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Expression of *PC*, *PCK1*, *PCK2*, *LDHB*, *FBP1* and *G6PC* genes in the liver of cows in the transition from pregnancy to lactation*

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The effect of the transition of dairy cows from pregnancy to lactation on the expression of genes encoding enzymes involved in hepatic glucose metabolism was studied. Six Holstein-Friesian heifers were used for this study. Liver samples were collected by biopsy on day 7 before expected parturition (-7) and days 3 and 21 after parturition (+3 and +21, respectively). The mRNA levels of pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase 1 (PCK1), phosphoenolpyruvate carboxykinase 2 (PCK2), lactate dehydrogenase B (LDHB), fructose-1,6-bisphosphatase 1 (FBP1) and glucose-6-phosphatase (G6PC) were measured using quantitative real-time PCR. The expression of PC and PCK2 mRNA on day 3 of lactation was significantly higher than that on day 7 before parturition (P<0.05) and slightly higher than on day 21 postpartum. The *LDHB* gene showed the highest expression level on day 3 of lactation, as compared with day 7 prepartum (P<0.001) and day 21 postpartum (P<0.001). No differences were shown in *PCK1, FBP1* and *G6PC* expression levels between pregnancy and early lactation.

KEY WORDS: dairy cow / gene expression / glucose metabolism / liver

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The transition from late pregnancy to early lactation is a time of physiological stress for high-yielding dairy cows. Reduced feed intake and, at the same time, increased needs for colostrum and milk synthesis during early lactation may lead to negative energy balance which may predispose the animals to health disorders, limit milk production and cause reproduction failures [Grummer 1995]. Daily requirements for glucose, amino acids, and fatty acids for an early lactating cow are 2.7, 2.0 and 4.5 times greater, respectively, than those needed for pregnancy [Bell 1995].

Glucose in ruminants derives mainly from the gluconeogenesis, primarily in the liver, which depends on the availability of glucogenic precursors [Young 1977]. The efficient gluconeogenesis is especially important in dairy cows in early lactation because it is the major pathway for maintaining glucose supply for the mammary gland [Huntington *et al*. 2006]. Apart from a substrate supply, the efficiency of enzymatic system involved in the gluconeogenesis is also crucial [Murondoti *et al*. 2004]. In highly productive cows, in which milk yield often exceeds 30-40 liters per day in the first two weeks of lactation, the liver metabolism has to be changed very fast from relatively slow prepartum to intensive in early lactation [Overton and Waldron 2004].

Remains an open question whether the enzymatic system of gluconeogenesis is immediately efficient postpartum and ready for such challenges. Therefore, changes of glucogenic enzymes activity in the liver during the transition from pregnancy to lactation might also be an important limiting factor in the adaptation of the liver glucose metabolism. Such changes may result from the increased expression levels of the relevant genes. In the study by Loor *et al*. [2005], a transcript profiling in the tissue samples obtained by liver biopsies of Holstein cows at days 65, 30, and 14 before parturition and days 1, 14, 28, and 49 postpartum were studied. The up-regulation of most genes from day 30 prepartum through day 1 postpartum, followed by down-regulation of many genes through day 28 of lactation, was observed. Agca *et al*. [2002] showed that the mRNA of PCK1, one of the most important enzymes of gluconeogenesis, was elevated in the liver of early-lactating cows, at the time when the increased activity of the enzyme was also observed [Mesbah and Baldwin 1983].

Increased mRNA levels of PC and G6PC in cows immediately after calving were shown by Greenfield *et al*. [2000], Murondoti *et al*. [2004] and Cedeno *et al*. [2008], indicating metabolic regulation during transition from pregnancy to early lactation in cows. Liver *PC* expression in periparturient cows was fourfold higher on day 1 postpartum than on day 14 prepartum [Greenfield *et al*. 2000] and was apparently negatively correlated with insulin concentration. Also in the study by Loor *et al*. [2006], PC mRNA was increased around parturition, and the increase was greater in cows fed restricted diet than those fed *ad libitum*. The changes in the transcription rate of the *PC* gene, and consequently the abundance of PC mRNA, were aimed to maintain synthesis of oxaloacetate for gluconeogenesis.

