THE PREDICATIVE VALUE AND CORRELATIONS OF TWO MILK INDICATORS IN MONITORING ENERGY METABOLISM OF TWO BREEDS OF DAIRY COWS*

O. Hanuš[1], T. Yong[2], J. Kučera[3, 4], V. Genčurová[4], A. Dufek[1], K. Hanušová[4], T. Kopec[3]

[1]AgriResearch Rapotín, Ltd., Vikýřovice, Czech Republic
[2]Anhui Agricultural University, Hefei, P. R. China
[3]Czech Fleckvieh Breeders Association, Prague, Czech Republic
[4]Research Institute for Cattle Breeding, Ltd., Rapotín, Czech Republic

Ketosis is a disorder of energy metabolism in dairy cows. It deteriorates milk yield, quality and reproduction and can have a fatal impact on animal. Milk acetone (AC) in combination with fat/crude protein (F/CP) ratio could represent good ketosis indicators. Prevention of dissease is very important in practice. 960 individual milk samples (MSs) from the Holstein (H) and Czech Fleckvieh (C) dairy cows (1 : 1), their whole lactations and summer and winter seasons of three years were analysed. Milk yield of seven included herds varied from 5,500 to 10,000 kg. AC values were logarithmically transformed. Impacts of breeder factors on AC and F/CP were evaluated by statistical parameters and regression analyse. AC levels differed insignificantly ($P > 0.05$) between H and C breed in whole lactation (1.87 and 1.70 mg.l^{-1} as geometrical means) but differed significantly ($P < 0.05$) in the first third of lactation (FTL; 2.20 > 1.72 mg.l^{-1}). F/CP did not differ between breeds. The significant differences were not recorded beween winter and summer seasons for both indicators. AC and F/CP are good indicators for monitoring and prevention of subclinical ketosis in dairy cows because of good correlations between them (0.23, 0.28 H, 0.19 C; $P < 0.001$), especially in FTL (0.33 /n = 329/, 0.36 H, 0.30 C; $P < 0.001$). Indicators correlated more in summer than in winter season (0.38 > 0.29, $P < 0.001$, in FTL). The closest relationship was found in H cows in FTL in summer (0.48, P < 0.001). Discrimination limits for subclinical ketosis, which were derived for F/CP via model according to relevant AC threshold (10 mg.l^{-1}) via suitable equations, could be 1.32 and 1.42 (H) and 1.27 and 1.52 (C) for the first and other lactations. Results could be used in practical advisory service for support of good milk quality and adequate dairy cow health, reproduction performance and longevity.

cow; milk; lactation; sub clinical ketosis; negative energy balance; acetone; fat/crude protein ratio; diagnostic threshold value; prediction

INTRODUCTION

Subclinical ketosis

Ketosis as a common disorder in productive dairy cow energy metabolisms is the result of energy defficiency mostly during the postpartum period. The disease arises mostly via the metabolic liberation of body fat reserves in a negative energy balance (NEB), in particular in the calving and postcalving period. Apart from milk yield losses, it can cause a fatal end to the suffering animal as well. Ketosis causes partial anorexia, depression and increased ketone levels in body fluids such as blood and urine including raised acetone levels in milk (Steger et al., 1972; Majewska, Rybczyňska, 1978; Unglaub, 1983; Andersson, 1984; Andersson, Lundström, 1984a, b; Gravert et al., 1986; Diekmann, 1987; Mottram, 1996; Mottram, Masson, 2001; Mottram et al., 2002). Blood and milk acetone are highly correlated (r = 0.96; Enjalbert et al., 2001). Prevention is the key for reducing the economic losses caused by ketosis. In this regard, prevention of the subclinical stages of ketosis through effective diagnosis and monitoring are of paramount importance (Andersson, 1988; Hanuš et al., 1999, 2001). For this purpose a number of milk indicators can potentially be used including fat content/crude protein content (F/CP) coefficient (Table 1; Heuer et al., 2001) and acetone (AC) levels in milk. The diagnostic levels (discrimination limits of ketones and AC) are still not uniform in the literature. These range from 2 to 41 mg.l^{-1} (from 0.03 to 0.7 mmol.l^{-1}; Gustafsson, Emanuelson, 1993) for milk AC though most studies describe from 7 to 23 mg.l^{-1} (from 0.12 to 0.4 mmol.l^{-1}; Gravert et al., 1986; Hanuš et al., 1999; Miettinen, 1995; Gasteiner, 2000). Milk ketone or AC discrimination (diagnostic threshold) limit determination is based on the definition of subclinical ketosis as ketosis without clinical signs in the cows. This is sometimes claimed to be a certain daily milk yield loss (Gustafsson, Emanuelson, 1993) during early lactation and sometimes as a certain body condition score loss (BCS; Hanuš et al., 1999). The

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Table 1. Interpretation of the fat content/crude protein content (F/CP) ratio in cow milk (modified according to: Agabriel et al., 1991, 1992; Bíro et al., 1992; Fürll et al., 1992; Geishauser, Ziebell, 1995; Schulz, 1997; Gasteiner, 2000).

