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EFFECT OF PERIPARTUM DIETARY ENERGY SUPPLEMENTATION ON THYROID HORMONES, INSULIN-LIKE GROWTH FACTOR-I AND ITS BINDING PROTEINS IN EARLY LACTATION DAIRY COWS

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The objective of this study was to examine the effect of dietary energy supplementation on hormones that are considered to be the main signals of a shift in energy balance around parturition. Sixty dry cows, 15 days before calving, were chosen and divided into two eaqual groups: control and experimental (GLY). Both groups were fed a standard ration balanced in accordance to the stage of the productive-reproductive cycle. Additionally, each cow in the GLY group was given glycerol based dietary energy supplementation (250 mL daily during the dry and 300 mL daily during the lactation period), which provided additional 9.30 MJ NEL during the dry and 13.95 MJ NEL during the early lactation period. Milk production was measured on days 30 and 60 of lactation and milk production was significantly higher in GLY compared to control group at day 60 of lactation ($p<0.05$). Service period and insemination index were used as reproductive outcome parameters. Average service period in the control group was significantly longer than in the GLY group ($p<0.05$). Average insemination index in the control group was not significantly different than the index obtained for the GLY group.

Blood samples were taken before the begining of the experiment (15 days before parturition), and at days 7, 30 and 60 of lactation. Concentrations of thyroid hormones, IGF-I, relative abundance of IGFBP-2, IGFBP-3 and IGFBP-4, concentrations of total protein and albumin in the blood were measured. Results showed that at days 7 and 30 after parturition, T_4 concentrations were significantly higher ($p<0.001$, respectively) in GLY than in the control group, while T_3 concentrations were significantly higher in GLY group only at day 7 after parturition ($p<0.001$). IGF-I concentrations and IGFBP-3 abundance were significantly higher in the GLY compared to the control group in all three examined postpartum periods. IGFBP-2 and IGFBP-4 concentrations were higher in GLY compared to the control group in all three examined postpartum periods, but the difference was significant

only on day 60 after parturition ($p<0.01$, respectively). Concentrations of total protein and albumin were significantly higher in GLY compared to the control group in all three examined periods after parturition. Based on these results it can be concluded that peripartum dietary energy supplementation prevent cows' exposure to severe negative energy balance, preserves synthetic activity of hepatocytes and consequently has a positive impact on milk production and reproductive performances in dairy cows.

Key words: dairy cows, energy supplementation, IGF-I, IGFBPs, thyroid hormones

INTRODUCTION

High-yielding dairy cows cannot consume sufficient nutrients in early lactation to support the level of milk yield. Peak milk production, at about 8 to 10 weeks postpartum, occurs earlier than maximum energy intake, causing cows to be in a negative energy balance (NEB). To compensate for the NEB, the dairy cow mobilizes body fat reserves in the form of non-esterified fatty acids (NEFA). Mobilized NEFA are taken up mainly by the liver and are either oxidized in the mitochondria to produce energy or exported in the form of TAG-rich very low density lipoproteins (VLDL). When the uptake of NEFA by the liver exceeds their disposal through oxidation or export as VLDL, fatty liver syndrome develops to different extend (Grummer, 1993). Fatty liver usually provokes other metabolic diseases and reproductive problems in lactating cows that are initially derived from a state of NEB during the early lactation period.

During the post partal period of NEB, substantial changes in the endocrine system also occur, characterized by an increase in growth hormone and a decrease in insulin, thyroid hormones and insulin like growth factor-I (IGF-I). Blood IGF-I is bound to specific binding proteins (IGFBPs) which affect the transport and bioactivity of IGF-I and its half life in blood plasma (McGuire et al., 1992). Use of Western ligand blot analyses has shown that IGFBPs migrate with apparent molecular masses of 25, 28, 35 and 45 – 53 kDa for IGFBPs 4, 1, 2 and 3 respectively (Cohick et al. 1992; Kirovski et al., 2008). Studies have demonstrated that IGFBP-3 binds most of the immunoreactive IGF-I (Baxter and Martin, 1989) and is regulated by growth hormone and IGF-I itself (Zapf et al., 1989). Simmons et al. (1994) reported an increase in IGFBP-2 levels just after parturition while IGFBP-3 remains lower. These variations in hormones and their binding proteins at late dry and early lactation periods in high yielding dairy cows reflect the variation in energy balance.

A possible way to overcome NEB is to add gluconeogenic precursors in cows' diet. The search for suitable gluconeogenic precursors has focused on three- and four-carbon compounds that share common properties related to their palatibility, ease of mixing with grain-based concentrates, reasonable cost and resistance to microbial fermentation in the rumen. Three classes of compounds have been extensively investigated: (1) amino acids; (2) mono- and dicarboxylic

acids; and (3) alcohols. Group 1 includes methionine for its action as a methyl donor and its role in α-lipoprotein synthesis (Socha et al., 2005) and glucogenic amino acids such as aspartate and glutamate (van Knegsel et al., 2005). Group 2 includes compounds such as propionate and succinate (Peters and Eliot, 1984). A common disadvantage of group 1 and 2 compounds is that if they are used in their free acid form they lower rumen pH and if they are used as sodium salts they interfere with the electrolyte balance, i.e., the Na : K ratio. Group 3 compounds, the alcohols, do not have these disadvantageous properties and seem to be the most promising for general application. Consumed orally, glycerol is metabolized in the rumen and liver to intermediates of gluconeogenesis and glycolysis with the potential to alleviate postpartal NEB (Osman et al., 2010).

The objective of this study was to examine the influence of glycerol based energy dietary supplementation on the IGF system and thyroid hormone status as main endocrine indicators of energy status of early lactation dairy cows.

