# REFERENCE AND INDIRECT INSTRUMENTAL DETERMINATION OF BASIC MILK COMPOSITION AND SOMATIC CELL COUNT IN VARIOUS SPECIES OF MAMMALS*

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Analytical results of milk samples (MSs) are important for animal genetical improvement and health state and for control of milk food chain quality. Beside reference analyses first of all the results of effective indirect instrumental methods are important for mentioned purposes. These have to be calibrated according to reference method results for assurance of reliable results. Aim of this paper was to perform calibration of indirect methods for measurement of indicators of various biological kinds of milk and examine their suitability to reciprocal measurement of other milk kinds. Cow (CW), sheep (SP), goat (GT) and woman (WN) MSs were measured via reference and indirect methods on contents of fat (F), protein (P), lactose (L) and somatic cell count (SCC). The relevant calibrations of indirect methods were carried out according to reference results. The same MSs were measured on their own and also on foreign calibrations as calibration validation. The correlations of specific calibrations varied mostly from 0.976 to 0.999. The results showed the necessity of specific calibrations according to milk kind for reliable results of milk composition via infra-red (IR) analysis. The results of species irrelevant calibrations provided mostly unreliable results for F, P and L. For instance CW calibration underestimated P in GT and SP milk (by 0.30 and 0.26%). In the case of necessity the WN milk would be measured for F and P via GT and CW calibration with a relatively reliable result. Specific calibrations of IR method (milk F, P and L) are necessary in terms of biological kind of milk for reliable result assurrance. The correlations from 0.997 (CW) to 1.0 (GT) between reference and instrumental results at the same calibration adjustment according to CW milk for SCC. It is possible to measure the used biological milk kinds by one instrumental adjustment for SCC with reliable results.

cow; goat; sheep; human milk; analytical method; reference; calibration; components; somatic cell count

## INTRODUCTION

### Importance of basic milk analyses

The last forty years are characterized by development of effective instrumental methods in dairy analyses. Routine laboratories use the results of such procedures to series analyses for control of milk food chain quality, for milk payment and for genetical improvement of animals and control of their nutrition and health state. Main milk composition as fat (F), crude protein (P) and lactose (L) or total solids and solids non fat contents are determined mostly via infra-red (IR) instrumental analysis (Barbano, Dellavalle, 1987; Barbano, Clark, 1989) in mid or near IR spectrum. Somatic cell count (SCC) as general indicator of milk hygiene and udder health state (as mastitis indicator) is determined mostly via fluoro-opto-electronical (FOE) instruments.

### Development of milk indirect instrumental analyses

Theoretical and technical aspects of IR milk analysis were studied and described previously by more authors (Goulden, 1964; Biggs, 1979a; Kerkhof Mogot et al., 1982; Grappin, 1987; Hanuš et al., 1995). The classical optical filter technology is commonly used in mid IR instruments (MIR) (Ardö, 1979) and whole IR spectrum with Fourier's transformations in mid (MIR-FT) or near IR procedure (Sato et al., 1985, 1987; Kukačková et al., 2000; Tsenkova et al., 2000; Jankovská, Šustová, 2003; Kráčmar et al., 2004; Šustová et al., 2006, 2007). MIR-FT technology is indeed more modern but filter MIR technology is forever broadly used in particular in North America milk laboratory systems. In SCC determination the instruments work on FOE basis with disc rotation (FOE-DR) (Ardö, 1982; Miller et al., 1986) or flow cytometry (FOE-FC)

