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METAANALYSIS OF KETOSIS MILK INDICATORS IN TERMS OF THEIR THRESHOLD ESTIMATION

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Abstract

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Real time analyses of main milk components are attended in milking parlours today. Regular day information without delay is advantageous. Farmers can know milk composition every day. They can calculate milk energy quotients, identified subclinical ketosis in early lactation of dairy cows and thus improve ketosis prevention and avoid economical losses. Aim was to improve the estimation reliability of thresholds of milk indicators of energy metabolism for subclinical ketosis detection and its prevention support by metaanalysis. This can have higher result reliability than individual studies. Results of similar papers were analysed. These were focused on ketosis indicators in milk (acetone (AC) and milk energy quotients (fat/crude protein, F/CP; fat/lactose, F/L)) and their thresholds for subclinical ketosis. Methods for threshold derivation were specified: – statistically to reference procedure; – calculation according to relevant data frequency distribution; – qualified estimation; – combinations of mentioned procedures. This was as weight source. Variability in AC subclinical ketosis cut–off values was high (78.5%) and in ketosis milk quotients was low (from 5 to 8%). The value 10.57 $mg.l^{-1}$ could be the validated estimation of milk AC cut–off limit for subclinical ketosis identification. Similarly the milk quotients F/CP and F/L 1.276 and 0.82. The F/CP F/L relationship is closer in 1st third of lactation (0.89; $P < 0.001$) than in whole lactation (0.86; $P < 0.001$). This could be one of proofs of ability for subclinical ketosis identification because the majority of cases occurs in early lactation. The improved estimations of thresholds of milk indicators in early lactation for subclinical ketosis can be used at this technological innovation. Combined use of both quotients could bring an improvement of regular diagnosis of subclinical ketosis.

dairy cow, health, individual milk sample, acetone, milk energy quotients, subclinical ketosis identification, ketosis prevention

Ketosis as disorder of energy metabolism of mammal females reduces milk yield (Emanuelson and Andersson, 1986; Andersson, 1988; Gustafsson and Emanuelson, 1996; Duffield, 2000; Duffield *et al.*, 2009) and reproduction performance of dairy cows (Fig. 1; Duffield, 2000; Gasteiner, 2000; Duffield *et al.*, 2009). Animal can also die in this case.

Ketosis, milk yield and cow reproduction

Ketosis can threaten milk quality in terms of its processing (Hanuš *et al.*, 1993). This disorder occurrence is linked with higher levels of ketones in body liquids. Ketosis is caused by higher output of nutrients due to lactation as compared to their lower input by feeding. Cow in early lactation (Wood *et al.*, 2004; Manzenreiter *et al.*, 2013) is not able to take into enough nutrients for its high milk yield. That is why ketosis rises especially in high yielding dairy cows (Januš and Borkowska, 2013).

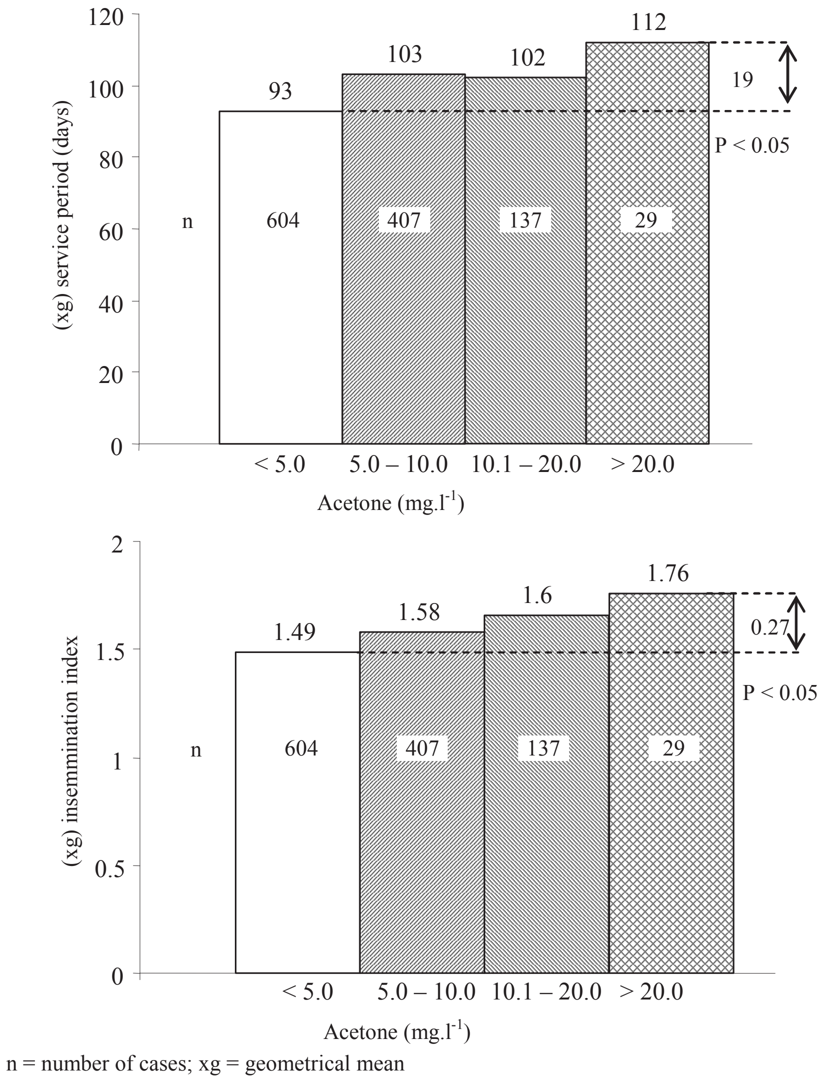
Ketones are product of body fat catabolism in early lactation in time of energy malnutrition. Miettinen (1994) found out correlation –0.47 ($P < 0.001$) between milk acetone and yield. Therefore ketones are seen as undesirable metabolites. Some of them are metabolised and other (for instance acetone) go out of blood and body as a rule through urine, breath, sweat and milk (Andersson and Lundström, 1984 a, b; Enjalbert *et al.*, 2001). That is also reason why there are high positive correlations between blood and milk acetone (Steger *et al.*, 1972; Andersson, 1984, 0.96; Andersson and Lundström, 1984 b, 0.88; Gravert *et al.*, 1986, 0.98; Enjalbert *et al.*, 2001, 0.96; all $P < 0.001$). This body fat destroying phenomenon can lead to reduction of liver metabolic function.