The objective of the present study was to collect more information on expression of six key genes involved in hepatic glucose metabolism: pyruvate carboxylase (*PC*),

phosphoenolpyruvate carboxykinase 1 (*PCK1*), phosphoenolpyruvate carboxykinase 2 (*PCK2*), lactate dehydrogenase B (*LDHB*), fructose-1,6-bisphosphatase 1 (*FBP1*) and glucose-6-phosphatase (*G6PC*) in the transition period of Holstein dairy cows, from day 7 prepartum to days 3 and 21 postpartum. Our hypothesis assumes the existence of differences between pregnant and lactating cows in expression profiles of genes involved in hepatic gluconeogenesis and glucose metabolism that adapt glucose supply in early lactation.

Material and methods

Animals and experimental design

Experiment was carried out on 6 primiparous Holstein-Friesian (HF) cows, belonging to dairy herd of the Institute of Genetics and Animal Breeding PAS, Jastrzębiec. All cows were clinically healthy, kept in the same environment in a free-stall barn and fed the same total mixed ration (TMR), in which the concentration of ingredients covered demands for 40 kg milk daily [INRA, 2007].

Liver tissues were sampled *via* puncture biopsy, three times from each cow, under local anaesthesia: on day 7 before expecting calving (day -7; effective – day -7±1) and on days 3 and 21 after calving (days +3 and +21, respectively).The biopsy technique was adapted from Rabelo *at al*. [2005].The skin was clipped, scrubbed and swabbed before being anesthetized with 10 ml lidocaine. Biopsies were performed by an authorized veterinarian. Biopsied tissues were flash frozen in liquid nitrogen and stored at -80°C until analysed. All procedures involving cows were approved by the Local Ethics Commission (permission No. 99/2009 of 22.10.2009).

Gene expression

Primers. The mRNA levels for the following key hepatic glucogenic and glucose metabolism enzymes were measured: pyruvate carboxylase (PC; EC 6.4.1.1), phosphoenolpyruvate carboxykinase 1 (PCK1, PEPCK-C, cytosolic; EC 4.1.32), phosphoenolpyruvate carboxykinase 2 (PCK2, PEPCK-M, mitochondrial; EC 4.1.32), lactate dehydrogenase B (LDHB; EC 1.1.1.27), fructose-1,6-bisphosphatase (FBP1; EC 3.1.3.11) and glucose-6-phosphatase (G6PC; EC 3.1.3.9). The PCR primers for measuring expression of these genes with real-time PCR (qPCR) were designed using Primer 3 (http:// frodo.wi.mit.edu/primer3/), Oligo Analyzer (http://eu.idtdna.com/ analyzer/ applications/oligoanalyzer/) and bovine GenBank sequences. The sequence of primers, GenBank accession numbers, and estimated size of PCR products are listed in Table 1.

Table 1. Sequence of primers used in real-time PCR to measure expression of genes involved in hepatic glucogenesis and glucose metabolism

Gene	Primer	Sequence (5'→3')	Length of amplicon (bp)	GenBank Accession number
PC	PCEF1 PCER1	TGCTGATGGACACGACCT TCTGGAATGGGATGTTGG	230	NC_007330
PCK1	PCK1EF1 PCK1ER1	GGATGGAAAGTAGAGTGTGTGG CTTCTGGATGGTCTTGATGG	156	NC_007311
PCK2	PCK2EF1 PCK2ER1	CCACAATCCAGAGTAACACCA GGCACAAAAGCGAGAGTTG	179	NC_007308
LDHB	LDHBEF1 LDHBER1	GCATAAGATGGTGGTTGAGA TGACACTGGGTGAATCCTC	133	NC_007303
FBP1	FBP1EF1 FBP1ER1	ATGGAAGAGGGCAGGAAG GACACAAGAACACAGGTAGCA	236	NC_007306
G6PC	G6PCEF1 G6PCER1	GTTGTGGTTGGGATTCTGG AACAGGAAGCAGGTGATGAG	189	NC_007317

RNA isolation, reverse transcription and real-time PCR quantification

Total RNA was extracted from the liver samples using NucleoSpin® RNA II (Macherey-Nagel, France) according to the manufacturer's protocol. The resulting RNA pellets were dissolved in RNase-free water (60 µl). The quantity and quality of the purified RNA was measured using Nanodrop® ND-1000 spectrophotometer (Wilmington, USA), and the RNA integrity number (RIN) was measured with the Agilent 2100 Bioanalyzer (USA). Only those samples with A_{260}/A_{280} ratios between 1.8 and 2.0 and RIN 8.8 to10.0 were used for further measurements.