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(Heeschen et al., 1994; Gunasekera et al., 2003). The mentioned procedures usually combine chemical and physical principles or some of them have clearly physical principle. That is why it is necessary to calibrate them according to reference method results (Grappin, Jeunet, 1974, 1975, 1976; Biggs, 1978, 1979a, b; Kerkhof Mogot et al., 1982; Lintner et al., 1984; Sjaunja, 1984a; Szijarto, Barnum, 1984; Sjaunja, Andersson, 1985; Vines et al., 1986; Grappin, 1987; Biggs et al., 1987; Ng-Kwai-Hang et al., 1988; Barbano et al., 1991; Arndt et al., 1991; Barbano, Lynch, 1992; Grappin, Lefier, 1993; Hanuš et al., 1993a, b, c, 1995). Reference methods have a direct character. The calibrations have to be repeated regularly in relevant intervals. In general, there are more effects, which can influence the calibration and results, season, feeding of animals (Franke et al., 1977), reparation of basic parts of instrument, age of samples and their possible lipolysis (Sjaunja, 1984c; Hanuš et al., 1992), their preservation (Ardö, 1979; Ng-Kwai-Hang, Hayes, 1982) and treatment (Sjaunja, 1984b, c; Sjaunja et al., 1984; Coleman, Moss, 1989). Some of effects are called interferential effects. One of them could be also animal species, it means biological kind of milk. Instrumental calibrations in routine laboratories are performed most frequently for cow milk. In the case that other biological kinds of milk are analysed, the specific calibrations are performed as far as the number of samples is economically significant. The analyses of other biological milk kinds on traditional cow calibration are carried out occasionally as well. Of course, such a solution could cause unreliable results sometimes. The importance of other milk analyses, for example in goats and sheep herd, is growing up day by day (Grappin, 1987; Hanuš et al., 1996; Antunac et al., 2001; Kráčmar et al., 2004; Šustová et al., 2006; Genčurová et al., 2008; Macek et al., 2008). Similar situation is with control of mammary gland health state and milk hygiene and quality by SCC (Grappin, 1987) in goats, sheep including human milk (Paape, Keller, 1985).

### Aim of the work

Various biological, physiological, chemical, physical, technical and technological reasons can cause that in some cases it could be possible to measure various milk kinds via cow IR or FOE instrumental calibration and in other cases not. Because of the mentioned facts, the goal of this paper was to perform calibration of indirect methods for measurement of indicators of various biological kinds of milk and examine their suitability to reciprocal measurement of other milk kinds.

## MATERIAL AND METHODS

### Analytical methods and instruments

As reference methods according to ČSN 57 0530 and ČSN EN ISO 13366-1 were used: acido-butyro-metric Gerber's method for fat (F) determination (%, g.100 $g^{-1}$); Kjeldahl's method via 2 200 Kjeltec Auto Distillation (Foss Tecator AB, Sweden) for crude protein (P; %, g.100 $g^{-1}$) determination (Hanuš et al., 1995); polarimetric method via polarimeter Carl Zeiss (Jena, Germany) for lactose monohydrate (L; %, g.100 $g^{-1}$) determination; direct microscopical method (light microscope Meopta, Czech Republic) for somatic cell count (SCC) determination (in thousands.$ml^{-1}$).

Two analytical instruments with indirect measurement principles (ČSN ISO 8196-2) were used: MIR apparatus MilkoScan 133B (Foss Electric, Denmark) for milk composition determination (F, P and L) according to ČSN 57 0536; FOE-DR apparatus Fossomatic 90 (Foss Electric, Denmark) for SCC investigation according to ČSN EN ISO 13366-2.

### Milk samples

The individual milk samples (MSs) of four mammal's species were obtained simultaneously: cow milk (CW; *Bos primigenius* f. *taurus*, L, 1758) from Holstein and Czech Fleckvieh breed, $n$ = 10; sheep milk (SP; *Ovis aries*, L, 1758) from Tsigai breed, $n$ = 10; goat milk (GT; *Capra aegagrus* f. *hircus*, L, 1758) from White short-haired breed, $n$ = 6; woman (mother) milk (WN; *Homo sapiens sapiens*, L, 1758), $n$ = 5. Small volumes of woman MSs had to be decanted together because of obtaining of necessary milk amount for all analyses. All samples were preserved by $K_2Cr_2O_7$ (1.3 mg.$ml^{-1}$ ) and stored in refrigerator (4 °C) before analyses.

