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Original Scientific Article

ASSOCIATIONS BETWEEN THE FAT TO PROTEIN RATION IN MILK, HEALTH STATUS AND REPRODUCTIVE PERFORMANCE IN DAIRY CATTLE

Ožbalt Podpečan[1]*, Janko Mrkun[2], Petra Zrimšek[2]

[1]Savinian Veterinary Policlinic, Celjska c. 3/a, 3310 Žalec, [2]Clinic for Reproduction and Horses, Veterinary Faculty, Gerbičeva 60, 1000 Ljubljana, Slovenia

*Corresponding author, E-mail: ozbalt.podpecan@gmail.com

Summary: The aim of the present study was to evaluate pregnancy rates of 232 dairy cows in relation to fat to protein ratio (FPR) in milk, using survival analysis. Pregnancy rates of cows inseminated within 90 and 120 days postpartum in a group of clinically healthy cows were 38 and 68 %, respectively. Lower pregnancy rates are observed in groups of cows with ketosis and reproductive disorders, 44 and 28 % for pregnancy rate within 120 days. The highest correlation between FPR and calving to conception interval (CC) was observed between 30 and 60 days postpartum ($r = 0.411$, $P < 0.001$). Diagnostic evaluation of FPR using ROC (receiver operating characteristics) analysis showed that FPR at 1.37 discriminates cows with CC below and above 120 days with an accuracy of 71 %. Survival curves for the subgroups of animals with FPR below or above 1.37 differed significantly in the case of clinically healthy cows, where CC in subgroups were 87 ± 28 and 122 ± 42 days, respectively. Although survival curves for subgroups for cows with diseases did not differ significantly we observed longer CC in all subgroups with FPR > 1.37 than in subgroups with FPR < 1.37. In all groups pregnancy rates within 90 and 120 days were lower in subgroups with FPR > 1.37 than in subgroups with FPR < 1.37. Therefore, FPR can be used by bovine practitioners to predict fertility problems in dairy herds.

Key words: dairy cows; fat to protein ratio in milk; calving to conception interval; ROC analysis; survival analysis

Introduction

Increased fat mobilization in a period of negative energy balance (NEB) coupled with decrease in dry matter and energy intake is shown in higher milk fat concentration and lower milk protein concentration in the postpartum period of dairy cows (1). In the last fifteen years a correlation between energy balance in the early postpartum period and milk composition has been reported, using different parameters such as fat to protein ratio (FPR), protein to fat ratio, fat/lactose quotient, milk yield and milk protein concentration (2, 3, 4, 5). Buttchereit *et al.* (6) showed FPR to be a suitable indicator of the energy status in postpartum dairy cows. FPR indicates low energy balance more reliably than body condition score (BCS) (7). A FPR threshold of 1.4 during the first month of lactation is commonly used by veterinary practitioners as a marker of NEB (8). FPR is also used as a diagnostic tool to estimate

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some metabolic disorders such as subclinical and clinical ketosis (1, 7).

Geishauser *et al.* (9) found that FPR in the first test milk could be useful as a predictor for subsequent abomasal displacement. Clinical mastitis appears most commonly in the first 30 days postpartum (10). Increased incidence of mastitis during early lactation or at peak production could result from a severe NEB (11, 12) severity of induced *E coli* mastitis has been related to some blood metabolite concentrations characteristic of NEB (13). Animals exposed to severe NEB suffer from impaired reproductive performance (14, 15, 16) which can be shown as absence of oestrus signs, delayed onset of cyclicity, failure to conceive at 1st artificial insemination (AI) and finally in prolonged calving to conception interval. Moreover, FPR was recently assessed as a predictor of calving to conception interval (CC) of individual cows, using fixed thresholds (17).

The aim of the present study was to evaluate practical use of milk and medical data records in association with reproductive performance in high yielding dairy cows. We evaluated pregnancy rates of dairy cows in relation to FPR in milk, using survival analysis. First, a complete receiver operating characteristics (ROC) analysis, was performed to provide an index of accuracy by demonstrating the limits of the test's ability to discriminate between healthy cows pregnant at day 90 (or day 120) after calving or not (18). Groups of clinically healthy cows and cows with ketosis, clinical mastitis or fertility problems were compared using Kaplan-Meier survival curves.

