

## CONCENTRATION OF BLOOD HSP70 AND ITS RELATION WITH LIPIDE MOBILISATION AND KETOGENESIS IN DAIRY COWS DURING PERIPARTURIENT PERIOD\*

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*SUMMARY: Heat shock proteins (HSP) are a family of stress-responsive proteins, which has important role in cell protection from different stressors. Cows suffer from metabolic stress during early lactation, which is characterized with lipolysis increased and ketogenesis. The aim of this study is to measure HSP70 concentration in blood during periparturient period, and examine relationship between HSP70 concentration, lipolysis and ketogenesis. The experiment included 20 Holstein-Friesian cows. Blood samples were taken by venepuncture of v.coccigea at week before calving and 1, 2, 4 and 8 weeks after calving. Concentration of HSP70 in blood is significantly higher in early lactation in comparison with week before calving. Also, higher concentration of NEFA and BHB were found in first and second weeks after lactation. HSP70 are in positive correlation with NEFA and BHB. Partially, correlations in first and second week after calving were stronger (when there is higher lipid mobilization and ketogenesis) then overall correlation, but it loses in fourth and eight weeks after calving. We can conclude that increases HSP70 concentration in first two weeks after calving is depend of level of lipid mobilization and ketogenesis. Metabolic adaptation as lipid mobilisation and ketogenesis upregulates HSP70 in dairy cows during early lactation.*

**Keys words:** cows, HSP70, lipolysis, ketogenesis.

### INTRODUCTION

Heat shock proteins (HSP) are a family of stress-responsive proteins that modulate cell function and contribute to protein homeostasis (Asea, 2008). HSP show some important functions: protection against apoptotic stimuli, assistance in de novo folding

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of polypeptides, and prevention of protein misfolding and aggregation (Atalay et al. 2009; Mayer and Bukau 2005). Intracellular role of HSP is well-known. Certain results show an increase in the blood concentration of HSP70 in the various actions of stress and disease (De Maio, 2011). HSP are primarily released into blood during tissue injury, causing cell lysis or necrosis (Kimura et al. 2004; Lancaster and Febbraio 2005), but other results demonstrated increase concentration of HSP70 by exocytotically release in response to stressors which are not definitive reasons for cell death and necrosis (Fleshner and Johnson 2005; Johnson and Fleshner 2006).

Cows suffer from metabolic stress during early lactation, which is characterized with negative energy balance, lipolysis, ketogenesis, and dominant inflammation response (Sordillo et al., 2009; Cincović et al. 2012; Đoković et al., 2014). To date, only a limited number of studies investigated HSP70 concentration in dairy cows during periparturient period and its relation with metabolic stress. Kristensen et al. (2004) showed that Hsp72 is present in plasma from female dairy cattle and that the age and stage of lactation affect the concentration level. Results obtained from Catalani et al. (2010) provided the first evidence on dairy cows that both cellular and circulating Hsp72 are subjected to changes during the periparturient period.

The aim of this study is to measure HSP70 concentration in blood during periparturient period, and examine relationship between HSP70 concentration, lipolysis and ketogenesis.

## MATERIAL AND METHODS

*Animals:* The experiment included 20 Holstein-Friesian cows. Cows were kept in equal environmental and technology condition.

*Laboratory analysis:* Blood samples were taken by venepunction of v.coccigea at week before calving and 1, 2, 4 and 8 weeks after calving. Blood samples were immediately transported to the laboratory. HSP70 concentration was measured in blood serum using a ELISA method (Cusabio, Ch) at Rayto ELISA reader. Concentration of NEFA and BHB was measured by standard biochemical kits (Randox, UK) using a Rayto spectrophotometer.

*Statistics model:* Difference in blood HSP70 concentration was analysed using a ANOVA model with post-hoc LSD test. Relationship between NEFA, BHB and HSP70 was examined by testing of Pearson's correlation coefficient. Relationship between HSP70 concentrations at different week of observation was analysed Pearson's correlation coefficient.

## RESULTS AND DISCUSSION

Concentration of HSP70 in blood was from 2.55 ng/ml in week before calving to 4.11 ng/ml in second week after calving. Concentration of HSP70 was significantly lower in week before calving in comparison to postpartal weeks, but difference in concentration of HSP70 at different week postcalvig was not observed. Results are present in Table 1. Correlation between HSP70 in different week was positive. Results are present in Table 2. Our results are in line with results obtained from Kristensen et al. (2004) and Catalani et al. (2010). Kristensen et al. (2004) were not observed statistically significant

difference between HSP concentrations in early and mid lactation, but concentration in early lactation showed tendency to be higher. Molvarec et al. (2007) reported that serum concentration of HSP72 is lower in pregnant than in non-pregnant women.