### Design of experiment

All CW, SP, GT and WN milk samples were investigated via introduced reference methods in duplicates on the above mentioned indicators (F, P, L and SCC). The reference results were obtained in this way. The instrument MilkoScan 133B (MIR) as an indirect method was calibrated according to reference results to CW, SP, GT and WN calibration in accordance with ČSN 57 0536. After calibration all MSs were measured on their own calibration as validation in terms of biological kind and then reciprocally on foreign calibrations. It means the system CW milk reference – CW milk IR calibration and CW, SP, GT and WN milk IR measurement and reciprocally in the same way for all mammal's species. CW, SP, GT and WN samples were measured on SCC via Fossomatic 90 with one adjustment, which originated from cow milk.

### Statistical evaluation

Basic statistical characteristics of MS sets in consideration of biological kind of milk, method and indicator were calculated, such as arithmetical means ($x$), average differences ($d$) and their standard deviations ($sd$). Because of no existence of normal data frequency distribution in current somatic cell count sets the SCC data were logarithmicaly transformed in our evaluation and as a conse-

quence of this fact also geometrical means (*xg*) were used. Pair *t*-test was used for evaluation of SCC differences as well. Reference results and results obtained by indirect methods were compared and evaluated by linear regression (G r a p p i n , 1987). Relevant regression equations (slope and bias), coefficients of correlation (*r*) and residual standard deviations (*sr*) were calculated via Microsoft Excel programme.

## RESULTS AND DISCUSSION

### Evaluation of methods for milk composition determination in various milk kinds

Results MIR-FT calibrations on various biological kinds of milk are shown in Table 1. Correlations (from 0.976 to 0.999) are good with the exception of lactose in mother milk calibration. This result was realy poor. The residual standard deviations are mostly acceptable excepting lactose for SP and WN milk. These are higher. In WN it was caused probably by small value range in calibration set of MSs (Table 2).

The results of validation measurements of calibrations according to various sets of milk in terms of mammal species are shown in Table 2 as compared to reference values. These were made immediately after calibration performance. As it is seen very well, the best accordance of validation results with reference results is mostly on relevant calibration (underlined characteristics), it means CW or GT milk measured by CW or GT calibration, and so on. These results are mostly in accordance with previous relevant specifications for acceptable calibration (B i g g s , 1979a; G r a p p i n , 1987; ČSN 57 0536). In reciprocal instrumental results the accordance with reference values is much worse, for instance SP milk by CW or WN calibration. One of larger differencies against reference value is in protein at GT analyses by CW MIR calibration (–0.30%; Table 2). This difference was lower (–0.16%) but in accordance in terms of trend in our previous paper (H a n u š et al., 1996). Z e n g (1996) found out –0.27% in similar case. However, similar difference is for SP analyses by CW calibration (–0.26%; Table 2). It means that real GT SP protein results are underestimated by MIR CW calibration measurement very essentially. This fact could be explained by G r a p p i n ' s (1987) opinion that it is caused by essentially lower citrate concentration in GT milk as compared to CW milk, which have IR absorption band near instrumental protein wave length.

Previously mentioned facts mean that because of interferential effects of species specific milk matrix it is not suitable to perform the MIR milk measurement by foreign calibrations. The specific calibration for every milk kind is necessary. This opinion is in accordance with Z e n g (1996). There are more reasons for such conclusion. One of them could be also this fact that averages and value ranges of sets of reference MSs for mammal species are mutually markedly different because of seasonal effect of sampling as consequence of stage of lactation which is influenced by seasonal births in reproduction physiology of small ruminants. Of course, such effect does not exist in dairy cows under our geographical circumstances in contrast to New Zealand for instance. Also the ranges of reliability of relevant calibration lines play important role in this explanation and conclusion.

