

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

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## 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 peak 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 total (5 aylık ölçümlerin 4'üne belirlenen lokomasyon 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 total inekler muhtemelen enerji ve protein dengesizliğine bağlı olarak anlamsız derece daha düşük üre konsantrasyonuna, ALP ve daha yüksek kolesterol ve trigliserid konsantrasyonuna sahipti. Total ve sağlıklı ineklerde glikoz konsantrasyonu benzerlik göstermekteydi. Sağlıklı ineklerde düşük LDH aktivitesi ile birlikte benzer ortalama glikoz konsantrasyonu sağlanırken total 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



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## 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  $\leq 2.5$  (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; Silage 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  $\geq 4$ ) or non-lame (a locomotion score  $\leq 3$ ). Lameness was indicated by a LS  $>3$  from the 2<sup>nd</sup> to 6<sup>th</sup> month of lactation according to at least 4 of 5 monthly measurements.

### Milk Production

The average milk yield in the previous lactation was  $7794 \pm 1210$  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 and compared 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>0.8mmol/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 97.5<sup>th</sup> 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).

## DISCUSSION

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

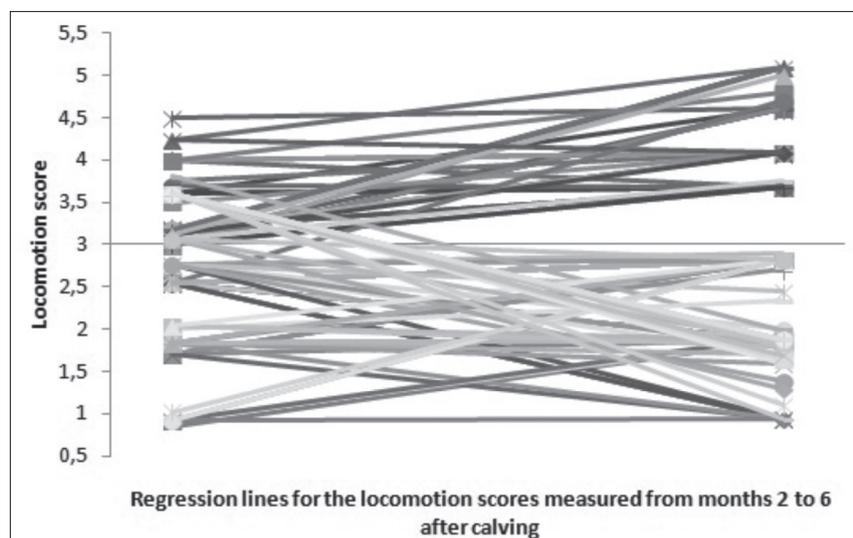
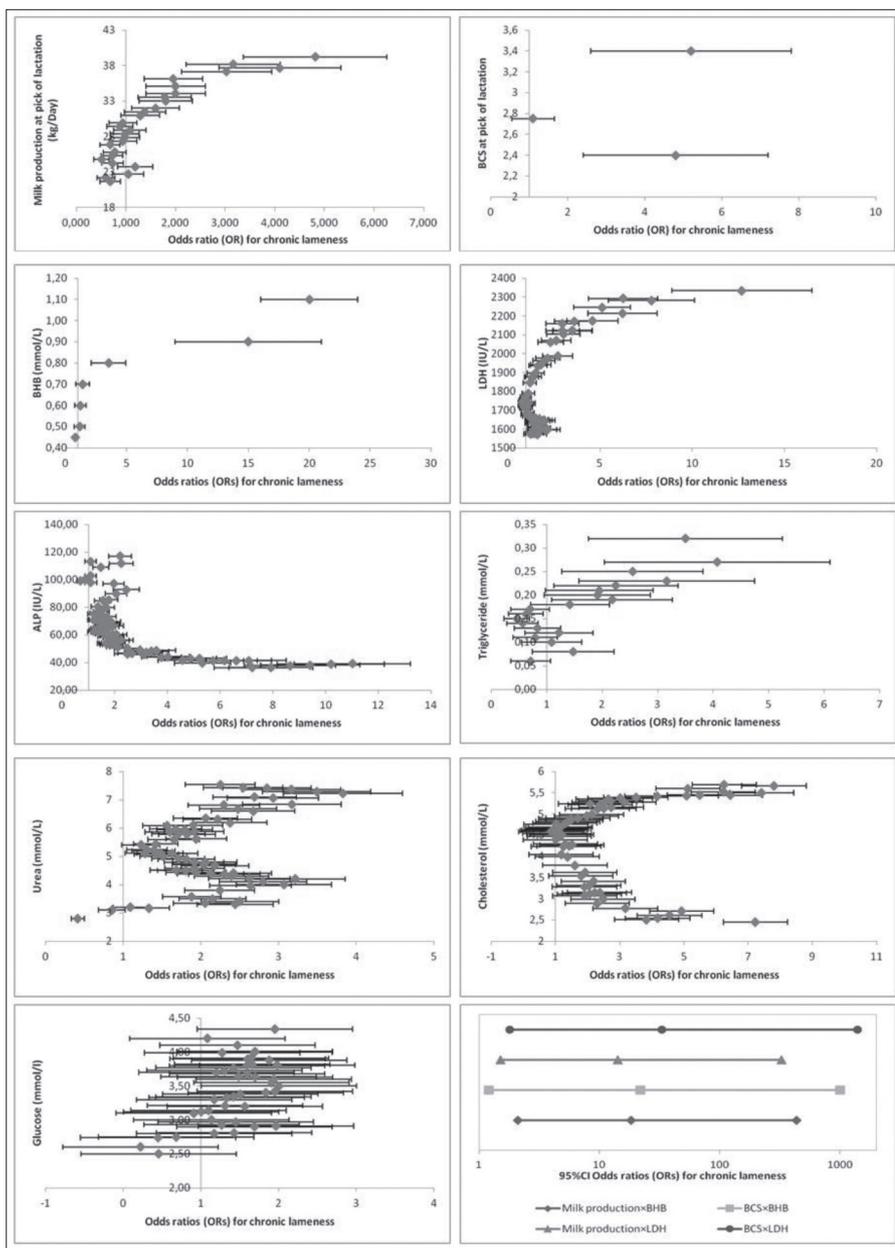


