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Cut off value of metabolic blood and milk parameters for prediction of lameness developing in Holstein-Friesian cows

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ABSTRACT
100 Holstein–Friesian cows were enrolled from dry perod to six months of lactation. The cows were selected from population of dry cows, LS 3 or lower, free from other serious diseases. A 27 cows in our research developed lameness during lactation. Retrospectively we determined which were the critical values of milk production, composition of produced milk and blood metabolic parameters in early lactation which increase risk for lameness developing. The ROC analysis show that cows with milk production of ≥36.00 kg., milk fat of ≥4.24% and milk proteins of ≥3.47% in the third month of lactation (70-80. days) are more likely to develop lameness. The tested values show that the decline in the concentration of milk urea increases the specificity and decreases sensitivity for detection on the lame cows. Cows with milk urea concentration of <3.95 mmol/l at 6-8 week of lactation are more likely to develop lameness compared to cows with a lower concentration of milk urea .The blood metabolite parameters show significant predictive value for lameness developing. The cows with glucose ≥3,91 mmol/l, LDH ≥2123 U/l, ALP ≥92,86 U/l, total cholesterol <2,6 mmol/l, triglycerides concentration <0,11 mmol/l, BHB concentration ≥0,90 mmol/l, and urea 3,30 mmol/l, at onset of lactation were much more likely to develop lameness during lactation. In conclusion milk production, composition of produced milk and blood metabolite parameters in early lactation can be used for prediciton of lameness event at dairy cows.

KEY WORDS
cows, milk production, locomotion scoring, metabolic profile, lameness.

Introduction

Lameness is the third most common reason for involuntary culling of dairy cows after infertility and mastitis (Enting et al., 1997). Lameness is a painful condition (Whay et al., 1997) and has a negative impact on milk production (Warnick et al., 2001).

Lameness aetiology is multifactorial and complex. The factors influencing lameness include: housing conditions (Zebeli et al., 2015), social influence, stage of lactation and pregnancy or calving (Knott et al., 2007), and high yielding (Bicalho et al., 2008). Bicalho et al. (2009) in a cross sectional study, found a greater risk of lameness and claw horn disruption lesions developing in cows with lower body condition score (BCS) and lower digital cushion thickness (DCT). Most metabolic diseases (milk fever, ketosis, retained placenta, and displacement of abomasum) of dairy cows occur within the first weeks of lactation. Furthermore, some other metabolic diseases that manifest clinically later in the lactation (eg. laminitis) can be traced back to metabolic insults that occurred during early lactation (Bertoni and Trevisi, 2013). At this period a drastic metabolic, immune and endocrine changes, are happening which lead cows to be susceptible to diseases (Lacetera et al., 2005).

The purpose of the current study was to detect cut-off value of metabolic and milk parameters in early lactation to predict lammenes in cows.

Material and method

Animals and study design – This is observational, prospective cohort study. A prospective study was designed, in which 118 Holstein–Friesian cows were enrolled. The cows were selected from population of dry cows, LS 3 or lower and free from other serious diseases. The cows were regularly examined and tracked from dry perod to six months of lactation. During this period, 11 cows that developed severe diseases were excluded and another 7 cows were moved because of farm management decisions. After all exclusions, 100 cows remained in the final group. The cows were situated on a dairy farm with a cubicle housing system. They were milked twice a day and fed a total mixed ration based on alfalfa hay, sugar beet pulp, corn silage, and concentrate. The average milk yield at the previous lactation was 7794±1210 kg/305 days. All cows were under the competent and permanent supervision of an employed veterinarian, with daily veterinary examination. Cows were divided in two groups depending of lameness developing in lactation as non lame (LS 1, 2 or 3) and lame group (LS 4 or 5). Retrospectively we determined which were the critical values of milk production, composition of produced milk and metabolic status in early lactation which increase risk for lameness developing.

Metabolic blood parameters - 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 hours) 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 Beta-hydroxybutyrate (BHB) was measured by ketone meter (FreeStyle Optium - Abbot Germany).

