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CROSS VALIDATION OF CUT–OFF LIMIT ESTIMATIONS FOR MILK INDICATORS OF SUBCLINICAL KETOSIS

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Abstract

HANUŠ OTO, ROUBAL PETR, SAMKOVÁ EVA, FALTA DANIEL, CHLÁDEK GUSTAV, VYLETĚLOVÁ KLIMEŠOVÁ MARCELA: *Cross validation of cut–off limit estimations for milk indicators of subclinical ketosis.* Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 2013, LXI, No. 6, pp. 1661–1667

Real time analyses of main milk components are attended in milking parlours today. Breeders can know milk composition regularly. Energy (ketosis) milk quotients such as fat/crude protein (F/CP) and fat/lactose (F/L) could be essential for ketosis prevention and losses which can be linked with it. Aim was to do a reciprocal validation of reliability of milk indicators for subclinical ketosis (SK) identification. There were: dairy cows on 1st and other lactations free of clinical and superior subclinical mastitis; 3 herds of Czech Fleckvieh (CF) breed, 3 herds of Holstein (H) breed and 1 herd with CF and H; 329 individual milk samples from summer and winter season and 1st third of lactation. Average milk yield varied from 5,500 to 10,000 kg per lactation. Thresholds of F/CP and F/L were estimated ($P < 0.05$) according to SK milk acetone cut–off limit ($\geq$ 10 mg.l^{-1}). These were: for first lactation 1.27 (CF) and 1.32 (H), for other lactations 1.52 (CF) and 1.42 (H) in F/CP; for first lactation 0.84 (CF) and 0.84 (H), for other lactations 0.87 (CF) and 0.85 (H) in F/L. They were used for cross validation ($P < 0.001$) in the same order: 1.251, 1.31, 1.31 and 1.383 in F/CP; 0.831, 0.821, 0.989 and 0.852 in F/L. Validated cut–off limits are quite similar to their original values. It confirmes good reliability of original limits. The validated cut–off limits of milk indicators (F/CP and F/L) for SK in early lactation can be used at technological innovation in animal husbandry. Use of both quotients could improve the regular investigation of SK in practice.

dairy cow, individual milk sample, milk composition, energy quotient, subclinical ketosis identification

Ketosis is energy metabolism disorder (Andersson, 1984) of mammal females. It reduces milk yield, quality and reproduction performance of dairy cows (Gustafsson and Emanuelson, 1996; Gasteiner, 2000; Duffield *et al.*, 2009). It can have also fatal course (Hanuš *et al.*, 2001) for animal in clinical case. Prevention of subclinical form of ketosis (SK) is very important from practical point of view (Fig. 1), therefore its regular identification is essential. Prevention can be carried out by various forms of nutrition modifications in early lactation such as fast energy or hepatoprotective matters supplementation into cow feeding ration (Emery *et al.*, 1964; Miettinen, 1995; Gasteiner, 2003; Tedesco *et al.*, 2004; Coskun *et al.*, 2012). In this sense blood, urine and milk indicators and their thresholds and interpretations are important (Heuer *et al.*, 2000; Enjalbert *et al.*, 2001; de Roos *et al.*, 2007; Hanuš *et al.*, 2001, 2011 a, b; Van der Drift *et al.*, 2012). Real time analyses of main milk components are attended in milking parlours today. Breeders can know milk composition regularly (Mottram *et al.*, 2002). According to literature sources the milk indicators such as acetone content and fat/crude protein (F/CP) and fat/lactose (F/L) quotient could be successfuly used in the SK diagnosis (Duffield *et al.*, 1997; Geishauser and Ziebell, 1995; Steen *et al.*, 1996; Geishauser *et al.*, 1997; Heuer *et al.*, 2001; Reist *et al.*, 2002; Van Knegsel *et al.*, 2010; Siebert and Pallauf, 2010; Manzenreiter *et al.*, 2013; Hanuš *et al.*, 2013). In this sense the first third of lactation period is important (Januš and Borkowska, 2013) as Manzenreiter *et al.* (2013) found 80% of ketosis cases occurrence in 1st 50 days and 35% of the positive diagnoses in 1st 10 days of lactation.