Interpretation:	Too low F/CP	Suitable F/CP	Too high F/CP
– in relation to the nutritional physiology of dairy cows (individual milk samples):			
• for Holstein dairy cows	< 1.05	from 1.05 to 1.18	> 1.18
• for combined and milk breeds (in Germany)	< 1.1 – shortage of structural fiber in feeding ration	from 1.1 to 1.6	> 1.6 – shortage of energy, risk of ketosis
– in relation to cheesemaking technology (bulk and tank milk samples):			
• milk in general in France	< 1.10	from 1.10 to 1.20 from 1.14 to 1.18 – the best technological values	> 1.20

BCS method has been evaluated in terms of predicting dairy cow NEB (Heuer et al., 1999; Gasteiner, 2003) in relation to ketosis prevention. Some authors have approached ketosis prevention via modification of the traditional or special (medication) nutrition in suspicious dairy cows in early lactation (Emery et al., 1964; Vojtíšek et al., 1991; Jagoš et al., 1991; Miettinen, 1995; Millward, Wijesinghe, 1998; Green et al., 1999; Gasteiner, 2003; Tedesco et al., 2004). Emergency resolution to a ketosis situation was experimentally achieved by treating the cows with glucose and insulin administration (Sakai et al., 1993).

There are now other good methods for dairy cow energy balance prediction for the practical purposes of prevention. One is body weight change (Heuer et al., 2001) and postpartum body condition scoring (Heuer et al., 1999) in commercial dairy herds as mentioned already is another. Fat dairy cow before parturition is an important ketosis predisposition (BCS > 4; Gasteiner, 2003). However, monitoring milk composition is also possible.

In general, the problems with ensuring sufficient energy nutritional sources for dairy cows in terms of natural and preserved fodder crop feedstuffs, especially in the less favourable areas are acknowledged. Milk acetone levels are not influenced significantly by altitude (geometrical mean 1.8 in lowland versus 1.7 $mg.l^{-1}$ in highland; Hanuš et al., 2005). Genetic (breed) aspects of ketosis and subclinical ketosis and milk acetone levels have been studied in a number of papers (Emanuelson, Andersson, 1986; Gravert et al., 1991; Mäntysaari et al., 1991; Hanuš et al., 2003; Wood et al., 2004). Use of milk AC during lactation as an energy state indicator for genetic improvement of feed receipt and energy utilization in dairy cows has been recommended (Brandt et al., 1985; Diekmann, 1987; Gravert et al., 1986, 1991).

Regeneration of dairy cow's metabolism

The regenerative ability of living matter is a very important physiological phenomenon and undoubtedly linked to longevity. The loss of the ability to regenerate determines the extinction of live animals and finally their death. This rule good holds for both natural conditions and house and domestic animals which are slaughtered as a rule, as well. The regeneration ability in terms of the plant and animal kingdom is shown in Fig. 1 (hypothetical rendering). The high regeneration ability of plants and lower animals with lower degrees of life matter ordering is generally well known. Acomparable expression, for cells tissues and organs in higher animals or vertebrates and mammals, is shown in Fig. 2 (hypothetical graph). The higher the cell, tissue and organ specialisation (life matter specialisation), the lower the biological regeneration ability.

People often work with mammals in the context of animal production. Milk production is connected with the biological manifestation of the mammals directly. The significance of the risk of cow production disorders as it is reflected in milk production results from both (Figs 1 and 2). Production disorders, such as postpartum paresis, NEB, ketosis, fatty liver syndrome, liver steatosis, nitrogen-energy nutrition imbalance (nitrogen metabolism overloading), alkalosis, acidosis, caused by unsatisfactory fulfillment of the dairy cow life demands and from errors of nutrition can influence the liver, which is the main metabolic organ of the organism, in a negative way.

The production disorders cause and mutually influence each other. The problems start usually with milk fever (Geishauser et al., 1997, 1998). Placenta retention, ketosis, steatosis, abomasum distortion, mastitis, alkalosis, acidosis and laminitis very often follow. The developmental direction and causal sequences of these disorders depend on the initial deviation of the metabolic balance in high yielding dairy cows. A whole complex of reactions is accompained by peripartum immunosupression usually which apart from metabolic changes of early lactation and nutritional mistakes of farmer can also be caused by large shifts in blood serum hormone levels.

The liver, with only moderate regenerative ability (Fig. 2) can be damaged in irreversible ways by some production disorders. In the case, when an animal does not die immediately, its progeny will be permanently reduced and the animal will be more susceptible to infectious diseases such as mastitis and metritis. For this reason preventing liver damage in dairy cows requires thorough attention. In this regard, modern procedures for regular analyses of individual milk indicators can accomplish monitoring the health status and production disorder pre-

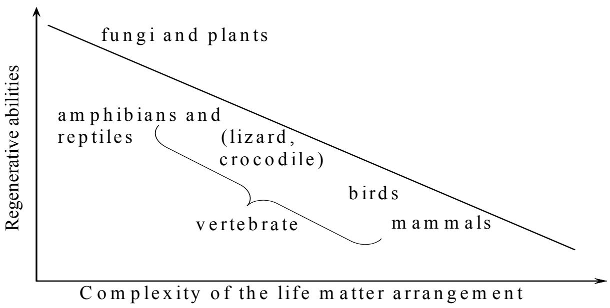

Fig. 1. Regeneration in biology – restoration of damaged integrity and functions at a level of biological taxonomy
Regenerative ability differs and generally increases with lower arrangement of life matter or biological empires according to orders, classes, species and strains as biological taxonomical units.

