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The Analysis of Variability of Indicators Associated with Prevalence of Subclinical Ketosis/Acidosis in Dairy Cattle

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Summary

Metabolic disorders of dairy cows, primarily ketosis and acidosis, are one of the most significant problems in dairy herd management. Numerous studies have shown that metabolic disorders induce economic losses for milk producers due to treatment costs, reduced milk production, impaired reproduction, and ultimately increased animal excretion rates. Given the above, and for the purpose of sustainable management of the dairy herd, it is extremely important to detect as early as possible the cows that are at risk of subclinical metabolic disorders and to prevent the development of the clinical form in time. On dairy farms, milk recording is performed every month, which includes the collection of data on the productivity of dairy cattle that are in the system of breeding and selection work. The results of milk recording together with the pedigree data, the basis for calculating the breeding value of cattle and for the implementation of selection in accordance with the breeding program of each breed, also allow the breeder to improve dairy herd management and timely detection of metabolic disorders in the subclinical phase using precision dairy farming technologies. The daily content of milk fat and protein represents the basis for estimating the prevalence, with the optimal values of the ratio of milk fat and protein being 1.1 - 1.5, while the deviation from the same indicates the prevalence of acidosis or ketosis. The aim of this study was to determine the prevalence of subclinical disorders of Simmental and Holstein cows depending on the season by applying precision dairy farming technologies- that is, the results of milk recording. Our logical control dataset consisted of over 1.6 million of test day records (Holstein cows: 805,247 records/ 69,368 animals/ 4,998 farms; and Simmental cows: 845,514 records/ 78,540 animals/ 7,242 farms). Conducted analysis indicated that daily milk production, stage of lactation, parity, age at first calving, milk recording, and cattle breed significantly affected daily fat and protein content as well as F/P ratio. Also, determined higher values of F/P ratio in winter period indicates higher ketosis prevalence risk, while lower values of F/P ratio in summer period indicates higher acidosis prevalence risk. Finally, in order to more precisely predict the ketosis / acidosis prevalence in dairy cows based on milk recording data, factor that significantly affects the variability of daily fat and protein content, and consequently the fat to protein ratio as well as the prevalence risk of metabolic disorders should be taken into account.

Key words

metabolic disorders, dairy cows, precision dairy farming, milk recording

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Introduction

Precision dairy farming implies set of different technologies, tools and methods based on usage of various devices for optimisation of dairy cattle farm. Precision devices could be used for measuring the indicators of animals related to their physiological, behavioural, and production state. The technologies of precision farming are used in order to increase animal's productivity, to enable early detection of possible disease/disorder, and finally to decrease the costs of production process through adequate preventive health measures and farming optimisation. Many of these technologies, such as data-loggers, pedometers, automatic oestrus detection monitors, daily body weight measurements, as well as milk recording (daily milk quantity and quality), are already used by dairy producers. The milk recording implies animal identification, collection of data referring to certain animal, and determination of animal's daily milk production in terms of yield and contents. Milk recording data (daily milk yield and contents) enables fast indication of potential imbalance, as well as determination of possible disorder / disease of individual animal.

Dairy cows could experience various disorders during the transition period as a consequence of changes in nutrition, negative energy balance, decreased feed intake, rapid weight loss, or hypocalcemia. LeBlanc (2010) emphasised that the metabolic disorders (up to 50%) mainly occurred at the beginning of lactation (first 2 weeks). Furthermore, the source of stress could be environmental factors such as regrouping (Mulligan and Doherty, 2008), or inappropriate (micro)climatic conditions (Broucek et al., 2007). The ketosis as well as acidosis represents one of the most common disorders in lactating dairy cows.