Therefore, aim of this paper was to carry out a reciprocal validation of reliability of milk indicators such as F/CP and F/L for subclinical ketosis (SK) identification in early lactation. This can contribute also to an improvement of estimation reliability of thresholds of main milk indicators of dairy cow energy metabolism for SK detection and its prevention.

MATERIAL AND METHODS

Animals and individual milk samples

The individual milk samples were taken over three years (MSs; n = 329). The milk was sampled regularly in the summer (August and September) and winter (February and March) season. Twelve healthy (clinical and superior subclinical mastitis free) dairy cows with above average for herd milk yield from each herd (from 5,500 to 10,000 kg per lactation) were included in the one sample set (herd group). These groups represented an average number of lactations in relation to the relevant herd and important period of first third of lactation as mentioned Januš and Borkowska (2013) in terms of physiological point of view regarding cow energy metabolism balance. It means that all animals were in first third of lactation. The MSs were taken in the morning and evening milkings by flow samplers (Tru–Test) similar to the official milk recording system.

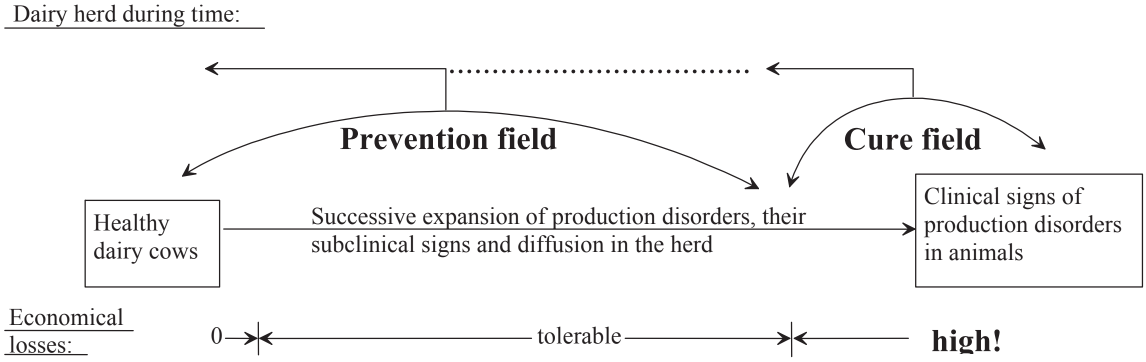
Seven dairy herds were included in the investigation and represented two cattle breeds: the Czech Fleckvieh (CF) with dual purpose, 3 herds; the Holstein (H) dairy breed, 3 herds; 1 herd with CF and H breed. Breeds were included in approximately two halves in the total set of MSs. The herds presented the whole profile of the nourishment condition scale in the Czech Republic. Different but typical varieties of dairy cow nourishment, feeding rations and systems were applied in the herds: – alfalfa silage with maize silage in the lowland areas; – clover–grass silage, grass silage with maize silage and grass pasture in the highland areas. The concentrates were fed according to milk yield and nutrition demand standards. The nutritional and feeding systems were well balanced between breeds and keeping areas although the nutrition levels may not have been exactly comparable between breeds and herds. The average milk yield varied in range from 5,500 to 10,000 kg per lactation.

Analyses of milk samples

The MSs were transported in cold state ($\leq$ 10 °C) in thermoboxes to milk laboratory. The investigated milk indicators were as follows: fat (F) content (in g/100 ml = %); crude protein (CP) content (in g/100 g = %); lactose (L) content (lactose monohydrate in g/100 g = %); milk acetone concentration (AC, in mg.l^{-1}). The F and L contents were determined using the instrument MilkoScan 133B (Foss Electric, Denmark), which was regularly calibrated according to the reference methods. The CP content was determined by the reference Kjeldahl's method. The AC content was investigated by spectrophotometry at 485 nm wavelength with the salicylaldehyde (Vojtíšek, 1986) using the Spekol 11 instrument (Carl Zeiss Jena, Germany) which was calibrated by samples with AC concentration from 1 to 20 mg.l^{-1}.

