Kafkas Univ Vet Fak Derg
23 (5): 721-727, 2017
DOI: 10.9775/kvfd.2017.17593

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
Online Submission: http://submit.vetdergikafkas.org

Research Article

Milk Production, Body Condition Score and Metabolic Parameters at the Peak of Lactation as Risk Factors for Chronic Lameness in Dairy Cows

Mimi RISTEVSKI [1] Bojan TOHOLJ [2] Marko CINCOVIĆ [2] Plamen TROJAČANEC [3] Jože STARIČ [4] Ozren SMOLEC [5]

[1] Veterinary Faculty, "Sv. Kliment Ohridski" University of Bitola, MACEDONIA
[2] Department of Veterinary Medicine, Faculty of Agriculture, University of Novi Sad, SERBIA
[3] Faculty of Veterinary Medicine, University of Ss. Cyril and Methodius, Skopje, MACEDONIA
[4] Clinic for Reproduction and Large Animals - Section for Ruminants, Veterinary Faculty, University of Ljubljana, SLOVENIA
[5] Clinic for Surgery, Faculty of Veterinary Medicine, Zagreb, CROATIA

Article Code: KVFD-2017-17593 Received: 18.03.2017 Accepted: 08.04.2017 Published Online: 09.06.2017

Citation of This Article

Ristevski M, Toholj B, Cincovic M, Trojacanec P, Staric J, Smolec O: Milk production, body condition score and metabolic parameters at the peak of lactation as risk factors for chronic lameness in dairy cows. *Kafkas Univ Vet Fak Derg,* 23 (5): 721-727, 2017. DOI: 10.9775/kvfd.2017.17593

Abstract

The objective of this case-control study was to examine the milk production, body condition score and metabolic profiles at the peak of lactation as risk factors for chronic lameness present in cows during the first six months of lactation. A total of 100 Holstein-Friesian cows were enrolled in the study, out of which 30 were classified as lame (a locomotion score (LS) >3 according to 4 of 5 monthly measurements) and 70 exhibited no signs of clinical lameness (LS ≤3). The cows with milk production above 30.9 kg/day showed a higher risk for chronic lameness (OR=1.9, a 95% confidence interval (CI)=1.2-4.5), and the risk peaked at a milk production of 39.1 kg/day (OR=4.8, CI=2.1-8.8). A suboptimal BCS <2.5 or >3 at the peak of lactation increased the probability of lameness in the exposed group of cows (OR=4.9, CI=2.2-8). The cows were exposed to higher risk factors for chronic lameness under the following circumstances: BHBA>0.8 mmol/L (OR=3.5, CI=1.2-9.9), LDH>1900 IU/L (OR=2.3, CI=1.4-5.9), and triglycerides>0.22 mmol/L (OR=2.2, CI=1.5-2.9). The interaction between two risk factors showed a higher OR for developing chronic lameness in comparison with a single-factor exposure: BCS × BHBA (OR=22, CI=1.2-1000), BCS×LDH (OR=33, CI=1.8-1400), milk production × BHBA (OR=18.24, CI=2.1-433) and milk production×LDH (OR=14.2, CI=1.5-327). Lameness cows exposed to risk factor showed un-significant lower concentration of urea, ALP and higher concentration of cholesterol and triglycerides probably due to energy and protein malnutrition. Glucose concentrations were similar in healthy and lameness cows. Same mean concentration of glucose was maintained with decrease of LDH activity in healthy cows, but with increase LDH in lame cows, probably due to high glycolysis. Metabolic adaptation in pick of lactation and its relation with lameness need further research.

Keywords: *Cows, Lameness, BCS, Milk production, Metabolic parameters*

Sütçü İneklerin Laktasyon Pikinde Kronik Topallığın Risk Faktörleri Olarak Süt Üretimi, Vücut Kondisyon Skoru ve Metabolik Parametreler