Ketosis represents a metabolic disorder that can occur both in clinical and subclinical forms. According to Gillund et al. (2001), clinical ketosis most frequently occurs in high producing cows at the beginning of lactation (2nd - 7th week) as a consequence of unbalanced nutrition and farm management. The prevalence of ketosis could vary due to breed, parity, season and herd-related factors (Dohoo and Martin, 1984; Rajala-Schultz et al., 1999). Furthermore, the occurrence of clinical ketosis induces results in economic losses of farm through treatment costs, decreased milk production, impaired reproduction efficiency, and increased involuntary culling (Rajala and Gröhn, 1998; Suthar et al., 2013). Since subacute ruminal acidosis (SARA) is an increasing problem in high productive dairy cows, the monitoring of animals and prevention have become extremely important. The highest prevalence of SARA was determined in early lactation and in animals at peak dry matter (DM) intake (Dirksen et al., 1985; Bramley et al., 2008; O'Grady et al., 2008). The occurrence of both disorders induces significant farm costs and early detection is one of the key conditions for enabling sustainable business. A precondition for timely detection of the possible occurrence and prevention of the development of more severe disorder phase is a quick, simple, and accurate determination based on already available data, the usage of test-day records (TDR). The usage of TDR is much more cost effective and non-invasive when compared to specific diagnostic (Duffield et al., 1997; Eicher, 2004). TDR include information regarding the daily milk yield,

fat and protein content and fat to protein ratio (F/P ratio). Gravert (1991) indicated that the ideal values of F/P were in $< 1, 1.25 >$, while Duffield et al. (1997) set 1.33 as upper margin. Furthermore, the F/P ratio > 1.4 indicates energy deficit and, if ketone bodies are present, subclinical ketosis (Haas and Hofirek, 2004). The $F/P \geq 1.5$ could be defined as indicator of ketosis prevalence risk, while $F/P < 1.0$ represents indicator of acidosis prevalence risk. Furthermore, the F/P ratio together with cow's daily milk production indicate prevalence of metabolic disorders (acidosis, ketosis). The $F/P \geq 1.5$ in cows that yielded between 33 to 50 kg/day represents an indicator of subclinical ketosis, while $F/P < 1.0$ in cows that yielded between 20 to 43 kg/day represents an indicator of subclinical acidosis (Eicher, 2004).

Given that a number of factors affect variability of daily milk contents, the aim of this study was to determine the prevalence of subclinical disorders of Simmental and Holstein cows depending on the season by applying precision dairy farming technologies, which means the results of milk recording.

Materials and Methods

Test-day records of Holstein and Dairy Simmental cows collected during the five-year period (January/2008 – December/2012) were used in the statistical analysis. Test-day records were collected during the regular milk recording in Croatia, performed according to the alternative milk recording method (AT4 / BT4) on dairy cattle farms on monthly basis (every four weeks). The alternative milk recording method implies measuring and sampling of milk during the evening or morning milkings. In Croatia, milk recording is performed by the field officers of the Croatian Agency for Agriculture and Food while the milk samples are analysed in the Central Laboratory for Milk Quality Control. In the laboratory milk samples are analysed according to the accredited laboratory methods using infrared spectrophotometry for determination of fat and protein content in milk. Furthermore, Milcoscan FT6000 is used for determination of the milk composition.

During the logical data control the following limitation for test-day records was set; lactation stage (> 5 days and < 500 days), parity (> 1 and < 7), age at first calving (> 21 and < 36 months), breed (Holstein, and Simmental). Furthermore, test-day records with missing information regarding parity, breed, and missing or nonsense daily milk traits according to standards of ICAR (ICAR standards, 2017) were deleted from the dataset. The whole logical control dataset contained over 1.6 million of test day records (Holstein cows: 805,247 records/ 69,368 animals/ 4,998 farms; and Simmental cows: 845,514 records/ 78,540 animals/ 7,242 farms).

Regarding the parity, animals were divided into four classes: I., II., III., and IV. (animals in fourth and higher lactations) and regarding the recording date, test-day records were divided into twelve recording months: January, ..., December.

Basic statistical parameters of daily milk yield, daily fat and protein content, as well as fat to protein ration are presented in Table 1.