Data statistical processing

Ketosis milk quotients (F/L and F/CP) were calculated. Their SK cut–off values were determined by SK milk acetone cut–off limit ($\geq$ 10 mg.l^{-1}; according to: Emery *et al.*, 1964; Vojtíšek, 1986; Heuer *et al.*, 2001; Hanuš *et al.*, 2001, 2011 a; de Roos *et al.*, 2007) in our relevant previous papers (Hanuš *et al.*, 2011 b, 2013). The original AC values were log transformed (de Roos *et al.*, 2007) as the data were not normally distributed. In this case, the arithmetic mean is not suitable parameter for representation of these data sets in all cases. Standard statistical



1: *Pattern of prevention setting at reduction of milk yield losses of dairy cows cused by production disorders – especially by metabolic dysbalancies and milk secretion disorders* methods were used to evaluate the results (means, standard deviations and coefficient of variation). Linear and non linear regression were used for the correlations using relevant regression equations. In subclinical ketosis threshold validation of F/CP and F/L the procedure of reciprocal estimation (cross calculation) according to linear regression results was used. Data were processed using Microsoft Excel programme.

RESULTS AND DISCUSSION

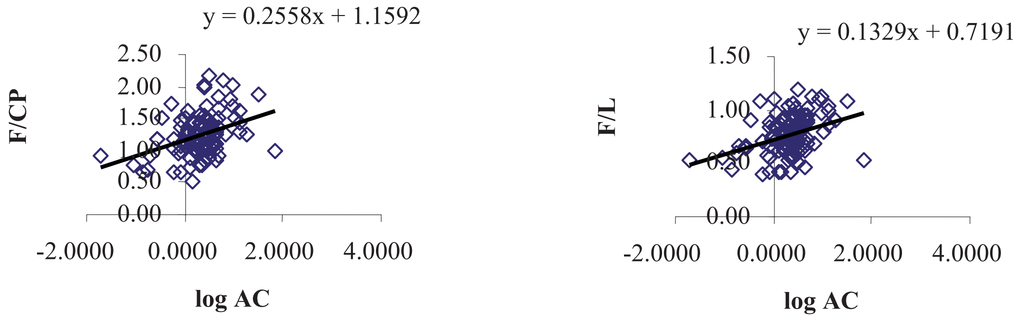
Milk acetone threshold and F/CP and F/L threshold estimation

In Tab. I, there are shown main statistical characterictics of evaluated milk indicators according to breed and number of lactation. These have quite typical values for breeds and conditions in the country. SK cut–off values for milk quotients F/L and F/CP were calculated in relation to milk acetone (≥ 10 mg.l^{-1}) as mentioned already. It was carried out using regression equations ($P < 0.05$) such as these are shown in Fig. 2 and Tab. II. As maximum through regression analysis, there is possible to

I: *Main statistical characteristics of evaluated milk indicators in first third of lactation*

Data set	n	MY x ± sd	AC x ± sd	log AC x ± sd xg	F x ± sd	CP x ± sd	L x ± sd	F/CP x ± sd	F/L x ± sd
All	329	28.65 ± 7.86	3.31 ± 5.38	0.2894 ± 0.460 1.95	3.86 ± 0.97	3.20 ± 0.33	5.01 ± 0.20	1.21 ± 0.308	0.77 ± 0.200
CF 1st lact.	30	24.75 ± 5.38	2.43 ± 3.17	0.2274 ± 0.338 1.69	3.67 ± 0.71	3.36 ± 0.23	5.12 ± 0.15	1.09 ± 0.215	0.72 ± 0.152
H 1st lact.	51	27.74 ± 7.36	3.98 ± 5.66	0.3855 ± 0.429 2.43	3.70 ± 1.02	3.12 ± 0.35	5.01 ± 0.22	1.19 ± 0.311	0.74 ± 0.215
CF other l.	133	29.22 ± 8.22	2.79 ± 3.04	0.2368 ± 0.474 1.73	4.02 ± 1.06	3.28 ± 0.33	4.99 ± 0.19	1.23 ± 0.314	0.81 ± 0.217
H other l.	115	29.42 ± 7.95	3.84 ± 7.42	0.3237 ± 0.480 2.11	3.81 ± 0.89	3.09 ± 0.31	5.00 ± 0.20	1.24 ± 0.316	0.76 ± 0.178