Özet

Bu olgu-kontrol çalışmasının amacı ineklerde laktasyonun pik yaptığı ilk altı ayı süresince kronik topallığın risk faktörleri olarak süt üretimi, vücut kondisyon skoru ve metabolik parametrelerin incelenmesidir. Çalışmada toplam 100 adet Holstein-Friesian inek kullanıldı. Bu ineklerin 30'u topal (5 aylık ölçümlerin 4'üne belirlenen lokomosyon skoru (LS) >3'e göre) olarak sınıflandırılırken 70'i klinik olarak topallık göstermemekteydi (LS ≤3). 30.9 kg/gün'den daha fazla süt üretimine sahip olan inekler topallık için daha yüksek risk gösterirken (OR=1.9, 95% güvenlik aralığı (CI)=1.2-4.5) süt üretimi 39.1 kg/gün'de risk pik yaptı (OR=4.8, CI=2.1-8.8). Laktasyon pikinde suboptimal BCS <2.5 veya >3 maruz kalan ineklerde topallık olasılığını artırdı (OR=4.9, CI=2.2-8). İnekler belirtilen şartlarda kronik topallık için daha fazla risk faktörü gösterdiler: BHBA>0.8 mmol/L (OR=3.5, CI=1.2-9.9), LDH>1900 IU/L (OR=2.3, CI=1.4-5.9) ve trigliseridler>0.22 mmol/L (OR=2.2, CI=1.5-2.9). İki risk faktörünün etkileşmesi tek faktöre maruz kalanlarla karşılaştırıldığında kronik topallığın gelişmesi için daha yüksek OR gösterdi BCS × BHBA (OR=22, CI=1.2-1000), BCS×LDH (OR=33, CI=1.8-1400), süt üretimi × BHBA (OR=18.24, CI=2.1-433) ve süt üretimi×LDH (OR=14.2, CI=1.5-327). Risk faktörlerine maruz kalan topal inekler muhtemelen enerji ve protein dengesizliğine bağlı olarak anlamsız derece daha düşük üre konsantrasyonuna, ALP ve daha yüksek kolesterol ve trigiliserid konsantrasyonuna sahipti. Topal ve sağlıklı ineklerde glikoz konsantrasyonu benzerlik göstermekteydi. Sağlıklı ineklerde düşük LDH aktivitesi ile birlikte benzer ortalama glikoz konsantrasyonu sağlanırken topal ineklerde artmış LDH mevcuttu. Bu durum muhtemelen yüksek glikolizise bağlıydı. Laktasyonun pik döneminde metabolik adaptasyon ve topallık ile ilişkisi daha fazla çalışmaya ihtiyaç olduğunu göstermektedir.

Anahtar sözcükler: *İnek, Topallık, BCS, Süt üretimi, Metabolik parametreler*

İletişim (Correspondence)
+381 21 4853516
mcincovic@gmail.com

INTRODUCTION

After infertility and mastitis, lameness is the third most common reason for involuntary culling of dairy cows [1]. Lameness is a painful condition which adversely affects longevity, fertility and milk production. Moreover, lameness has been classified as the most representative animal-based indicator of compromised welfare in dairy cattle [2-5]. The etiology of lameness is complex and multifactorial. The factors influencing lameness include the following: housing conditions, social interactions/influence, stages of lactation, pregnancy or calving and high yielding [6-9].

In a cross-sectional study, Bicalho et al.[10] found a greater risk of lameness and claw horn disruption lesions developing in cows with lower body condition scores (BCS) and lower digital cushion thickness (DCT). Cows with a low BCS ≤2.5 (on a scale from 0 to 5) are more likely to be treated for lameness in 0 to 2 or >2 to 4 months following such a score [11]. This result supports the hypothesis that a low BCS is correlated with reduced digital cushion thickness, which can be associated with claw horn disruption lesions [12]. Our previous study showed that BCS assessment is a suitably strong predictor of lameness in fat cows, but in normal or thin cows, lameness prediction required the combination of both BCS and ultrasound measurement of subcutaneous fat deposit [13].

Zhang et al.[14] demonstrated higher values of inflammatory cytokines, beta-hydroxybutyrate (BHBA) and nonesterified fatty acid (NEFA) in the transition period prior to a clinical diagnosis of lameness in early lactation. Some other metabolic diseases, which become clinically manifest later in lactation (e.g. laminitis), can be traced back to metabolic insults that occurred during early lactation [15]. Days in milk (DIM) and lameness are significantly interrelated, thus lameness is most common in the first 100 DIM [16]. A relation between the metabolic status, milk production, a BCS and lameness in later lactation has not been argued in the literature. The purpose of the present study is to examine the milk production, body condition scores and metabolic profiles, as well as their mutual interaction, at the peak of lactation as risk factors for chronic lameness developing during the subsequent lactation period.

MATERIAL and METHODS

Animals

A total of 100 Holstein-Friesian cows were enrolled in a case-control study from the group with a high prevalence of lameness. The cows were milked twice a day and fed a total mixed ration, according to NRC recommendations [17]: Hay 4.5 kg; Concentrate mixture 9.95 kg; Straw 0.30 kg; Sugar beet 0.7 kg; Haylage, 7.0 kg; Sylage 20 kg; Provided DM 21.5 kg; Crude protein 3440 g; Digestible crude protein 2250 g; Percentage of protein 16.5 kg/SM; Total energy 144; NEL 6.65(MJ/kg DM); CF 17.2%; ADF 21.7%; NDF 36%; Ca 0.85%; P 0.50%; NaCl 0.30%.

Locomotion Score

The locomotion score was assessed using a 1-5 lameness scoring system (LS) as argued by Sprecher et al.[18]. This system uses a 1-5 numerical scale where a score of 1 denotes sound locomotion, 2 and 3 indicates clinically unimportant changes in gait, and a score of 4 to 5 describes different severities of clinical lameness. In the present study, the cows were described as lame (a locomotion score ≥4) or non-lame (a locomotion score ≤3). Lameness was indicated by a LS >3 from the 2nd to 6th month of lactation according at least to 4 of 5 monthly measurements.

Milk Production

The average milk yield in the previous lactation was 7794±1210 kg/305 days. All the cows were supervised daily by a veterinarian. Milk production was recorded by a farm software program. In this research, the peak of milk production was used as a risk factor. Cows reach the peak of milk production between the sixth and eighth week of lactation.