According to obtained results, in the case of necessity, it would be possible to obtain the restricted reliable results as compared to reference values only via reciprocal IR measurement of SP milk by CW MIR calibration for L, CW milk by GT calibration for L and GT milk by WN calibration for F between species of ruminants. In the case of incidental analyses of mother (woman) milk, there is not possible to suppose the existence of specific instrument calibrations in relevant laboratories because of eco-

Table 1. Result values of performed calibrations of MIR-FT for various biological milk kinds

| Biological milk kind (sample number) | Milk component | Calibration equation | | Relation between reference and instrumental method | |
|---|---|---|---|---|---|
| | | slope | bias | *r* | *sr* |
| CW | F | 1.179 | –0.15 | 0.995*** | 0.052 |
| | P | 0.999 | 0.30 | 0.998*** | 0.015 |
| (10) | L | 1.008 | –0.06 | 0.952*** | 0.059 |
| SP | F | 1.122 | 0.41 | 0.984*** | 0.211 |
| | P | 1.056 | 0.22 | 0.996*** | 0.096 |
| (10) | L | 0.912 | 0.26 | 0.966*** | 0.264 |
| GT | F | 1.053 | 0.55 | 0.996*** | 0.107 |
| | P | 1.050 | 0.40 | 0.999*** | 0.059 |
| (6) | L | 0.919 | 0.43 | 0.996*** | 0.077 |
| WN | F | 1.135 | 0.25 | 0.981*** | 0.175 |
| | P | 0.959 | 0.30 | 0.976*** | 0.023 |
| (5) | L | 0.789 | –0.06 | $0.318^{ns}$ | 0.266 |

*r* = correlation coefficient, *sr* = residual standard deviation, CW = cow milk, SP = sheep milk, GT = goat milk, WN = woman (mother) milk, F = fat, P = crude protein, L = lactose monohydrate; valid also for Tables 2 and 3 and Figs 1 and 2; statistical significance: *, ** and *** = $P < 0.05$, $P < 0.01$ and $P < 0.001$, ns = $P > 0.05$

Table 2. Results of measurements of component contents in milk of various mammals by reference and indirect instrumental methods after MIR calibration on individual milk kinds