From hypothetical point of view this is possible to include ketosis (subclinical and clinical) into time row of production disorder occurrence in early lactation as possible consequence of postpartum paresis (of course in link with nutritional energy deficiency) and possible cause of abomasum displacement (Geishauser *et al.*, 1997 a, b), mastitis and placenta retention with other reproduction disorders (Duffield, 2000; Duffield *et al.*, 2009; Fig. 1). Some of these negative health consequences are caused due to ketosis immunosuppression (Duffield, 2000; Gasteiner, 2000).

Especially subclinical ketosis identification in practice is important for its prevention (Duffield, 2000; Gasteiner, 2000). There is possible hormonal treatment by insulin for cow health improvement (Gasteiner, 2000). For instance also the feeding of glucoplastic and hepatoprotective matters such as propyleneglycol, natriumpropionate, niacin, carnitine, lecithin, monensisn, sylimarin (Emery *et al.*, 1964; Jagoš *et al.*, 1991; Vojtíšek *et al.*, 1991; Miettinen, 1995; Green *et al.*, 1999; Gasteiner, 2000, 2003; Tedesco *et al.*, 2004; Hanuš *et al.*, 2001, 2011 a; Coskun *et al.*, 2012) can help in prevention. That is why these methods are essential for improvement of milk quality, dairy cow health and reproduction.

Importance of ketosis investigation in milk

Milk in contrast to blood or urine is very easy for the sampling. Ketones in milk (acetone and betahydroxybutyrate) are suitable for confident noninvasive monitoring and control of dairy cow



1: *The relation of acetone levels in dairy cows (individual milk samples) to some reproduction indicators in the first third of lactation (Říha and Hanuš, 1999; Hanuš et al., 2004)* nutrition and health state. During ketosis (lack of energy, it means blood glucose deficiency) milk fat content is increased due to body fat destroying and at the same time on the contrary the protein content is decreased (Diekmann, 1987; Geishauser and Ziebell, 1995; Steen *et al.*, 1996; Hansen, 1999; Gasteiner, 2000; Hana *et al.*, 2007; Siebert and Pallauf, 2010; van Knegsel *et al.*, 2010; van der Drift *et al.*, 2012).

The possibilities for identification of subclinical ketosis are better step by step in dairy herds. The infrared spectroscopy with Fourier's transformations (MIR–FT) which expands in laboratory practice makes possible regular determination of acetone in individual milk samples (Hansen, 1999; Heuer *et al.*, 2000; de Roos *et al.*, 2007; van Knegsel *et al.*, 2010; Hanuš *et al.*, 2011 a; van der Drift *et al.*, 2012). Next possibility is investigation of milk ketones by stable tests (Geishauser *et al.*, 1997 a; Hanuš *et al.*, 1999; Carrier *et al.*, 2004). Also changes in ratios of main milk components are usable in subclinical ketosis monitoring by calculation of ketosis milk quotients (fat/crude protein and fat/lactose; Geishauser and Ziebell, 1995; Duffield *et al.*, 1997; Steen *et al.*, 1996; Gasteiner, 2000; Reist *et al.*, 2002; van Knegsel *et al.*, 2010; Siebert and Pallauf, 2010; Hanuš *et al.*, 2011 b, 2013; Manzenreiter *et al.*, 2013). These components are regularly monthly investigated during milk recording in laboratories with MIR and MIR–FT technology. There is also possibility of daily measurement of individual milk samples (NIR technology) in sophisticated milking parlours. Regarding mentioned facts especially this last possibility is advantageous.

Therefore, aim of this paper was to improve the estimation reliability of thresholds of main milk indicators of dairy cow energy metabolism for subclinical ketosis detection and its prevention support by metaanalysis method.