Body Condition Score

A 1-5 BCS system [19], which incorporates a numerical scale where thin animals receive lower scores and fat animals higher scores, was employed in the study. All the cows were scored at the end of a dry period and on a monthly basis during the first six months of lactation.

Metabolic Profile

Blood samples were obtained from the *v. jugularis*, using sodium heparin vacutainers (Becton, Dickinson & Co, GB), during the second month of lactation, when cows achieve peak milk production. The samples were stored on ice (max. 2 h) until centrifugation (2000 g. for 20 min. at 4°C), after which plasma was harvested. The plasma samples were stored at -20°C until analysis. Concentration of plasma level of glucose, lactate dehydrogenase (LDH), alkaline phosphatase (ALP), total cholesterol, triglycerides, urea were measured by colorimetric reaction (Accent 200, P.Z. Cormay, S.A.) and blood level of BHBA was measured by ketone meter (FreeStyle Optium - Abbot Germany).

Statistics and Design

The milk production, LS and BCS were recorded at the peak of lactation, and blood samples were taken for the measurement of metabolic parameters. In order to classify the cows, locomotion scores were recorded throughout the first six months of lactation. At the end of month 6 of lactation, the cows were divided into two groups, lame and non-lame cows, depending on the LS observed during lactation. A linear regression line for the LS from months 1 to 6 of lactation will be displayed for each cow. The data are presented in 2x2 tables: lame cows, non-lame cows, cows with parameter values lower than the cut-off value, and cows with parameter values higher than the cut-off value. An odds ratio (OR) was calculated for each value of milk production, BCS and metabolic parameters in order to find the optimal cut-off point for those continuous parameters in binary outcomes. An OR was calculated was calculated between groups with normal BCS (2.5-3.0) and suboptimal BCS (BCS <2.5 or >3). Thereafter, the cows were divided into two groups: lame and healthy cows. Each group was divided into two groups: group with risk factor (significant parameters above the risk cut-off value) and group without risk factor. The difference between the mean values of measured parameters was confirmed using a t-test. Regression between glucose and LDH was examined in order to detect influence of glycolysis to maintaining glucose level in blood. For statistical purposes, the Analyse-it program (v3.90.70.) for Microsoft Excel was used.

RESULTS

A total of 30 cows were classified as lame (a locomotion score (LS) >3 according to 4 of 5 monthly measurements), whereas 70 exhibited no signs of clinical lameness (LS ≤3). The regression lines for the LS from months 2 to 6 of lactation are shown in *Fig. 1* with a clear distinction between lame and non-lame cows.

The cows with milk production above 30.9 kg/day showed a higher risk for chronic lameness (OR=1.9, a 95% confidence interval (CI)=1.2-4.5), and the risk peaked at a milk production of 39.1 kg/day (OR=4.8, CI=2.1-8.8). A suboptimal BCS <2.5 or >3 at the peak of lactation increased the probability of lameness in the exposed group of cows (OR=4.9, CI=2.2-8.0). The cows were exposed to higher risk factors for chronic lameness under the following circumstances: BHBA >0.8 mmol/L (OR=3.5, CI=1.2-9.9), LDH>1900 IU/L (OR=2.3, CI=1.4-5.9), and triglycerides >0.22 mmol/L (OR=2.2, CI=1.5-2.9). The ALP, cholesterol and urea concentrations showed significant ORs at higher values, i.e. the highest ORs at the most extreme parameter values. The glucose concentrations did not indicate the cut-off value with significant ORs (all the CIs contain 1). The maximum odds ratios (maxOR) for BHBA and LDH concentrations were obtained using the 97.5th percentile cut-off value: BHBA >0.9 mmol (maxOR=20) and LDH >2300 IU/L (maxOR=12.6). These cut-off values are only to be used for risk assessment on account of a very small number of animals with such parameter values in the blood. The interaction between two risk factors showed a higher OR for developing chronic lameness in comparison with a single-factor exposure: BCS×BHBA (OR=22, CI=1.2-1000), BCS×LDH (OR=33, CI=1.8-1400), milk production×BHBA (OR=18.24, CI=2.1-433), and milk production×LDH (OR=14.2, CI=1.5-327) *(Fig. 2)*.

When the cows are classified in the two categories (lame cows and cows with no signs of lameness), we conclude that the body condition, milk production, LDH and BHB are the most sensitive indicators of lameness, because their values are significantly changed in lame cows compared to the healthy cows and in healthy cows exposed to risk factors compared to the entirely negative control (healthy cows with no risk factors). On the other hand, there is a deviation in cholesterol, triglycerides, urea and ALP in a group of cows which is under a risk factor with lameness compared to the negative control *(Table 1)*.