Reverse transcription reactions were conducted using 2 µg of total RNA as a template with 0.5 µg of the oligo$(dT)_{15}$ Primer, 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT, 10 mM dNTP, 25 U RNasin, 200 U M-MLV RT (Promega, USA), in a final volume of 40 µl. Reverse transcription reactions were carried out at 70°C for 5 min and 4°C for 10 min, followed by 42°C for 60 min and 4°C for 10 min. The resulting first-standard cDNA was stored at -20°C until use for real-time PCR analysis.

Real-time PCR was performed in triplicates in 96-well plates using Power SYBR®Green PCR Master Mix (Applied Biosytsems, UK) in 7500 ABI PRISM apparatus (Applied Biosystems, USA). The following reagents were used for cDNA amplification in 25 µl of final volume: 10.7 µl of water, 0.4 µl of primers (10 µM), 1 µl of cDNA sample, and 12.5 µl of Power SYBR®Green PCR Master Mix. Standard curves were made for calculating the amplification efficiency. To prepare standards, pooled liver RNA from several cows and different time points was used to make cDNA. Serial dilutions were made from this cDNA stock to create a set of standards for the experiment. All samples were run on the same plate with five dilutions of

pooled cDNA (1x, 5x, 25x, 125x, 625x) and control sample with water, for the same gene. Amplification conditions for mRNA quantification were 95°C for 10 min and 40 cycles of 95°C for 15 s, 58°C for 30 s and 74°C for 40 s. A dissociation step was added to ensure that the desired amplicon was detected. The melting step was performed, consisting of 15 s at 95°C, 60 s at 60°C and slow heating at a rate of 0.1°C per second up to 95°C.

Amplification efficiencies were determined for the target genes by serial dilutions using the equation $E=10^{[-1/slope]}$. A total of 54 samples were analysed from 6 animals at three time points (days -7, +3, +21). Data expression levels were recorded as cycle threshold (Ct) values. The Ct values are representative of both the starting template copy number and the efficiency of amplification. Data were acquired using standard ABI prism 7000 SDS software (version 1.2.3).

The mRNA levels of the genes under study were expressed as the normalized ratio relative to ribosomal protein S9 (*RPS9*) gene, the reference gene which appeared the most stably expressed in our previous study (unpublished). The qPCR results for target genes were calculated using the mathematical formula for relative mRNA quantification in real-time PCR given by Pfaffl [2001].

Data were subjected to analysis of variance using PROC MIXED statement of the SAS (ver. 9.2). Statistical model included fixed effect of day of biopsy (-7, +3, +21). When the model was significant ($P<0.05$) means were separated using PDIFF option of the SAS adjusted for Tukey option [SAS, 2002].

Results and discussion

The present study was designed to evaluate the effect of transition of dairy cows from pregnancy to early lactation on expression of genes encoding six key hepatic enzymes involved in glucose metabolism (gluconeogenesis and glycolysis). The expression of PC and PCK2 mRNA on day 3 of lactation was significantly higher than on day 7 before parturition ($P<0.05$) and slightly higher than on day 21 postpartum (Fig. 1). The *LDHB* gene showed the highest expression level on day 3 of lactation, as compared with day 7 prepartum ($P<0.001$) and day 21 postpartum ($P<0.001$) (Fig. 2). No differences were shown in *PCK1*, *FBP1* and *G6PC* expression levels between pregnancy and lactation (Figs 1 and 2).