Milk sampling -The milk samples are taken in standard plastic bottles (Labena Ltd.) with inside diameter of 29 mm, using Waikato Speedsampler device (Waikato Milking Systems NZ Ltd). Milk fat and milk proteins contents were analyzed with MilkoScan FT 6000. To determine the concentration of milk urea, samples submitted to the lab were kept in refrigerators at 4 °C for 24 hours, and then centrifuged at 3000 rpm for 10 minutes. After centrifugation, the separated milk serum was obtained from samples of milk separation of fat that is removed from the surface with the vacuum pump. The concentration of urea in the milk serum was determined with photometer Merck KGaA, 64271 Darmstadt, Germany, and the Pharo 300 spectrophotometer Spectroquant® Merck. Germany, using a MUN test (milk urea nitrogen) by the enzyme urease. The content of milk urea was determined using enzyme kinetic method standard (Tietz, N.W., 1995).

Locomotion score - All cows were scored for locomotion in dry period and every month during six months of lactation, using the 1-5 locomotion scoring system (LS) of Sprecher et al. (1997). This system use a numerical scale 1-5 where, score of 1 denoted sound locomotion, and whereas a score 2 and 3 indicated a clinically non-important change in gait, and a score of 4 and 5 describes different severities of clinical lameness. In the current study, cows were described as lame (locomotion score >3) or non-lame (locomotion score ≤3).

Statistical analysis - Average values and standard deviations of milk production, composition of produced milk, LS and blood metabolic parameters, according to time frame (Table 1, Table 2) were calculated for the observed cows. At the end of sixt month of lactation a cows were divided in two groups and pronounced as lame or non lame depending by observed LS during lactation. The value of milk production, composition of produced milk and blood metabolite parameters which optimally divided cows with high and low risk of developing a lameness was determined using the cut-offs from ROC analysis. The linear correlation between lameness intesity in different months of lactation was determined using Pearson's correlation coefficient. Chi square test, sensitivity, specificity, positive and negative predictive values and relative risk for lameness developing were calculated for blood metabolic parameters, milk production and composition of produced milk.

Results and discussion

Test results show that the intensity of lameness in the dry period does not show a significant correlation with the intensity of lameness in lactation. However, an interesting finding is that during the lactation there is a positive correlation between the intensity of lameness from the first to the fifth month of lactation. (Table 1). Lameness in the third month of lactation showed the most intensive relationship and a positive

correlation with the score of lameness in 4th, 5th, and 6 th month of lactation.

Blood metabolic parameters in first month of lactation show significant predictive value for lameness developing. The cows with glucose concetration ≥3,91 mmol/l were 1,6 times more likely to develop lameness (Table 2, Fig. 1). The lactate dehidrogenase (LDH) in our research were sligthly increased which may be due to increased lipomobilisation in hepatocytes at the onset of lactation. Our research reveal that a cow with LDH ≥2123 U/l were 3,5 times more likely to develop lameness (Table 2, Fig. 1). Our results show that cow with alkaline phosphatase (ALP) level ≥92,86 U/l were 2,4 times more likely to develop lameness (Table 2, Fig. 1). In our research a total cholesterol concentration (4,23±1,26 mmol/l) is in physiological range. The cholesterol concentration indirectly reveal ability of liver to produce a VLDL (very low density lipoproteines). In case of limited ability of liver to produce a VLDL, the lipid infiltration occur, and blood cholesterol concentration drop to <2,6 mmol/l (Holtenius and Hjort, 1990). Our research show that cows with total cholesterol concentration ≥5,42 mmol/l at the onset of lactation were 4,2 times increased risk to develop lameness compared with cows with lower cholesterol level (Table 3, Fig. 1).

Table 1. The correlation between score of lameness in different periods of lactation
Tabela 1. Korelacija između skorova šepavosti u različitim periodima laktacije

r	LS 3rd. month	LS 4th. month	LS 5th. month	LS 6th. month
LS 2nd. month	0,260**	0,332**	0,316**	0,289**
LS 3rd. month	-	0,621**	0,503**	0,479**
LS 4th. month		-	0,437**	0,453**
LS 5th. month			-	0,522**

**p<0,01

Table 2. Descriptive statistic for parameters – median value and interquartile diference (IQR)
Tabela 2. Deskriptivna statistika ispitivanih parametara-medijana i interkvartilna razlika (IQR)