The glucose level was not statistically significantly different in the examined groups of cows. LDH activity and glucose concentration exhibit polynomial quadratic relation. In the lame cows, LDH activity increases from the lowest values up and peaks at the mean values of the glucose, and there on the LDH activity decreases and this relationship is statistically significant ($LDH=-634.5 \times GLU^2 + 4391.7 \times GLU - 5609.1$; R=0.365; P<0.05). However, in healthy cows LDH and glucose demonstrate an inverse relation compared to the lame cows, so that the lowest LDH activity is reached at the mean values of the glucose, and its activity increases with increasing and decreasing values of glucose, but this relationship was not statistically significant ($LDH = 196.74 \times GLU^2 - 1351.7 \times GLU + 4019.1$; R= 0.19; NS) *(Fig. 3)*.

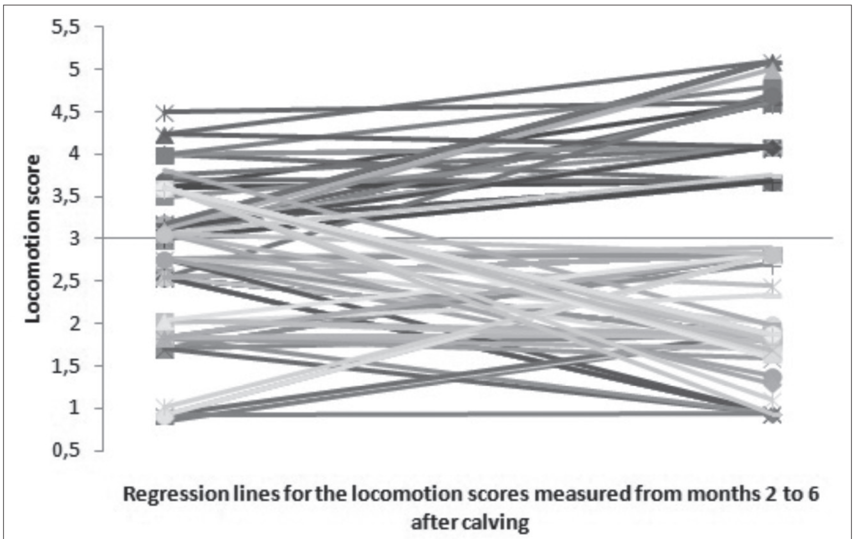

Fig 1. Lameness in dairy cows from months 2 to 6 of lactation

DISCUSSION

Milk production is an important risk factor for lameness. High milk production can cause lameness in