Material and methods

Animals and data

Records of 232 high yielding dairy cows (Holstein-Friesian), BVD and IBR-IPV free, in a period from April 2009 to June 2010 formed a basis for the study. Cows were kept in a free-stall barn system. The basic ration was composed of hay, grass and maize silage. According to the milk yield, protein concentrate (19 % digestible raw protein), roughly crushed maize grains and vitamin-mineral mixture were supplemented by a computerised feeding system. All cows could access basic ration and water *ad libitum* during the whole year in the stall. The voluntary waiting period of the herd was 80 days. They were inseminated by a well trained inseminator. Reproduction parameters, calving to 1st service (CFS), 1st service to conception (FSC) and calving to conception (CC) intervals were derived from farm records. All animals showing clinical signs of disease were examined and treated by bovine practitioners using standardized protocols. Treatment data were recorded for every animal individually. Animals were divided into 5 groups according to occurrence of different diseases in the first 90 days post partum (p.p.) (Table 1): Group 1: clinically healthy cows; Group 2: cows with clinical ketosis (diagnose based on clinical signs and milk tests with sodium nitoprosside); Group 3: cows with clinical mastitis; Group 4: cows with fertility problems (included gynaecological disorders such as retained placenta, puerperal metritis, cystic ovarian disease and endometritis); Group 5: other cows.

Milk sampling and analysis

Daily milk yield was measured and milk samples were collected at regular test days performed in a 30 day intervals in the post partum period at three stages: stage 1, 0 – 30 days post partum; stage 2, 30 – 60 days post partum; stage 3, 60 – 90 days post partum. Samples were conserved with sodium azide and sent, at outdoor temperature, to a dairy research laboratory. Protein and fat were analysed in milk samples using Fourier Transform Infrared Spectroscopy (CombiFoss 6000).

Statistical analysis

Groups of cows were compared with respect to CFS, FSC, CC, milk yield and FPR at stages 1, 2 and 3, using One way analysis of variance in the case of normal distribution of the data or Kruskal-Wallis one way analysis of variance on ranks in the case of non-normal distribution of data. When a significant difference among the groups was found, further pair-wise multiple comparisons were performed using the Holm-Sidak method (normal data distribution) or Dunn's method (non-normal data distribution).

FPRs in stages 1, 2 and 3 were compared within each group using One way repeated measures analysis of variance or Friedman repeated measures analysis of variance on ranks, according to the normal or non-normal distribution of the data, followed by pair-wise multiple comparison using the Holm-Sidak method and Tukey's test, respectively.

ROC analysis

First, a correlation between milk data records and reproductive parameters of clinically healthy cows was determined using Spearman rank correlation coefficient. 115 healthy cows were included in diagnostic evaluation of FPR. Receiver operating characteristics (ROC) analysis was used to evaluate FPR in stage 2 (30-60 days postpartum) to discriminate between cows with CCs above and below 120 days. Stage 2 was selected on the basis of the highest correlation between FPR and CC in stage 2, and the criterion value of 120 days was based on reproductive characteristics found in Slovenian dairy herds.

Sensitivity (proportion of cows with FPR below the cut-off value in cows with CC below 120 days) and specificity (proportion of cows with FPR above the cut-off value in cows with CC above 120 days) were calculated for all possible cut-off values (Analyse-it, General + Clinical Laboratory statistics, version 1.71). ROC curves, displaying true positive rate (sensitivity) against false positive rate (1-specificity) for the complete range of cut-off points were used to determine the cut off value that minimizes the sum of false negative and false positive results (19). This optimal cut off value is found closest to the upper left hand corner of the ROC curve. The selection was supported with the plot of sensitivity and specificity as a function of cut-off value, which provides a useful visualisation in selecting optimal cut-off values on the basis of the best balance of sensitivity and specificity (20). Area under the curve (AUC) provides an index of accuracy by demonstrating the limits of an FPR's ability to discriminate between cows with different CC (18).

Survival analysis

Differences in proportion of non-pregnant cows among groups of healthy cows, cows with ketosis, cows with reproductive disorders and cows with clinical mastitis were measured by Kaplan-Meier survival analysis (21). Kaplan-Meier survival curves were constructed and compared using Log Rank test following pair-wise multiple comparison using the Holm-Sidak method in the case of significant difference among the curves. Criteria of censored animals included cows that did not conceive until day 300 post partum and animals culled during the study. For each cow's group, survival curves for subgroups, divided by FPR at 1.37, were constructed and compared using Log rank test. Pregnancy rates were calculated as numbers of cows conceived within 90 and 120 days, divided by the total number of cows in a group (22).

Results

Milk data in lactating cows

Distribution of diseases and groups of cows are presented in Table 1. Reproductive performance and milk data in lactating cows are summarized in Table 2.