MATERIALS AND METHODS

Animals and treatments

Sixty dry cows (a month before expected calving) were chosen from a commercial dairy herd and included in the study. Cows were kept in a tie-stall barn for the duration of the experiment. Cows chosen for the study were paired by calving date, parity (mean parity 3, range from 1 to 5) and previous milk production (from 6500 to 7000 L of milk per 305 days in the previous lactation) into two groups. One member of each pair was assigned randomly to GLY or control group.

Animals were allocated to treatment on day 15 before expected calving and remained on their respective treatment until day 60 postpartum. During the examined period all cows were fed a standard ration (Tables 1 and 2). Each cow in the GLY group (n = 30) was given the energy supplement dispersed in the morning feed by a sprayer for the period of the experiment in a quantity of 250 mL during the dry and 300 mL during the lactation period. Energy supplement contained glycerol (60%), crude protein (3.4%), crude fat (0.8%), crude ash (4.4%), nitrogen free extract (14.7%), water (16.7%), sodium chloride (0.1%), betaine (30 g/kg), propionic acid (50 g/kg) and citric acid (50 g/kg) as additives (Energy Top, Biochem GMBH, Germany). Added glycerol provided additional 9.30 MJ NEL during the dry period and additional 13.95 MJ NEL during the early lactation period. Control animals (n = 30) did not receive any energy supplement. Milk production was measured on days 30 and 60 of lactation. Measurements were performed with an automatic milking machine "Delaval". The parameters used to evaluate reproductive performances were service period (days postpartum to conception) and insemination index (average number of inseminations per conception).

Blood sampling

Four blood samples were taken by jugular venipuncture from each animal: the first 15 days before expected calving (dry period), the second 7 days after calving (early puerperal period), the third 30 days after calving and fourth 60 days

after calving. Samples obtained using a sterilized needle were placed into tubes and allowed to clot spontaneously at room temperature. The serum was decanted, centrifuged at 3000×g, portioned into aliquots of 1.5 mL, and stored in polypropylene microtubes at -20°C until analysis. To compare blood metabolite concentrations without influence of daily rhythms, samples were taken 4 to 6 hours after morning feeding.

Table 1. Ingredients of cow's diets

Ingredient (kg/day)	During dry period	Until day 60 of lactation
Alfalfa hay	3.00	3.50
Wheat straw	2.60	–
Alfalfa haylage	3.50	1.00
Corn silage	10.00	17.50
Extruded fullfat soybeans	–	2.00
Sugar beet molasses	–	0.30
Dry sugar beat pulp	–	0.75
Corn grain meal	1.45	3.79
Barley grain	0.27	0.90
Sunflower meal	0.85	3.51
Wheat flour	0.50	0.90
Dicalcium phosphate	0.04	0.10
Calcium carbonate	0.04	0.24
Sodium chloride (iodized)	0.02	0.13
Vitamin mineral premix	0.03	0.10

Table 2. Chemical composition of cow's diets

Chemical composition	During dry period	Until day 60 of lactation
DM (kg/day)	11.80	22.10
Net energy oflactation (NEL) MJ/day	72.01	148.21
Metabolizeable protein (MP) g/day	940	2142
Rumen degradable protein (RDP) g/day	1110	2743
Rumen undegradable protein (RUP) g/day	388	1025
Crude fat (%DM)	2.6	4.7
Non-structural carbohydrates units	35.0	39.3
Acid detergent fibre (ADF) %DM	29.90	22.00
Neutral detergent fibre (NDF) %DM	44.70	33.20
NDF from forages %DM	39.2	20.60
Ca g/day	41	66
P g/day	32	25
K g/day	161	88

Laboratory methods

Serum total protein (TP) and albumin were measured by using the automatic analyzer biochemical kits (Bio-Merieux). Triiodothyroinine (T_3), thyroxine (T_4) and IGF-I concentrations in sera were measured by radioimmunoassay (RIA; INEP-Zemun, Serbia). Intra-assay coefficients of variation (CV) ranged from 3.1% to 7.2%. For the IGF-I RIA, binding proteins were removed by acid-ethanol treatment followed by cryoprecipitation.

Electrophoresis and immunoblotting of IGFBPs were performed as described by Hossenlopp et al. (1986). Serum samples were diluted 1:20 (for analysis of IGFBP-2 and -4) or 1:40 (for analysis of IGFBP-3), in 0.05 M sodium phosphate buffer, 0.15 M NaCl, pH 7.5 (PBS), mixed with an equal volume of reducing sample buffer (0.125 M Tris-HCl, 4% (w/v) SDS, 20% (v/v) glycerol, 10% (v/v) 2-mercaptoethanol, 0.01% (w/v) bromophenol blue; pH 6.8), boiled for 5 min and loaded onto the gels (30 μL). Samples were subjected to SDS-PAGE under non-reducing conditions (using a 10 % gel). Molecular mass markers (Bio-Rad Laboratories, Hercules, CA, USA) were run in parallel. Electrophoresis was performed in a Mini-PROTEAN 3 Cell (Bio-Rad Laboratories) at a constant voltage (150 V) for 1.5 h. Proteins were transferred to a nitrocellulose membrane (Protran, Whatman, PerkinElmer, Boston, USA) in a Mini-PROTEAN 3 Cell at a constant voltage of 25 V for 1 h. Nonspecific binding on membranes was prevented by immersing membranes in 0.01 M Tris-HCl buffer, 0.15 M NaCl, 0.1% (v/v) Tween-20, pH 7.4 (TBST) containing 5% nonfat dry milk, for 1 h. Membranes were left overnight at 4°C in TBST containing 1% nonfat dried milk and primary antibody: goat polyclonal anti-IGFBP-2 (sc-6002), anti-IGFBP-3 (sc-6004) or anti-IGFBP-4 (sc-6005) produced by Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA) at a final dilution of 1:1000. Membranes were further incubated with swine anti-goat IgG antibody coupled to horseradish peroxidase (Biosource, Camarillo, USA) diluted 1:10000 in TBST at room temperature for 1 h. Immunoreactive proteins were visualised by autoradiography using an enhanced chemiluminescence kit (Amersham, Little Chalfont, UK) containing luminol as a supstrate. The X-ray films and developing reagents were purchased from KODAK (Paris, France).