Gluconeogenesis and glycolysis are regulated on several levels, including substrate supply, activity of enzymes and product utilization [Overton and Waldron 2004]. They are under hormonal control (primarily insulin, glucagon, and growth hormone). Transcriptional regulation was shown relevant for regulating precursor entry into gluconeogenesis (propionate, alanine and other amino acids, lactate, and glycerol) [Aschenbach *et al*. 2010]. Increased activity of enzymes involved in these processes was the result of increased expression of their genes, which may be a part of adaptation of cow's metabolism to the huge demands for energy accompanying the transition to lactation. Four of the genes investigated in the present study, ie.

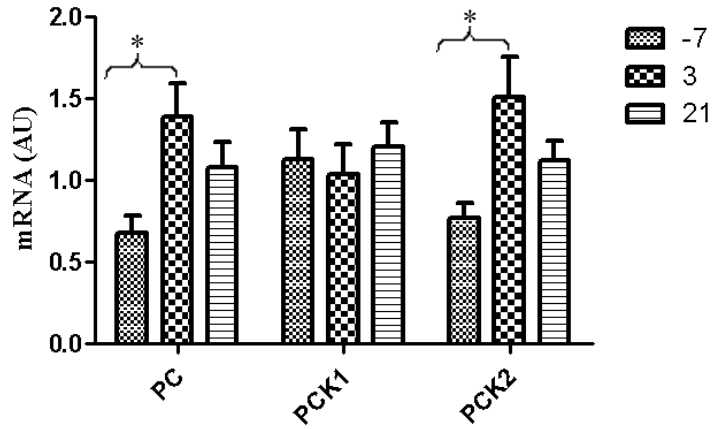

Fig. 1. Relative mRNA expression of three genes encoding glucogenic enzymes: PC, PCK1 and PCK2 in the liver of cows at the around-calving period: 7 days before and 3 and 21 days after parturition. Means of mRNA levels are shown in arbitrary units ± SEM. Differences statistically significant are shown – P<0.05 (*).

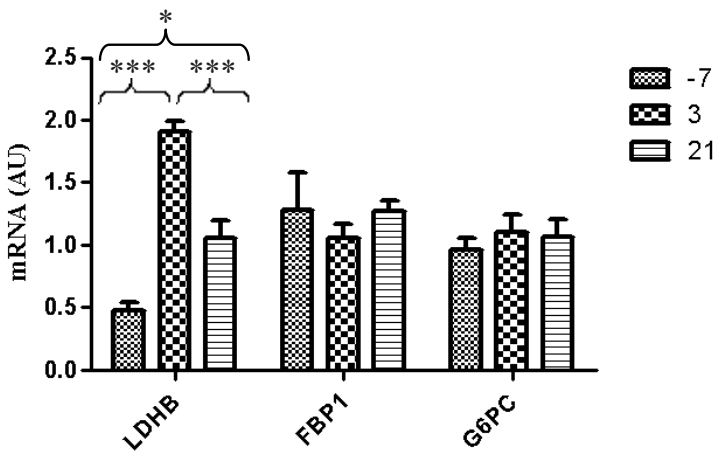

Fig. 2. Relative mRNA expression of three genes encoding glucogenic and glucose metabolism enzymes: LDHBR, FBP1 and G6PC in the liver of cows at the around-calving period: 7 days before and 3 and 21 days after parturition. Means of mRNA are shown in arbitrary units ± SEM. Differences statistically significant are shown – P<0.05 (*); P<0.001 (***).

PCK1, *PCK2*, *FBP1* and *G6PC* regulate the common glucogenic pathway, whereas *PC* regulates the entrance of propionate, lactate and glucogenic amino acids into this pathway [Jitrapakdee and Wallace 1999]. *LDHB* catalyses the conversion of lactic acid and NAD to pyruvic acid and NADH in the glycolysis pathway, thus providing energy for cells.

In most cases PC mRNA levels changed consistently with varying supply of glucose or other energy substrates in ruminants, as shown by Loor *et al*. [2006].