Cows that were culled for various reasons during the study are recorded in Table 3. Mean milk yield over all cows in the first 100 days was 3069 ± 716 kg (average ± SD) and did not differ between the groups of clinically healthy cows and cows with diseases ($P>0.05$). No significant difference in FPR between the groups is observed in stage 1 ($P>0.05$), whereas FPR in stages 2 and 3 differed among the groups ($P < 0.001$ and $P = 0.003$, respectively). FPR within the group of cows suffering from ketosis did not differ significantly between stage 1 and stage 3 FPR ($P > 0.05$), whereas in clinically healthy cows and cows with reproductive disorders or clinical mastitis FPR decreased from stage 1 to stage 3 with a difference close to statistical significance ($P=0.067$, $P=0.046$ and $P=0.048$ respectively).

Reproductive performance and comparison of CC among the groups

The CFS interval was calculated as 87 ± 32 days; a significant difference was observed among the two groups ($P=0.021$), corresponding only to the difference between cows with clinical mastitis and cows with fertility problems ($P<0.05$) (Table 2). The average CC in the lactating cows was 113 ± 49. A significant difference was observed among the groups according to the calving to conception (CC) interval ($P < 0.001$).

Table 1: Distribution of diseases and structure of groups of cows

Groups (N)	Subgroups (N)	disease				
		ketosis	EM	RP	Clinical mastitis	DA
Group 1: Clinically healthy cows (n=130)	/	–	–	–	–	–
Group 2: Cows with clinical ketosis (n=32)	15	+	–	–	–	–
	7	+	–	–	+	
	8	+	–	–	–	+
	1	+	+	+		–
	1	+	+	+	+	–
Group 3: Cows with clinical mastitis (n=32)	/	–	–	–	+	–
Group 4: Cows with fertility problems (n=36)	15	–	+	–	–	–
	11	–	–	+	–	–
	5	–	+	+	–	–
	2	–	+	_	+	–
	1	–	–	+	+	–
	1	–	+	+	+	–
	1	–		+		+
Group 5: Other cows (n=2)	/	–	–	–	–	+

Legend: EM: endometritis; RP: retained placenta; DA: abomasal displacement

Table 2: Reproductive performance and milk data records of dairy cows in different groups according to disease

Group	1	2	3	4	Total
	Healthy cows	Cows with clinical ketosis	Cows with clinical mastitis	Cows with fertility problems	
N	130	32	32	36	230
CC (days)	103 ± 39	132 ± 57	98 ± 46	153 ± 51	113 ± 49
FSC (days)	18 ± 29	50 ± 55	17 ± 33	53 ± 46	28 ± 40
CFS (days)	85 ± 31	85 ± 38	80 ± 27	100 ± 32	87 ± 32
Milk yield (kg)	3069 ± 716	3267 ± 693	3211 ± 797	2948 ± 714	3096 ± 726
FPR – stage 1	1.39 ± 0.31	1.48 ± 0.34	1.41 ± 0.27	1.45 ± 0.27	1.42 ±0.30
FPR – stage 2	1.35 ± 0.25	1.59 ± 0.30	1.37 ± 0.23	1.37 ± 0.25	1.39 ± 0.27
FPR – stage 3	1.30 ± 0.25	1.46 ± 0.19	1.29 ± 0.17	1.33 ± 0.18	1.32 ± 0.23

Legend:
CC: calving to conception interval;
FSC: 1st service to conception interval;
CFS: calving to 1st service interval;
FPR – stage 1: fat to protein ratio in milk between 0 and 30 days post partum;
FPR – stage 2: fat to protein ratio in milk between 30 and 60 days post partum;
FPR – stage 3: fat to protein ratio in milk between 60 and 90 days post partum.
Values are expressed as mean ± SD.

CC interval of clinically healthy cows and cows with clinical mastitis was comparable. Conversely, cows with ketosis and fertility problems required longer times to conceive (Table 2). Cows with clinical ketosis and fertility problems had a significant longer CC interval than those in the group of clinically healthy cows (P < 0.05). Significantly lower CC is observed in clinically healthy cows and cows with clinical mastitis than in cows with fertility problems (P<0.05) (Table 2).

The proportion of non-pregnant cows in different groups was evaluated using survival analysis (Fig. 1). Significant differences were observed among Kaplan-Meier survival curves for all groups (P=0.003). Pair-wise multiple comparisons showed significant differences between survival curves of clinically healthy cows and cows with fertility problems and between cows with clinical mastitis and cows with fertility problems (P<0.05). Survival curves for clinically healthy cows and cows with ketosis differ, but not significantly (P=0.0794). Pregnancy rates within 90 and 120 days postpartum in a group of clinically healthy cows were 38 and 68 %, respectively. Lower pregnancy rates within 120 days were observed in the group of cows suffering from ketosis and in the group of cows with reproductive disorders (44% and 28% respectively), whereas 69 % of cows with clinical mastitis conceived by day 120 postpartum.

