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## Association between white line disease and sole ulcers with certain milk components in Simmental cows

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**ABSTRACT:** Lameness is one of the high influence production illnesses in intensive dairy production farming, it reduces milk yield and can also negatively affect the quality of milk. Many factors can affect the production of milk components. Subsequently, breed, nutrition, milk yield, various metabolic disorders, and lameness can have an effect on the synthesis of milk components. White line disease and sole ulcers are widespread hoof diseases of cows in tied-holding systems. Albeit the main cause of lameness, associations between claw disorders of cows and variation of milk components haven't been widely studied in Simmental cows. The objective of our study was to investigate the effect of white line disease and sole ulcers on the percentage of milk components of Simmental dairy cows kept in the small households in Mačva locality, Serbia. For milk analysis were enrolled 36 cows in the study: affected by white line disease (n=12), sole ulcers (n=12), and healthy cows (n=12) in the early stage of lactation. Milk components (milk protein, fat, and non-fat dry matter) were analyzed using Lactoscan S. Significance of differences in milk component characteristics between white line disease, sole ulcers, and healthy groups were tested using a Kruskal-Wallis multiple comparisons test. The percentage of milk fat of cows affected by white line disease and cows affected by sole ulcers were significantly lower than those of non-lame cows: 3.80%, 3.69%, and 4.18%, respectively (both p<0.05). However, differences in the contents of milk protein and the contents of non-fat dry matter of cows affected by white line disease, sole ulcers, and in healthy cows were not significantly different (p>0.05). Our results indicate that hoof diseases of cows namely white line disease and sole ulcers, are associated with reduced significantly milk fat production in lame Simmental cows.

**Keywords:** claw disease; lameness; milk fat; non-fat dry matter; Simmental cows.

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## INTRODUCTION

Lameness causes reduced milk production, and reproductive disorders and has consequent economic losses (Warnick et al., 2001). Therefore it draws great attention to the in the dairy industry (Barker et al., 2010). Milk yield is correlated with lameness (Amory et al., 2008; Olechnowicz et al., 2010); it is decreased from 0.78 kg up to 5.5 kg per day, depending on the level of lameness and the stage of lactation (Onyiro et al., 2008). In Serbia, the frequency of white line disease and sole ulcers of Simmental cows are 18.5% and 15.7%, respectively (Ninković et al., 2021). The greatest reduction in milk yield of lame cows is caused by sole ulcers, white line disease, and interdigital phlegmon (Warnick et al., 2001). Metabolic changes in the digestive tract are key factors, that further trigger the occurrence of laminitis as the main inducer of white line disease and sole ulcers (Mulling, 2002; Holzhauer et al., 2008). Potential factors for the development of white line disease and sole ulcers include among others: stage of lactation, high milk yield, age, body condition, parity, and diet (Bicalho et al., 2009; Pavlenko et al., 2011). Nutritional diversities from optimal diet lead to ruminal disorders. The suboptimal ratio between roughage and concentrates increases the risk of developing subacute ruminal acidosis and rumen acidosis, resulting in an increased risk of lameness (Burger et al., 2017; Kitkas et al., 2019) and reduced milk fat content (Danscher et al., 2015). A linear relationship was found between the decrease in milk yield and the degree of lameness in cows in the early lactation phase (Hernandez et al., 2005).

Components of milk may be potential biomarkers for predicting lameness before the appearance of clinical signs of lameness (Antanaitis et al., 2021). Locomotion score is a useful tool for the determination of early lameness in cows (Sprecher et al., 1996). Literature on the effect of hoof diseases in cows and the impact on the components of milk is limited. This study aims to evaluate the association of sole ulcers and white line disease with the concentrations of certain milk components, during the first 100 days of lactation, in Simmental dairy cows.

## MATERIAL AND METHODS

### Study population

The study was performed during the winter and spring of 2021. A total of 36 Simmental dairy cows were enrolled, from two farms located in the municipality of Vladimirci, Mačva district, Western Serbia.