		Median	IQR
Glucose (mmol/l)	Healthy	3,3	0,9
	Lame	3,6	0,8
LDH (U/l)	Healthy	1850	480
	Lame	2080	550
ALP (U/l)	Healthy	45	5,5
	Lame	82	10,1
Total cholesterol (mmol/l)	Healthy	3,3	0,85
	Lame	4,8	1,02
Triglycerides (mmol/l)	Healthy	0,25	0,1
	Lame	0,12	0,12
BHB (mmol/l)	Healthy	0,61	0,2
	Lame	0,82	0,26
Urea (mmol/l)	Healthy	4,2	1,9
	Lame	5,9	2,1
Milk production (L) 70-80d.	Healthy	24,2	6,2
	Lame	31,5	5,3
Milk fat (%) 70-80 d.	Healthy	3,5	0,7
	Lame	4,05	0,8
Milk proteins (%) 70-80d.	Healthy	2,99	0,15
	Lame	3,25	0,18
Milk urea (mmol/l)	Healthy	4,2	0,7
	Lame	5,1	1,2

The average triglycerides concentration in our research 0,18±0,1 mmol/l, and were in range (Bertoni and Trevisi, 2013). Our results show that cow with triglycerides concentration <0,11 mmol/l were 1,5 times more likely to develop lameness (Table 2, Fig. 1). The average BHB in early lactation in our research were 0,72±0,23 mmol/l. Our research reveals that cows with BHB concentration ≥0,90 mmol/l in early lactation were 31 times more likely to develop lameness compared with cows with lower BHB concentration (Table 2, Fig. 1). In our research mean serum level of urea is 5,23±1,7 mmol/l. Our results reveals that cows with urea concentration lower than 3,30 mmol/l at onset of lactation were 2,4 times more likly to develop lameness (Table 2, Fig. 1)

Table 3. Influence of blood metabolite parameters, milk production and composition of produced milk on lameness developing
Tabela 3. Uticaj vrednosti krvnih parametara, proizvodnje i sastava proizvedenog mleka na razvoj hromosti

	Cut offs values	Sensitivity	Specificity
Glucose (mmol/l)	2,93	0,90	0,11
	≥3,91	0,16	0,89
LDH (U/l)	1568,2	0,90	0,19
	≥2123	0,25	0,91
ALP (U/l)	41,71	0,90	0,32
	≥92,86	0,19	0,91
Total cholesterol (mmol/l)	2,77	0,90	0,25
	≥5,42	0,29	0,91
Triglycerides (mmol/l)	<0,11	0,06	0,95
	0,32	0,96	0,10
BHB (mmol/l)	0,50	0,83	0,17
	≥0,90	0,32	0,98
Urea (mmol/l)	<3,30	0,19	0,91
	6,84	0,90	0,25
Milk production (L) 70-80d.	21,50	0,903	0,060
	≥36,00	0,161	0,940
Milk fat (%) 70-80 d.	3,08	0,903	0,149
	≥4,24	0,097	0,925
Milk proteins (%) 70-80d.	2,85	0,903	0,075
	≥3,47	0,226	0,925
Milk urea (mmol/l)	<3,95	0,323	0,910
	6,92	0,903	0,030