Diagnostic evaluation of FPR

The diagnostic ability of FPR was evaluated in the group of clinically healthy cows.

First, Spearman rank correlation coefficients were calculated between reproduction parameters, milk yield and FPR in all stages. Milk yield did not correlate with any of reproductive parameters, but significant correlations were observed with FPR in stage 1 (r=0.190, P=0.0325), stage 2 (r=0.279, P=0.0015) and stage 3 (r=0.200, P=0.0243).

CFS correlated with FPR in stage 1 (r=0.233, P=0.0108) and stage 2 (r=0.246, P=0.0072). No correlation was observed between CFS and FPR in stage 3 or between SP and FPR in stage 1 (P>0.05). Low correlations between FSC and FPR in stage 2 and stage 3 were observed (r=0.290 P=0.0017; r=0.254, P=0.0062); similar correlations were found between CC and FPR in stages 1 and 3 (r=0.274, P=0.0031; r=0.2565, P=0.0042). The strongest correlation between CC and FPR was observed in stage 2 (r=0.411, P<0.001). FPR in stage 2 was therefore further evaluated diagnostically.

Selection of optimal cut-off values of FPR

Area under the ROC curve (AUC) for the criterion value of 120 days post partum (AUC = 0.726; P<0.0001) indicates that FPR is valuable in distinguishing cows with different CC (Fig. 2).

The best balance between sensitivity and specificity is observed, with 71 % accuracy, at an optimal cut-off point of FPR = 1.37, corresponding to a sensitivity of 74 % and specificity of 68 %. 90 % sensitivity was found for the cut-off at 1.22, whereas cut-off at 1.53 provides over 85 % specificity of FPR (Fig. 3).

Comparison of CC according to FPR in healthy cows and cows with ketosis, fertility problems or clinical mastitis

Survival curves for subgroups, divided by FPR at 1.37, differed only for clinically healthy cows (P=0.007; Fig. 4A), showing CC intervals in subgroups with FPR below and above 1.37 were 87 ± 28 and 122 ± 42 days, respectively. Pregnancy rates within 90 or 120 days were higher in a subgroup of FPR < 1.37 than in a subgroup with FPR > 1.37. Subgroups of FPR < 1.37 has a pregnancy rate of 52 % at 90 days and 79 % at 120 days, whereas a subgroup of FPR > 1.37 has a pregnancy rate of 19 % at 90 days and 53 % at 120 days.

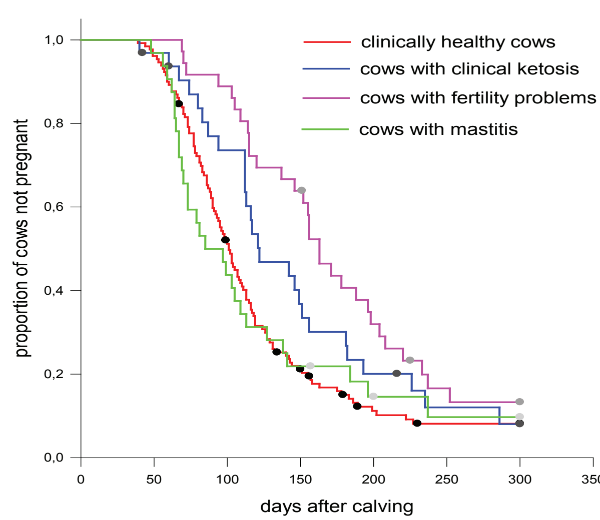

Figure 1: Kaplan-Meier survival analysis for the proportion of lactating dairy cows non pregnant, according to disease status

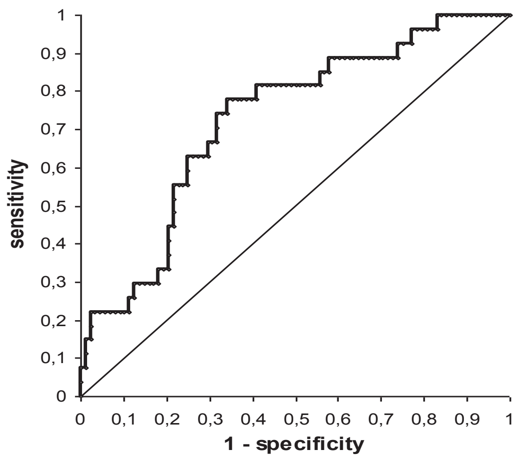

Figure 2: ROC curve of FPR for identifying healthy cows with pre-selected minimum of postpartum period at 120 days
Characteristics for the ROC curve are as follows: Area under the curve (AUC): 0.726, P < 0.0001