Films were scanned and analysed by densitometry using the ImageMaster TotalLab software, version 2.0 (Amersham, UK). The intensity of the protein bands was expressed in arbitrary units.

Statistical analysis

All results are expressed as means ± SD. Student t test was applied to identify differences between groups. The differences were considered significant at $p<0.05$.

RESULTS

Milk production and reproductive performances

On day 30 after calving, milk production in the control group of cows was 39.40± 4.31 L and 40.97±6.30 L in the GLY group of cows. The difference was not significant. At day 60 after calving milk production in the control group was

38.16±6.19 L and 41.70±3.84 L in the GLY group. The difference in milk production between groups was significant ($p<0.05$) at day 60 of lactation. It is important to emphasize that cows from the GLY group increased milk production from day 30 to day 60, while cows in control group decreased milk production during this period of time. Although differences between milk production between days 30 and 60 were not significant it is important to emphasize that peak of lactation was achieved at day 30 in the control and day 60 in the GLY group. Average service period in the control group of cows was 125.96±37.45 days and was significantly longer ($p<0.05$) than in the GLY group of cows (102.76±36.38 days). Average insemination index in the control group was 1.80±1.22 and was not significantly different from the value obtained in the GLY group (1.84±1.07).

Thyroid hormones

As presented in Figures 1 and 2, there were no differences in mean T_4 and T_3 concentrations between groups in the dry period and on day 60 of lactation, while T_4 concentrations were significantly greater in GLY than in the control group on days 7 and 30 of lactation ($p<0.001$). T_3 concentrations were significantly greater in GLY compared to the control group only on day 7 of lactation (2.38±0.37 mmol/L vs 1.77± 0.75 mmol/L; $p<0.001$). In the control group T_4 concentrations determined on days 7 and 30 of lactations (25.97±15.03 mmol/L and 44.63± 8.17 mmol/L) were significantly lower than in the dry period (58.47±8.39 mmol/L), while in the GLY group only T_4 concentrations determined on day 7 of lactation (43.00±17.49 mmol/L) were significantly lower than values determined in the dry period (58.87± 8.17 mmol/L). T_3 concentrations determined at all three postpartum periods were significantly lower than the values determined in the dry period in both groups of cows.

IGF-I

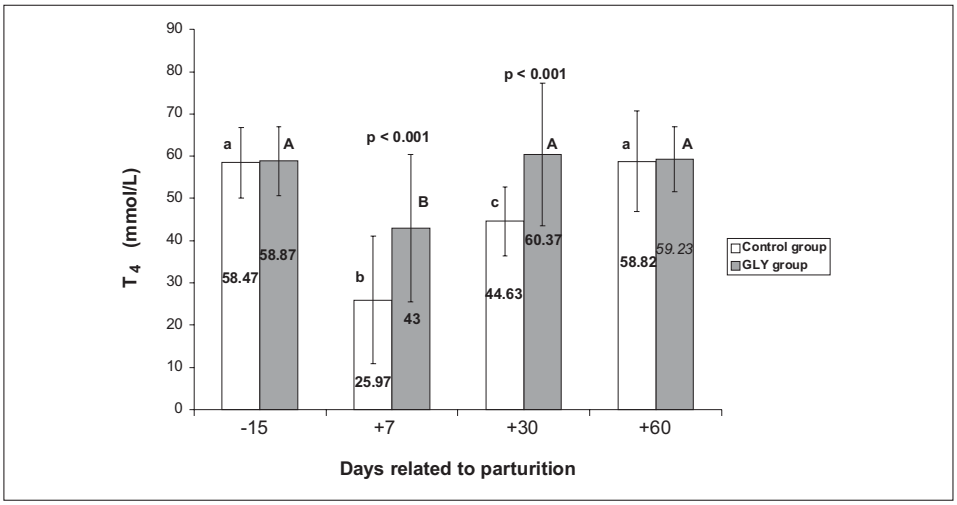

Figure 1. Average blood serum T_4 concentrations (X ± SD) in control and GLY group of cows at day 15 before and days 7, 30 and 60 after parturition

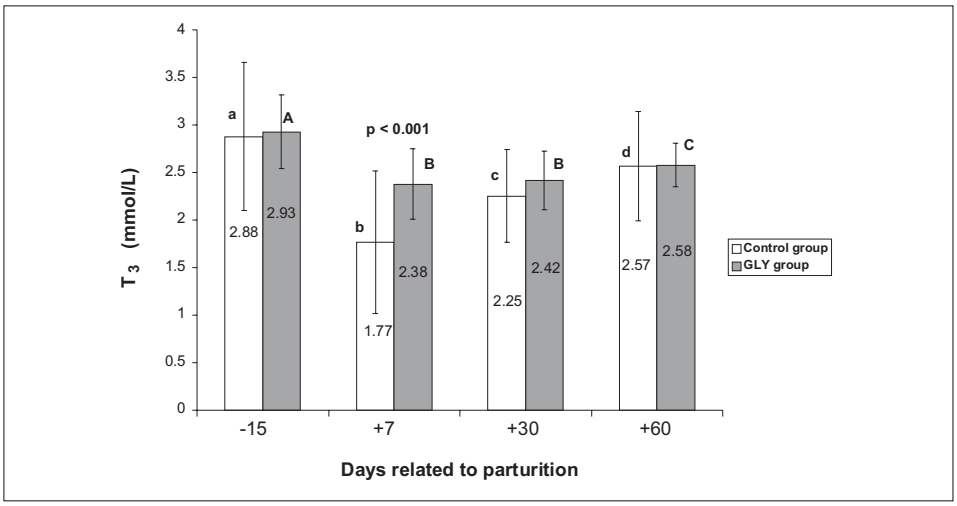

Figure 2. Average blood serum T_3 concentrations (X ± SD) in control and GLY group of cows at day 15 before and days 7, 30 and 60 after parturition

