as percentage (mean±standard deviation). Correlation coefficients were calculated using linear regression models to study relationships between variables.(Statgrafic Centurion,Statpoint Technologies Inc. Warrenton,Virginia, USA). Correlation coefitient between energy balance and change of body conditions score was also calculated by linear regression model.

Results and discussion

Transitional period is period with the most important nutrition, metabolic and endocrine challenges in dairy cows. Beginning of lactation in dairy cows is characterized by NEB which is accompanied by marked decrease in body condition score and significant changes of blood metabolites [Rukkwamusk *et al.* 1999, Jorritsma *et al.* 2003, Djokovic *et al.* 2007]. Since change of BCS reflects energy balance there is a recommendation for cows BCS scoring at least three times per year in order to avoid great oscillations in energy metabolism that may provoke fatty liver [Bobe *et al.* 2004, Grummer 2008]. The physiological range of body condition scores for high-yielding Holstein cows is 3.25 to 4.00 (dry period) and 3.25 to 3.75 (puerperium).

The values of mean BCS and and absolute BCS difference of examined cows in this experiment (n=20) at dry period and puerperium are presented at Table 2.

The data presented in Table 2 indicate that significant BCS loss in cows occurs between dry period and puerperium. This difference between mean BCS values was 0.25 and 0.87 for the L and H BCS loss group, respectively.

Table 2. The BCS values of experimental cows (mean BCS±SD) and absolute BCS difference (ΔBCS) between dry period and *puerperium*

Group	BCS (-2 wk)	BCS (+2 wk)	ΔBCS
Low BCS loss, L (N=9)	3.78^{A}±0.15	3.53^{A}±0.15	0.25
High BCS loss, H (N=11)	4.39^{B}±0.26	3.52^{B}±0.28	0.87

BCS – body condition score.
Means within row bearing the same superscript letter differ significantly at P<0.01.

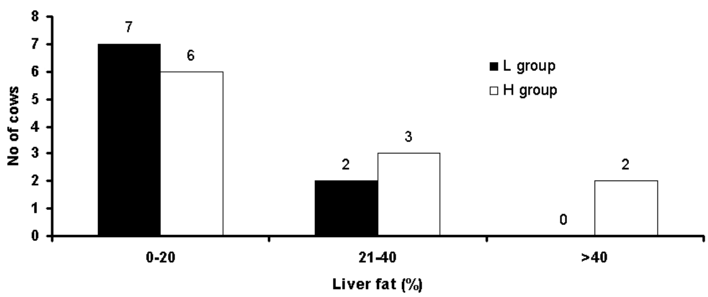

Fig. 1. Distribution of the liver fat content in L and H groups of dairy cows.

Liver fat content 2 weeks after partum in the H group was numerically higher than in the L group of dairy cows (23±17% *vs.* 17±10%), but there were no significant differences between means due to the high individual variation. Distribution of liver fat content in both groups is presented in Figure 1.

Table 3. Effects of time and group on selected blood serum metabolites

Metabolite	Class	Time in relation to parturition				P-value		
		-2 wk	-1 wk	+1 wk	+2 wk	time	group	time × group
NEFA (mmol/l)	H	0.267	0.426	0.549	0.642	**<0.0001**	0.115	**0.048**
	L	0.264	0.314	0.431	0.442			
Glucose	H	2.47	2.22	2.55	2.27	0.904	**<0.0001**	**0.007**
(mmol/l)	L	2.67	2.95	2.71	2.99			
TAG	H	0.434	0.385	0.346	0.346	**0.020**	**0.003**	0.360
(mmol/l)	L	0.348	0.329	0.337	0.309			
tBIL	H	6.165	6.950	7.470	7.323	0.056	**<0.0001**	0.878
(µmol/l)	L	4.249	4.719	5.874	4.919			

Grouped according to high (H >0.75) and low (L ≤0.75) ΔBCS
Bold values indicate significant effects.
NEFA – Non-esterified fatty acids; TAG – triacylglyceride; tBIL – total bilirubin.

Table 4. The difference in blood serum NEFA concentrations (mmol/l) between periods in both groups of dairy cows (increment index, H/L)

Period	L	H	Increment index (H/L)
-2 wk to -1 wk	0.050	0.159	3.18
-1 wk to +1 wk	0.117	0.123	1.05
+1wk to +2 wk	0.011	0.093	8.45

NEFA – non-esterified fatty acids.