MATERIAL AND METHODS

Analytical viewpoint and newly created database

Metaanalysis combines and evaluates previously published results about same problem in statistical way. Is it one of proof in terms of scientific verification hierarchy and in general very forceful verification in biology. Metaanalysis can improve a quantitative estimation reliability of results and conclusions as compared to individual studies.

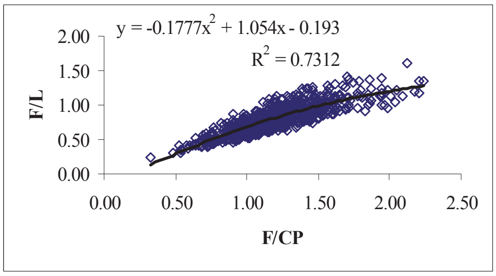
Scientific papers from last fifty years, which were specifically focused on evaluation of ketosis indicators in milk (such as acetone concentration and milk energy quotients (fat/crude protein, F/CP; fat/lactose monohydrate, F/L (F, CP and L in %)) and their thresholds (for subclinical ketosis identification), were studied and main results were collected and noted into newly created database (Tab. I and II). In case of necessity at using of various units of milk acetone concentration the relevant values were recalculated (to mg.l^{-1}) and thus unified. Database was completed by specification of method for threshold derivation. There were more possibilities as follows (Tab. I and II): a) – statistically according to reference procedure results; b) – calculation according to principles of relevant data frequency distribution; c) – qualified estimation according to frequency distribution of values or case study; d) – mutual combinations of previously mentioned procedures. This classification was used also as weight (degree of importance) for data evaluation (w for a = 3, b = 1, c = 2 and d = 2).

Additional statistic evaluation of F/L ratio

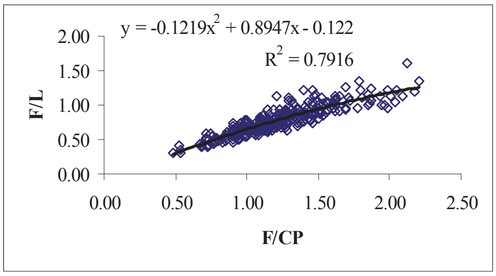
As additional evaluation to our previous results (Fig. 2) the energy milk coefficient F/L was calculated to originally introduced F/CP ratio in relevant data file under conditions which are briefly described in Tab. I, II and by paper Hanuš *et al.* (2011 b). The relationship between them was studied.

Data statistical treatment in metaanalysis

The mean values and their variabilities (sd) of acetone (AC) and log acetone (log AC), F/CP and



r = 0.8551 n = 960



r = 0.8897 n = 329

2: *Non-linear relationships between milk energy quotients F/CP (x axis) and F/L (y) during whole lactation and in the 1st third of lactation*

Individual milk samples; clinical and superior subclinical mastitis free primiparous and multiparous dairy cows; breeds Czech Fleckvieh (CF) and Holstein (H); 3 herds CF, 3 herds H and 1 herd CF and H; summer and winter season; n = 960 (whole lactation) and 329 (1st third of lactation); average milk yield varied in range from 5,500 to 10,000 kg per lactation.

I: *The survey of scientific sources and results about milk acetone (AC) concentrations and their thresholds for identification and diagnosis of subclinical ketosis of dairy cows*