^{a,b,c} – differences between concentrations in control group; values not sharing the same superscript are significantly different ($p<0.05$)

^{A,B,C} – differences between concentrations in GLY group; values not sharing the same superscript are significantly different ($p<0.05$)

$p<0.01$ and $p<0.001$ – difference between concentrations in control and GLY group at the same time point

As presented in Figure 3, serum IGF-I concentrations were similar in both groups of cows on day 15 before parturition (31.73±5.30 nmol/L in the control and 29.39±7.86 nmol/L in the GLY group). Peripartum glycerol administration significantly affected IGF-I levels. Namely, the mean IGF-I concentrations were significantly higher in cows treated with GLY than in the control group on day 7 (11.86±1.47 nmol/L in the control and 14.28±3.47 nmol/L in the GLY group; $p<0.001$), day 30 (16.42± 2.51 nmol/L in the control and 18.67±3.80 nmol/L in the GLY group; $p<0.01$) and day 60 after calving (17.80±4.69 nmol/L in the control and 24.39±7.86 nmol/L in the GLY group; $p<0.001$). In the control group, IGF-I concentrations decreased significantly from day 15 prepartum to day 7 after calving and than increased significantly until day 30. There was no significant difference in IGF-I concentrations between day 30 and day 60 after parturition. In GLY group, IGF-I significantly decreased from day 15 to day 7 after calving and thereafter significantly increased until day 60 of lactation.

IGFBP-2

An autoradiogram of an immunoblot for serum IGFBP-2 at day 15 before and days 7, 30 and 60 after parturition in GLY and control group of cows is presented in Figure 4 (representative samples for one cow in each group).

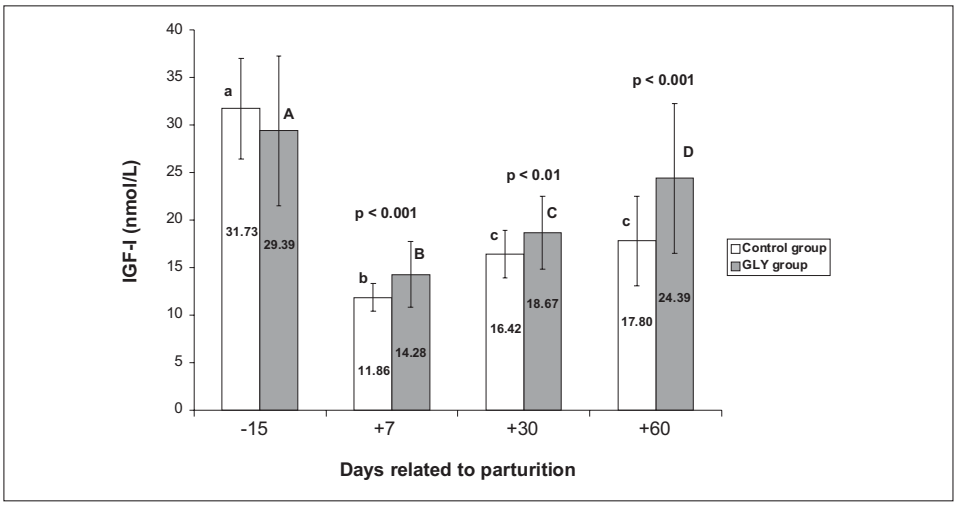

Figure 3. Average blood serum IGF-I concentrations (X±SD) in control and GLY group of cows at day 15 before and days 7, 30 and 60 after parturition
^{a,b,c} – differences between concentrations in control group; values not sharing the same superscript are significantly different ($p<0.05$)
^{A,B,C} – differences between concentrations in GLY group; values not sharing the same superscript are significantly different ($p<0.05$)
$p<0.01$ and $p<0.001$ – difference between concentrations in control and GLY group at the same time point

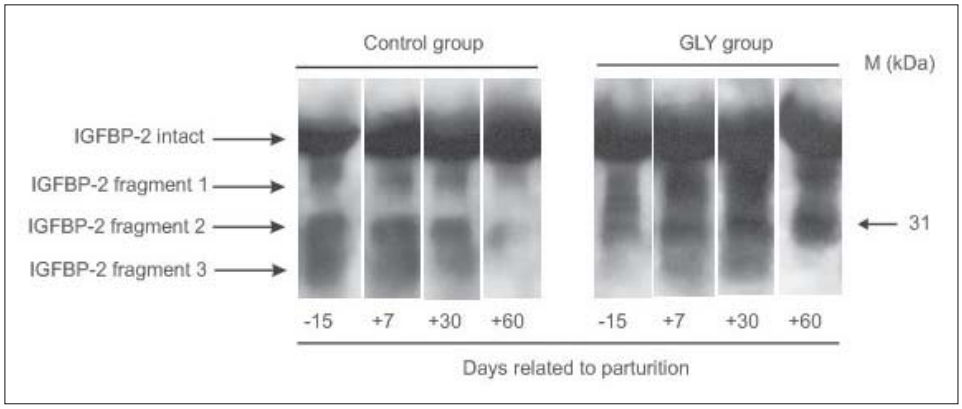

Figure 4. An autoradiogram of an immunoblot for serum IGFBP-2 at day 15 before and days 7, 30 and 60 after parturition in control and GLY group of cows

Intact IGFBP-2 (approximately 35 kDa) and three proteolytic fragments were detected in all samples. Relative abundance of intact IGFBP-2 is presented in Table 3, expressed in arbitrary densitometric units (ADU) per 10 μL of serum.