White *et al*. [2011] determined PC mRNA profile in liver tissues collected by biopsies from Holstein cows on days -28, +1, and +28 relative to calving, and showed that the expression of *PC* expression increased 6-fold at calving. Nuclei were isolated from the liver cells and used to determine the rates of *PC* gene transcription. These results indicated that the changes in the abundance of PC mRNA were due to corresponding changes in the rate of transcription of the bovine *PC* gene. Surprisingly, no changes in PC mRNA abundance were shown by van Dorland *et al*. [2009] in dairy cows around parturition. Consistently with most previous reports, in the present study, the mRNA levels of *PC* gene were higher on day 3 postpartum than on day 7 prepartum, which may reflect the necessity for adaptation to the source and availability of the gluconeogenic precursors. In dairy cows, liver adapts its metabolism by increasing capacity for gluconeogenesis from propionate, amino acids (mostly alanine) and lactate by increasing PC activity through changes in PC mRNA levels [Greenfield *et al*. 2000]. It may be speculated that the observed decrease in PC mRNA 21 days after calving might have contributed to a decreased deamination of amino acids, because PC is required for hepatic glucose synthesis from glucogenic amino acids [Velez and Donkin 2005], and/or the demand for glucose decreases at this time due to higher consumption of energy in the feed.

As shown in rats, cattle and other species, the phosphoenolpyruvate carboxykinase enzyme exists in two forms – cytosolic PCK1 (PEPCK-C) and mitochondrial PCK2 (PEPCK-M) [Hod *et al*. 1986], and they are distributed between these intracellular compartments. The activity of the cytosolic PCK1 and its transcript level were shown to be regulated by hormonal stimuli and nutrition, while PCK2 activity was shown not to be responsive to hormones or physiological state [Savon *et al*. 1993, Hanson and Reshef 2000]. We did not observe any significant changes in PCK1 mRNA expression in the periparturition period. However, in our study, significantly higher mRNA levels of PCK2 were detected on day 3 postpartum, as compared to day 7 prepartum. This is in contrary to the results of Agca *et al*. [2002] who observed an increase in mRNA level of PCK1 but not of PCK2 during the transition from pregnancy to lactation, between day 14 pre- and day 28 post-partum. The slight increase in the liver PCK mRNA on day 28 after calving was also observed by Greenfield *et al*. [2000], but their analyses did not distinguish between *PCK1* and *PCK2* transcripts. However, in another study [van Dorland et al. 2009], in accordance with our results, the multiparous dairy cows (Brown Swiss, Holstein, Fleckvieh) showed higher PCK2 mRNA on day 1 postpartum than before parturition, with no increase of the PCK1 transcript level at this time. These apparent discrepancies between different studies may result e.g. from the different methods used to measure mRNA levels (qPCR *vs*. Northern-blotting) and different days of pregnancy and lactation on which the liver samples were taken. The lack of the changes in our experiment in *PCK1* mRNA expression around calving suggests that the maximum attainable rate of gluconeogenesis may ultimately depend on the ratio of total PCK to PC activity, as suggested by Greenfield *et al*. [2000]. The increase in PC mRNA and no increase in PCK1 mRNA just after calving can be also

caused by the supply of amino acids derived from endogenous protein breakdown or possible release of fatty acids from adipose tissue shortly before and after parturition, as it was show by Taylor *et al*. [1971]. Agca *et al*. [2002] suggested that their results strengthen the action cytosolic form of PCK rather than mitochondrial one in the transition period. However we believe in another opinion that PCK2 is more than PCK1 suited for gluconeogenesis from lactate [Watford *et al*. 1981]; therefore, an increase in the level of PCK2 mRNA in our experiment may reflect increased utilization of this precursor at the transition to lactation.

In the present study, much higher mRNA expression level of LDHB was shown on day 3 postpartum comparing to day 7 before parturition and day 21 postpartum (Fig. 2); the differences were statistically significant $P<0.001$. Also Peter *et al*. [1987] showed an increase in the activity of LDHB from day 3 before parturition until day 3 postpartum.