Table 1. Basic statistical parameters of daily milk yield, daily fat and protein content, as well as fat to protein ration in regard to breed (Holstein and Simmental)

Variable	N	Mean	SD	CV	Minimum	Maximum
Holstein breed						
DMY	794640	21.75	9.09	41.80	3.00	96.00
FAT	780120	4.15	0.95	22.98	1.50	9.00
PROTEIN	785022	3.41	0.47	13.69	1.05	6.98
F/P	780075	1.22	0.28	22.69	0.25	4.59
Simmental breed						
DMY	844252	16.13	6.14	38.03	3.00	94.00
FAT	815276	4.15	0.90	21.70	1.50	9.00
PROTEIN	822557	3.46	0.47	13.54	1.13	7.00
F/P	815201	1.21	0.26	21.65	0.23	4.32

*DMY – daily milk yield (kg); FAT – daily fat content (%); PROTEIN – daily protein content (%); F/P – fat to protein ratio

For the evaluation of the effect of recording month on the variability of daily fat and protein content, as well as fat to protein ration in Holstein and Simmental cows the following statistical model was used:

$$y_{ijklmn} = \mu + b_1(d_i/305) + b_2(d_i/305)^2 + b_3 \ln(305/d_i) + b_4 \ln^2(305/d_i) + b_5 m_j + A_k + P + M_{ml}$$

where:

y_{ijklmn} – estimated trait (daily fat and protein content, as well as fat to protein ration);

μ – intercept;

b_1, b_2, b_3, b_4, b_5 – regression coefficients;

d_i – days in milk ($i = 5$ to 500 day) as the polynomial regressions by Ali and Schaeffer (1987);

m_j – daily milk yield ($j = 3.00$ to 96.00 kg);

A_j – fixed effect of age at first calving class j ($j = 21$ to 36 month);

P_l – fixed effect of parity l ($l = I., II., III.,$ and $IV.$);

M_m – fixed effect of recording month m ($m =$ January, ..., December);

e_{ijklmn} – residual.

The analysis was performed separately for each dairy cattle breed. The significance of the differences between the recording months was tested by Scheffe's method of multiple comparisons (using the PROC GLM procedure in SAS (SAS Institute Inc., 2019)).

Results

The analysis of variance showed that cow's daily productivity, stage of lactation, parity, age at first calving, and recording month statistically highly significantly ($P < 0.001$) affected daily fat and protein content as well as fat to protein ratio. The results of testing the significance of the differences in LSMs of analysed traits according to Scheffe's method of multiple comparisons are presented in Table 2. The LSMs of analysed traits differed statistically highly significantly ($P < 0.001$) due to the month of milk recording. In Holstein cows, higher daily fat content ($> 4.3\%$) was determined in the period from November to February with the highest determined value (4.376 %) in January. Also, the daily protein content was the highest in the defined period with the highest value in November (3.546 %). A similar trend was also determined in Simmental cows. In both breeds, the lowest values of daily fat and protein content were determined in summer period in July. Furthermore, the lowest value of fat to protein ratio (F/P) was observed in August in the amount of 1.193 in Holsteins and 1.173 in Simmental cows. Higher values of F/P were observed in winter period (November, December, and January) in both breeds.

Determined results indicate that daily fat and protein content as well as F/P ratio significantly vary due to recording month. Higher LSMs values of F/P ratio in winter period indicate higher ketosis prevalence risk, while lower LSMs values indicate higher acidosis prevalence risk during the summer period. Also, slightly higher LSMs of F/P ratio were determined in Holstein breed indicated higher ketosis prevalence risk in high yielding Holsteins.

Table 2. LSMs of daily fat and protein content, as well as fat to protein ration regarding the recording month separately for each breed (Holstein and Simmental)

Recording month	Holstein			Simmental		
	FAT	PROTEIN	F/P	FAT	PROTEIN	F/P
January	4.376A	3.503A	1.258A	4.373A	3.539A	1.245A
February	4.318B	3.487B	1.247B	4.319B	3.505B	1.242A
March	4.243C	3.437C	1.242B	4.213C	3.452C	1.229B
April	4.125D	3.388D	1.227C	4.096D	3.414D	1.209C
May	4.093E	3.363E	1.226C	4.021E	3.389E	1.196D
June	3.955F	3.294F	1.210D	3.951F	3.339F	1.193D
July	3.917G	3.258G	1.211D	3.886G	3.309G	1.184E
August	3.927G	3.314H	1.193E	3.901G	3.350F	1.173F
September	4.099E	3.420I	1.206D	4.038E	3.440G	1.182E
October	4.234C	3.522J	1.209D	4.234C	3.535A	1.205C
November	4.362A	3.546K	1.237C	4.348A	3.575H	1.224B
December	4.312B	3.534L	1.227C	4.351A	3.572H	1.227B