Table 3. Relative abundance of intact IGFBP-2 bands (X ± SD) in control and GLY group of cows at day 15 before and days 7, 30 and 60 after parturition

IGFBP-2 (ADU/10 μL)				
Days related to parturition	-15	+7	+30	+60
Control	12.44 ± 1.19^{a}	14.26 ± 1.71^{ab}	14.86 ± 2.17^{b}	11.28 ± 2.11^{a}
GLY	12.88 ± 1.91^{A}	14.90 ± 1.88^{B}	16.65 ± 1.93^{C}	14.08 ± 2.11*^{AB}

^{a,b,c} – differences between concentrations in control group (small letter); values in rows not sharing the same superscript are significantly different ($p<0.05$)
^{A,B,C} – differences between concentrations in GLY group (capital letter); values in rows not sharing the same superscript are significantly different ($p<0.05$)
* – difference between concentrations in control and GLY group at the same time period ($p<0.05$)

The intensity of the protein band corresponding to IGFBP-2 did not differ between groups on day 15 before and days 7 and 30 after parturition. On day 60 after parturition the relative abundance of IGFBP-2 was significantly higher in GLY compared to the control group of cows ($p<0.05$).

IGFBP-3

An autoradiogram of an immunoblot for serum IGFBP-3 on day 15 before and days 7, 30 and 60 after parturition in GLY and control group of cows is presented in Figure 5 (representative samples).

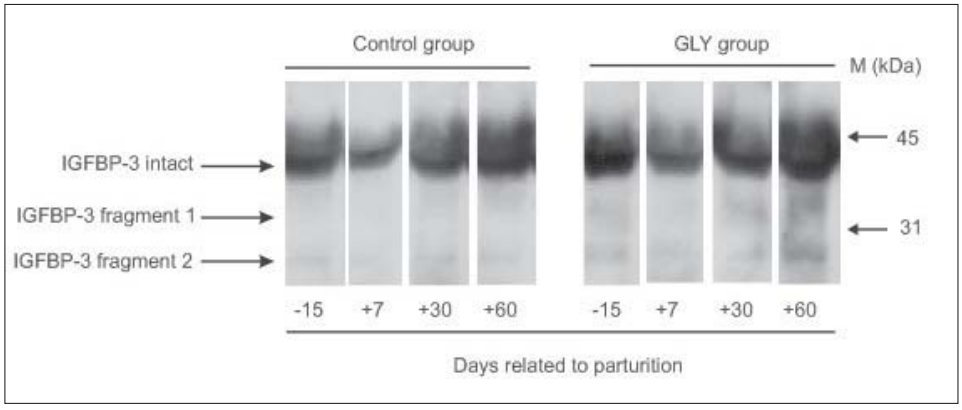

Figure 5. An autoradiogram of an immunoblot for serum IGFBP-3 at day 15 before and days 7, 30 and 60 after parturition in control and GLY group of cows

In Figure 5, intact IGFBP-3 (approximately 45 kDa) and two proteolytic fragments are seen. Relative abundance of intact IGFBP-3 is presented in Table 4, expressed in arbitrary densitometric units (ADU) per 10 μL of serum

Table 4. Relative abundance of intact IGFBP-3 bands (X ± SD) in control and GLY group of cows at day 15 before and days 7, 30 and 60 after parturition

IGFBP-3 (ADU/10 μL)				
Days related to parturition	-15	+7	+30	+60
Control	8.26 ± 1.39^{a}	5.84 ± 2.02^{b}	8.61 ± 1.25^{a}	13.50 ± 2.06^{c}
GLY	8.68 ± 1.75^{A}	8.13 ± 1.59*^{A}	11.33 ± 1.85**^{B}	17.05 ± 2.03**^{C}

^{a,b,c} – differences between concentrations in control group (small letter); values in rows not sharing the same superscript are significantly different ($p<0.05$)
^{A,B,C} – differences between concentrations in GLY group (capital letter); values in rows not sharing the same superscript are significantly different ($p<0.05$)
* – difference between concentrations in control and GLY group at the same time period ($p<0.05$)
** – difference between concentrations in control and GLY group at the same time period ($p<0.01$)

Statistical analysis has demonstrated that, except on day 15 before parturition, animals in GLY group exhibited higher relative abundance of IGFBP-3 compared to the control group ($p<0.05$ for day 7 and $p<0.01$ for days 30 and 60 after parturition).

IGFBP-4

An autoradiogram of an immunoblot for serum IGFBP-4 on day 15 before and days 7, 30 and 60 after parturition in GLY and control group of cows is presented in Figure 6.