| Milk kind | Comp. | Reference method (R) | | Method MIR, CW | | Difference MIR – R | | Method MIR, SP | | Difference MIR – R | | Method MIR, GT | | Difference MIR – R | | Method MIR, WN | | Difference MIR – R | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *n* | | *x* | *sd* | *x* | *sd* | *x* | *sd* | *x* | *sd* | *x* | *sd* | *x* | *sd* | *x* | *sd* | *x* | *sd* | *x* | *sd* |
| CW | F | 3.75 | 0.537 | 3.76 | 0.532 | 0.01 | 0.0499 | 4.13 | 0.501 | 0.38 | 0.0629 | 4.06 | 0.478 | 0.31 | 0.0770 | 4.03 | 0.511 | 0.28 | 0.0592 |
| | P | 3.33 | 0.212 | 3.33 | 0.206 | 0.00 | 0.0169 | 3.45 | 0.213 | 0.12 | 0.0169 | 3.60 | 0.219 | 0.27 | 0.0204 | 3.20 | 0.195 | –0.13 | 0.0230 |
| 10 | L | 4.84 | 0.193 | 4.85 | 0.152 | 0.01 | 0.0660 | 4.72 | 0.140 | –0.12 | 0.0729 | 4.91 | 0.144 | 0.07 | 0.0730 | 3.79 | 0.123 | –1.05 | 0.0850 |
| SP | F | 7.76 | 1.177 | 7.59 | 1.227 | –0.17 | 0.2280 | 7.74 | 1.147 | –0.02 | 0.1980 | 7.48 | 1.091 | –0.28 | 0.2240 | 7.72 | 1.166 | –0.04 | 0.2110 |
| | P | 6.20 | 1.056 | 5.94 | 0.989 | –0.26 | 0.1090 | 6.21 | 1.033 | 0.01 | 0.1020 | 6.34 | 1.037 | 0.14 | 0.0978 | 5.72 | 0.943 | –0.48 | 0.1450 |
| 10 | L | 2.55 | 1.027 | 2.47 | 1.098 | –0.08 | 0.2850 | 2.57 | 0.983 | 0.02 | 0.2676 | 2.73 | 0.999 | 0.18 | 0.2650 | 1.92 | 0.856 | –0.63 | 0.2970 |
| GT | F | 4.84 | 1.142 | 4.63 | 1.271 | –0.21 | 0.1760 | 4.96 | 1.184 | 0.12 | 0.1223 | 4.85 | 1.129 | 0.01 | 0.1080 | 4.88 | 1.212 | 0.04 | 0.1379 |
| | P | 4.36 | 1.681 | 4.06 | 1.588 | –0.30 | 0.1170 | 4.23 | 1.685 | –0.13 | 0.0668 | 4.37 | 1.670 | 0.01 | 0.0730 | 3.91 | 1.521 | –0.45 | 0.1710 |
| 6 | L | 3.73 | 0.905 | 3.57 | 0.987 | –0.16 | 0.1190 | 3.55 | 0.894 | –0.18 | 0.0794 | 3.74 | 0.899 | 0.01 | 0.0830 | 2.78 | 0.763 | –0.95 | 0.1616 |
| WN | F | 3.93 | 0.897 | 3.67 | 0.910 | –0.26 | 0.1620 | 4.15 | 1.878 | 0.22 | 0.1488 | 3.98 | 0.805 | 0.05 | 0.1640 | 3.99 | 0.869 | 0.06 | 0.1520 |
| | P | 1.46 | 0.108 | 1.52 | 0.108 | 0.06 | 0.0148 | 1.57 | 0.107 | 0.11 | 0.0228 | 1.71 | 0.112 | 0.25 | 0.0110 | 1.47 | 0.105 | 0.01 | 0.0250 |
| 5 | L | 5.72 | 0.280 | 7.30 | 0.069 | 1.58 | 0.3070 | 6.94 | 0.052 | 1.22 | 0.2926 | 7.15 | 0.063 | 1.43 | 0.3056 | 5.71 | 0.045 | –0.01 | 0.1870 |
| | | | | A | | | | B | | | | C | | | | D | | | |

Comp. = component, *x* = arithmetical mean, *d* = mean difference, *sd* = standard deviation; valid also for Table 3; underlined = $|d| < 0.10$ and $sd < 0.12$; A, B, C and D = results of calibration on various kinds of milk (for CW, SP, GT and WN in the same order) and measurement results for various kinds of milk

Table 3. Results of somatic cell count (SCC, thousands.ml$^{-1}$) determination via reference (R) and instrumental (FOE-DR) method in milk of various species of mammals before and after logarithmical transformation (ln, natural basis)

| Milk kind (*n*) | Original values | | | | | | After ln transformation | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Method R | | Method F | | Difference F – R | | Method R | | | Method F | | | Difference F – R | |
| | *x* | *sd* | *x* | *sd* | *x* | *sd* | *xg* | *x* | *sd* | *xg* | *x* | *sd* | *x* | *sd* |
| CW (10) | 204 | 140 | 229 | 135 | 25*** | 12 | 166 | 5.1119 | 0.6630 | 196 | 5.2778 | 0.5701 | 0.1659*** | 0.1075 |
| SP (10) | 3115 | 5773 | 2873 | 5134 | –242$^{ns}$ | 695 | 1125 | 7.0251 | 1.2780 | 1013 | 6.9209 | 1.3280 | –0.1042$^{ns}$ | 0.1578 |
| GT (6) | 2338 | 4280 | 2309 | 4184 | –29$^{ns}$ | 120 | 493 | 6.2010 | 1.6339 | 474 | 6.1606 | 1.6702 | –0.0404$^{ns}$ | 0.1800 |
| WN (5) | 120 | 89 | 98 | 61 | –22$^{ns}$ | 29 | 98 | 4.5802 | 0.5923 | 85 | 4.4440 | 0.5025 | –0.1362* | 0.1049 |