*DMY – daily milk yield (kg); FAT – daily fat content (%); PROTEIN – daily protein content (%); F/P – fat to protein ratio

LSMs marked with different letters differ statistically with high significance ($P < 0.001$)

Discussion

Fats in milk are synthesized from carbohydrates by the action of rumen microorganisms. They are formed mainly from acetic acid and to a lesser extent from butyric acid. An important source for the synthesis of milk fats are also body fats, which are mostly used in the first third of lactation when productive needs are greatly increased. The physiologically optimal interval of fat values in milk is 3% - 5%. Lower or higher values are an indicator of certain metabolic disorders. Low values of milk fat indicate the possibility of acidification of the rumen or acidosis and inflammation of the mucous membrane of the rumen. In addition, they may indicate fertility disorders, rennet dislocation, decreased resistance to food mycotoxins, decreased ability to consume feed, and hoof disease. Exceptionally, a low fat content may be due to an incorrect order of the meal (first feeding concentrate) or general malnutrition of animals. Factors that can cause a decrease in the percentage of milk fat (Palmquist et al., 1993; Doreau et al., 1999) are: increased milk production, reduced feed particle size, feeding with too much starch (> 28% of the total meal), heat stress, nutrition with the addition of polyunsaturated fatty acids (linoleic and linolenic) as free oils. High levels of milk fat at the beginning of lactation are often the result of excessive depletion of body reserves characteristic of highly productive heads. A high value of milk fat in cow after calving is an indicator of digestive disorders and is correlated with loss of appetite, ketosis, rapid weight loss, reduced milk yield, permanent liver damage, rennet dislocation, mastitis and various other infections. Furthermore, high values of milk fat at the end of lactation are expected to give

the decrease in milk yield, so they are not an indicator of digestive disorders. Factors that may increase milk fat content are as follows (Palmquist et al., 1993; Doreau et al., 1999): increased proportion of voluminous feeds, more frequent feeding, decreased fitness or weight loss, feeding with recommended oilseed levels (< 2.5 kg), feeding with a higher proportion of saturated fats such as palmitic (c 16:00) and stearin (c 18:00). Furthermore, the fat content of milk is influenced by numerous factors such as breed, order and stage of lactation, season (calving, milk recording), milking frequency, udder health, nutrition, i.e. energy supply and the proportion of voluminous feed in the meal, and individual characteristics of animal (Hargrove and Gilbert, 1984; Arsov, 1986; Keown et al., 1986; Erdman and Varner, 1995; Klei et al., 1997; Weiß et al., 2002).

The protein content in milk is a consequence of nutrition (supply of digestible protein in the meal), season (lower content is characteristic of summer season), order and stage of lactation, breed, udder health, and individual characteristics of each cow (Hargrove and Gilbert, 1984; Arsov, 1986; Keown et al., 1986; Murphy and O'Mara, 1993; Erdman and Varner, 1995; Klei et al., 1997; Eicher et al., 1999; Weiß et al., 2002). Proteins in milk are mostly synthesized from amino acids resorbed in the small intestine (microbial proteins synthesized in the rumen) and to a lesser extent from body reserve proteins. The optimal value of protein content in milk in the interval is 3.2% - 3.8%. A low protein content indicates a lack of digestible protein and energy in the meal, while too high a protein content indicates the general nutrition of the animal.