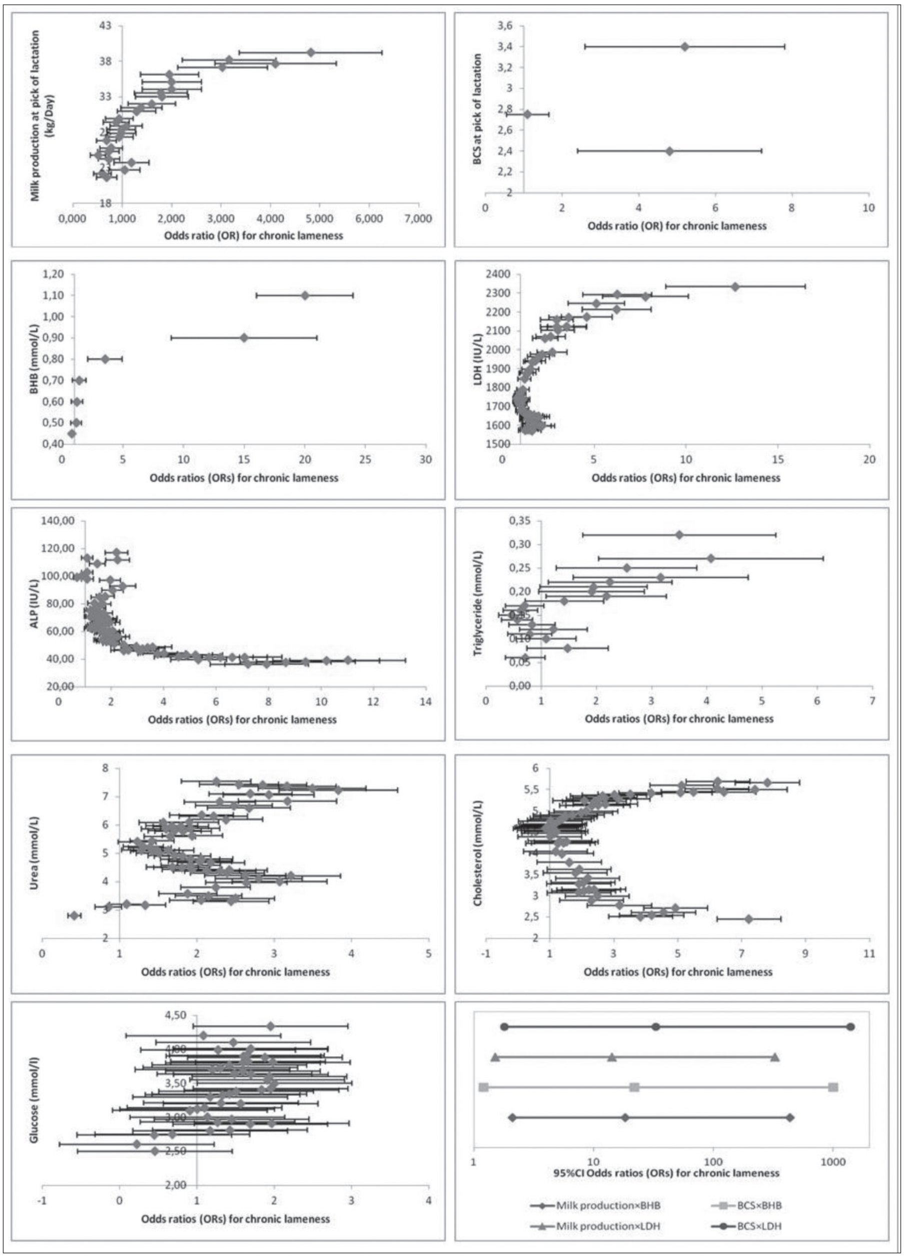

Fig 2. Odds ratios (ORs) for chronic lameness in the function of milk production, BCS, metabolic parameters in the blood and their combination

cows [20]. Bicalho et al.[9] showed that lame cows produced 3.02±0.23 kg more milk prior to lameness in comparison with the control group. Using body fat reserves for milk production entails mobilizing fat from many tissues, including the digital cushion [8]. Other studies have also revealed a connection between a lower BCS and lameness [6,10,12,21], as well as a connection between a higher BCS and a higher LS [22]. In a prospective longitudinal study, Randall et al.[23] found that a low BCS predisposes cows to lameness and that maintaining a BCS ≥2.5 is optimal for reducing the risk of a lameness event.

High concentrations of BHBA and LDH in cows at the peak of lactation indicate high odds of chronic lameness. According to Reist et al.[24] there is a strong correlation between the BHBA concentration and the energy balance. Zhang et al.[14] found higher concentrations of NEFA and BHBA in the serum within the first week of diagnosis and at four weeks postpartum. The concentration of BHBA in the aforementioned study was about 0.85 mmol/L within the first week of diagnosis, which is 0.05 mmol/L higher than our cut-off value. Cows with a higher concentration of BHBA in early lactation are at great risk for developing other diseases such as abomasal displacement and ketosis [15,25]. Oetzel [26] categorizes hyperketonemia (due to high milk production) as type 1 ketosis which occurs in cows 3 to 6 weeks post-calving on account of the highest milk energy outflow at this time. These cows usually do not have calving difficulties and a negative energy balance in early lactation, but underfeeding (in relation to the milk energy output) leads to the negative energy balance with a lipid mobilization and higher ketosis. Cows with high locomotion scores have a lower dry matter intake [27]. Type 1 ketosis and a decreased dry matter intake in lame cows could account for higher odds of lameness when cows are exposed to two risk factors (high milk production or a low BCS with a high BHBA concentration) in comparison with a single-factor exposure. Consequently, a low energy balance at the peak of lactation can predict the development of lameness during a longer period of lactation.

A high LDH concentration is an important risk factor for lameness. A high LDH concentration could be a consequence of a higher BHBA concentration affecting the liver. Furthermore, cows stand longer during lameness with consequently higher muscle loads. High muscle loads lead to high lactate production, which is reduced by LDH in muscle tissues. LDH is not an organ-specific enzyme as it is found in large concentrations in the muscles, heart, kidneys and liver, and is released during acute inflammations of those organs. Moreover, the activities of LDH in the blood are closely correlated with the degree of fatty infiltration of the liver [28]. The LDH cut-off value in

Table 1. *Metabolic profile, body condition scores and milk production in lame cows with different numbers of risk factors, and in healthy cows*

Parameter	Lame Cows with Risk Factor	Lame Cows without Risk Factor	Healthy Cows with Risk Factor	Healthy Cows without Risk Factor
Glucose (mmol/L)	3.52±0.51^{a}	3.6±0.42^{a}	3.5±0.48^{a}	3.41±0.42^{a}
LDH (IU/L)	2150±224.8^{A}	1812±192.3^{B}	1725±101.5^{B}	1657.82±231.7^{B}
ALP (IU/L)	49.1±18.3^{a}	61.3±11.5^{a}	51.4±18.1^{a}	60.33±21.2^{a}
Urea (mmol/L)	4.49±1.12^{A}	7.2±0.91^{B}	4.5±0.95^{B}	6,51±1.7^{A}
Cholesterol (mmol/L)	4.68±1.62^{a}	4.51±1.5^{a}	4.11±1.3^{a}	4.01±0.98^{a}
Triglycerides (mmol/L)	0.21±0.09^{a}	0.19±0.08^{b}	0.17±0.09^{c}	0.18±0.1^{d}
BHBA (mmol/L)	1.2±0.11^{A}	1.1±0.09^{A}	0.96±0.1^{B}	0.61±0.11^{B}
Suboptimal BCS (%)	90^{A}	0	5^{B}	5^{B}
Milk production (L)	33.34±5.35^{A}	30.5±2.9^{B}	29.87±3.1^{B}	26.90±3,14^{B}