n = number of cases; x = arithmetic mean; sd = standard deviation; xg = geometric mean; MY = milk yield in kg/day; AC = acetone concentration in milk (mg.l^{-1}); F = milk fat content (g.100ml^{-1}, %); CP = milk crude protein content (g.100g^{-1}, %); L = lactose monohydrate (g.100g^{-1}, %); F/CP and F/L quotient.



$R^2 = 0.1512$, r = 0.3888 $R^2 = 0.1289$, r = 0.359

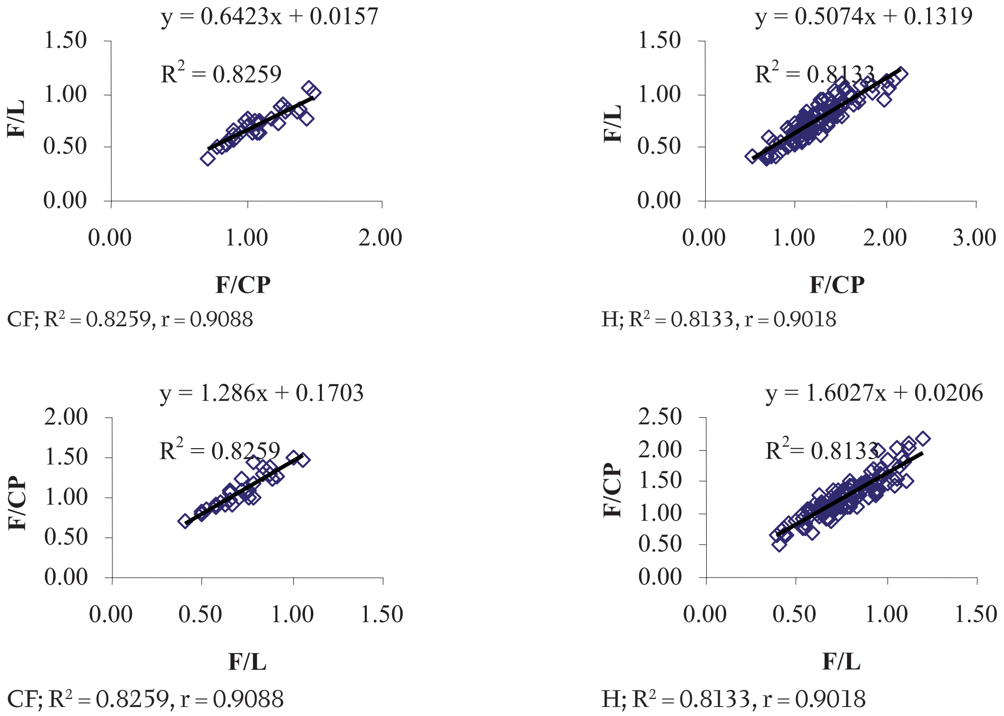
2: *The examples of regression relationships between log AC and ketosis milk quotients F/CP and F/L for Holstein breed and other (2nd and next) lactations in 1st third of lactation ($P < 0.05$; Tab. II)*

II: *The relationships between milk indicators log AC (x) and F/CP and F/L (y) in first third of lactation according to breed and number of lactation ($P < 0.05$)*

Data set	Equation form	
	log AC × F/CP	log AC × F/L
CF first lactation	$y = 0.2323x + 1.0412$	$y = 0.1534x + 0.6834$
H first lactation	$y = 0.2111x + 1.108$	$y = 0.1598x + 0.6815$
CF other lactations	$y = 0.0919x^2 + 0.1829x + 1.1571$	$y = 0.0856x + 0.7855$
H other lactations	$y = 0.2558x + 1.1592$	$y = 0.1329x + 0.7191$