The optimal values of fat and protein ratio (F/P) in milk are 1.1 - 1.5. The variation in the value of the ratio is small in healthy animals in good condition. Changes in the F/P ratio are mainly caused by inadequate nutrition, animal disease/disorder, or inappropriate environmental conditions (Duffield, 2004; Eicher, 2004). The F/P ratio > 1.5 is most often associated with the occurrence of disorders in cows with high milk fat content (decreased appetite, rennet dislocation, ketosis (Duffield et al., 1997), rapid weight loss, reduced milk yield, permanent liver damage, fertility disorders, mastitis and various other infections). The F/P ratio higher than 1.5 is most often the result of excessive consumption of body reserves of highly productive animals at the beginning of lactation. The F/P ratio < 1.1 is most often the result of inadequate meal structure (too much concentrated feed) and too fast transition from one type of meal to another. Furthermore, an F/P ratio lower than 1.5 is most commonly associated with disorders determined in cows with low milk fat content.

This research indicates that daily production level, stage of lactation, parity, age at first calving, and month of milk recording are statistically highly significant ($P < 0.001$) in affecting daily fat and protein content as well as F/P ratio. Furthermore, the values of analysed traits vary due to animal breed. The research results indicate that the prevalence of metabolic disorders significantly vary during the year with indicated higher ketosis prevalence risk in the winter period, as well as higher acidosis prevalence risk during the summer period. Furthermore, slightly higher ketosis prevalence risk has been indicated in Holstein cows.

Conclusions

In order to determine the prevalence of subclinical disorders of Simmental and Holstein cows depending on the season, the precision farming methodology that is indirect assessment of milk recording data was applied. Conducted analysis showed a significant effect of daily milk production, stage of lactation, parity, age at first calving, milk recording, and cattle breed on the variability of daily fat and protein content as well as F/P ratio.

Furthermore, the analysis indicated that daily fat and protein content as well as F/P ratio significantly vary due to recording month with the higher values of F/P ratio in winter period indicating higher ketosis prevalence risk, and lower values of F/P ratio in summer period indicating higher acidosis prevalence risk. Since numerous factors affect the variability of daily fat and protein content, and consequently the fat to protein ratio as well as the prevalence risk of metabolic disorders, these factors should be taken into account when predicting the ketosis/acidosis prevalence in dairy cows based on milk recording data.