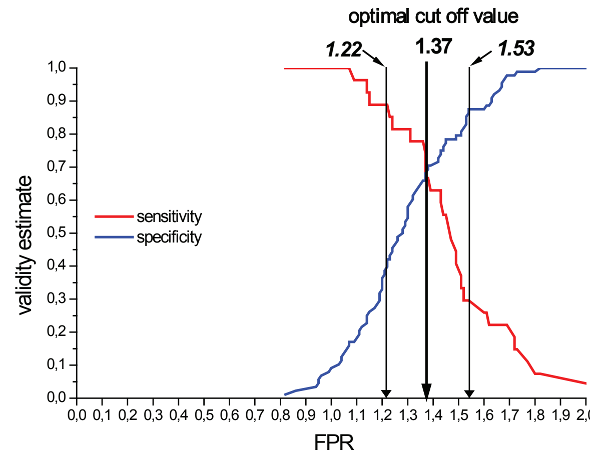

Figure 3: Plot of diagnostic parameters of FPR according to pregnancy status at 120 days post partum for the selection of optimal cut-off values

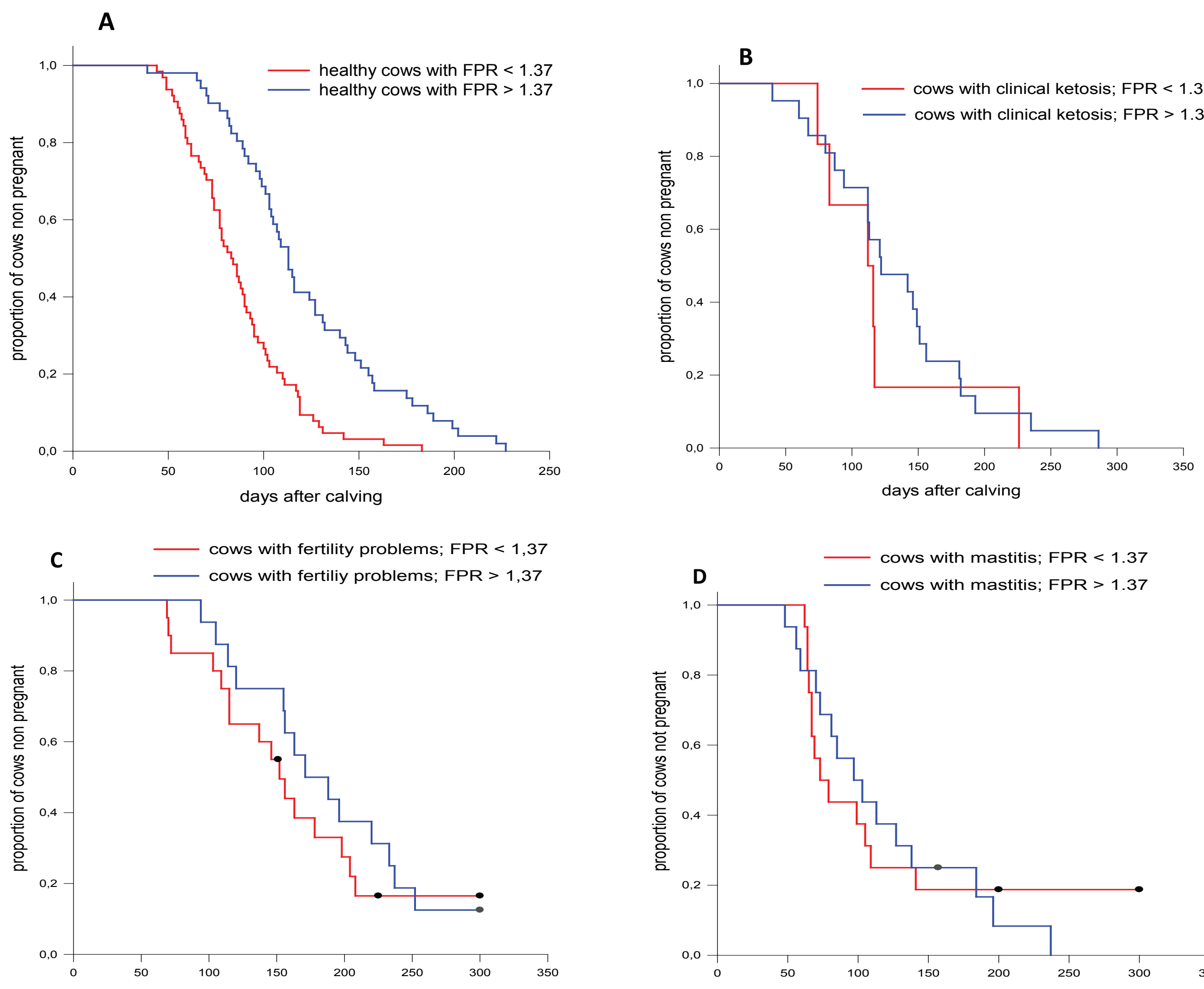

Figure 4: Kaplan-Meier survival analysis for the proportion of cows clinically healthy cows (A), cows with clinical ketosis (B), cows with fertility problems (C) and cows with clinical mastitis (D) non pregnant, according to the cut off value of FPR at 1.37