III: *The reciprocal (mutual) relationships between milk indicators F/CP and F/L in first third of lactation according to breed and number of lactation (P < 0.001)*

Data set	Equation form	
	F/CP × F/L	**F/L × F/CP**
CF first lactation	y = 0.6423x + 0.0157	y = 1.286x + 0.1703
H first lactation	y = 0.5947x + 0.0358	y = 1.2447x + 0.2645
CF other lactations	y = 0.623x + 0.042	y = 1.307x + 0.1729
H other lactations	y = 0.5074x + 0.1319	y = 1.6027x + 0.0206



3: *The examples of relationships between ketosis milk indicators F/CP and F/L for Czech Fleckvieh (CF) on 1st lactation, Holstein (H) on other lactations in 1st third of lactation (P < 0.001; Tab. III)*

IV: *The results of reciprocal estimation of cut–off values for milk indicators such as F/CP and F/L ratio for the purpose of diagnosis of dairy cow subclinical ketosis in first third of lactation*

Milk indicator	Previous cut–off limit (A)	Specification	Validation of estimation of cut–off limit (B)
F/CP	1.27	CF first lactation	0.831 F/L
	1.32	H first lactation	0.821 F/L
	1.52	CF other lactations	0.989 F/L
	1.42	H other lactations	0.852 F/L
F/L	0.84	CF first lactation	1.251 F/CP
	0.84	H first lactation	1.31 F/CP
	0.87	CF other lactations	1.31 F/CP
	0.85	H other lactations	1.383 F/CP

A = according to equations in Tab. II (10 $mg.l^{-1}$); B = according to equations in Tab. III regarding A value

explain 16.3% and 12.9% of variations in F/CP and F/L quotient by variations in log AC values both for H breed and other lactation in 1st third of lactation. Obtained cut–off limits were as follows: for first lactation 1.27 (CF) and 1.32 (H), for other lactations 1.52 (CF) and 1.42 (H) in F/CP; for first lactation 0.84 (CF) and 0.84 (H), for other lactations 0.87 (CF) and 0.85 (H) in F/L. Consequently these were used for cross validation by mutually reciprocal calculation according to relevant equations which showed statistically significant relationships (*P* < 0.001; Tab. III and IV and Fig. 3).

Reciprocal estimation of F/CP and F/L threshold by cross calculation

The study of F/CP×F/L correlation between energy (ketosis) milk indicators and their threshold cross calculation is one of the first cases of such type of cut–off value validation regarding their mutual relationship according to approachable research literature sources. There are relatively close relationships between milk indicators (F/CP and F/L) under various conditions in terms of breed and lactation number (Fig. 3 and Tab. III). Therefore as maximum, there could be explained 82.6% of variations in F/L quotient by variations in F/CP values for CF breed and first lactation in 1st third of lactation. Of course, as expected, these relations are logically closer because of using of the same fat values in calculation of F/CP and F/L. Results of cross calculation are shown in Tab. III and IV: for first lactation 1.251 (CF) and 1.31 (H), for other lactations 1.31 (CF) and 1.383 (H) in F/CP; for first lactation 0.831 (CF) and 0.821 (H), for other lactations 0.989 (CF) and 0.852 (H) in F/L. As these results are compared to previous cut–off limits which were stated according to milk AC cut–off limit there is possible to state that these validated cut–off limits are quite similar to their original values. There are only two small exceptions (differences) and these are: 1.52 and 1.31 for F/CP; 0.87 and 0.989 for F/L; both in CF and other lactations. This fact confirmes good reliability of original limit estimations. The means in pairs of relevant cut–off limit estimations, which means original estimation (according to log AC, Tab. II and IV) and validation of estimation (mutual cross calculation, Tab. III and IV), could be practically usable.