Literature source	Health and metabolic state of dairy cows and their environmental conditions	Declared milk AC threshold (in $mg.l^{-1}$)	AC result in experiment, case study and field observation, x ± sd (in $mg.l^{-1}$)	Number of cases (samples, cows), n	Way of milk AC threshold derivation	Note
Emery *et al.*, 1964	subclinical ketosis	17.4	–	–	(a, w = 3)	acetone + acetacetate
Steger *et al.*, 1972	subclinical ketosis	(0.17 – 0.25, (0.21) $mmol.l^{-1}$) 12.2	–	–	(a, w = 3)	acetone
Andersson, 1984, 1988	hyperketonemic cows	(0.4 $mmol.l^{-1}$) 23.2	48.7 ± 49.3	111	according to results of suspicious animals (a, w = 3)	cows in 1st 8 weeks of lactation
Andersson and Lundström, 1984 a	clinic healthy dairy cows	–	4.1 ± 4.6	328	–	cows in 1st 8 weeks of lactation
Andersson and Lundström, 1984 b	hyperketonemic cows	–	18.6 ± 225.6	104	–	3rd – 8th lactation week
Andersson and Emanuelson, 1985	prevalence of hyperketonemie 8.9, 4.7, 1.1% in three months	23.2	–	3 087	accepted from literature and confirmed by results (d (a and b), w = 2)	cows in 1st 3 months of lactation, Swedish dairy cows from 126 herds
Gravert *et al.*, 1986	cows with controlled nutrition and normal (commercial herds)	23.2	6.4 7.0 4.6	10 010 1 106 614	accepted from literature and confirmed by results (d (a and c), w = 2)	according to breeds (H, F and Angler), cows in 1st 4 months of lactation
Vojtíšek, 1986	physiological with trend to physiolog. acetonemia	7	–	–	accepted from literature and confirmed by own experience (c, w = 2)	up to 10th week after calving
Diekmann, 1987	normal herds and herds with higher milk acetone content	(0.21 $mmol.l^{-1}$) 12.2	4.6 13.3	28 and 29	according to comparison of lactation energy requirement and intake (a, w = 3)	experience from advisory service about dairy cow nutrition and health
Kvíz and Hofman, 1990	AC over physiological range	2 – 6 (4)	–	–	(c, w = 2)	experience from advisory service about dairy cow nutrition and health
Vojtíšek *et al.*, 1991	ketosis cows	7.9	20.1 ± 9.7 1.5 ± 0.4	48	(a, w = 3)	acetone + acetacetate, according to experiment stadium
Gravert *et al.*, 1991	normal (commercial herds)	(0.25 $mmol.l^{-1}$) 14.5	17.4 ± 1.7	5 320	accepted from literature and confirmed by results (d (a and c), w = 2)	H herds
Šrámek *et al.*, 1992	normal (commercial herds)	–	5.6 ± 4.5	3 724	–	individual milk samples, 1st third of lactation
Hanuš *et al.*, 1993	normal (commercial herds)	–	4.89 ± 3.5	358	–	bulk milk samples
Miettinen, 1994	4 healthy cows and 6 were overfed (20%) before calving and underfed (25%) to induce ketosis	(0.05 $mmol.l^{-1}$) 2.9	3.9 ± 1.0 46.9 ± 17.3	172	from AC change induced by cow feeding towards ketosis (a, w = 3)	individual milk samples, 6 – 64 days post partum, Finish Ayrshire cows
Gustafsson and Emanuelson, 1996	hyperketonemic cows	(0.7 $mmol.l^{-1}$) 40.7	–	11 690	according to milk losses (a, w = 3)	individual milk samples
Hanuš *et al.*, 1999	selection of healthy problematic dairy cows	10	9.26 (± 13.31) > 6.36 (± 6.96); 6.01 > 4.41 xg	1 559 5 193	according to body condition losses, higher and lower (d (a and c), w = 2)	individual milk samples

Literature source	Health and metabolic state of dairy cows and their environmental conditions	Declared milk AC threshold (in mg.l^{-1})	AC result in experiment, case study and field observation, x ± sd (in mg.l^{-1})	Number of cases (samples, cows), n	Way of milk AC threshold derivation	Note
Hansen, 1999	hyperketonemic cows	40.7	–	58	from literature and confirmed by own analytical indirect and reference method results (c, w = 2)	individual milk samples
Hanuš *et al.*, 2001 and 2011 a	selection of ketotic and healthy cows using rapid stable tests	5 and 7 (6)	44.2 ± 49.1 (19.5 xg)	76	according to stable test result (d (combination a and c), w = 2)	summer and winter threshold
Heuer *et al.*, 2001	7 cows were overfed (20%) before calving and 7 were fed according to requirements, group 1 had higher body condition by 1	(0.3 mmol.l^{-1}) 17.4	26.1 ± 40.0	180	from literature and confirmed by own analytical indirect and reference method results (c, w = 2)	from 1st to 15th week of lactation, still good prediction
Hanuš *et al.*, 2003	normal (commercial herds)	–	2.29 : 2.21, xg; 1.99 : 1.9, xg; 2.16 : 1.99, xg; 2.44 : 1.25, xg	96 and 96; 96 and 96; 382 and 386; 94 and 98	–	individual milk samples (H and CF)
de Roos *et al.*, 2007	normal (commercial herds)	(0.15 mmol.l^{-1}) 8.7	8.5	1 080	from literature and confirmed by own analytical indirect and reference method results (c, w = 2)	from 217 herds, early lactation, multiparous dairy cows
van Knegsel *et al.*, 2010	selection of nonketotic and hyperketonemic cows	(0.07 mmol.l^{-1}) 4.06	–	618 milk samples, 69 dairy cows	diagnostic reference using plasma BHB ≥ 1.2 mmol.l^{-1} (a, w = 3)	individual milk samples, early lactation
Sojková *et al.*, 2010 a	normal (commercial herds)	–	2.95 ± 5.5, 1.72 xg; 3.3 ± 4.34, 2.01 xg	234 and 224	–	individual milk samples, 1st third of lactation, higher and lower milk yield (H)
Sojková *et al.*, 2010 b	normal (commercial herds)	–	2.61 ± 2.67, 1.76 xg; 2.63 ± 2.51, 1.67 xg	213 and 265	–	individual milk samples, first third of lactation, higher and lower milk yield (CF)
Hanuš *et al.*, 2011 b	normal (commercial herds)	2.4 and 2.1 (H); 3.3 and 2.5 (CF); (2.6)	3.31 ± 5.38, 1.95 xg; 2.86 ± 3.91, 1.79 xg	327 and 960	according to F/CP (1.18 and 1.6) threshold (a, w = 3)	individual milk samples, 1st lactation and other lactations, 1st third of lact. and whole lact. (H and CF)
van der Drift *et al.*, 2012	normal (commercial herds), 11.2% hyperketonemia prevalence	AC no defined, only as regression model with F/CP and AC	–	1 678	reference as plasma BHB ≥ 1.2 mmol.l^{-1}	cows between 5 and 60 days in milk, from 118 randomly selected farms