Table 3: Reproductive performance of dairy cows in different groups according to the diseases

	Groups			
	1	2	3	4
	Healthy cows	Cows with clinical ketosis	Cows with clinical mastitis	Cows with fertility problems
total group				
N	130	32	32	36
cows censored (%)	12	16	13	17
mean CC (days)	103 ± 39	132 ± 57	98 ± 46	153 ± 51
pregnancy rate at 90 days (%)	38	22	50	8
pregnancy rate at 120 days (%)	68	44	69	28
subgroup; FPR < 1.37				
N	73	6	16	20
cows censored (%)	12	0	19	20
mean CC (days)	87 ± 28	121 ± 54	82 ± 24	136 ± 48
pregnancy rate at 90 days (%)	52	33	56	15
pregnancy rate at 120 days (%)	79	83	75	35
subgroup; FPR > 1.37				
N	57	26	16	16
cows censored (%)	11	19	6	13
mean CC (days)	122 ± 42	135 ± 59	111 ± 56	171 ± 50
pregnancy rate at 90 days (%)	19	19	44	0
pregnancy rate at 120 days (%)	53	35	63	19

Legend: FPR: fat to protein ratio; CC: calving to conception interval

Survival curves for subgroups for cows with diseases did not differ significantly (P>0.05) (Fig. 4: B, C, D), although longer CC in all subgroups with FPR > 1.37 than in subgroups with FPR < 1.37 were observed (Table 3).

Discussion

The aim of this study was to evaluate fat to protein ratio (FPR) in milk for the first three months of lactation and find a threshold which is most appropriate to predict reproductive performance of dairy cows. Results of many studies in the past decade have shown significant correlations between body condition score, energy balance, mobilization of body reserves, blood metabolite concentrations, milk traits and different production and reproductive disorders (3, 4, 6, 15, 23).

Our results demonstrated poor reproductive performance in cows with ketosis and reproductive disorders. They had significantly longer CFS and CC intervals than healthy cows and cows with clinical mastitis. Pregnancy rates were calculated as the proportion of cows conceived within 90 or 120 days (22). A lower proportion of pregnant cows were observed in groups with ketosis and fertility problems, while the proportion of cows with clinical mastitis conceiving up to day 120 postpartum is similar to that for healthy ones. Prolonged CC intervals in cows with clinical ketosis were probably related to exposure to NEB, which can be caused by either high milk yield or displaced abomasum. Cows in NEB are subject to increased risk of clinical mastitis (12). The group of mastitic cows in the first three months post partum, contrary to our expectation, did not differ significantly from the clinically healthy one in FPR, CFS, FSC or CC intervals. Reasons may be found in rapid response to treatment, better management and care of treated animals, but also in the statistically smaller number of mastitic cows included in the study compared to healthy ones.

Buttchereit *et al.* (6) showed FPR as a suitable indicator of the energy status of dairy cows during the most critical period for their metabolic constitution. FPR is also used as a diagnostic tool for estimating nutritional imbalance and some metabolic disorders such as subclinical or clinical ketosis (1, 7). Therefore in our study FPR was evaluated by correlation with reproduction parameters and its association with certain diseases.

The strongest correlations were observed between FPR and milk yield, CFS, FSC and CC intervals in stage 2. In our previous study (17) the strongest correlations were observed in stage 3, but clinically healthy and diseased animals were not differentiated. From the diagnostic point of view, the strongest correlations in stage 2 enable us to predict animals at risk before the voluntary waiting period ends. Although FPRs were calculated for only three lactation stages, the tendency to decrease is evident in all groups and comparable to the results of Buttchereit *et al.* (6).

ROC analysis showed that the optimal cut-off value at 1.37 in our study allowed discrimination between cows with CC above 120 days and cows with CC below 120 days with an accuracy of 71 %. High sensitivity of FPR was found at a cut-off value of 1.22, which enabled around 90 % correct identification of cows with CC lower than 120 days. On the other hand, cows with FPR more than 1.53 were over 85 % correctly identified as cows with CC above 120 days.