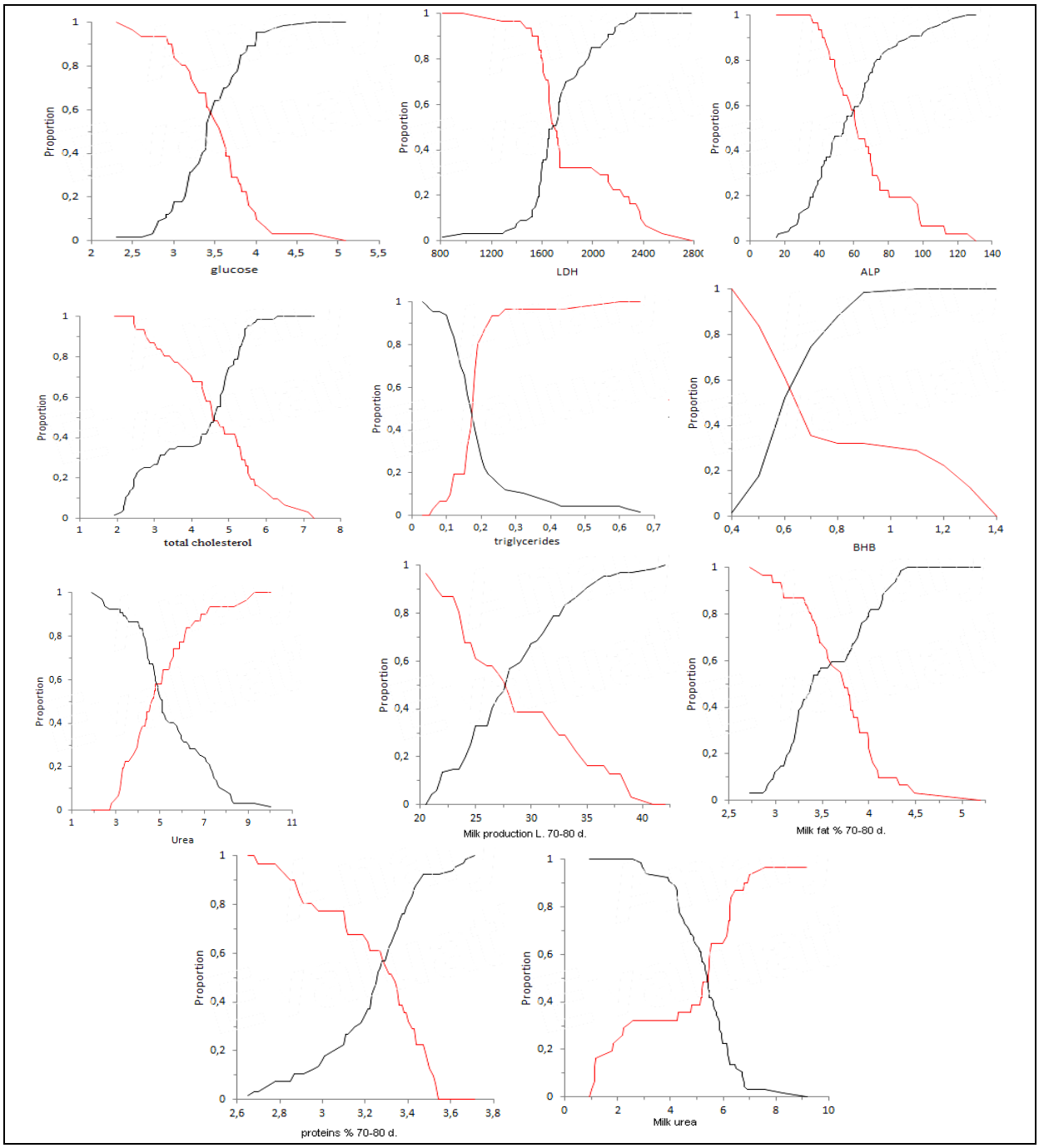

Figure 1. Specificity (blue line) and Sensitivity (red line) for glucose, LDH, ALP, total cholesterol, triglycerides, BHB, urea, milk production, milk fat, milk proteins and milk urea as predictor of lameness developing.

Slika 1. Specifičnost (plava linija) i senzitivnost (crvena linija) za glukozu, LDH, ALP, ukupni holesterol, trigliceride, BHB, ureu, proizvodnju mleka, mlečnu mast, proteine i ureu kao prediktore za razvoj hromosti

Blood metabolite parameters in our research has a strong predictive value for lameness developing. Glucose concetration ≥3,91 mmol/l seems to be critical in our research were 1,6 times more likely to develop lameness O'Driscoll et al. (2015) found that lame cows tended to have glucose levels higher than sound cows (3.13 ± 0.09 vs. 2.89 ± 0.12 mmol/L; P ≤ 0.1). The increasing of