AC = acetone; BHB = beta–hydroxybutyrate; H = Holstein (Black Spotted); F = Fleckvieh; CF = Czech Fleckvieh; x ± sd = arithmetic mean ± standard deviation; xg = geometric mean; threshold derivation: a) - statistically according to reference procedure results; b) - calculation according to principles of relevant data frequency distribution; c) - qualified estimation according to frequency distribution of values or case study; d) - mutual combinations of previously mentioned procedures; this classification was used as weight for evaluation (w for a = 3, b = 1, c = 2 and d = 2).

II: *The list of research sources and results about milk ketosis (energy) quotients (fat/crude protein (F/CP) and fat/lactose monohydrate (F/L)) and their cut–off values for investigation of subclinical ketosis of dairy cows*

Literature source	Health and metabolic state of dairy cows and their environmental conditions	Declared milk ketosis quotient threshold	Result in experiment, case study and field observation, x ± sd	Number of cases (samples, cows) n	Way of milk energy quotient threshold derivation	Note
about F/CP:						
Geishauser and Ziebell, 1995	27 DA case herds and 27 control herds without DA	1.4	–	54	with DA the F/CP was higher than in herds without DA (b, w = 1)	27 H herds
Duffield *et al.*, 1997	prevalence of ketosis in early lactation (65 days) was 14.1%	0.75 CP/F = 1.33 F/CP	–	1 333	diagnostic reference using plasma BHB ≥ 1.2 mmol.l^{-1} (a, w = 3)	93 farms
Geishauser *et al.*, 1997 b	27 abomasum displacement (DA) case herds as ketosis consequence	1.4	–	27	F/CP ≥ 1.4 there was 8.6 time higher likelihood for DA occurrence (b, w = 1)	milk tested 18 to 23 days
van Knegsel *et al.*, 2010	selection of nonketotic and hyperketonemic cows	1.5	1.48 ± 0.09	618 milk samples, 69 dairy cows	diagnostic reference using plasma BHB ≥ 1.2 mmol.l^{-1} (a, w = 3)	individual milk samples, early lactation
Siebert and Pallauf, 2010	normal (commercial herds)	1.5 and 1.33	1.22 ± 0.2	104 167 (H)	literature reference and statistical derivation (b, w = 1)	52 farms
Hanuš *et al.*, 2011 b	normal (commercial herds)	1.32 and 1.42 (H); 1.27 and 1.52 (CF); (1.38)	1.21 ± 0.31; 1.19 ± 0.27	327 and 960	according to AC (10 mg.l^{-1}) threshold (a, w = 3)	individual milk samples, 1st lactation and other lactations, 1st third of lact. and whole lact. (H and CF)
Manzenreiter *et al.*, 2013	control and ketosis cows	1.33 (F)	1.28 and 1.47 (F); 1.38 and 1.67 (H)	932 cases, 629 F cases; 1 372 ketose and 144 643 milk control cases (F)	according to veterinary doctor acetonemia diagnosis and literature references (a, w = 3)	92% of ketosis cases in 1st 100 lactation days, 80% in 1st 50 days
about F/L:						
Steen *et al.*, 1996	normal (commercial herds)	1.0	–	800 and 1 368	with increased AC decreased L and stable CP (a, w = 3)	43 and 82 herds (lowland and highland)
Reist *et al.*, 2002	high yielding dairy cows	1.0	–	90	estimation of energy balance by F/L (a, w = 2)	from 1st to 10th week of lactation (H)
Manzenreiter *et al.*, 2013	control and ketosis cows (groups)	0.9	0.87 and 0.99 (all breeds)	932 cases, 629 F cases; 1 367 ketose and 144 238 milk control cases (F)	according to veterinary doctor acetonemia diagnosis and literature references (a, w = 3)	53 veterinary practice fields and 1 133 diagnosen for acetonemia
Hanuš *et al.*, 2013	normal (commercial herds)	0.84 and 0.85 (H); 0.84 and 0.87 (CF); (0.85)	0.82 ± 0.31 (1st third of lactation); 0.80 ± 0.27 (whole lactation)	327 and 960	according to AC (10 mg.l^{-1}) threshold (a, w = 3)	individual milk samples, 1st lactation and other lactations, 1st third of lact. and whole lact. (H and CF)

F = fat %; CP = crude protein %; L = lactose %; F/CP = fat%/crude protein%; F/L = fat%/lactose monohydrate%; DA = displaced abomasum;.

F/L in individual milk samples and their estimated thresholds for subclinical ketosis were included into study from previous scientific papers. AC log transformation was used because of no normal frequency distribution of values in AC data files. Mentioned fact is typical for health milk indicators such as AC and somatic cell count (Hanuš *et al.*, 1999, 2011 a, b). This treatment makes also possible to use the geometrical means in case of AC values. Specifically modified version of metaanalysis was used for collected paper data treatment. In general, remote values were canceled in created data files on the basis of Grubbs' test (5%) as step of metaanalysis limitation. Statistical parameters were calculated from newly created database. The regression analysis was used for evaluation of relationship between F/CP and F/L. The Microsoft Excel was used for calculation and graphs.