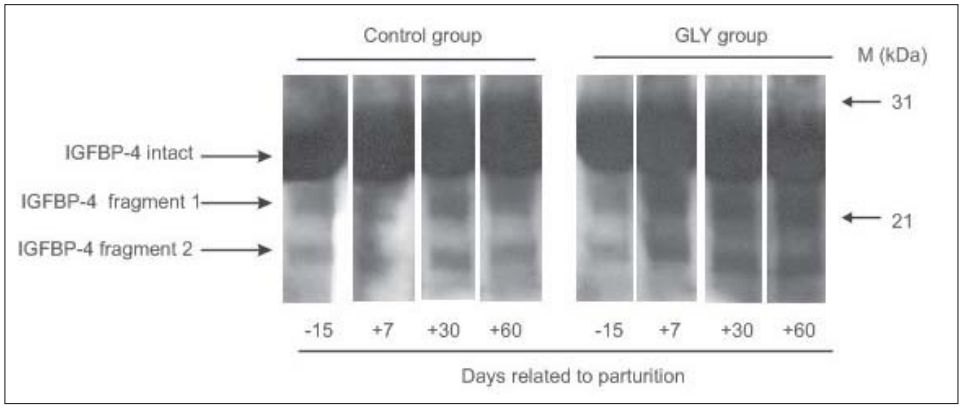

Figure 6. An autoradiogram of an immunoblot for serum IGFBP-4 at day 15 before and days 7, 30 and 60 after parturition in control and GLY group of cows

In the case of IGFBP-4, intact molecule (approximately 25 kDa) and two fragments were detected in all samples. Relative abundance of intact IGFBP-4 is presented in Table 5, expressed in arbitrary densitometric units (ADU) per 10 μL of serum.

Table 5. Relative abundance of intact IGFBP-4 bands (X ± SD) in control and GLY group of cows at day 15 before and days 7, 30 and 60 after parturition

IGFBP-4 (ADU/10 μL)				
Days related to parturition	-15	+7	+30	+60
Control	14.70 ± 2.25^{a}	15.02 ± 2.56^{ab}	17.57 ± 2.49^{bd}	18.13 ± 2.25^{cd}
GLY	14.58 ± 1.89^{A}	17.48 ± 2.27^{B}	20.95 ± 3.25^{BC}	$21.29 \pm 3.16^{*C}$

^{a,b,c,d} – differences between concentrations in control group (small letter); values in rows not sharing the same superscript are significantly different ($p<0.05$)
^{A,B,C} – differences between concentrations in GLY group (capital letter); values in rows not sharing the same superscript are significantly different ($p<0.05$)
* – difference between concentrations in control and GLY group at the same time period ($p<0.05$)

The amount of IGFBP-4 was greater postpartum in the GLY group than in the control, but statistical significance was found only for day 60 after calving ($p<0.05$).

Total protein and albumin concentrations

Concentrations of total protein and albumin are presented in Figures 7 and 8, respectively. Total protein concentrations in both groups of cows were similar

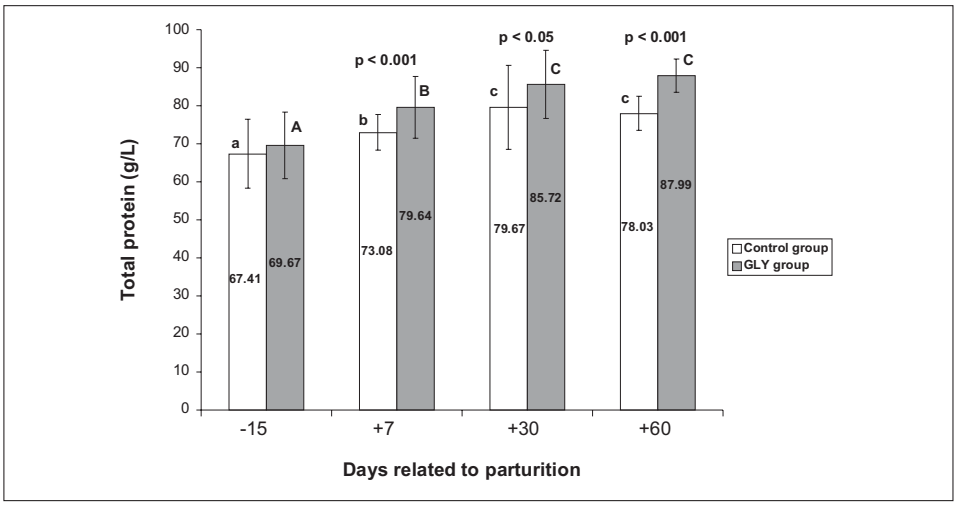

Figure 7. Average blood serum total protein concentrations (X±SD) in control and GLY group of cows at day 15 before and days 7, 30 and 60 after parturition
^{a,b,c} – differences between concentrations in control group; values not sharing the same superscript are significantly different ($p<0.05$)
^{A,B,C} – differences between concentrations in GLY group; values not sharing the same superscript are significantly different ($p<0.05$)
$p<0.05$ and $p<0.001$ – difference between concentrations in control and GLY group at the same time point

before treatment (67.41±9.05 g/L in the control and 69.67±8.79 g/L in the GLY group). Seven days after parturition total protein concentrations were significantly higher in GLY compared to the control group (79.64±8.22 g/L vs 73.08±4.77; $p<0.001$) and remained significantly higher until the end of the experiment ($p<0.05$ and $p<0.001$ on days 30 and 60 after parturition, respectively). Total protein concentrations significantly increased from day 15 before to day 30 after parturition in both groups, and did not significantly change from day 30 to day 60 after parturition.

Albumin concentrations followed the pattern of total protein alteration. They were similar before treatment (28.22±6.06 g/L in the control and 30.26±4.94 g/L in the GLY group) and seven days after parturition albumin concentrations significantly increased in GLY group compared to the control (37.35±5.04 g/L vs 30.86±5.24; $p<0.001$). The difference in albumin concentrations between two groups was significant until the end of the experiment ($p<0.01$ and $p<0.001$ on days 30 and 60 after parturition, respectively). In both groups the most intensive increase in albumin concentration occurred from day 15 before to day 7 after parturition. Within the control group, albumin concentrations significantly increased only from day 15 before to day 7 after parturition and thereafter did not significantly change. Concentrations of albumin continued to increase, but slightly and not significantly.