glucose level is generated trought increased level of cortisole caused by exposure to stress due to lameness event. Almeida et al. (2008) reported that lame cows had cortisol levels that were 49% higher than sound. The lactate dehidrogenase (LDH) in our research were sligthly increased. Our research reveal that a cow with LDH ≥2123 U/l were 3,5 times more likely to develop lameness. ALP level ≥92,86 U/l increase lameness event risk for 2,4 times. ALP is main indicator of osteoblaste activity and increased activity of this enzyme can be observed during dry period and anabolic processes in bones, while decreasing occur at onset of lactation during catabolism. Cows with total cholesterol concentration ≥5,42 mmol/l at the onset of lactation were 4,2 times in higher risk for lameness developing. The cholesterol concentration indirectly reveal ability of liver to produce a VLDL (very low density lipoproteines). In case of limited ability of liver to produce a VLDL, the lipid infiltration occur, and blood cholesterol concentration drop to <2,6 mmol/l (Holtenius and Hjort, 1990). The average Triglycerides concentration <0,11 mmol/l increase lameness risk for 1,5 times. The lower concentration of triglycerides is commonly in weeks following calving because of hepatic accumulation, which indicate a decreased capability of liver to produce lipoproteins and release them in blood stream (Bertoni and Trevisi, 2013; Đoković et al., 2009). The BHB concentration has a very strong predictive value (BHB≥0,90 mmol/l, increase risk for 31 times). Glucose, NEFA, and BHB are useful as energy indicators, but mainly at the end of pregnancy and in the first weeks of lactation, or in the case of great and prolonged energy deficiencies, the latter being an infrequent occurrence in dairy cows (Bertoni and Trevisi, 2013; Cincović, 2013; Ospina et al., 2010a; Ospina et al., 2010b). According by Reist et al. (2002) there is a strong correlation between BHB and energy balance (r =−0,451). The cows with lower level of BHB in early lactation are in great risk for developing other diseases like abomasal displacement and ketosis (Chapinal et al., 2012a). Serum urea concentration lower than 3,30 mmol/l increase 2,4 times risk for lameness developing. In simmilar research O'Driscol et al. (2015) found that lame cows had lower urea levels than sound (4.35 ± 0.27 vs. 5.17 ± 0.26 µmol/L; P ≤ 0.05). Serum urea levels have been observed to be lower in sheep and beef cattle that are submitted to stressors such as isolation, restraint, and transportation (Galyean et al., 1981; Apple et al., 1993). The lower serum urea may be due to disturbance in energy and protein metabolism due to insufficient food intake at the onset of lactation. The analysis of the test results of the milk production and composition of produced milk show that the cow with the milk production of ≥36.00 kg are 3,029 times more likely to develop lameness compared to cows with a lower milk production. Cows that produce milk fat of ≥4.24% in the third month of lactation are 1,329 times more likely to develop lameness compared to cows with a small amount of milk fat. Cows with the production of milk proteins of ≥3.47% in the third month of lactation are 3,617 times more likely to develop lameness compared to cows with a small amount of protein in milk in peak of lactation (Table 2). Cows with milk urea concentration of <3.95 mmol/l at 6-8 week of lactation are 4,841 times more likely to develop lameness compared to cows with a higher concentration of milk urea (Table 2).

Conclusions

In conclusion milk production, composition of produced milk and blood metabolite parameters in early lactation can be used for prediction of lameness event at dairy cows. We recommended use of cut-off value with high specificity (about 90%) because if the test result for a highly specific test is positive we can be nearly certain that cow will suffer from lameness.

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Kritične vrednosti metaboličkih parametara krvi i mleka značajne za predikciju nastanka hromosti kod holštajn-frizijske rase krava

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SAŽETAK

Ispitivanje je vršeno na 100 Holštajn-Frizijskih krava od peroda zasušenja do šestog meseca laktacije. Krave su bile izabrane iz populacije zasušenih krava sa LS 3 ili manje, slobodne od ostalih bolesti. Hromost u toku laktacije u našem istraživanju javila se kod 27 krava. Retrospektivno smo utvrdili koje su kritične vrednosti proizvodnje mleka, sastava mleka i metabolički parametri krvi u ranoj laktaciji koji povećavaju rizik za nastanak hromosti. ROC analiza je pokazala da krave sa proizvodnjom mleka od ≥36.00 kg, mlečne masti ≥4.24% i sadržajem proteina u mleku od ≥3.47% u trećem mesecu laktacije (70-80. dana) imaju veću mogućnost za nastanak hromosti. Rezultati ispitivanja su pokazali da pad koncentracije uree u mleku povećava specifičnost i smanjuje senzitivnost za otkrivanje hromih krava. Krave sa koncentracijom uree od <3.95 mmol/l u 6-8 nedelji laktacije imaju veću mogućnost za nastanak hromosti od krava sa manjom koncentracijom uree u mleku. Krave sa vrednostima glukoze ≥3,91 mmol/l, LDH ≥2123 U/l, ALP ≥92,86 U/l, ukupni holesterol <2,6 mmol/l, koncentracije triglicerida <0,11 mmol/l, koncentracije BHB ≥0,90 mmol/l i uree 3,30 mmol/l na početku laktacije su imale mnogo veću verovatnost za nastanak hromosti za vreme laktacije.

KLJUČNE REČI

krave, mlečnost, bodovni sistem dijagnostike hromosti, metabolički profil, hromost.

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