It appears that the results of optimal cut-off values of FPR differ among studies due to the numbers of animals or herds included in the studies, their general nutrition status, lactation time frame and the statistical methods used (1, 7, 8, 17, 24). Nevertheless it is clear that cows with FPR values above 1.4 in early postpartum are at high risk of NEB-dependent disorders such as ketosis, displaced abomasum and fertility problems (1).

Survival curves calculated for subgroups of animals with FPR below and above 1.37 differed significantly only in the case of clinically healthy cows. According to survival curves, pregnancy rates within 90 and 120 days were higher in the subgroup of FPR < 1.37 than in the subgroup with FPR > 1.37 (19 and 53 %). This indicates that even some clinically healthy cows (FPR>1.37) are exposed to intensive NEB.

Although survival curves for subgroups for cows with diseases did not differ significantly (P>0.05), pregnancy rates within 90 and 120 days in all groups were lower in subgroups with FPR > 1.37 than in subgroups with FPR < 1.37. The reason that no significant difference is observed between subgroups of cows with ketosis could be the small number of cows with FPR < 1.37. However, longer CC intervals were observed in all subgroups with FPR > 1.37 than in subgroups with FPR < 1.37. It can thus be concluded that FPR is strongly associated with the pregnancy rate in NEB-related diseases such as clinical ketosis, whereas in cows suffering from other diseases the increased FPR contributes to prolonged CC, but the effect is not as strong as in cows with clinical ketosis.

In our previous study it was shown that FPR in milk could be an indicator of the ability of a cow to adapt to the demands of milk production and reproduction in the post partum period, resulting in prolongation of the latter (17). It is also in accordance with the fact that the rate of mobilization of body reserves is directly related to the postpartum interval to first ovulation and to lower conception rate (25).

The present study clearly demonstrates that milk data records (e.g. FPR) and medical data can be used by bovine practitioners to analyse and to predict some fertility problems, mainly failure to conception and address metabolic disorders such as ketosis more quickly. The results presented here offer a simple and useful tool for assessing energy balance in a dairy herd in order to predict reproductive performance.

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References

1. Eicher R. Evaluation of the metabolic and nutritional situation in dairy herds diagnostic use of milk components. Med Vet Quebec 2004; 34 (1): 36–8.

2. Čejna V, Chladek G. The importance of monitoring changes in milk fat to protein ratio in Holstein cows during lactation. J Centr Eur Agric 2005; 4: 539–46.

3. Heuer C, Van Straalen CWM, Schukken YH, Dirkzwager A, Noordhuizen JPMT. Prediction of energy balance in a high yielding dairy herd in early lactation: model development and precision. Livest Prod Sci 2000; 65: 91–105.

4. Reist M, Erdin D, von Euw D, et al. Estimation of energy balance at the individual and herd level using blood and milk traits in high-yielding dairy cows. J Dairy Sci 2002; 85: 3314–7.

5. Steen A, Osteras O, Gronstol H. Evaluation of bulk milk analyses of aceton, urea and the fat-lactose-quotient as diagnostic aids in preventive veterinary medicine. J Vet Med 1996; 43: 261–9.

6. Buttchereit N, Stamer E, Junge W, Thaller G. Evaluation of five curve models fitted for fat: protein ratio of milk and daily energy balance. J Dairy Sci 2010; 93: 1702–12.

7. Heuer C, Schukken YH, Dobbelaar P. Postpartum body condition score and results from the first test milk as predictors of disease, fertility, yield and culling in commercial dairy herds. J Dairy Sci 1999; 82: 295–304.

8. Cook NB, Oetzel GR, Nordlund KV. Modern techniques for monitoring high producing dairy cows 1. Principles of herd-level diagnoses. In Practe 2006; 28: 510–5.

9. Geishauser T, Leslie K, Duffield T, Edge V. Fat/protein ratio in first DHI test milk as test for displaced abomasums in dairy cows. J Vet Med 1997; 44: 265–70.

10. Barkema HW, Schukken YH, Lam TJ et al. Incidence of clinical mastitis in dairy herds grouped in three categories by bulk milk somatic cell counts. J Dairy Sci 1998; 8(1): 411–9.

11. Leslie KE, Duffield TF, Sandals D, Robinson E. The influence of negative energy balance on udder health. In: Proceedings of the 2nd International Symposium on Mastitis and Milk Quality. Vancouver, Canada: 2001:19–9.

12. Suriyasathaporn W, Heuer C, Noordhuizen-Stassen EN, Schukken YH. Hyperketonemia and the impairment of udder defence: a review. Vet Res 2000; 31: 397–412.

13. Van Werven T. The role of leucocytes in bovine *Echerichia coli* mastitis: Ph.D. thesis. Utrecht: University of Utrecht, The Netherlands, 1999: 45–51.