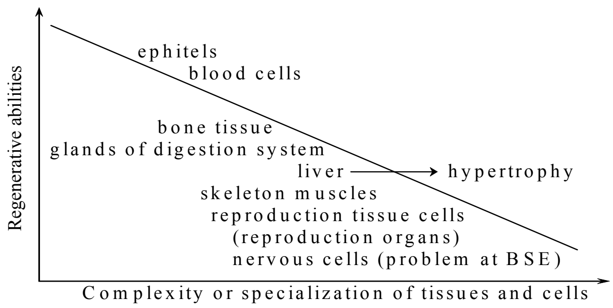

Fig. 2. Regeneration of animal cells, tissues and organs in mammals
Regenerative ability differs and generally decreases with increased complexity or specialization of cells, tissues and organs. Regeneration means compensation and restoration of damaged tissue by tissue, which is equivalent in terms of physiology. Compensatory hypertrophy means cell increase beyond normal organ size. This is found in liver under specific circumstances but there is a quite different situation in liver and ovaries when cells are infiltered by fat and lose their physiological functions (ketosis, liver steatosise, fatty liver syndrome).

vention in dairy cows to a great extent routinely. According to the changes, it is very often possible to assess the character of the hazard and predict the consequent production disorders.

The aim of this study was: – to carry out an analysis of the relationships between the F/CP coefficient and acetone (AC) concentration, as the main milk indicators related to ketosis; – to contribute to improving milk indicator discrimination limit estimation to better identification and diagnosis subclinical ketosis; – to specify the optimal conditions for using these indicators (F/CP and AC).

MATERIALS AND METHODS

Design of the investigation

The individual milk samples were taken over three years (MSs; n = 960). The milk was sampled regularly in the summer (August and September) and winter (February and March) season. Twelve healthy (mastitis free) dairy cows with above average for herd milk yield from each herd were included in the one sample set. These represented an average number of lactations in relation to the herd and whole lactation profile in terms of average day in milk. 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 Cattle (C) is a breed with combined milk and beef yield (on the Simmental basis) and Holstein (H) dairy breed. The two 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 per lactation varied in range from 5,500 and 10,000 kg. It was between the mean and high level of milk yield in the country.

Analyses of the individual milk samples

The MSs were transported in cold state (< 10 °C) in thermoboxes to accredited dairy laboratory of the institute. The investigated milk indicators were as follows: fat (F) content (in g/100 ml = %); crude protein (CP) content (in g/100 g = %); milk acetone concentration (AC, in mg.l^{-1}). The F content was determined using the instrument MilkoScan 133B (Foss Electric, Denmark), which was regularly calibrated according to the reference method (standard ČSN 57 0536, by the Gerber's acidobutyrometrical method for fat content, according to the standard ČSN 57 0530). The CP content was determined by the reference Kjeldahl's method according to standard ČSN 57 0530. The AC content was investigated by spectrophotometry at 485 nm wavelength. The acetone was absorbed into an alkaline solution of KCl with the salicylaldehyde by 24 hours microdiffusion (O'Moore, 1949; Vojtíšek, 1986; Vojtíšek et al., 1991) in special glass vessels (at 20 °C in darkness). The Spekol 11 instrument (Carl Zeiss Jena, Germany) was calibrated by the five points on the standard scale with increased acetone concentration in the calibration samples from 1 to 20 mg.l^{-1}. However, other ketone and acetone detection methods were and still are under the development (Geishauser et al., 1997; Hansen, 1999; Hanuš et al., 1999, 2001; Heuer et al., 2000; Carrier et al., 2004; Roos et al., 2006) and at first of all the stable tests and FT (Fourier transformation) infrared spectroscopy.

Statistical procedures

Standard statistical methods were used to evaluate the results (means, standard deviations and coefficient of variation). Differences between groups were tested in terms of breed (as population is important factor (B i e d e r m a n n et al., 2003, 2004), lactation number and season using Student's *t*-test. Linear and non linear regression were used for the correlations using relevant regression equations in the same groups. Correlation and regression coefficients were the most considered test statistics.

The original AC values were log transformed (M e l o u n, M i l i t k ý, 1992, 1994; R o o s et al., 2006; J a n ů et al., 2007; H a n u š et al., 2007) as the data were not normally distributed. In this case, the arithmetical mean is not suitable parameter for representation of these data sets in all cases. It made possible the standard statistical testing for work with geometrical means as well.

RESULTS AND DISCUSSION

The differences within milk indicators in dependence on some factors

The mean values of the milk indicators are shown in Table 2. They are distributed into two groups for whole lactation and the first third of lactation (FTL). There were no significant differences between investigated milk indicators such as F/CP ratio and AC concentration between whole lactation and FTL (Table 2). Logically, significantly lower crude protein content was found in the FTL (Table 2). This is explainable as the NEB which very probably existed in these dairy cows. Also the milk fat and AC were slightly lower and higher (both more variable) in terms of the facts relating to this stage of lactation.

The differences in terms of breed, number of lactation and season for milk F/CP ratio and AC are shown in Table 3. During the whole lactation these differences were mostly non significant. Significant differences were found in the FTL. Significantly higher concentrations of AC were found in the H breed at the beginning of than the C breed. The F/CP ratio was significantly higher in the multiparous dairy cows compared to the first lactation. This was observed at the beginning and during whole lactation (Table 3). The differences between seasons were not significant for all indicators (Table 3). This does not coincide with our previous findings (H a n u š et al., 1999) when we found significantly higher AC contents during winter season. This could be due to that dairy cows in the previous investigation were fed by silage during the winter season and a mixture of silage with green forage during the summer season. In contrast, the dairy cows in the present investigation were fed a totally mixed ration of preserved fodder components (silages) continuously the whole year. Significantly higher AC contents in the winter season (from the first investigation) could be caused by feeding more ketogenic feedstuff during the winter. It means in a secondary way.