Concentration of NEFA was higher in first week after calving. Statistically higher concentration of BHB was observed in first and second weeks after calving. Results are present in Table 3. Positive correlation was observed between HSP70, NEFA and BHB through all weeks. Results are present in Table 4. Partially, correlations in first and second week after calving were stronger than overall correlation, but it loses in fourth and eight weeks after calving. We can conclude that increases HSP70 concentration in first two weeks after calving is depend of level of lipid mobilization and ketogenesis. In early lactation cows are in negative energy balance, with metabolic stress and inflammation. Eitam et al. (2009) reported that a prolonged low-energy diet promoted Hsp response with a significant increase of Hsp90 but unchanged levels of Hsp70 mRNA in white blood cells, and a lower expression of Hsp70 in milk somatic cells. In human with diabetes was founded increased concentration of HSP70. Same authors showed inverse correlation between fasting glucose concentration and HSP70 (Nakhjavani et al., 2010). Liver lipidosis and insulin resistance are typical findings during early lactation. Increased HSP70 concentration could be protective response to this process. Namely, intracellular HSP72 alleviated insulin resistance and reduced fat accumulation in the hepatocytes (Morino et al. 2008). Lipid mobilisation and ketogenesis upregulated HSP70 in dairy cows during early lactation.

Table 1: Concentration of HSP70 in blood during periparturient period

	-1	1	2	4	8
HSP70	2.55±0.6 <sup>a</sup>	3.77±0.5 <sup>b</sup>	4.11±0.4 <sup>b</sup>	3.8±0.4 <sup>b</sup>	3.9±0.5 <sup>b</sup>

<sup>a,b,c</sup> Values with different superscripts, within the row, significant differ ( $p < 0.05$ ).

Table 2: Correlation between HSP70 concentrations in different weeks

	1	2	4	8
-1	0.49*	0.45*	NS	NS
1	/	0.56**	NS	NS
2	/	/	0.46*	0.45*
4	/	/	/	NS

\* $p < 0.05$ ; \*\* $p < 0.01$

Table 3: Concentrations of NEFA and BHB during periparturient period

	-1	1	2	4	8
NEFA	0.35±0.09 <sup>a</sup>	0.68±0.12 <sup>b</sup>	0.49±0.15 <sup>a</sup>	0.41±0.1 <sup>a</sup>	0.29±0.07 <sup>a</sup>
BHB	0.51±0.12 <sup>a</sup>	0.73±0.15 <sup>b</sup>	0.91±0.2 <sup>b</sup>	0.43±0.1 <sup>a</sup>	0.47±0.08 <sup>a</sup>

<sup>a,b,c</sup> Values with different superscripts, within the row, significant differ ( $p < 0.05$ ).

Table 4: Correlation of HSP70 with NEFA and BHB during all time of observation and partially for each week

	All time	-1	1	2	4	8
Hsp70:NEFA	0.44*	0.33	0.62**	0.51*	0.25	0.19
Hsp70:BHB	0.5*	0.37	0.51*	0.57**	0.3	0.1

\*p<0.05; \*\*p<0.01

### CONCLUSION

Concentration of HSP70 in blood is significantly higher in early lactation in comparison with week before calving. Also, higher concentration of NEFA and BHB were found in first and second weeks after lactation. HSP70 are in positive correlation with NEFA and BHB. Partially, correlations in first and second week after calving were stronger (when there is higher lipid mobilization and ketogenesis) then overall correlation, but it loses in fourth and eight weeks after calving. We can conclude that increases HSP70 concentration in first two weeks after calving is depend of level of lipid mobilization and ketogenesis. Metabolic adaptation as lipid mobilisation and ketogenesis upregulates HSP70 in dairy cows during early lactation.

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## **KONCENTRACIJA HSP70 U KRVI I NJEGOVA VEZA SA LIPIDNOM MOBILIZACIJOM I KETOGENEZOM KOD KRAVA U PERIPARTALNOM PERIODU**

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### **Izvod**

Proteini toplotnog udara (heat shock proteins, HSP, eng.) predstavlja familiju proteina čija koncentracija raste prilikom delovanja različitih stresora na organizam jedinke. Tokom rane laktacije krave pate od metaboličkog stresa koji se karakteriše povećanom lipidnom mobilizacijom i ketogenezom. Cilj ovog rada je da se ispita veza između koncentracije HSP70 sa neesterifikovanim masnim kiselinama (NEFA) i beta-hidroksibutiratom (BHB) kod krava u peripartalnom periodu. U ogled je uključeno 20 krava Holštajn-frizijske rase. Krv je uzorkovana punkcijom v.coccigea u nedelji pre teljenja, zatim u prvoj, drugoj, četvrtoj i osmoj nedelji posle teljenja. Koncentracija HSP70

je bila značajno viša u nedeljama posle teljenja u odnosu na nedelju pre teljenja. Viša koncentracija NEFA i BHB nađena je u prvoj i drugoj nedelji posle teljenja u odnosu na ostale periodu. Koncentracija HSP70 pozitivno korelira sa vrenostima NEFA i BHB. Parcijalna korelacija pokazuje da su veze jače u prvoj i drugoj nedelji posle teljenja, što je period kada su lipidna mobilizacija i ketogeneza naizraženije. Koncentracija HSP70 u prve dve nedelje posle teljenja je zavisna od nivoa lipidne mobilizacije i ketogeneze. Metabolički stres koji se karakteriče lipidnom mobilizacijom i ketogenezom povećava koncentraciju HSP70 u krvi tokom rane laktacije.

**Ključne reči:** krave, HSP70, lipoliza, ketogeneza.

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