14. Loefler SH, de Vries MJ. Schukken YH. The effects of time of disease occurrence, milk yield and body condition on fertility of dairy cows. J Dairy Sci 1999; 82: 2589–604.

15. Opsomer G, Grohn YT, Hertl J, Coryn M, Deluyker H, de Kruif A. Risk factors for postpartum ovarian dysfunction in high producing dairy cows in Belgium: a field study. Theriogenology 2000; 53: 841–57.

16. Podpečan O, Kosec M, Cestnik V, Čebulj-Kadunc N, Mrkun J. Impact of negative energy balance on production and fertility in Slovenian brown-breed dairy cows. Acta Vet Beogr 2007; 57 (1): 69–79.

17. Podpečan O, Mrkun J, Zrimšek P. Diagnostic evaluation of fat to protein ratio in prolonged calving to conception interval using receiver operating characteristic analyses. Reprod Domest Anim 2008; 43: 249–54.

18. Zwieg MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem 1993; 39/40: 561–77.

19. Greiner M, Pfeiffer D, Smith RD. Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests. Prev Vet Med 2000; 54: 23–41.

20. Weiss HL, Niwas S, Grizzle WE, Piyathilake C. Receiver operating characteristic (ROC) to determine cut-off points of biomarkers in lung cancer patients. Dis Markers 2003-2004; 19(6): 273–8.

21. Petrie A, Watson P. Statistics for veterinary and animal science. Oxford: Blackwell Science, 1999: 168–81.

22. Yusuf M, Nakao T, Ranasinghe RMS, et al. Reproductive performance of repeat breeders in dairy herds. Theriogenology 2010; 73: 1220–9.

23. Vanholder T, Leroy J, Dewulf J, et al. Hormonal and metabolic profiles of high yielding dairy cows prior to ovarian cyst formation on first ovulation postpartum. Reprod Domest Anim 2005; 40: 460–9.

24. Duffield TF, Kelton DF, Leslie KE, Lissemore K, Lumsden JH. Use of test day milk fat and milk protein to predict subclinical ketosis in Ontario dairy cattle. Can Vet J 1997; 38: 713–8.

25. Butler WR, Smith RD. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. J Dairy Sci 1989; 72: 767–83.

POVEZAVA MED RAZMERJEM MAŠČOB IN BELJAKOVIN V MLEKU, ZDRAVSTVENIM STATUSOM IN REPRODUKCIJSKO SPOSOBNOSTJO KRAV MOLZNIC

O. Podpečan, J. Mrkun, P. Zrimšek

Povzetek: V raziskavi smo z analizo preživetja ovrednotili deleže brejosti 232 krav mlečne pasme v povezavi z razmerjem med maščobami in proteini (koeficient M/B) v mleku. Delež brejih krav v obdobju 90 oziroma 120 dni po porodu je bil 38 oziroma 68 %. Pri kravah s ketozo in reprodukcijskimi problemi smo po 120 dneh po porodu ugotovili nižji delež brejih krav in sicer 44 oziroma 28 %. Najvišjo korelacijo med poporodnim premorom (PP) in razmerjem M/B smo ugotovili v obdobju med 30 in 60 dnevi po porodu ($r = 0{,}411$; $P < 0{,}001$). Na podlagi diagnostičnega vrednotenja razmerja M/B z uporabo krivulj ROC (receiver operating characteristics) smo ugotovili, da razmerje M/B pri 1,37 z 71 % zanesljivostjo loči krave s poporodnim premorom pod 120 dnevi in nad tem obdobjem. Krivulje preživetja za podskupine krav z razmerjem M/B nad 1,37 in pod 1,37 so se statistično značilno razlikovale pri zdravih kravah, kjer je povprečni poporodni premor znašal 87 ± 28 dni za krave z razmerjem M/B pod 1,37 in 122 ± 42 dni za krave z razmerjem nad 1,37. Krivulje preživetja za omenjene podskupine se pri kravah z različnimi boleznimi niso statistično značilno razlikovale, čeprav smo pri vseh skupinah opazili daljši poporodni premor pri kravah, ki so imele razmerje M/B višje od 1,37. Delež brejih krav v obdobju med 90 in 120 dnevi po porodu je bil pri vseh skupinah višji v podskupini z razmerjem M/B pod 1,37 v primerjavi s kravami, kjer je bilo razmerje M/B višje od 1,37. Rezultati raziskave nam dokazujejo, da je razmerje M/B lahko v pomoč veterinarjem praktikom pri predvidevanju težav s plodnostjo v čredah krav mlečnih pasem.

Ključne besede: krave mlečnih pasem; razmerje med maščobami in proteini v mleku; poporodni premor; analiza ROC; analiza preživetja