H a n u š et al. (1999) also suggested significantly higher average AC levels in the dairy cows with the onset of the NEB (high body condition score losses during FTL) in comparison to dairy cows with normal postpartum body condition losses ($P < 0.001$), where the arithmetic means were $9.3 \pm 13.3 > 6.4 \pm 7.0$ mg.l^{-1} and corresponding geometrical means were $6.0 > 4.4$ mg.l^{-1} (n = 1,559 and 5,193). The difference was 2.9 mg.l^{-1}. Ř í h a and H a n u š (1999) and H a n u š et al. (2000) also reported significant ($P < 0.05$) worsening of reproductive performance in dairy cows (service period by 19 days and insemination index by 0.27) in relation to increase in AC (from < 5 to > 20 mg.l^{-1}). Lactation is part of reproduction and a very important economical factor (B e z d í č e k et al., 2007, 2008). This could be endangered by ketosis, of course. Similar negative subclinical ketosis impacts were also mentioned by K a u p p i n e n (1984), A n d e r s s o n and E m a n u e l s o n (1985), H a r a s z t i and Z ö l d a g (1990), M i e t t i n e n (1994, 1995) and G a s t e i n e r (2000). These studies show the connection of high AC with worsening dairy cow health state in terms of reproduction and energy metabolism in terms of NEB.

Mutual relationships between acetone concentration and F/CP ratio in milk

Most of the investigated relationships F/CP ratio × log AC concentration were significant. It is clear (Tables 4 and 5) that all relationships were positive and closer during FTL (Table 5) compared to whole lactation (Table 4). These relations were investigated to explain the impacts of various factors such as breed, stage and number of the

Table 2. Basic statistics for investigated milk indicators

Data set	*n*	MY *x* ± *sd*	AC *x* ± *sd*	log AC *x* ± *sd* *xg*	F *x* ± *sd*	CP *x* ± *sd*	F/CP *x* ± *sd*
Whole lactation	960	26.41 ± 7.995	2.86 ± 3.919	0.2521 ± 0.457 1.79	3.94 ± 0.897	3.32 ± 0.349	1.19 ± 0.266
First third of lactation	327	28.65 ± 7.862	3.31 ± 5.384	0.2894 ± 0.46 1.95	3.86 ± 0.973	3.20 ± 0.333	1.21 ± 0.308
Statistical significance of difference (*t*-values)		4.40 ***	1.62 ns	1.27 ns	1.36 ns	5.44 ***	1.13 ns

n – number of cases, *x* – arithmetical mean, *sd* – standard deviation, *xg* – geometrical mean, MY = milk yield in kg/day, AC = acetone concentration in milk (mg.l^{-1}), F – milk fat content (g.100 ml^{-1}, %), CP – milk crude protein content (g.100 g^{-1}, %); statistical significance: ns = $P > 0.05$, * $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, t = t-test value, abbreviations are valid for all tables and figures

Table 3. Mean values for acetone content and F/CP ratio in whole lactation and in first third of lactation

Set	Whole lactation							First third of lactation						
	n	AC	log AC	F/CP	AC	log AC	F/CP	*n*	AC	log AC	F/CP	AC	log AC	F/CP
		x ± *sd*	*x* ± *sd* *xg*	*x* ± *sd*	*t*-value				*x* ± *sd*	*x* ± *sd* *xg*	*x* ± *sd*	*t*-value		
C breed only	477	2.62 ± 2.584	0.2316 ± 0.453 1.70	1.18 ± 0.259	1.94 ns	1.38 ns	0.58 ns	163	2.72 ± 3.058	0.235 ± 0.451 1.72	1.2 ± 0.302	1.96 *	2.13 *	0.88 ns
H breed only	483	3.11 ± 4.882	0.2724 ± 0.461 1.87	1.19 ± 0.274				166	3.88 ± 6.911	0.3427 ± 0.464 2.20	1.23 ± 0.314			
First lactation only	272	2.96 ± 4.069	0.2488 ± 0.467 1.77	1.16 ± 0.239	0.46 ns	0.14 ns	2.10 *	81	3.41 ± 4.926	0.327 ± 0.403 2.12	1.15 ± 0.282	0.19 ns	0.84 ns	2.03 *
Other lactations	688	2.83 ± 3.86	0.2535 ± 0.454 1.79	1.2 ± 0.276				248	3.28 ± 5.534	0.2771 ± 0.478 1.89	1.23 ± 0.314			
Winter season	480	2.95 ± 3.731	0.238 ± 0.493 1.73	1.2 ± 0.246	0.71 ns	0.96 ns	1.16 ns	158	3.05 ± 4.149	0.2475 ± 0.4800 1.77	1.23 ± 0.267	0.84 ns	1.62 ns	0.88 ns
Summer season	480	2.77 ± 4.1	0.2663 ± 0.418 1.85	1.18 ± 0.285				171	3.55 ± 6.317	0.3300 ± 0.4390 2.14	1.21 ± 0.342			
C first lactation	130	2.36 ± 2.423	0.1799 ± 0.474 1.51	1.15 ± 0.22				30	2.43 ± 3.174	0.2274 ± 0.338 1.69	1.09 ± 0.215			
C other lactations	347	2.71 ± 2.639	0.251 ± 0.444 1.78	1.2 ± 0.271				133	2.79 ± 3.04	0.2368 ± 0.474 1.73	1.23 ± 0.314			
H first lactation	142	3.5 ± 5.081	0.3119 ± 0.454 2.05	1.17 ± 0.256				51	3.98 ± 5.664	0.3855 ± 0.429 2.43	1.19 ± 0.311			
H other lactations	341	2.94 ± 4.795	0.2559 ± 0.464 1.80	1.2 ± 0.281				115	3.84 ± 7.42	0.3237 ± 0.48 2.11	1.24 ± 0.316			
C winter season	238	2.8 ± 3.033	0.2015 ± 0.521 1.59	1.18 ± 0.251				83	2.55 ± 3.148	0.1607 ± 0.506 1.45	1.22 ± 0.291			
C summer season	239	2.43 ± 2.032	0.2617 ± 0.372 1.83	1.19 ± 0.266				80	2.9 ± 2.971	0.3121 ± 0.373 2.05	1.19 ± 0.313			
H winter season	242	3.11 ± 4.309	0.2739 ± 0.463 1.88	1.22 ± 0.239				75	3.6 ± 4.995	0.3435 ± 0.432 2.21	1.25 ± 0.237			
H summer season	241	3.11 ± 5.406	0.2709 ± 0.46 1.87	1.16 ± 0.303				91	4.11 ± 8.181	0.342 ± 0.492 2.20	1.21 ± 0.366			