RESULTS AND DISCUSSION

Milk acetone threshold for subclinical ketosis

The results from various experiments, case studies and field observations are shown in Tab. I. There are specified conditions under which the relevant milk acetone thresholds for subcilinical ketosis identification were obtained such as calculation according to reference procedure results including those which were derived from database or case study by qualified estimation. Variability in professional AC subclinical ketosis cut–off values was high (Tab. I and III; vx cca 78%). As breed is mostly not significant effect on milk acetone concentration in milked dairy cows (Hanuš *et al.*, 2003, 2011 b; Sojková *et al.*, 2010 a, b) and genetic effects as heritability and genetic variance on milk AC are low (Wood *et al.*, 2004) so breeds were not included in evaluation separately.

According to here performed metaanalysis results (Tab. III and IV) this is possible to take into account the validated estimation of milk AC cut–off value for subclinical ketosis identification 10.57 mg.l^{-1} (geometric mean of previous scientific estimations) in milk laboratory and recording system. Unglaub (1983) stated acetone physiological concentration and also its risk subclinical ketosis threshold 7 mg.l^{-1} for first 10 weeks of lactation in German dairy cows. However, the historical variability about this threshold was stated from 2 to 41 mg.l^{-1} (from 0.03 to 0.7 mmol.l^{-1}; Tab. I). This was stated using various reference procedures. The highest cut–off limit was derived along defined decrease of milk yield in early lactation (Gustafsson and Emanuelson, 1996). However, both marginal values could be over– or undervalued. On the basis of previous results (Hanuš *et al.*, 1999) it is possible to keep as threshold for subclinical ketosis estimation the values > 15 for milk ketones and > 10 mg.l^{-1} for acetone. Nevertheless, most of studies described cut–off value from 7 to 23 mg.l^{-1} (from 0.12 to 0.4 mmol.l^{-1}), Gravert *et al.* (1986), Miettinen (1995) and Gasteiner (2000). Despite it and also therefore, the metaanalysis of mentioned results (Tab. I) could be advantageous. In our previous papers (Hanuš *et al.*, 2011 a, b) the winter and summer cut–off limits 10 and 7 mg.l^{-1} were used. Considering winter feeding of ketogenic roughage like silages and summer pasture and pertinent higher animal movement linked with it the used summer threshold was lower.

Under presupposition of acceptance of mentioned relationships (Andersson (1984, 1988), Andersson and Lundström (1984 a, b), Andersson and Emanuelson (1985) and Vojtíšek (1986)) it is possible to deduce that ratio between contents of acetone in milk and urine, which is determined by physiological–pathological principles and their mutual ratio combinations, could be cca from 1:10 to 1:35 in dependence on health state of organism. The mentioned fact is equal to 100 and 350 mg.l^{-1} of acetone in urine along limit of subclinical ketosis 10 mg.l^{-1} of milk acetone. Currently the MIR–FT calibrations for milk ketones (acetone) determination are tested (van Knegsel *et al.*, 2010). These would make possible regular AC monitoring. De Roos *et al.* (2006) evaluated the sensitivity (70%), specificity (95%) and percentage of false positive (27%) and false negative (7%) findings regarding method ability to identify subclinical ketosis of dairy cows on the basis of reading of acetone, acet–acetate and beta–hydroxybutyrate in individual milk samples using calibrated MIR–FT method (MilkoScan FT 6000).

Also Hansen (1999) carried out work about milk acetone and animal ketosis investigation using calibrated infrared spectroscopy on MIR–FT principle. He found out acceptable reliability for dairy cow classification into two groups (healthy and suspected of ketosis) in samples which ranged from 0 to do 2.8 mM of acetone with determination coefficient 0.81 and rightness 0.27 mM. Similarly Heuer *et al.* (2001) who found out threshold for subclinical ketosis as value from 0.4 to 1.0 mM. In terms of reproduction performance the number of artificial inseminations per pregnancy increased by 0.14 for each unit increase in the natural logarithm to the concentration of acetone measured at first artificial insemination (Waldmann *et al.*, 2003).