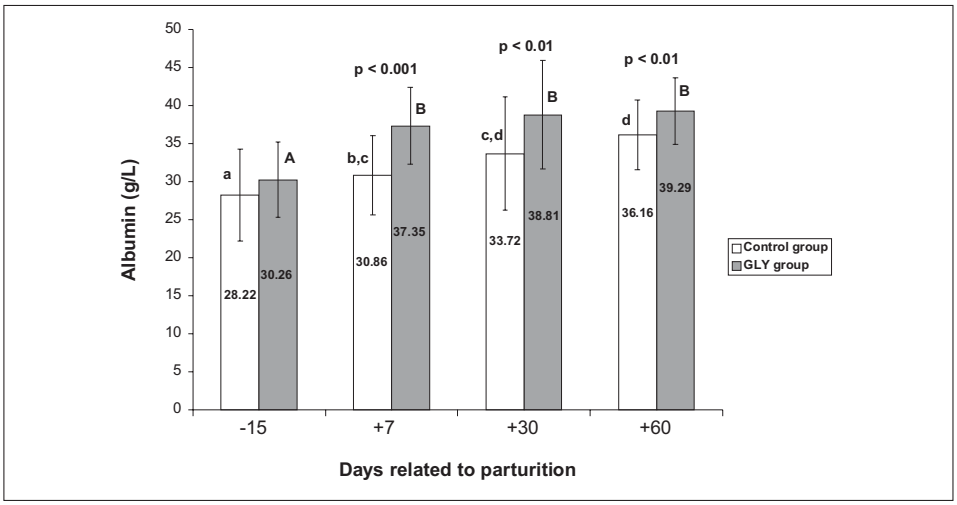

Figure 8. Average blood serum albumin concentrations (X±SD) in control and GLY group of cows at day 15 before and days 7, 30 and 60 after parturition

^{a,b,c} – differences between concentrations in control group; values not sharing the same superscript are significantly different ($p<0.05$)

^{A,B,C} – differences between concentrations in GLY group; values not sharing the same superscript are significantly different ($p<0.05$)

$p<0.01$ and $p<0.001$ – difference between concentrations in control and GLY group at the same time point

DISCUSSION

The objective of this study was to examine the effect of dietary energy supplementation on hormones that are considered to be the main signals of a shift in energy balance close to parturition. Our results showed that feeding cows with an additional energy source succeeded to avoid severe postpartum blood IGF-I and thyroid hormone depression. Namely, after parturition high yielding dairy cows are exposed to NEB (Grummer et al., 2010). Thyroid hormones are positively correlated with energy balance in dairy cows during the early lactation period (Stojić et al., 2001), and their concentrations decrease after calving. Reduction of thyroid hormone concentrations after calving is an adaptive mechanism to NEB and beginning of lactation (Nikolić et al., 1997; Pezzi et al., 2003). If reductions are pronounced or not synchronized with other metabolic and endocrine adjustments, the decrease may cause a metabolic disturbance that leads to increased lipolysis of adipose tissue and triglyceride accumulation in parenchymal tissues, especially in the liver, may be provoked. Therefore, if postpartum hypothyroid state is pronounced it may become an important etiopathogenetic factor underlying different metabolic diseases (Nikolić et al., 1997; Šamanc et al. 2010). Accordingly, it may be speculated that less severe depression of thyroid hormone concentrations after parturition in GLY group of cows may decrease the risk of metabolic disorders.

NEB in early lactation is usually associated with low serum IGF-I concentrations resulting from depressed synthesis of IGF-I by the liver (Sharma et al., 1994). As expected, serum IGF-I concentrations were decreased from dry to early lactation period in both groups of cows. Anyway, average IGF-I concentration was significantly higher in GLY compared to control group on day 7 after calving meaning that dietary energy supplement prevented extreme NEB. GLY treatment also resulted in a much earlier rise in postpartum IGF-I which is, probably, related to improved energy status of these cows. It is known that IGF-I circulates in the blood bound to several specific binding proteins. Immunoblotting results obtained in this study are in agreement with those published by Cochick et al. (1992). The major IGFBP species is IGFBP-3, which in most physiological conditions binds more than 75% of the circulating IGF-I. Association with IGFBPs increases IGF-I half-life, as compared with free IGF-I, and IGFBPs are thought to regulate the bioavailability of IGF in target tissues (Kostecka and Blahovec, 2002). In accordance with findings of Sharma et al (1994) and Formigoni et al. (1996), our results have shown that serum IGFBP-3 levels, like those of IGF-I, were the lowest just after parturition, whereas IGFBP-2 and IGFBP-4 levels increased at the same time. After calving IGF-I increased to a greater extent in GLY group reaching approximately 80 % of the prepartum level on day 60, whereas in the control group IGF-I level reached only 50 % of the initial value on day 60. On day 30 after calving levels of IGFBP-2 and IGFBP-4 were further elevated, but also the concentration of IGFBP-3 started to increase. On day 60 postpartum levels of IGFBP-3 were drastically augmented, whereas levels of IGFBP-2 began to drop and those of IGFBP-4 remained unchanged. Glycerol supplemented diet induced an earlier increase in serum IGFBP-3 concentrations and to a greater extent,

similarly to the effect it had on IGF-I. This result is in accordance with literature data (Formigoni et al., 1996).

From day 7 to day 60 of lactation IGFBP-2 was not significantly lower in the group that received the energy supplement, as it was expected based on literature data (Formigoni et al., 1996). Namely, IGFBP-2 is negatively correlated with plasma IGF-I levels (Sharma et al., 1994) and it would be expected that IGFBP-2 decreases more rapidly in GLY than in control group from day 7 to day 60 postpartum. Lower IGFBP-2 is usually consistent with a more positive energy balance in dairy cows. Greater abundance of IGFBP 2 in GLY group compared to the control group of cows on day 60 of lactation probably indicates enhanced synthesis of this IGFBP in liver cells stimulated by additional energy supplementation.