Both were tie-stall farms, having a concrete stall base with deep litter straw beds. Cows' selection was based on a known history of lameness, to represent those affected by either white line disease (n=12) or sole ulcers (n=12). The remaining 12 cows were clinically healthy and served as a control group. All cows in the study were between second and fifth lactation, in the early stage of lactation (<100 days in milk), and had an average milk yield from 5800 to 6400 kg. The average daily milk yield was 24L. To be enrolled, all 36 cows were tested and found negative for the California mastitis test on the day of milk sampling. They were fed corn silage, alfalfa hay, and a concentrated mixture containing 16% protein and a vitamin-mineral supplement of 2%. The milk samples were collected on the days of the scheduled claw trimming, before trimming, and during the morning milking.

### Locomotion score

The level of lameness of the enrolled cows was measured by performing a locomotion score. The cows were observed standing as they walk, using a five-point scale (1-non lame, 2-mildly lame, 3-moderately lame, 4-lame, and 5-severely lame) (Sprecher et al., 1997). Locomotion scores were collected before claw trimming.

### Milk sample analysis

Milk samples were collected after proper disinfection of the teat surface with 70% ethanol, aseptically, in separate sterile containers. Immediately after collection, they were placed in the icebox and carried to the laboratory where they were kept at 4 °C until analysis. Concentrations of milk fat, protein and non-fat dry matter content were measured using a Lactoscan S Advanced (Milkotronic Ltd, Bulgaria).

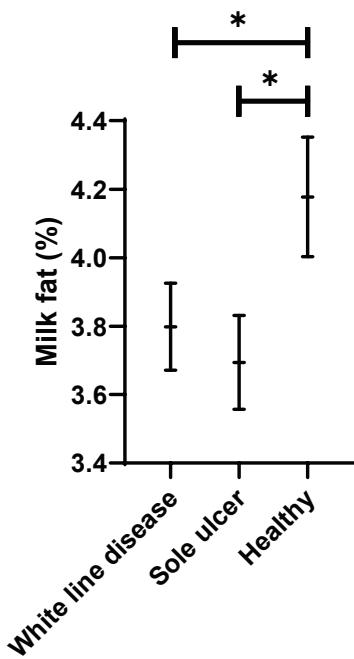
### Statistical analysis

Descriptive statistics and significance of differences in milk component characteristics between white line disease, sole ulcers, and healthy groups were tested. The collected data are ordinal values obtained by ranking the illness degree, also the Shapiro-Wilk test results revealed normal distribution scores in WLD scores, not on SU scores, therefore a nonparametric test set for inferential data analysis was chosen a Kruskal-Wallis multiple comparisons test, followed by Dunn's post hoc individual analysis in GraphPad® Prism® 6 software. Probabilities of  $p \leq 0.05$  were considered significant.

## RESULTS

Contents of milk fat, protein, and non-fat dry matter during the first 100 days postpartum in healthy, white line disease and sole ulcers groups of Simmental cows are presented in Table 1.

The mean milk fat content in the group of cows with white line disease was 3.80%, while in cows with sole ulcers was 3.69%. Both groups had significantly lower contents of milk fat compared with the healthy cows (Fig. 1), the difference among milk fat percentages was higher between groups of healthy cows and sole ulcers ( $p<0.0001$ ) than between of healthy cows and white line disease group ( $p=0.001$ ). There was not any significant difference among groups of lame cows ( $p=0.0665$ ). The mean ( $\pm SD$ ) lameness score of cows affected by white line disease was  $2.17 \pm 0.72$  (median 2) and by sole ulcers  $2.83 \pm 0.94$  (median 3). The distribution of lameness score in cows of white line disease and sole ulcers groups is shown in Table 2.



**Figure 1:** Statistical differences in milk fat (%) between groups of cows affected by white line disease, sole ulcer and healthy cows. Significance levels  $p \leq 0.05$  (\*)

**Table 1:** Descriptive statistics of milk components (%) of the cows of the 3 groups of the study

Parameter	Protein content			Non-fat dry matter			
	Group	WLD	SU	Healthy	WLD	SU	Healthy
<b>Group size</b>	12	12	12	12	12	12	12
<b>Min</b>	3.32	3.30	3.34	9.03	9.09	9.12	
<b>Median</b>	3.46	3.45	3.45	9.16	9.19	9.21	
<b>Max</b>	3.57	3.55	3.56	9.26	9.25	9.29	
<b>Range</b>	0.25	0.25	0.22	0.23	0.16	0.17	
<b>Mean</b>	<b>3.46</b>	<b>3.44</b>	<b>3.46</b>	<b>9.15</b>	<b>9.18</b>	<b>9.21</b>	
<b>SD</b>	0.073	0.068	0.069	0.068	0.048	0.049	
<b>Variation coef.</b>	2.12%	1.99%	1.99%	0.741%	0.524%	0.536%	
<b>SE</b>	0.021	0.020	0.020	0.020	0.014	0.014	