C – Czech Fleckvieh, H – Holstein, AC – milk acetone concentration, F/CP – fat/crude protein ratio, log – decimal logarithm, *n* – number of cases, *x* – arithmetical mean, *xg* – geometrical mean, *sd* – standard deviation; ns, *, ** and *** = statistical significance = $P > 0.05$, < 0.05, < 0.01 and < 0.001

Table 4. The relationships between milk indicators F/CP (*x*) and log AC (*y*) over whole lactation according to breed, number of lactation and season

Set	Number of samples	Equation form	Coefficient or index of correlation	Coefficient of determination (%)	Note
Whole sample set	960	$y = 0.3939x - 0.2157$	0.23***	5.3	Fig. 3, I
C breed only	477	$y = 0.2372x^2 - 0.2894x + 0.2261$	0.19***	3.5	
H breed only	483	$y = -0.0905x^2 + 0.6998x - 0.4263$	0.28***	7.8	
First lactation only	272	$y = 0.4354x - 0.2563$	0.22**	5.0	
Other lactations	688	$y = 0.116x^2 + 0.0861x - 0.0252$	0.24***	5.5	
Winter season	480	$y = 0.3846x - 0.2232$	0.19***	3.7	
Summer season	480	$y = 0.406x - 0.2113$	0.28***	7.7	
C first lactation	130	$y = 0.4341x - 0.3172$	0.20*	4.1	
C other lactations	347	$y = 0.3082x^2 - 0.5203x + 0.4094$	0.18*	3.4	
H first lactation	142	$y = 0.411x - 0.1705$	0.23**	5.4	
H other lactations	341	$y = 0.4938x - 0.3363$	0.30***	9.0	
C winter season	238	$y = 0.1399x^2 + 0.1976x - 0.2348$	0.27***	7.0	
C summer season	239	$y = 0.3504x^2 - 0.7987x + 0.6913$	0.13ns	1.8	
H winter season	242	$y = -0.2461x^2 + 0.8369x - 0.3666$	0.11ns	1.2	
H summer season	241	$y = 0.6486x - 0.4843$	0.43***	18.3	

C – Czech Fleckvieh, H – Holstein; statistical significance: ns = $P > 0.05$, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

Table 5. The relationships between milk indicators F/CP (x) and log AC (y) in first third of lactation according to breed, number of lactation and season

Set	Number of samples	Equation form	Coefficient or index of correlation	Coefficient of determination (%)	Note
Whole sample set	329	$y = 0.4857x - 0.3002$	0.33***	10.6	Fig. 3, II
C breed only	163	$y = 0.2453x^2 - 0.21x + 0.111$	0.30***	8.9	Fig. 3, III
H breed only	166	$y = -0.2176x^2 + 1.1012x - 0.6588$	0.36***	13.0	Fig. 3, IV
First lactation only	81	$y = 0.4721x - 0.2179$	0.33**	10.9	Fig. 3, V
Other lactations	248	$y = 0.5068x - 0.3481$	0.33***	11.1	Fig. 3, VI
Winter season	158	$y = 0.6315 \ln x + 0.1305$	0.29***	8.2	
Summer season	171	$y = 0.4935x - 0.263$	0.38***	14.7	
C first lactation	30	$y = -1.3603x^2 + 3.6254x - 2.0497$	0.41*	16.6	Fig. 4, I
C other lactations	133	$y = 0.3262x^2 - 0.4353x + 0.2483$	0.30***	9.0	Fig. 4, III
H first lactation	51	$y = 0.0887x^2 + 0.17x + 0.0494$	0.29*	8.5	Fig. 4, II
H other lactations	115	$y = -0.3557x^2 + 1.5397x - 1.0048$	0.40**	16.3	Fig. 4, IV
C winter season	83	$y = 0.6449x - 0.6246$	0.37***	13.8	
C summer season	80	$y = 0.2697x^2 - 0.4326x + 0.4199$	0.25*	6.0	
H winter season	75	$y = 0.3208 \ln x + 0.2783$	0.14ns	2.0	
H summer season	91	$y = -0.1441x^2 + 1.0226x - 0.6644$	0.48***	22.8	Fig. 5

According to Table 4

lactation (Kauppinen, 1983) and season (Rauramaa, Rajamäki, 1988; Hanuš et al., 2004). The facts confirm that both milk indicators are good parameters of the energy state of the metabolism in precisely the hazardous period for dairy cows (in FTL; *r* 0.33 > 0.23; Tables 5 and 4 and Fig. 3, II and I). It is a positive conclusion.