Different superscript means significant difference at level: ^{a,b,c} *P<0.05;* ^{A,B,C} *P<0.01*

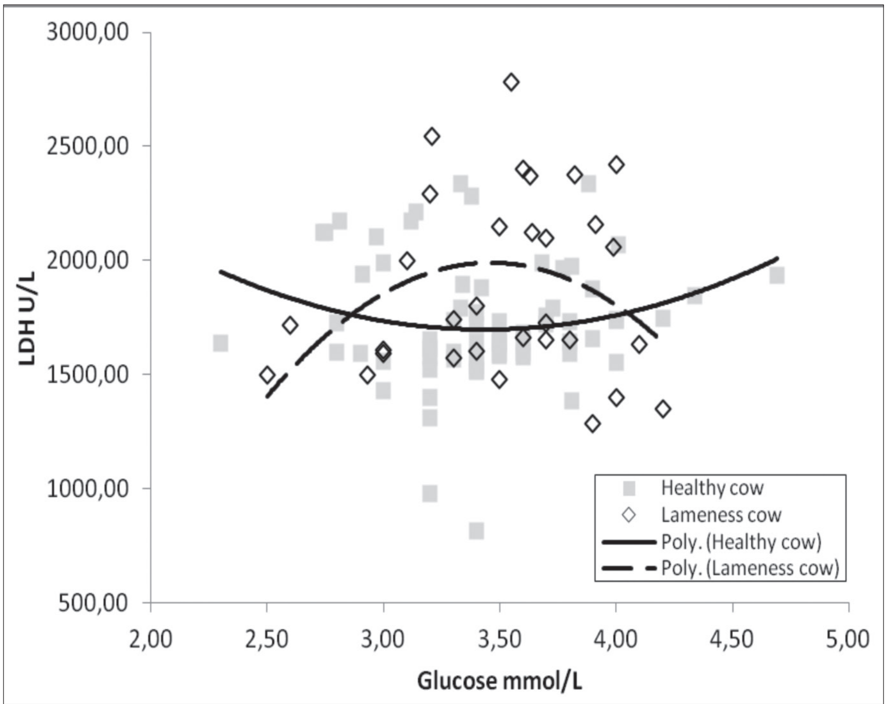

Fig 3. Regression lines between glucose concentration and LDH in healthy and lame cows

this research is approximate to the mean value of LDH in healthy cows during early lactation [29].

A high risk for lameness occurs when the concentrations of cholesterol and urea are either at high or low levels. The cholesterol concentration indirectly reveals the ability of the liver to produce VLDLs (very low density lipoproteins) and decreased cholesterol and triglycerides are sign of fatty liver [30,31]. Gross et al.[32] showed that change in lipid metabolic parameters during starvation is depends on lactation stage. During early lactation we have decrease of cholesterol and triglyceride concentration, but in later period concentration of this parameters increase during feed restriction. Yeruham et al.[33] found lower concentrations of cholesterol and urea, and higher blood activities of ALP and LDH in heifers associated with an excessive carbohydrate intake. Decrease concentration of urea could be in relation with malnutrition in proteins and lower ALP could be sign in low protein intake or hypophosphatemia.

LDH and BHBA are metabolites which together with milk production and body condition score help in the assessment of risk for the development of lameness. Lame cows have either prolonged time standing or prolonged time lying when compared to healthy cows [34]. These changes can lead to changes in glucose metabolism in muscle and the result is an increase in the production of lactate. The later was confirmed by Zhang et al.[14] who found elevated plasma lactate in lame cows. On the other hand, lactate is always the end product of cell glycolysis and is accompanied by high activity of LDH [35]. Lactate is then transported to the liver through the bloodstream where in the process of gluconeo-genesis it is again transformed into glucose that flows into the muscles and other tissues, a process known as the Cori cycle. However, in dairy cows the udder is the preferred site to use glucose, so it is possible that the produced glucose goes to the udder where it is used directly. As a result, the muscle tissue continues to utilize glycolysis that further increases the lactate. In support of this speculation speaks the fact that the relation of LDH and glucose is significantly different and shows the inverse relationship in healthy and lame cow.

In cows that have BHBA values higher than 0.8 mmol/L, the risk for lameness increases. Elevated levels of BHBA indicate negative energy balance and the use of fat for energy. Although the changes in the BHBA value is small compared to the healthy controls, it is known that BHBA increases in cows in late lactation as a result of underfeeding, but not as much as in early lactation [36]. Also, in the late lactation there is no rapid decline of glucose as in early lactation [36], which renders glucose not a significant risk factor.

In conclusion, this study demonstrated that high milk production, a low or high BCS, a high BHBA concentration and a high LDH concentration are important risk factors for developing chronic lameness. Furthermore, it was demonstrated that the interaction between milk production, a BCS and metabolic parameters (high BHBA and LDH concentrations) poses a higher risk for developing chronic lameness in dairy cows in comparison with the exposure to a single risk factor. Metabolic adaptation in pick of lactation and its relation with lameness need further research.