Table 5. Energy balance and milk yield in two groups of cows and its relation to ΔBCS

Group	EB1 (-2 wk)	EB2 (+2 wk)	ΔEB (EB1-EB2)	Milk production in first 14 day (l/day)
Low BCS loss, L (N=9)	19.3±8.8	10.1±23.3	-9±14.4	21±3.2
High BCS loss, H (N=11)	17.1±9.5	6.1±15.5	-11±13.3	24±4.5
Correlation with ΔBCS	-0.51*	-0.59**	0.73**	0.3

BCS – body condition score; EB – energy balance.
*P<0.05; **P<0.01.

Effects of time, group and time × group interactions for blood serum metabolites profiles for NEFA, glucose, TAG and tBIL are presented in Table 3. The difference in blood serum NEFA concentrations between periods in both groups of dairy cows is presented in Table 4.

The correlation coefficients for selected parameters are presented in Figure 2.

Relationship between energy balance and change in body condition score is presented in Table 5.

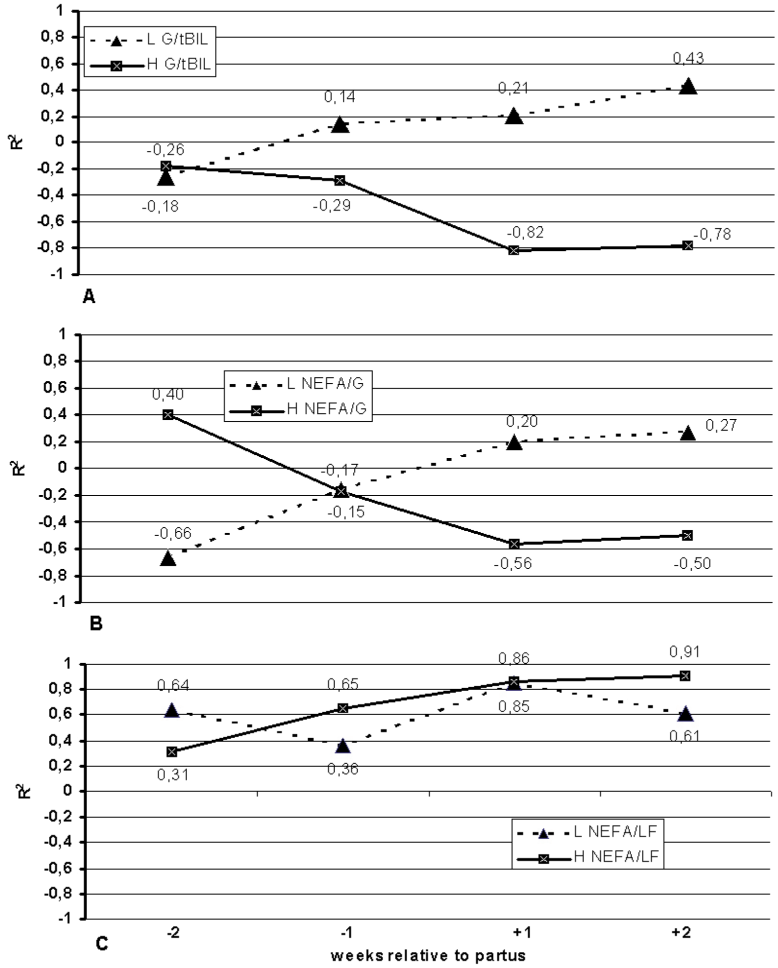

Fig. 2. The correlation coefficients (R^2) for selected parameters during peripartal period in the two groups of dairy cows; A – correlation between glucose and total bilirubin G/tBIL; B – between non-esterified fatty acids and glucose NEFA/G; C – between non-esterified fatty acids and liver fat NEFA/LF.
L – low ΔBCS; H – high ΔBCS; G – glucose; tBIL – total bilirubin; NEFA – non esterificated fatty lipids.