The relationships F/CP × log AC were regularly closer in the H breed. This means that predicting and indicating the energy state during lactation in terms of nutritional physiology could be more reliable in this breed (Tables 4 and 5 and Fig. 3, III and IV). The relationship F/CP × log AC was not essentially influenced by number of lactation (Tables 4 and 5, *r* 0.22 / 0.24 and 0.33 / 0.33; Fig. 3, V and VI) but was influenced by season (Table 4 and 5, *r* 0.28 > 0.19 and 0.38 > 0.29). Therefore estimating the lactation energy state (ketosis indication) using the indicators described could be more reliable during the summer feeding period.

The relationships, which could lead to more exact estimations of the discrimination limits (DLs) for subclinical ketosis indication during the FTL are shown in Fig. 4 (from I to IV; Table 5). The corresponding reciprocal relationships are demonstrated in Table 6. The closest relationship F/CP × log AC was found in the H breed during the summer feeding period ($r = 0.48$, $P < 0.001$; Table 5;

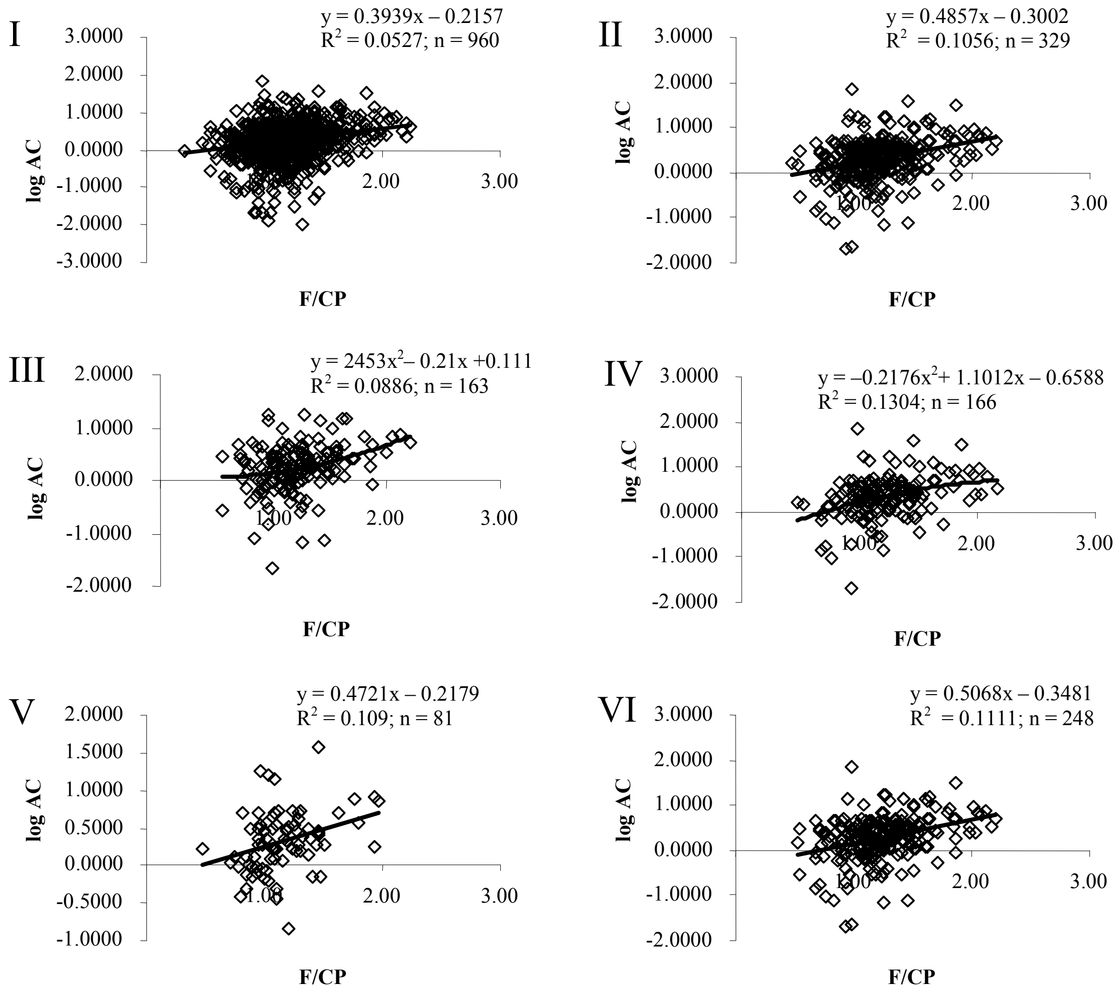

Fig. 3. The relationships between milk indicators F/CP ratio and acetone concentration (log AC) over the whole lactation (I) and in first third (from II to VI) of dairy cow lactation (according to Tables 4 and 5)