Acknowledgement

This research is financed from following bilateral projects: (Serbia-Croatia) Improvement of the Diagnostics and Therapy of Lameness in Horses and Cattle, (Serbia-Slovenia) Laboratory Markers of the Metabolic Status of Cows in Early Lactation" and MPNTR31062.

REFERENCES

1. Enting H, Kooij D, Dijkhuizen AA, Huirne RBM, Noordhuizen-Stassen EN: Economic loses due to clinical lameness in dairy cattle. *Livest Prod Sci,* 49, 259-267, 1997. DOI: 10.1016/S0301-6226(97)00051-1

2. Whay HR, Waterman AE, Webster AJ: The association between locomotion, nociceptive threshold and foot lesions in dairy heifers during the peripartum period. *Vet J*, 154, 155-161, 1997. DOI: 10.1016/S1090-0233(97)80053-6

3. Booth CJ, Warnick LD, Grohn YT, Maizon DO, Guard CL, Janssen D: Effect of lameness on culling in dairy cows. *J Dairy Sci,* 87, 4115-4122, 2004. DOI: 10.3168/jds.S0022-0302(04)73554-7

4. Warnick LD, Janssen D, Guard CL, Grohn YT: The effect of lameness on milk production in dairy cows. *J Dairy Sci,* 84, 1988-1997, 2001. DOI: 10.3168/jds.S0022-0302(01)74642-5

5. Whay HR, Main DCJ, Green LE, Webster AJF: Assessment of the welfare of dairy cattle using animal-based measurements: Direct observations and investigation of farm records. *Vet Rec*, 153, 197-202, 2003. DOI: 10.1136/vr.153.7.197

6. Espejo LA, Endres MI: Herd-level risk factors for lameness in high-producing Holstein cows housed in freestall barns. *J Dairy Sci,* 90, 306-314, 2007. DOI: 10.3168/jds.S0022-0302(07)72631-0

7. Galindo F, Broom DM: The relationships between social behaviour of dairy cows and the occurrence of lameness in three herds. *Res Vet Sci,* 69, 75-79, 2000. DOI: 10.1053/rvsc.2000.0391

8. Green LE, Hedges VJ, Schukken YH, Blowey RW, Packington AJ: The impact of clinical lameness on the milk yield of dairy cows. *J Dairy Sci*, 85, 2250-2256, 2002. DOI: 10.3168/jds.S0022-0302(02)74304-X

9. Bicalho RC, Warnick LD, Guard CL: Strategies to analyze milk losses caused by diseases with potential incidence throughout the lactation: A lameness example. *J Dairy Sci,* 91, 2653-2661, 2008. DOI: 10.3168/jds.2007-0744

10. Bicalho RC, Machado VS, Caixeta LS: Lameness in dairy cattle: A debiliating disease or a disease of debiliating cattle? A cross-sectional study of lameness prevalence and thickness of the digital cushion. *J Dairy Sci*, 92, 3178-3184, 2009. DOI: 10.3168/jds.2008-1827

11. Green LE, Huxley JN, Banks C, Green MJ: Temporal associations between low body condition, lameness and milk yield in a UK dairy herd. *Prev Vet Med,* 113, 63-71, 2014. DOI: 10.1016/j.prevetmed.2013.10.009

12. Toholj B, Cincović M, Stevančević M, Spasojević J, Ivetić V, Potkonjak A: Evaluation of ultrasonography for measuring solar soft tissue thickness as a predictor of sole ulcer formation in Holstein-Friesian dairy cows. *Vet J*, 199, 290-294, 2014. DOI: 10.1016/j.tvjl.2013.11.005

13. Ristevski M, Toholj B, Cincović M, Boboš S, Trojačanec P, Stevančević M, Ozren S: Influence of body condition score and ultrasound-determined thickness of body deposit in Holstein-Friesian cows on the risk of lameness developing. *Kafkas Univ Vet Fak Derg,* 23, 69-75, 2017. DOI: 10.9775/kvfd.2016.15851

14. Zhang G, Hailemariam D, Dervishi E, Deng Q, Goldansaz SA, Dunn SM, Ametaj BN: Alterations of innate immunity reactants in transition dairy cows before clinical signs of lameness. *Animals*, 5, 3, 717-747, 2015. DOI: 10.3390/ani5030381

15. Bertoni G, Trevisi E: Use of the liver activity index and other metabolic variables in the assessment of metabolic health in dairy herds. *Vet Clin North Am: Food Anim Pract*, 29, 413-431, 2013. DOI: 10.1016/j.cvfa.2013.04.004

16. Capion N, Thamsborg SM, Enevoldsen C: Prevalence and severity of foot lesions in Danish Holstein heifers through first lactation. *Vet J*, 182, 50-58, 2009. DOI: 10.1016/j.tvjl.2008.05.026

17. NRC Nutrient Requirements of Dairy Cattle. National Academy Press, Vashington DC, 2001.

18. Sprecher DJ, Hostetler DE, Kaneene JB: A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. *Theriogenology*, 47, 1179-1187, 1997. DOI: 10.1016/S0093-691X(97)00098-8

19. Ferguson JD: Implementation of a body condition scoring program in dairy herds. Feeding and managing the transition cow. *Proc. Penn. Annu. Conf., Univ. of Pennsylvania, Center for Animal Health and Productivity,* Kennett Square, PA.1996.