Different IGFBPs are produced by different cell types: IGFBP-3 in Kupffer and endothelial cells, IGFBP-2 in hepatocytes, while IGFBP-4 predominantly in hepatocytes. IGFBP-2 is additionally synthesized in many other tissues like osteoblasts. It may be expected that energy supplementation affects different cell types differently. IGF-I and IGFBP production is modulated by hormones in a specific manner at the level of their target tissues. Insulin is an important negative regulator of IGFBP-2 production in the liver but not in bone cells (Boni-Schnetzler et al., 1990). Triiodothironine positively regulates the expression of IGFBP-2 mRNA in hepatocytes, and it also induces the expression of the phosphoenolpyruvate carboxykinase (PEPCK) gene in an adult rat hepatocyte cell line (Pan et al., 1990). This enzyme is rate limiting for hepatic gluconeogenesis and is down regulated by insulin. Thus, the regulation of PEPCK and IGFBP-2 mRNA expression in liver cells may be connected. Data on circulating IGFBP-4 concentrations in dairy cows is lacking. Increased total protein and albumin concentrations in GLY group compared to the control confirm that livers of GLY cows were stimulated to synthesize proteins, most of which was albumin (Braun et al., 1986). In general, postpartal increase of total protein concentration may be the result of enhanced protein synthesis or consumption of a protein rich diet. As both our study groups ingested similar nutrients in respect to protein content, it may be concluded that higher TP and albumin concentrations, and also IGFBP-2 and IGFBP-4, in GLY group resulted from an increased protein synthesis in the liver.

In conclusion, dietary energy supplementation in peripartal dairy cows improved IGF and thyroid status in the postpartal period, implicating that these cows probably did not suffer from severe negative energy balance. Additionally, energy supplementation provoked postpartal synthesis of IGFBP-2, IGFBP-3 and IGFBP-4 suggesting that synthetic capacity of the liver is maintained even in the period when this organ is under great stress. Consequently, milk production was enhanced and reproductive performance improved in cows that received dietary energy supplement.

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UTICAJ ENERGETSKOG DODATKA U ISHRANI NA KONCENTRACIJU TIREOIDNIH HORMONA, INSULINU SLIČNOG FAKTORA RASTA-I I NJEGOVIH VEZUJUĆIH PROTEINA U KRVI KRAVA TOKOM RANE LAKTACIJE

KIROVSKI DANIJELA, SLADOJEVIĆ Ž, STOJIĆ V, VUJANAC I, LAZAREVIĆ M, RADOVANOVIĆ ANITA, SAVIĆ Đ i NEDIĆ OLGICA

SADRŽAJ

Cilj ovog rada je bio da se ispita uticaj energetskog dodatka u ishrani krava na koncentraciju hormona u krvi koji su u periodu oko teljenja glavni pokazatelji promena u energetskom statusu. U tu svrhu, petnaest dana pre teljenja odabrano je 60 krava koje su podeljene u dve jednake grupe: kontrolnu i oglednu (GLY). Obe grupe krava su dobijale identičan obrok usklađen sa proizvodno reproduktivnim ciklusom. Dodatno, kravama GLY grupe je tokom poslednje dve nedelje zasušenja i do 60. dana laktacije dodavan energetski dodatak na bazi glicerola (250 mL dnevno tokom zasušenja odnosno 300 mL nakon teljenja), obezbeđujući dodatnih 9,30 MJ NEL tokom zasušenja, odnosno 13,95 MJ NEL tokom rane laktacije. Kod svih krava je izmerena prosečna dnevna proizvodnja mleka 30. i 60. dana lakatcije, koja je kod GLY grupe bila značajno viša 60. dana ($p<0,05$). Kao pokazatelji reproduktivnog statusa korišćeni su servis period i indeks osemenjavanja. Servis period je bio značajno duži kod kontrolne u odnosu na GLY grupu ($p<0,05$), a vrednost indeksa osemenjavanja se nije značajno razlikovala između grupa. Uzorci krvi krava uzeti su neposredno pre početka ogleda, odnosno 15 dana pre teljenja, kao i 7, 30. i 60. dana laktacije i u njima je određivana koncentracija tireoidnih hormona, IGF-I, relativna zastupljenost IGFBP-2, IGFBP-3 i IGFBP-4, koncentracija ukupnih proteina i albumina. Rezultati su ukazali da je 7. i 30. dana nakon teljenja GLY grupa imala značajno višu koncentraciju T_4 ($p<0,001$, po-

jedinačno) u odnosu na kontrolu, dok je koncentarcija T_3 bila značajno viša kod GLY grupe 7. dana nakon teljenja ($p<0,001$). Koncentracija IGF-I i zastupljenost IGFBP-3 je bila značajno viša u krvi krava GLY grupe u odnosu na kontrolu u sva tri ispitivana perioda posle teljenja. Zastupljenost IGFBP-2 i IGFBP-4 je bila viša u krvi krava GLY u odnosu na kontrolnu grupu u sva tri ispitivana perioda postapartalno ali je ova razlika bila značajna jedino 60. dana lakatcije ($p<0.01$, pojedinačno). Koncentracija ukupnih proteina i albumina je, takođe, bila značajno viša u krvi krava GLY grupe u odnosu na kontrolnu grupu u sva tri ispitivana perioda posle teljenja. Na osnovu dobijenih rezultata može se zaključiti da energetski dodatak u ishrani sprečava izloženost krava izrazitom negativnom energetskom bilansu, održava sintetsku funkciju hepatocita i posledično ima pozitivan uticaj na mlečnost i reproduktivne pokazatelje.