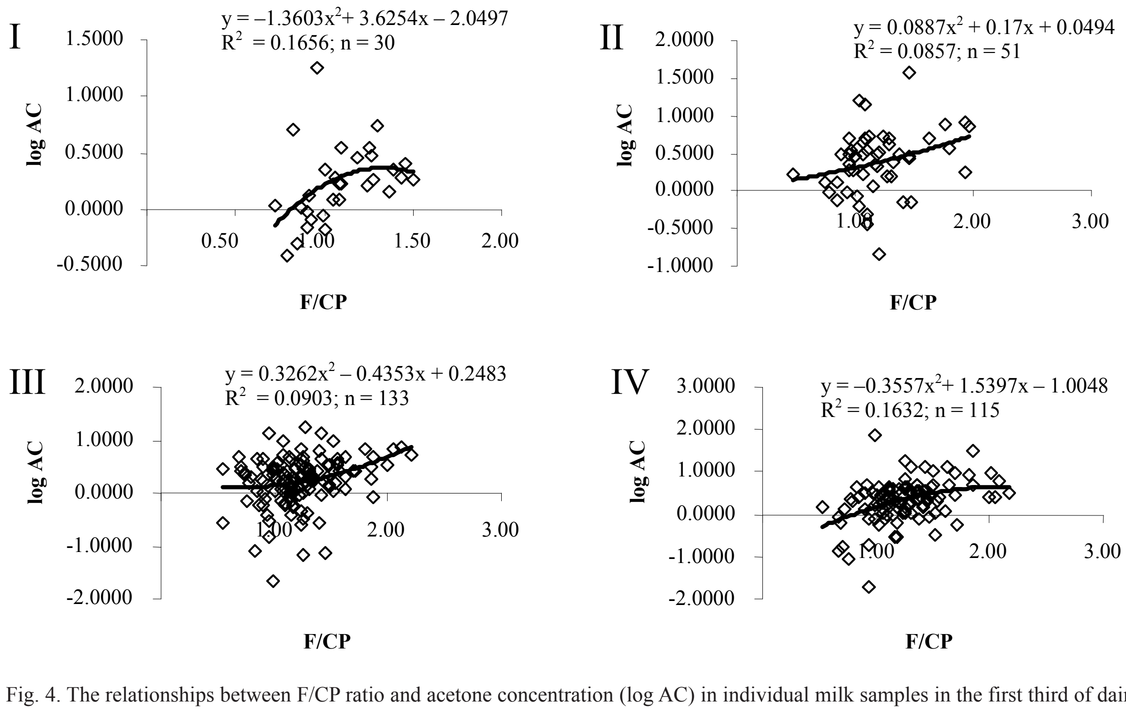

Fig. 4. The relationships between F/CP ratio and acetone concentration (log AC) in individual milk samples in the first third of dairy cow lactation (according to notes in Table 5)

Table 6. The relationships between milk indicators log AC (x) and F/CP (y) and F/CP (x) and log AC (y) in first third of lactation according to breed and number of lactation

Set	Equation form	
	log AC × F/CP	F/CP × log AC
C first lactation	$y = 0.2323x + 1.0412$	$y = 0.5725x - 0.399$
H first lactation	$y = 0.2111x + 1.108$	$y = 0.0887x^2 + 0.17x + 0.0494$
C other lactations	$y = 0.0919x^2 + 0.1829x + 1.1571$	$y = 0.4272x - 0.287$
H other lactations	$y = 0.2558x + 1.1592$	$y = -0.3557x^2 + 1.5397x - 1.0048$

Table 7. The results of reciprocal estimation of discrimination limits for milk indicators such as aceton content and F/CP ratio for the purpose of diagnosing low dairy cow energy metabolism in first third of lactation

Milk indicator	Previous discrimination limit	Specification	Estimated improved discrimination limit
AC	10 mg.l^{-1}	C first lactation	1.27 F/CP
	10 mg.l^{-1}	H first lactation	1.32 F/CP
	10 mg.l^{-1}	C other lactations	1.52 F/CP
	10 mg.l^{-1}	H other lactations	1.42 F/CP
F/CP	C 1.60	C first lactation	3.3 AC
	H 1.18	H first lactation	2.4 AC
	C 1.60	C other lactations	2.5 AC
	H 1.18	H other lactations	2.1 AC

According to equations in Table 6

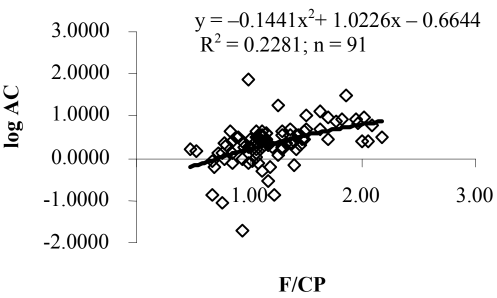

Fig. 5. The closest relationship between F/CP ratio and acetone concentration (log AC) in individual milk samples in Holstein dairy cows during their first third of lactation and summer sampling period (according to note in Table 5)

Fig. 5). This means that 22.8% of all variations in the AC content are explainable by variations in the F/CP ratio.

The discrimination limit estimation for milk indicators for the energy metabolism hazard

The DLs of AC and for F/CP ratio have been described in previous papers. These were done in terms of the subclinical ketosis and NEB diagnosis in the dairy cows, AC from 7 to 23 mg.l^{-1} (G r a v e r t et al., 1986; M i e t t i n e n , 1995; G a s t e i n e r, 2000) and F/CP according to Table 1. E n j a l b e r t et al. (2001) stated that this milk AC diagnostic threshold value (DL) could be about 0.2 mmol.l^{-1} (11.6 mg.l^{-1}). H a n u š et al. (1999, 2001) used from 7 to 10 mg.l^{-1} depending on season (summer and winter). Furthermore, the F/CP ratio is also considered a reliable indicator of milk useability for cheesemaking (Table 1). It may be possible to improve somewhat the estimations of DLs for both indicators via derivation. This could be made more exact by reciprocally puting these DLs into equations used for calculations in this analysis. Such improvement could be understood in terms of the reliability increasing of the DLs for the diagnosis of energy metabolism disorders in dairy cows. Also H e u e r et al. (2001) reported an improvement of NEB prediction with test-day information (for instance F/CP) as feasible.