Another glucogenic enzyme, FBP1, releases fructose 6-phosphate from the gluconeogenic pathway, which is first converted to glucose 6-phosphate, and then to glucose by the G6PC [Pilkis and Granner 1992]. However, the substrate for G6PC is not only available from glucogenic precursors but also from the hydrolysis of the liver glycogen. Increased levels of G6PC mRNA and the enzyme activity in cows immediately after calving were previously observed by Murondoti *et al*. [2004] and Cedeno *et al*. [2008]. However, the changes were not always related with mRNA abundance, indicating that release of glucose from gluconeogenesis could be regulated on the posttranscriptional level, according to actual glucose status [Al-Trad *et al*. 2010, Aschenbach *et al*. 2010]. Also in our studies, no increase was observed in mRNA levels of FBP1 and G6PC after calving, thus indicating that the regulation of glucose metabolism in cows during transition from pregnancy to lactation may not depend on the gene regulation on the transcriptional level.

In summary, our results showed that changes in mRNA expression of three genes occurred in the liver of cows being in the transition period. Increased expression level was shown of PC, PCK2 and LDHB mRNAs on day 3 postpartum comparing with day 7 before parturition and day 21 of lactation. Thus, our results show that the mobilisation of liver tissues to synthesize and metabolize huge amounts of glucose at the transition period may proceed with the increase of the expression of some liver enzymes, at least on the gene transcription level. However, the changes are relatively small (with the exception of LDHB), less than may be expected from the major changes in the liver metabolism accompanying the onset of lactation. Moreover, no statistically significant differences were shown in PCK1, FBP1 and G6PC expression levels between pregnancy and lactation.

REFERENCES

1. AGCA C., GREENFIELD R.B., HARTWELL J.R., DONKIN S.S., 2002 – Cloning and characterization of bovine cytosolic and mitochondrial PEPCK during transition to lactation. ***Physiological Genomics*** 11(2), 53-63.
2. AL-TRAD B., WITTEK T., PENNER G.B., REISBERG K., GABEL G., FURLL M., ASCHENBACH J.R., 2010 – Expression and activity of key hepatic gluconeogenesis enzymes in response to increasing intravenous infusions of glucose in dairy cows. ***Journal of Animal Science*** 88, 2998-3008.
3. ASCHENBACH J.R., KRISTENSEN N.B., DONKIN S.S, HAMMON H.M., PENNER G.B., 2010 – Gluconeogenesis in dairy cows: the secret of making sweet milk from sour dough. ***IUBMB Life*** 62, 869-877.
4. BELL A.W., 1995 – Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. ***Journal of Animal Science*** 73, 2804-2819.
5. CEDENO E.M., KOSER S.L., DONKIN S.S., 2008 – Quantification of glucose-6-phosphatase mRNA abundance in liver of transition dairy cows. ***Journal of Dairy Science*** 91(Suppl. 1), 424. (Abstr.).
6. GREENFIELD R.B., CECAVA M.J., DONKIN S.S. 2000 – Changes in mRNA expression for gluconeogenic enzymes in liver of dairy cattle during the transition to lactation. ***Journal of Dairy Science*** 83, 1228–1236.
7. GRUMMER R.R., 1995 – Impact of changes in organic nutrient metabolism on feeding the transition dairy-cow. ***Journal of Animal Science*** 73, 2820-2833.
8. HANSON R.W., RESHEF L., 1997 – Regulation of phosphoenolpyruvate carboxykinase (GTP) gene expression. ***Annual Review of Nutrition*** 66, 581-611.
9. HOD Y., COOK J.S., WELDON S.L., SHORT J.M., WYNSHAWBORIS A., HANSON R.W., 1986 – Differential expression of the genes for the mitochondrial and cytosolic forms of phosphoenolpyruvate carboxykinase. ***Annals of the New York Academy of Sciences*** 478, 31-45.
10. HUNTINGTON G.B., HARMON D.L., RICHARDS C.J., 2006 – Sites, rates, and limits of starch digestion and glucose metabolism in growing cattle. ***Journal of Animal Science*** 84 Suppl: E14-24.
11. JITRAPAKDEE S., WALLACE J.C., 1999 – Structure, function and regulation of pyruvate carboxylase. ***Biochemical Journal*** 340, 1-16.
12. LOOR J.J., DANN H.M., EVERTS R.E., OLIVEIRA R., GREEN C.A., GURETZKY N.A., RODRIGUEZ-ZAS S.L., LEWIN H.A., DRACKLEY J.K., 2005 – Temporal gene expression profiling of liver from periparturient dairy cows reveals complex adaptive mechanisms in hepatic function. ***Physiological Genomics*** 23, 217-226.
13. LOOR J.J., DANN H.M., GURETZKY N.A.J., EVERTS R.E., OLIVEIRA R., GREEN C.A., LITHERLAND N.B., RODRIGUEZ-ZAS S.L., LEWIN H.A., DRACKLEY J.K., 2006 – Plane of nutrition prepartum alters hepatic gene expression and function in dairy cows as assessed by longitudinal transcript and metabolic profiling. ***Physiological Genomics*** 27, 29-41.
14. MESBAH, M.M., BALDWIN R.L., 1983 – Effects of diet, pregnancy, and lactation on enzyme-activities and gluconeogenesis in ruminant liver. ***Journal of Dairy Science*** 66(4), 783-788.
15. MURONDOTI A., JORRITSMA R., BEYNEN A.C., WENSING T., GEELEN M.J.H. 2004 – Activities of the enzymes of hepatic gluconeogenesis in periparturient dairy cows with induced fatty liver. ***Journal of Dairy Research*** 71, 129-134.
16. OVERTON T.R., WALDRON M.R., 2004 – Nutritional management of transition dairy cows: strategies to optimize metabolic health. ***Journal of Dairy Science*** 87, 105-119.
17. PETER A.T., BOSU W.T.K., MACWILLIAMS P., GALLAGHER S., 1987 – Peripartal changes in serum alkaline-phosphatase activity and lactate-dehydrogenase activity in dairy-cows. ***Canadian Journal of Veterinary Research - Revue Canadienne De Recherche Veterinaire*** 51(4), 521-524.