*xg* = geometrical mean, *n* = number of cases, R = reference, F = Fossomatic (FOE-DR)

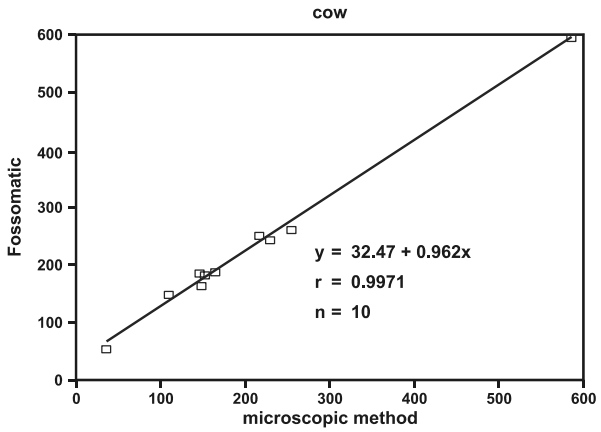
nomical reasons. Therefore, according to the mentioned results it could be possible to carry out such routine instrumental analyses via WN milk measurement by GT MIR calibration for F content (probably because of mutually comparable fat content levels and size of native fat globules), WN milk measurement by CW calibration for P content and WN milk measurement only by specific calibration for L with acceptable result reliability level.

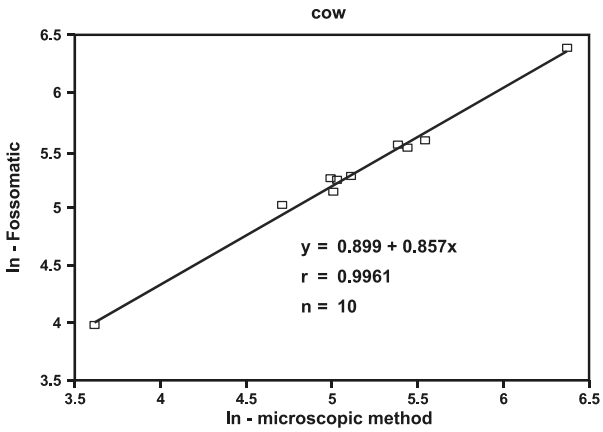
In general, there are two main reasons for specific calibration of MIR according to biological origin of MSs: 1) large difference (for example CW and GT milk from SP milk) between calibration and measured averages in terms of biological MS sets, for example CW and GT milk from SP milk – a technical reason; 2) too different interference effects exist between milk matrix of biological milk kinds, for instance between CW and GT for protein, which is given by lower citrate concentration in goat milk as compared to cow (Grappin, 1987) – a biological (physiological) reason.

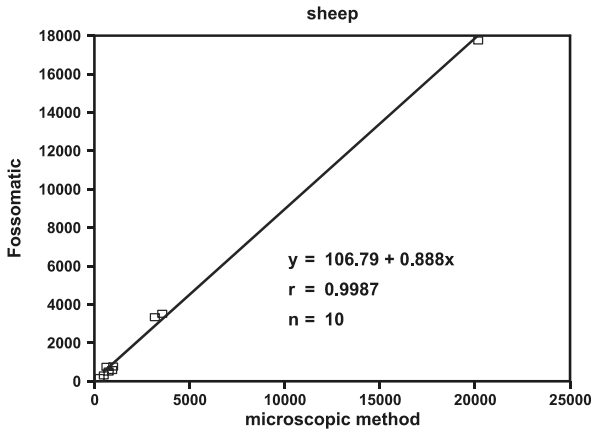
**Evaluation of methods for somatic cell count determination in various milk kinds**