References

- Ali, T. E., Schaeffer, L. R. (1987). Accounting for Covariances among Test Day Milk Yields in Dairy Cows. *Can J Anim Sci* 67: 637-644
- Arsov, A., Golc, S., Kastelic, D. (1986). Higiensko pridobivanje mleka. Knjižica za pospeševanje kmetijstva. Ljubljana, ČZP Kmečki glas, p.125
- Bramley, E., Lean, I.J., Fulkerson, W.J., Stevenson, M.A., Rabiee, A.R., Costa, N.D. (2008). The Definition of Acidosis in Dairy Herds Predominantly Fed on Pasture and Concentrates. *J Dairy Sci* 91 (1): 308-321
- Broucek, J., Ryba, S., Mihina, S., Uhrincat, M., Kisac, P. (2007). Impact of Thermal-Humidity Index on Milk Yield under Conditions of Different Dairy Management. *J Anim Feed Sci* 16: 329-344. doi: 10.22358/jafs/66755/2007
- Dirksen, G., Liebich, H., Mayer, H. (1985). Adaptive Changes of the Ruminant Mucosa and Functional and Clinical Significance. *Bovine Practice* 20: 116-120. doi: 10.21423/bovine-vol1985no20p116-120
- Dohoo, I. R., Martin, S. W. (1984). Subclinical Ketosis: Prevalence and Associations with Production and Disease. *Can J Comp Med* 48: 1-5
- Doreau, M., Chilliard, Y., Rulquin, H., Demeyer, D. I. (1999). Manipulation of Milk Fat in Dairy Cows. In *Recent advances in animal nutrition* (ed. PC Garnsworthy and J Wiseman), pp. 81-109. Nottingham University Press, Nottingham, UK.
- Duffield, T. F., Kelton, D. F., Leslie, K. E., Lissemore, K., Lumsden, J. H. (1997). Use of Test Day Milk Fat and Milk Protein to Predict Subclinical Ketosis in Ontario Dairy Cattle. *Can Vet J* 38: 713 - 718.
- Duffield, T.F. (2004). Monitoring strategies for metabolic disease in transition dairy cows. *World Association for Buiatrics. Proceedings of World Buiatrics Congress, Québec, Canada, July 11-16, 2004.*
- Eicher, R. (2004). Evaluation of the Metabolic and Nutritional Situation in Dairy Herds: Diagnostic Use of Milk Components. *World Association for Buiatrics. Proceedings of World Buiatrics Congress, Québec, Canada, July 11-16, 2004.*
- Eicher, R., Bouchard, E., Bigras-Poulin, M. (1999). Factors affecting milk urea nitrogen and protein concentrations in Quebec dairy cows. *Prev Vet Med* 39: 53 - 63.
- Gillund, P., Reksen, O., Gröhn, Y. T., Karlberg, K. (2001). Body Condition Related to Ketosis and Reproductive Performance in Norwegian Dairy Cows. *J Dairy Sci* 84: 1390-1396. doi: 10.3168/jds.S0022-0302(01)70170-1
- Gravert, H. O. (1991). Indicators for Assessment of Energy Balance in High-Yielding Cows. *Monatsh Veterinarmed* 46: 536-537
- Haas, D., Hofirek, B. (2004). The Diagnostic Importance of Milk Components for a Human and Cows' Health, CUA Prague, *Proceedings of Contributions to Milk Day: 26-29.*
- Hargrove, G. L., Gilbert, G. R. (1984). Differences in Morning and Evening Sample Milkings and Adjustment to Daily Weights and Percents. *J Dairy Sci* 67: 194-200. doi: 10.3168/jds.S0022-0302(84)81284-9
- International Committee for Animal Recording, ICAR (2017). Section 2 - Guidelines for Dairy Cattle Milk Recording.
- Keown, J. F., Everett, R. W., Empet, N. B., Wadell, L. H. (1986). Lactation Curves. *J Dairy Sci* 69: 769-781.
- Klei, L. R., Lynch, J. M., Barbano, D. M., Oltenacu, P. A., Lednor, A. J., Bandler, D. K. (1997). Influence of Milking Three Times on Milk Quality. *J Dairy Sci* 80: 427-436
- LeBlanc, S. (2010). Assessing the Association of the Level of Milk Production with Reproductive Performance in Dairy Cattle. *J Reprod Develop* 56: S1 - S7. doi: 10.1262/jrd.1056S01
- O'Grady, L., Doherty, M. L., Mulligan, F. J. (2008). Subacute Ruminant Acidosis (SARA) in Grazing Irish Dairy Cows. *Vet J* 176 (1): 44-49. doi: 10.1016/j.tvjl.2007.12.017
- Palmquist, D. L., Beaulieu, A. D., Barbano, D. M. (1993). Feed and Animal Factors Influencing Milk Fat Composition. *J Dairy Sci* 76: 1753 - 1771. doi: 10.3168/jds.S0022-0302(93)77508-6
- Rajala, P. J., Gröhn, Y. T. (1998). Effects of Dystocia, Retained Placenta, and Metritis on Milk Yield in Dairy Cows. *J Dairy Sci* 81: 3172-3181
- Rajala-Schultz, P. J., Grohn, Y. T., McCulloch, C. E. (1999). Effects of Milk Fever, Ketosis and Lameness on Milk Yield in Dairy Cows. *J Dairy Sci* 82: 288-294. doi: 10.3168/jds.S0022-0302(99)75235-5
- SAS User's Guide (2019). Version 9.4 Edition. SAS Institute Inc. Cary, NC.
- Suthar, V. S., Canelas-Raposo, J., Deniz, A., Heuwieler, W. (2013). Prevalence of Subclinical Ketosis and Relationships with Postpartum Diseases in European Dairy Cows. *J Dairy Sci* 96: 2925-2938. doi: 10.3168/jds.2012-6035
- Weiß, D., Hilger, M., Meyer, H. H. D., Bruckmaier, R. M. (2002). Variable Milking Intervals and Milk Composition. *Milchwissenschaft*, 57(5): 246 - 249