The physiologically acceptable loss in body condition between dry period and puerperium is up to 0.7 points [Kim and Suh 2003, Šamanc *et al*. 2010]. Previous results indicate that the obese cows had marked BCS loss (0.85 points) between dry period and *puerperium* [Kim and Suh 2003, Šamanc et *al.* 2010]. Our results indicate that both groups of cows showed statistically significant loss in BCS. The average BCS loss in the second group (H) was 0.87 points, almost the same value as previously reported for cows with severe fatty liver [Šamanc *et al*. 2010]. Most of the cows from both groups had liver fat content below 20%. However, tree cows from H group had moderate and 2 cows severe hepatic lipidosis, indicating tendency in that group of cows towards the pathological degree of liver fat accumulation (Fig. 1). Mobilization of body lipids is a major metabolic determinant of peripartal period in dairy cows. In high producing dairy cows lipomobilization during peripartal period could reach pathological range disturbing liver's morphological and functional integrity. One of the factors affecting level of lipomobilization is obesity in dry cows (BCS >4.0). Our data indicate similar levels of NEFA in blood serum appearing 2 weeks before partum (-2 wk) in H and L group of dairy cows. One week postpartum there was an increase in blood serum NEFA concentration that was more pronounced in H group of dairy cows. The difference in LS means of NEFA between -2 wk and -1 wk in the group H was 3.18 times higher than in group L. There was similar difference between -1 wk and +1 wk in blood serum NEFA levels in both groups (0.117 and 0.123 in the groups L and H, respectively). Serum NEFA continued to increase in group H until +2 weeks, whereas no corresponding increase was observed in group L after +1 week. According to these data it seems that process of lipomobilization started earlier before partum in group H compared to group L, being most intensive between 7 and 14 days postpartum. Higher serum NEFA concentration in the H group and positive correlation between blood serum NEFA and degree of liver fat accumulation indicate the importance of BCS in the pathogenesis of fatty liver in dairy cows. This is confirmed with the correlation between blood serum NEFA and degree of liver fat accumulation. In the group H there was high positive correlation during three time periods, with highest value at +2 wk (r=0,910, Figure 2C). At the group L dairy cows there were oscillations in correlation coefficients during investigating periods, and only at +1 wk there were similar correlation between NEFA and liver fat accumulation as in L and H groups. It seems that for high degree of liver lipid accumulation it is necessary that lipomobilization has to start earlier before partum and maintain high level until two weeks postpartum (reach maximal intensity at 7-14 days after partum). This is demonstrated by increased BCS loss occurring in the H group of dairy cows during transition period. Highest increase in blood plasma NEFA was found in Red Holstein cows that lost >0.75 points of BCS during transition period [Busato *et al.* 2002]. NEFA was the parameter that most closely reflected the BCS losses, supporting earlier findings of it's usefulness in diagnosing herd problems [Stengarde *et al.* 2008]. Cut-off points for plasma NEFA at the end of pregnancy is 0.4 mmol/l, and for lactating cows in early lactation 0.7 mmol/l [Whitaker 2004, Le Blanc 2010]. An increase in blood serum NEFA concentration is indicator of high level of lipid mobilisation from the adipose tissue. In the severe cases of fatty liver there is an increase of blood serum NEFA concentration to the level of more than 1 mmol/l [Kalaitzakis *et al.* 2007].

Recent complex study of the gene networks in liver of periparturient dairy cows with the nutrition-induced ketosis confirmed high NEFA and low blood serum glucose concentrations during feed restriction protocol [Loor *et al.* 2007] Our results indicate that the blood serum glucose concentration is continuously lower in high BCS loss cows with significant difference observed at one week before and two weeks postpartum.

This result could be explained by lower gluconeogenic capacity of liver cells in high BCS loss cows that is in accordance with the results of Loor *et al*. [2007]. Inability of early lactation dairy cows to satisfy an increase mammary gland glucose demands induces a decrease of blood serum glucose concentration, since mammary gland glucose utilization is almost completely independent on blood glucose concentration [Djokovic *et al.* 2007, Stengarde *et al.* 2008]. Early experimental models of induced ketosis in dairy cows showed a significant decrease of blood glucose concentration [Mills *et al*. 1986]. Results of latter studies have also found a decrease in blood glucose concentration during induction of ketosis at the beginning of lactation [Veenhuizen *et al*. 1991, Djokovic *et al*. 2007]. Hepatic *in vitro* gluconeogenic capacity decreased significantly for ketosis induction protocol cows when clinical ketosis was detected [Grummer 2008]. Contrary to those results Drackley *et al.* [1991] found no significant changes in blood plasma glucose concentration in similar model of ketosis induction. Investigation of metabolic profiles in herds with ketosis and abomasal displacement history showed that differences between herds in NEFA and insulin levels were not reflected in glucose levels [Veenhuizen *et al.* 1991]. This indicates that blood glucose may be not a sensitive measure of energy status, probably because glucose is subject to tight homeostatic control as previously concluded by Herdt [2000]. Investigating relationship between BCS loss and metabolic parameters [Kim and Suh 2003] have not found significant differences between blood serum glucose concentrations at dry period, calving or 1st month of lactation. In the same experimental model as in our study Busato *et al*. [2002] found lowest blood serum glucose concentration in cows with high BCS loss at 2 and 4 weeks after partus. The majority of NEFA present in blood lipoproteins of dairy cows are of intestinal (dietary) rather than hepatic origin [Pogson *et al.* 1983, Pulen *et al.* 1989]. One of the factors that may contribute to the development of fatty liver in dairy cows is the slow rate of TAG export as VLDL.