20. Deluyker HA, Gay JM, Weaver LD, Azari AS: Change of milk yield with clinical diseases for a high producing dairy herd. *J Dairy Sci*, 74, 436-445, 1991. DOI: 10.3168/jds.S0022-0302(91)78189-7

21. Gudaj R, Brydl E, Posta J, Komlosi I: Effect of lameness on milk production in Holstein-Friesian farms in Hungary. *Allattenyesztes Takarmanyozas* 61, 66-77, 2012.

22. Onyiro OM, Offer J, Brotherstone S: Risk factors and milk yield losses associated with lameness in Holstein-Friesian dairy cattle. *Animal,* 2, 1230-1237, 2008. DOI: 10.1017/S1751731108002279

23. Randall LV, Green MJ, Chagunda MGG, Mason C, Archer SC, Green LE, Huxley JN: Low body condition predisposes cattle to lamenness: An 8 year old study of one dairy herd. *J Dairy Sci,* 98, 3766-3777, 2015. DOI: 10.3168/jds.2014-8863

24. Reist M, Erdun D, Von Euw D, Tschemperlin K, Leuenberger H, Chilliard Y, Hammon HM, Morel C, Philipona C, Zbinden Y, Kuenzi N, Blum JW: Estimation of energy balance at the individual and herd level using blood and milk traits in high-yielding dairy cows. *J Dairy Sci*, 85, 3314-3327, 2002. DOI: 10.3168/jds.S0022-0302(02)74420-2

25. Ospina PA, Nydam DV, Stokol T, Overton TR: Association between the proportions of sampled transition cows with increased nonesterified fatty acids and beta-hydroxybutyrate and disease incidence, pregnancy rate, and milk production at the herd level. *J Dairy Sci*, 93, 3595-3601, 2010b. DOI: 10.3168/jds.2010-3074

26. Oetzel GR: Herd-level ketosis - diagnosis and risk factor. *Preconference Seminar 7C: Dairy Herd Problem Investigation Strategies: Transition Cow Troubleshooting American Association of Bovine Practitioners 40th Annual Conference,* September 19, Vancouver, BC, Canada, pp.67-91, 2007.

27. Norring M, Häggman J, Simojoki H, Tamminen P, Winckler C, Pastell M: Short Communication: Lameness impairs feeding behavior of dairy cows. *J Dairy Sci*, 97, 4317-4321, 2014. DOI: 10.3168/jds.2013-7512

28. Pechova A, Llek J, Halouzka R: Diagnosis and control of the development of hepatic lipidosis in dairy cows in the peri-parturient period. *Acta Vet Brno,* 66, 235-243, 1997.

29. Cozzi G, Ravarotto L, Gottardo F, Stefani A, Contiero B, Moro L, Brscic M, Dalvit P: Short communication: Reference values for blood parameters in Holstein dairy cows: Effects of parity, stage of lactation, and season of production. *J Dairy Sci,* 94, 3895-3901, 2011. DOI: 10.3168/jds.2010-3687

30. Van den Top M, Wensing T, Gellen J, Wentink H, van't Klooster T, Beynem A: Time trends of plasma lipids and enzymes synthesizing hepatic triaciglycerol during postpartum development of fatty liver in dairy cows. *J Dairy Sci*, 78, 2208-2220, 1995. DOI: 10.3168/jds.S0022-0302(95)76848-5

31. Grummer R: Etiology of lipid related metabolic disorders in periparturient dairy cows. *J Dairy Sci*, 76, 3882-3896, 1993. DOI: 10.3168/jds.S0022-0302(93)77729-2

32. Gross J, Kessler E, Albrecht C, Bruckmaier R: Negative energy balance in dairy cows depends on the lactational stage. *Plos One*, 10, e0121956, 2015. DOI: 10.1371/journal.pone.0121956

33. Yeruham I, Avidar Y, Bargai U, Adin G, Frank D, Perl S, Bogin E: Laminitis and dermatitis in heifers associated with excessive carbohydrate intake: skin lesions and biochemical findings. *J South African Vet Assoc*, 70 (4): 167-171, 1999.

34. Olechnowicz J, Jaskowski J: Behavior of lame cows: A review. *Veterinarni Medicina*, 56 (12): 581-588, 2011.

35. Rogatzki M, Ferguson B, Goodwin L, Gladden B: Lactate is always the end product of glycolysis. *Frontiers in Neuroscience*, 9, 1-7, 2015. DOI: 10.3389/fnins.2015.00022

36. Bjerre-Harpoth V, Friggens N, Thorup V, Larsen T, Damgaard B, Ingvartsen K, Moyes K: Metabolic and production profiles of dairy cows in response to decreased nutrient density to increase physiological i mbalance at different stages of lactation. *J Dairy Sci*, 95, 2362-2380, 2012. DOI: 10.3168/jds.2011-4419