Milk energy (ketosis) quotient thresholds for subclinical ketosis

In Tab. II there are specified conditions under which the relevant milk energy quotient thresholds for subcilinical ketosis identification were obtained. While the professional variability in scientific estimations of AC thresholds (Tab. I and III) was high (78.5%), the variability in cut–off limits of ketosis milk quotients (Tab. II and III; F/CP and F/L) was low (from 5 to 8%). This fact shows on particularity and scientific difficultness of subclinical ketosis definition in terms of occurrence of its specific signs and balance of professional viewpoints. As breed is important factor for its influence on main milk composition (Hanuš *et al.*, 2003; Sojková *et al.*, III: *The results of metaanalysis of individual study results (from Tab. I and II) about milk ketosis indicators (acetone (AC) and energy quotients (F/CP and F/L)) and estimation of their validated cut–off limits for possibility to investigate the subclinical ketosis of dairy cows*

Milk indicator	Type of evaluation	Original n	x ± sd	vx	n after Grubbs' test	x ± sd	vx	CI = sd × 1.64, one side limitation (95%)	Validated estimation of cut–off limit, x – CI
AC	single	19	14.6 ± 11.4	78.1	19	14.6 ± 11.4	78.1	18.7	–
AC	weighted	47	14.4 ± 11.3	78.5	47	14.4 ± 11.3	78.5	18.5	–
F/CP	single	8	1.396 ± 0.071	5.1	8	1.396 ± 0.071	5.1	0.116	1.28
F/CP	weighted	16	1.391 ± 0.07	5.0	16	1.391 ± 0.07	5.0	0.115	1.276
F/L	single	4	0.938 ± 0.075	8.0	4	0.938 ± 0.075	8.0	0.123	0.815
F/L	weighted	11	0.932 ± 0.068	7.3	11	0.932 ± 0.068	7.3	0.112	0.82

Milk AC in mg.l^{-1}; n = number of cases; CI = confidence interval (95%); vx = coefficient of variability in %.

2010 a, b), two main milked breeds (Fleckvieh (F) and Holstein (H)) were taken into account in this evaluation including Sweden red spotted. However H showed lower fat and also protein than F. That is why F/CP ration could be similar between H and F for the same degree of health state. So threshold evaluation was performed together (F and H). In spite of it there is still tendency to use a little bit different values according to breed.

According to here performed metaanalysis results (Tab. III) this is possible to take into account the validated estimation of cut–off value to subclinical ketosis identification of energy milk quotients as follows: for F/CP 1.276 (weighted value); for F/L 0.82 (weighted value). This could be important in milk laboratory and recording system. Of course, it can be important in current modern analytical systems as sophisticated milking parlours with real time measurement of milk composition as well.

Ketosis mainly (80%) occurs during the first 50 days of lactation and about 35% of the positive diagnoses were made during the first 10 days of lactation (Manzenreiter *et al.*, 2013). It means that practical utilization of results from month sampling of milk recording is given because of fact that for 49% of the positively diagnosed dairy cows no milk recording (sampling) was conducted within a relevant time period before ketosis was diagnosed. Manzenreiter *et al.* (2013) estimated that only 30% of ketosis occurrence can be successfuly investigated under mentioned conditions. In practice there are often missing the milk recording results in the important time of early lactation. It could be changed by daily milk composition analyses in modern sophisticated milking parlours with NIR technology.

Duffield *et al.* (2009) found significant impact of hyperketonemia of dairy cows on their greater fat percentage and less protein percentage on first test day in milk recording. A similar impact on milk composition stated previously also Geishauser and Ziebell (1995). The threshold value 1.5 for F/CP quotient at subclinical ketosis was used by van Knegsel *et al.* (2010). On the other hand Duffield *et al.* (1997) reported 1.3. The sensitivity for diagnose of hyperketonemic dairy cows was 66%. This is lower as compared to milk beta–hydroxybutyrate and acetone (80%) by MIR–FT with relevant cut–off values 23 and 70 µmol.l^{-1}.

Steen *et al.* (1996) and Reist *et al.* (2002) found the reliability of F/L value (threshold 0.9) for subclinical and clinical ketosis identification as more efficient regarding F/CP value. Similar result was concluded by Manzenreiter *et al.* (2013). They found 66.7% of right diagnose in ketosis group and in control group 37.5% of F/L values were over 0.9 as false positive findings. Considering ketosis diagnose it is too late at clinical signs occurrence. In terms of effective prevention and treatment, the diagnose of subclinical state is very important (Duffield *et al.*, 1997; Duffield, 2000; Gasteiner, 2000; Hanuš *et al.*, 2004, 2011 a, b). Part of ketosis prevention could be to indetify its subclinical period. Subclinical ketosis can threaten milk quality. That is why ketosis identification methodical improvement is important.

Siebert and Pallauf (2010) mentioned the F/CP threshold reduction from 1.5 to 1.33. Therefore Manzenreiter *et al.* (2013) found 60.9% of right ketosis diagnose (F/CP over 1.33 in ketosis group) and in control group there was 38.5% of false positive findings (no ketosis group with F/CP over 1.33 (Fleckvieh)). The diagnosis with F/CP 1.33 was better regarding 1.5. Also Hanuš *et al.* (2011 b) confirmed F/CP threshold value 1.27 and 1.32 (first third of lactation, first lactation) and 1.52 and 1.42 (first third of lactation, other lactations, Czech Fleckvieh and Holstein) using model regression calculation according to reference procedure (milk acetone).