Our results indicate that blood serum TAG concentration decreases during transition period in both groups of cows. Significant differences observed in TAG levels in high BCS loss cows at two weeks before partum and low BCS loss cows postpartum . This could be explained by decreasing ability of hepatic cells to export TAG during the transition period, which is more pronounced in high BCS loss cows. Study of van Dorland *et al.* [2009] also indicate a decrease of blood plasma TAG concentration in healthy cows with low and high blood plasma BHB level during transition period.

Liver enzymes activity and tBIL concentration correlate well with the degree of fatty liver accumulation [Kalaitzakis *et al*. 2007]. Our previous results of tBIL concentration in ketotic cows [Gvozdić *et al*. 2009] indicate changes in liver fat content from GdL 2 to GdL 3 ("*Grades der Leberverfetting*", GdL), based on the data presented by Kalaitzakis *et al.* [2007]. Busato *et al.* [2002] have also found highest plasma bilirubin concentration in cows with high BCS loss one week before partus. However, our results indicate a much higher bilirubin concentrations in both groups of cows (L and H). Furthermore, blood serum bilirubin level could vary between dairy cattle breeds, and Šamanc *et al.* [1992], reported significantly different tBIL in healthy fresh Friesian and Holstein heifers (4.85±1.7 and 6.99±1.9 μmol/l, respectively). Our results showed that bilirubin concentration was continuously higher in high BCS loss cows, indicating possible impaired liver cells bilirubin metabolism that could start as early as two weeks before parturition. Correlation between variables showed that blood serum NEFA concentration increse is followed by the increase of bilirubin and decrease of blood serum glucose concentration in transition dairy cows. Since bilirubin and NEFA share common hepatic metabolic pathways [Qualmann *et al.* 1995] similar changes in their blood serum concentrations are understandable. Busato *et al*. [2002] have also found significant increase of bilirubin and NEFA concentrations in high BCS loss cows. The results of Šamanc *et al*. [1992] indicated that blood serum bilirubin concentration should be more carefully investigated regarding the differences between breeds.

Holstein cows with high ΔBCS (≥0.75) between dry period and early lactation have increased blood serum NEFA, TAG and tBIL concentrations and lower blood serum glucose concentration during transition period compared to the low ΔBCS cows. Our results indicate high level of lipid mobilization from adipose tissue, possible decreased liver cells TAG export and gluconeogenic ability and impaired bilirubin metabolism in Holstein dairy cows with high ΔBCS.

Presented results show high variation in energy balance according to body weight and milk production. Energy balance did not show statistically proven differences between two experimental groups. This result is in relation to the previously referred results [Banos *et al.* 2005]. In this study cows with higher body condition score produced more milk in first two weeks of lactation (statistically non-significant) which are in relation to the previous results [Roche *et al.* 2007]. Intensity of change the body condition score ΔEB across the time in both groups is in strong correlation with changes in energy balance ΔBCS. In cows with higher BCS depression in dry mater intake [Roche *et al.* 2008], more resistance to insulin [Holtenius and Holtenius 2007] and higher level of oxidative stress [Bernabucci *et al.* 2005] were found. All of those biological factors could affect loss in body condition and consequently specific metabolic adaptation. However, Busato *et al.* [2002] showed that cows with optimal BCS (3.25) had the best metabolic status if they did not loss too much body reserves and when develop numerous metabolic adaptations.

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