In the scientific literature sources, there are more SK definitions and more ways of derivations of SK cut–off limits. Some of them are defined by volume of milk yield losses in early lactation (Gustafsson and Emanuelson, 1996; Duffield *et al*., 2009). The others are linked with sure reduction of body condition score in postpartum period (Gasteiner, 2000; Beran *et al*., 2012). However, most of them are described by high blood, urine or milk AC (ketone) level (Andersson, 1984; Hansen, 1999; Heuer *et al*., 2000; Enjalbert *et al*., 2001; Hanuš *et al*., 2001, 2011 a, b; de Roos *et al*., 2007; Van der Drift *et al*., 2012), sometimes with a relevant note about clinical signs. The results of this cross validation are comparable with research results of other authors as follows: Duffield *et al*., 1997, F/CP 1.33; Geishauser and Ziebell, 1995 and Geishauser *et al*., 1997, F/CP 1.4; Van Knegsel *et al*., 2010, F/CP 1.5; Siebert and Pallauf, 2010, F/CP 1.33 and 1.5; Manzenreiter *et al*., 2013, F/CP 1.33; Steen *et al*., 1996, F/L 1.0; Reist *et al*., 2002, F/L 1.0; Manzenreiter *et al*., 2013, F/L 0.9.

This reciprocal estimation of cut–off limits by cross validation has confirmed following general facts: – milk component AC and component quotients F/CP and F/L as ketosis indicators are in usable and good relationships in early lactation for diagnostical possibilities in case of SK; – confirmation of ketosis milk quotient thresholds by cross calculation shows good agreement; – simultaneously combined use of all milk indicators could be diagnostically advantageous because of cross validation results.

CONCLUSION

The methods of real time analysis of main milk components (fat, protein, lactose, solids non fat) and somatic cell count are introduced into milking parlours in practice last time. In this way the breeders can know milk composition regularly. Therefore they could investigate and identified SK occurrence in early lactation of cows due to use of milk quotients and control ketosis prevention. This could protect farm economy against incidental losses. Higher operation activity of such system is advantageous. Here validated estimations of thresholds (1st lact. 1.251 (CF) and 1.31 (H), other lact. 1.31 (CF) and 1.383 (H) in F/CP; 0.831 (CF) and 0.821 (H), 0.989 (CF) and 0.852 (H) in F/L) of milk indicators for SK in early lactation could be used for animal husbandry economy improvement. Combined use of both quotients could be more efficient in regular diagnosis of SK.

SUMMARY

Real time analyses of main milk components and properties (fat, protein, lactose, solids non fat and somatic cell count) are attended in milking parlours today. Regular day information without delay is advantageous as compared to month interval of regular milk recording. In this way the breeders can know milk composition regularly. Energy (ketosis) milk quotients such as fat/crude protein (F/CP) and fat/lactose (F/L) could be essential for ketosis prevention and losses which can be linked with it. Aim was to do a reciprocal validation of reliability of milk indicators for subclinical ketosis (SK) identification. There were: primiparous and multiparous dairy cows which were clinical and superior subclinical mastitis free; 3 herds of Czech Fleckvieh (CF) breed, 3 herds of Holstein (H) breed and 1 herd with CF and H; 329 individual milk samples from summer and winter season and 1st third of lactation. Average milk yield varied from 5,500 to 10,000 kg per lactation. Thresholds of F/CP and F/L were estimated ($P < 0.05$) according to SK milk acetone cut–off limit ($\geq$ 10 mg.l^{-1}). These were: for first lactation 1.27 (CF) and 1.32 (H), for other lactations 1.52 (CF) and 1.42 (H) in F/CP; for first lactation 0.84 (CF) and 0.84 (H), for other lactations 0.87 (CF) and 0.85 (H) in F/L. Consequently these were used for cross validation by mutually reciprocal calculation according to relevant equations ($P < 0.001$):

for first lactation 1.251 (CF) and 1.31 (H), for other lactations 1.31 (CF) and 1.383 (H) in F/CP; for first lactation 0.831 (CF) and 0.821 (H), for other lactations 0.989 (CF) and 0.852 (H) in F/L. These validated cut–off limits are quite similar to their original values. It confirmes good reliability of original limits. The validated estimations of thresholds of studied milk indicators (F/CP and F/L) in early lactation for SK can be used at technological innovation (milk recording and real time measurement systems) in animal husbandry. Combined use of thresholds of both quotients (F/CP and F/L) could improve regular investigation results of SK in practice as well.

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