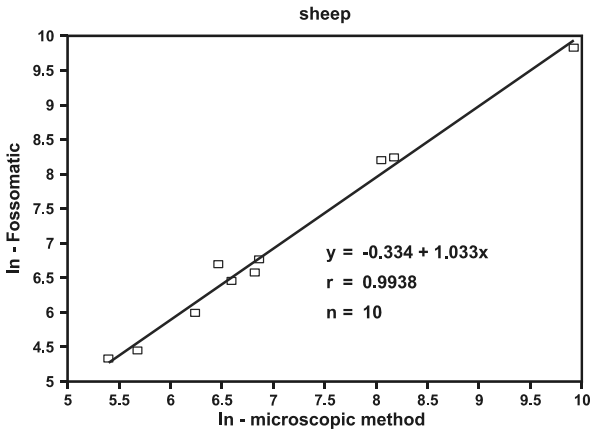
Reference and instrumental results of SCC are shown in Table 3 and Figs 1 and 2. The instrumental (FOE-DR) values obtained via one apparatus adjustment (by cow milk) are in good accordance with SCC reference values for all milk kinds (Table 3). It is valid in spite of this fact that SP and GT milk samples showed markedly higher SCC average values with high variability as compared to cow and woman milk. After logarithmic transformation the mean differences (indirect result – reference result) were sigificant for cow and mother milk ($P < 0.001$ and $< 0.05$), but these differences could be marked as practically negligible in terms of interpretation aspects. The mentioned facts are confirmed by linear regression in Figs 1 and 2. Correlation coefficients varied from 0.997 (cow milk) to 1.0 (goat milk).

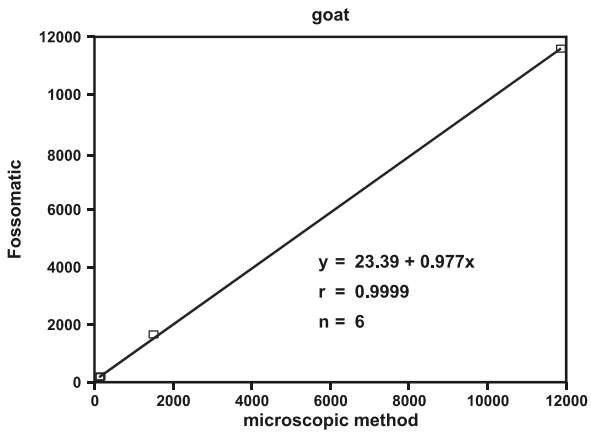
As conclusion from the mentioned facts we can state that the SCC analyses are possible for more biological kinds of milk without previous specific instrument calibration with obtaining of reults with acceptable reliability.