Abbreviations: WLD - White line disease, SU-Sole ulcer SD-Standard deviation SE- Standard error

a, b - statistically significant difference between groups marked with the same letter

**Table 2.** Distribution of locomotion score in cows with lameness in sole ulcer (SU) and white line disease (WLD) groups

	Score distribution								Central tendency and variability			
	1	2	3	4	5	N	Min	Max	Range	Mean	Std. Deviation	Coefficient of variation (%)
SU	8%	25%	42%	25%	0%	12	1	4	3	2.833	0.937	33.09
WLD	17%	50%	33%	0%	0%	12	1	3	2	2.167	0.718	33.13

Abbreviations: WLD - White line disease, SU-Sole ulcer, Std.- Standard, N-observations per group

## DISCUSSION

It's a widely recognized that lameness is associated with an overall decrease in milk yield (Hernandez et al., 2005; Archer et al., 2010); particularly cows affected with sole ulcers showed a significant reduction in milk yield in comparison with healthy cows (Charfeddine et al., 2017). Five main factors could influence the content of milk components: breed, nutrition, stage of lactation, milk yield, and subclinical ruminal acidosis (Alsaftli, 2020). Higher ratios of silage in the meal lead to decreased rumen pH, consequently leading, again, to subacute rumen acidosis (Amory et al., 2008) and consequently to reduced milk fat percentage in milk dairy cows (Malekhhahi et al., 2016). Negative energy balance during the first 50 days of lactation contributes to the development of lameness (Alawneh et al., 2014). The milk composition analysis in the present study showed a significant difference in milk fat percentage between cows having either white line disease or sole ulcers compared with non-lame cows. Our notice is supported in the study by Antanaitis et al., (2021) contents of milk fat of lame cows decrease 3rd day before a diagnosis of lameness. In agreement with our result, in one study Zhang et al., (2019) found that cows with lameness had lower content of milk fat than non-lame cows, while no significant differences in milk fat between lame and non-lame cows were detected in another (Olechnowicz and Jaskowski, 2012). The presence of subacute ruminal acidosis in cows leads to a significant decrease in milk fat contents (Kitkas et al., 2019). However, Singh et al., (2018) reported non-significant variation in the contents of milk components between lame and non-lame Indian cross-bred cows. Our findings further agree with the results of studies by (Pavlenko et al., 2011) who found no significant differences between cows with white line disease and/or sole ulcers in comparison with healthy cows in the protein content of milk, but there are data finding milk protein to be significantly lower in lame cows (Olechnowicz and Jaskowski, 2012). Our results showed no significant differences in contents of non-fat dry matter in milk

between cows affected with white line disease and/or sole ulcers and non-lame cows. However, a study by Petrović et al., (2006) reported an average content of non-fat dry matter in raw cows' milk of only 8.56%, though the difference from non-fat dry matters obtained in our study may be influenced by breed. The authors consider that diet of silage increased contents may start the chain of events resulting in sole diseases. In our study, lameness scores were associated with lower milk quality and decreased contents of milk fat in lame cows compared non-lame cows, therefore a locomotion score is a useful tool for early identification of lameness and minimizing losses in milk production and milk components.

## CONCLUSIONS

There were significantly lower milk fat contents in cows affected by white line disease and sole ulcer than in healthy cows. Contents of protein and non-fat dry matter in cows affected by white line disease or by sole ulcers were non-significantly different from those of healthy cows. Determining the lameness score is crucial for preventing losses in milk production and thus production milk of components. The results of this case warrant further investigation on a larger group of cows including research that should be done on a larger number of cows to take into account the interaction between cows that are clinically lame and the influence of diet on the content of milk components.

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## CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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