18. PFAFFL M.W., 2001 – A new mathematical model for relative quantification in real-time RT-PCR. ***Nucleic Acids Research*** 29(9), e45.
19. PILKIS S.J., GRANNER D.K., 1992 – Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. ***Annual Review of Physiology*** 54, 885-909.
20. RABELO E., REZENDE R.L., BERTICS S.J., GRUMMER R.R., 2005 – Effects of pre- and postfresh transition diets varying in dietary energy density on metabolic status of periparturient dairy cows. ***Journal of Dairy Science*** 88, 4375-4383.
21. SAVON S., HAKIMI P., HANSON R.W., 1993 – Expression of the genes for the mitochondrial and cytosolic forms of phosphoenolpyruvate carboxykinase in avian liver during development. ***Biology of the Neonate*** 64(1), 62-68.
22. TAYLOR P.H., WALLACE J.C., KEECH D.B., 1971 – Gluconeogenic enzymes in sheep liver - intracellular localization of pyruvate carboxylase and phosphoenolpyruvate carboxykinase in normal**,** fasted and diabetic sheep. ***Biochimica et Biophysica Acta*** 237, 179-191.
23. VAN DORLAND H.A., RICHTER S., MOREL I., DOHERR M.G., CASTRO N., BRUCKMAIER R.M., 2009 – Variation in hepatic regulation of metabolism during the dry period and in early lactation in dairy cows. ***Journal of Dairy Science*** 92, 1924-1940.
24. VELEZ J.C., DONKIN S.S., 2005 – Feed restriction induces pyruvate carboxylase but not phosphoenolpyruvate carboxykinase in dairy cows. ***Journal of Dairy Science*** 88, 2938-2948.
25. WATFORD M., HOD Y., CHIAO Y.B., UTTER M.F., HANSON R.W., 1981 – The unique role of the kidney in gluconeogenesis in the chicken - the significance of a cytosolic form of phosphoenolpyruvate carboxykinase. ***Journal of Biological Chemistry*** 256, 23-27.
26. WHITE H.M., KOSER S.L., DONKIN S.S., 2011 – Bovine pyruvate carboxylase 5′ untranslated region variant expression during transition to lactation and feed restriction in dairy cows. ***Journal of Animal Science*** 89, 1881-1892.
27. YOUNG J.W., 1977 – Gluconeogenesis in cattle – significance and methodology. ***Journal of Dairy Science*** 60, 1-15.