Relationship between milk ketosis indicators F/CP and F/L

Because of possibility for simultaneous use of milk energy quotients (F/CP and F/L) as subclinical ketosis indicators the relationship between them was studied. Such type of evaluation has not been carried out up to now. In Fig. 2, there are two correlation indexes for 1st third of lactation and whole lactation. There is possible to explain 79.2% of variability in F/L by variability in F/CP values in 1st third of lactation. These relationships are logically closer due to use of the same fat values in calculation of both quotients. As far as the F/CP F/L

IV: *The result of metaanalysis of individual study results (from Tab. I) about milk acetone (AC, in form log AC) and estimation of its validated cut–off limit for possibility to investigate the subclinical ketosis of dairy cows*

Milk indicator	Type of evaluation	n	x ± sd log AC	AC xg as validated estimation of cut–off limit
AC	single	19	1.035747 ± 0.357972	10.86
AC	weighted	47	1.024048 ± 0.361384	10.57

Milk AC in mg.l^{-1}; n = number of cases; xg = geometric mean.

relationship is a little bit closer in 1st third of lactation (Fig. 2; 0.89; $P < 0.001$) than in whole lactation (0.86; $P < 0.001$) this fact could be allowed to be one of proofs of ability for ketosis identification in both indicators because of Manzenreiter *et al.* (2013) finding of majority of ketosis occurrence in early lactation. That result could be promising for future real time diagnostical systems in animal husbandry. Further, as it already has beeen mentioned, there was the much higher variability in scientific sources for proposed AC cut–off limits (Tab. I and III; from 78.5%) as compared to lower for F/CP and F/L quotient thresholds (Tab. II and III; from 5 to 8%). That is also reason, why geometric mean was used as estimation of milk AC cut–off limit in metaanalysis (Tab. I and IV). According to known information that fact is highly probable that F/L quotient is less susceptible to breed impact as F/CP. Therefore it is recommended to use F/CP cut–off value a little bit higher (by 0.02–0.05) for F and lower for H breed (Hanuš *et al.*, 2011 b) than generally derived limit (Tab. III) in the practice diagnostical systems.

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. This fact means that every day there is possibility for farmers to know milk composition, calculate milk energy quotients, investigate and identified subclinical ketosis occurrence in early lactation of cows in their dairy herds and thus improve prevention against ketosis and avoid economical losses which are demonstrably linked with it. This all is possible to carry out with better efficiency than previously because every day information is preferable as compared to month interval at regular milk recording and delay by 2 or 4 days from sampling to result obtaining. Higher operation possibility of this system is visible. The improved estimations of thresholds (acetone and F/CP and F/L quotient) of studied milk indicators in early lactation for subclinical ketosis by metaanalysis (10.57 mg.l^{-1} and 1.276 and 0.82) can be successfuly used at this mentioned technological innovation in animal production. Also combined use of both quotients could bring an improvement of regular diagnosis of subclinical ketosis. From research point of view as the high variability was in literature sources for proposed AC thresholds and low in F/CP and F/L quotient tresholds the metaanalysis cut–off value estimation is more important in AC case.

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. Farmers can know milk composition every day. Therefore they can calculate milk energy quotients, investigate and identified subclinical ketosis occurrence in early lactation of cows in their dairy herds and thus improve prevention against ketosis and avoid economical losses which are linked with it. Therefore, aim of this paper was to improve the estimation reliability of thresholds of main milk indicators of dairy cow energy metabolism for subclinical ketosis detection and its prevention support by metaanalysis. This method can have higher result reliability than individual studies. Results of papers, which were focused on mentioned topic, were collected, classified and newly statistically analysed. There were scientific papers focused on evaluation of ketosis indicators in milk (acetone (AC) and milk energy quotients (fat/crude protein, F/CP; fat/lactose, F/L)) and their thresholds for subclinical ketosis. Methods for threshold derivation were specified: – a) statistically according to reference procedure results; – b) calculation according to principles of relevant data frequency distribution; – c) qualified estimation according to frequency distribution of values or case study; – d) mutual combinations of previously mentioned procedures. This classification was used as weight for data (w: a = 3, b = 1, c = 2, d = 2). Modified version of metaanalysis was used for data treatment. Remote values were canceled in created data files on the basis of Grubb's test (5%). Variability in professional AC subclinical ketosis cut–off values was high (78.5%). This is possible to take the value 10.57 mg.l^{-1} as the validated estimation of milk AC cut–off value (geometric mean) for subclinical ketosis identification. While the professional variability in scientific estimations of AC thresholds was high, the variability in cut–off limits of ketosis

milk quotients was low (from 5 to 8%). This fact shows on particularity and scientific difficultness of subclinical ketosis definition. This is possible to take the values of milk quotients F/CP and F/L 1.276 and 0.82 as the validated estimations of cut–off limits to subclinical ketosis identification. As far as the F/CP F/L relationship is closer in 1st third of lactation (0.89; $P < 0.001$) than in whole lactation (0.86; $P < 0.001$) this fact could be allowed to be one of proofs of ability for subclinical ketosis identification because the majority of cases occurs in early lactation. The improved estimations of thresholds of studied milk indicators in early lactation for subclinical ketosis by metaanalysis can be used at technological innovation (milk recording and real time measurement systems) in animal production. Also combined use of both quotients (F/CP and F/L) could bring an improvement of regular diagnosis of subclinical ketosis.

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