The DLs 10 mg.l^{-1} for milk AC and 1.60 and 1.18 (Table 1; C and H) for F/CP ratio were substituted into prediction equations for FTL (Table 6) and their reciprocal equivalents. The calculated values are shown in Table 7 as the mutually altered estimations of the DLs. These are specified for the FTL according to breeds and numbers of lactations. The altered DL estimation for the F/CP ratio could be 1.27 and 1.52 for C breed on the first and other lactations. Similarly it is equal to 1.32 and 1.42 for H dairy cows. This means milder in the first case and more benevolent in the second case compared to the literature sources mentioned (Table 1). In the reciprocal procedure further estimated DLs for the AC could be 3.3 and 2.5 mg.l^{-1} in the C dairy cows and 2.4 and 2.1 mg.l^{-1} in the H breed. While the new estimations of the F/CP ratio are different from the originally proposed values (1.60 and 1.18) only by –12.8% (C) and 16.1% (H) at the new DL estimations for the milk AC these are different essentially by –71.0% (C) and –77.5% (H). The differences are caused probably by the fact that original proposals were directly linked with degree of the subclinical ketosis, which was simultaneously estimated by some other appropriate method. Therefore our estimations of the DLs for the AC according to the previously proposed F/CP ratios could be probably too strict, which means lower. Despite this fact, our estimations of the DLs for the F/CP ratios according to the previously proposed AC levels could be more reliable than the original values. These are likewise mutually closer on average for both breeds or 1.39 for C (1.27 and 1.52) and almost coincident 1.37 for H (1.32 and 1.42).

CONCLUSIONS

Milk acetone level and F/CP ratio are good indicators for monitoring and prevention checks of subclinical ketosis in dairy cows owing to the high correlations between them, especially in FTL period. Milk AC levels differed insignificantly between Holstein and Czech Fleckvieh breed in whole lactation but differed significantly in FTL. Discrimination limits of F/CP according to relevant milk AC threshold for subclinical ketosis were estimated via modelling. The results could be used in a practical advisory service to ensure milk quality and adequate dairy cow health, reproduction performance and longevity.

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HANUŠ, O. – YONG, T. – KUČERA, J. – GENČUROVÁ, V. – DUFEK, A. – HANUŠOVÁ, K. – KOPEC, T.
(Agrovýzkum Rapotín, Česká republika; Anhui Agricultural University, Hefei, Čínská lidová republika; Svaz chovatelů českého strakatého skotu, Praha, Česká republika; Výzkumný ústav pro chov skotu, Rapotín, Česká republika):

Predikční hodnota a korelace dvou mléčných ukazatelů v monitoringu energetického metabolismu dvou plemen dojnic.

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Ketóza je porucha energetického metabolismu dojnic, zhoršuje mléčnou užitkovost, kvalitu mléka a reprodukci a může mít fatální dopad na zvíře. Aceton v mléce (AC) v kombinaci s poměrem tuk/hrubé bílkoviny (F/CP) může být dobrým ukazatelem ketózy. Prevence onemocnění je v praxi velmi významná. Bylo analyzováno 960 individuálních vzorků mléka (MSs) od plemen (1 : 1) dojnic holštýnské (H) a české strakaté (C) z jejich celé laktace a letní a zimní sezony za tři roky. Mléčná užitkovost sedmi zahrnutých stád kolísala od 5 500 do 10 000 kg. Hodnoty AC byly logaritmicky transformovány. Pomocí statistických parametrů a regresní analýzy byly hodnoceny vlivy chovatelských faktorů na AC a F/CP. Hladiny AC se mezi plemeny H a C lišily nevýznamně ($P > 0{,}05$) v celé laktaci (1,87 a 1,70 mg.l^{-1} jako geometrické průměry), ale lišily se významně ($P < 0{,}05$) v první třetině laktace (FTL; 2,20 > 1,72 mg.l^{-1}). Poměr F/CP se mezi plemeny nelišil. Významné rozdíly nebyly konstatovány pro oba ukazatele mezi zimní a letní sezonou. AC a F/CP jsou dobré ukazatele pro monitoring a prevenci subklinické ketózy u dojnic pro jejich dobrý korelační vztah (0,23, 0,28 H, 0,19 C; $P < 0{,}001$), zejména v FTL (0,33 /n = 329/, 0,36 H, 0,30 C; $P < 0{,}001$). Ukazatele korelovaly více v letní než zimní sezoně (0,38 > 0,29, $P < 0{,}001$, v FTL). Nejtěsnější vztah byl nalezen u krav H v FTL v létě (0,48, $P < 0{,}001$). Diskriminační limity pro subklinickou ketózu, které byly odvozeny od F/CP modelově podle relevantního prahu AC (10 mg.l^{-1}) pomocí vhodných rovnic, by mohly být pro první a další laktace 1,32 a 1,42 (H) a 1,27 a 1,52 (C). Výsledky mohou být použity pro podporu dobré kvality mléka a odpovídající zdravotní stav krav, jejich reprodukci a dlouhověkost v praktickém poradenském servisu.

kráva; mléko; laktace; subklinická ketóza; negativní energetická bilance; aceton; poměr tuk/hrubé bílkoviny; diagnostická prahová hodnota; předpověď

Contact Address:

Oto H a n u š, Agrovýzkum Rapotín, Výzkumníků 267, 788 13 Vikýřovice, Česká republika, tel.: +420 583 392 157, e-mail: oto.hanus@vuchs.cz