Fig 1. Lameness in dairy cows from months 2 to 6 of lactation



**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  $3.02 \pm 0.23$  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  $\geq 2.5$  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.

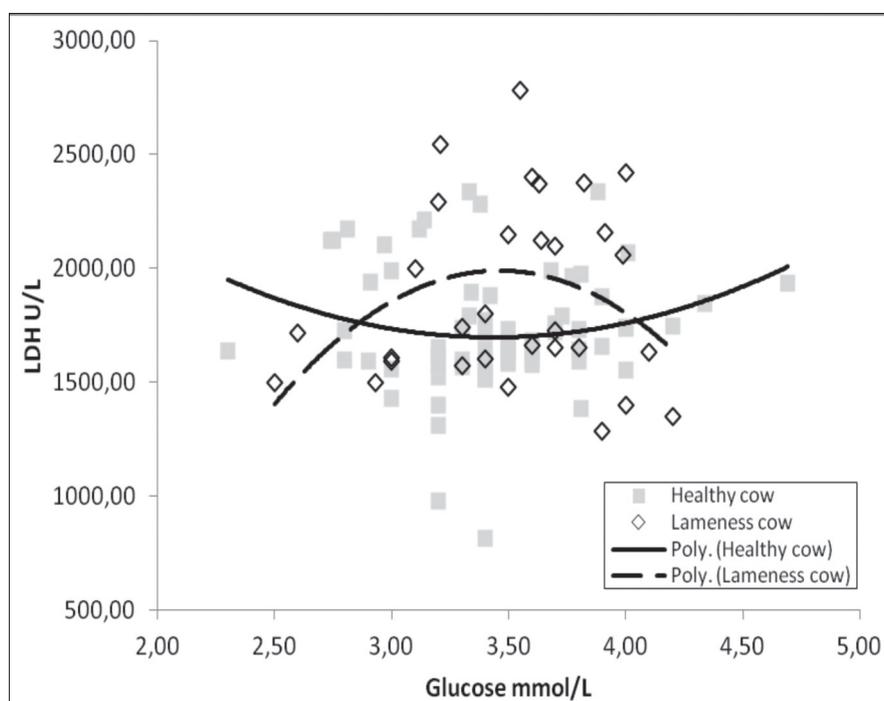
during a longer period of lactation.

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 <sup>a</sup>	3.6±0.42 <sup>a</sup>	3.5±0.48 <sup>a</sup>	3.41±0.42 <sup>a</sup>
LDH (IU/L)	2150±224.8 <sup>A</sup>	1812±192.3 <sup>B</sup>	1725±101.5 <sup>B</sup>	1657.82±231.7 <sup>B</sup>
ALP (IU/L)	49.1±18.3 <sup>a</sup>	61.3±11.5 <sup>a</sup>	51.4±18.1 <sup>a</sup>	60.33±21.2 <sup>a</sup>
Urea (mmol/L)	4.49±1.12 <sup>A</sup>	7.2±0.91 <sup>B</sup>	4.5±0.95 <sup>B</sup>	6.51±1.7 <sup>A</sup>
Cholesterol (mmol/L)	4.68±1.62 <sup>a</sup>	4.51±1.5 <sup>a</sup>	4.11±1.3 <sup>a</sup>	4.01±0.98 <sup>a</sup>
Triglycerides (mmol/L)	0.21±0.09 <sup>a</sup>	0.19±0.08 <sup>b</sup>	0.17±0.09 <sup>c</sup>	0.18±0.1 <sup>d</sup>
BHBA (mmol/L)	1.2±0.11 <sup>A</sup>	1.1±0.09 <sup>A</sup>	0.96±0.1 <sup>B</sup>	0.61±0.11 <sup>B</sup>
Suboptimal BCS (%)	90 <sup>A</sup>	0	5 <sup>B</sup>	5 <sup>B</sup>
Milk production (L)	33.34±5.35 <sup>A</sup>	30.5±2.9 <sup>B</sup>	29.87±3.1 <sup>B</sup>	26.90±3.14 <sup>B</sup>

Different superscript means significant difference at level: <sup>a,b,c</sup> P<0.05; <sup>A,B,C</sup> 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 <sup>[29]</sup>.

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 <sup>[30,31]</sup>. Gross et al. <sup>[32]</sup> 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. <sup>[33]</sup> 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 <sup>[34]</sup>. 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. <sup>[14]</sup> 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 <sup>[35]</sup>. Lactate is then transported to the liver

through the bloodstream where in the process of gluconeogenesis 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<sup>[36]</sup>. Also, in the late lactation there is no rapid decline of glucose as in early lactation<sup>[36]</sup>, 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.

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