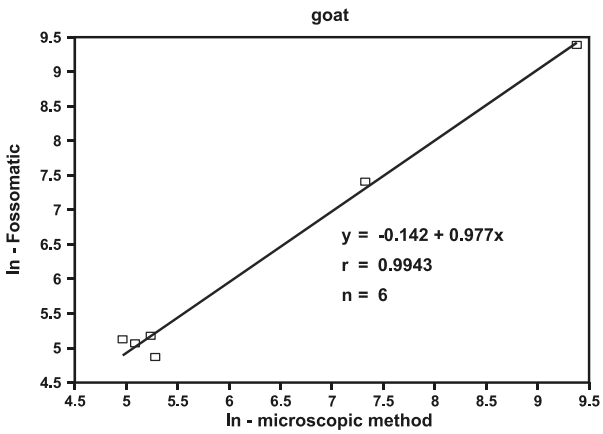


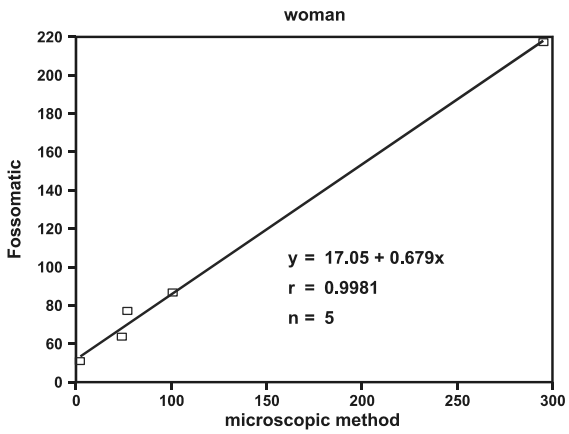



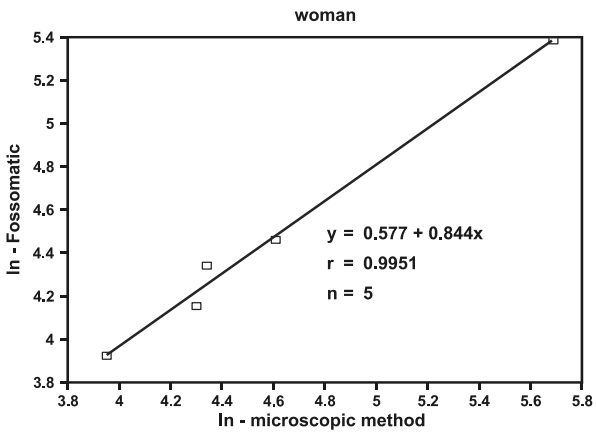




Fig. 1. Relationships between reference and instrumental determination of somatic cell count (SCC; ths./ml) in various kinds of mammal's milk

Fig. 2 Relationships between reference and instrumental determination of somatic cell count (ln SCC; ths./ml) after logarithmical transformation in various kinds of mammal's milk

This finding does not agree with results by Zeng (1996) and Zeng et al. (1999). They found higher goat SCC by 27.0 and 24.5% in the case of Fossomatic calibration by cow SCC standards. According to our results for SCC investigation there is not necessity for calibration change according to milk sample biological origin. Probably the somatic cells in various biological milk kinds are mutually more similar than corresponding milk matrices and related interference effects in the case of IR analysis. This fact enables easier analytical control of mammary gland health state by SCC counting (Paape, Keller, 1985) via same instrumental adjustment in various species of mammals.

## CONCLUSIONS

From methodical point of view: – it is necessary to perform the specific calibrations of IR instrumental method (milk fat, protein and lactose measurement) in terms of biological kind of milk because of reliable result assurrance; – it is possible to measure the used biological milk kinds by one instrumental adjustment for somatic cell count with reliable results.

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**Referenční a nepřímé přístrojové stanovení základního složení mléka a počtu somatických buněk u různých druhů savců.**

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Výsledky analýz vzorků mléka (MSs) jsou důležité pro šlechtění a zdraví zvířat a pro kontrolu kvality mléčného potravinového řetězce. Vedle referenčních analýz jsou pro daný účel důležité především výsledky efektivních nepřímých instrumentálních metod. Tyto musí být pro zajištění věrohodných výsledků kalibrovány podle výsledků metod referenčních. Cílem práce bylo provést kalibrace nepřímých metod na měření ukazatelů různých biologických druhů mléka a posoudit jejich vhodnost k recipročnímu měření jiných druhů mléka. Referenčními a nepřímými metodami byly měřeny vzorky kravského (CW), ovčího (SP), kozího (GT) a ženského (WN) mléka na obsahy tuku (F), bílkovin (P) a laktózy (L) a na počet somatických buněk (SCC). Podle referenčních výsledků byly provedeny příslušné kalibrace nepřímých metod. Tytéž MSs pak byly měřeny na svých a recipročně i na cizích kalibracích jako validace kalibrace. Korelace specifických kalibrací kolísaly většinou od 0,976 do 0,999. Výsledky ukázaly nutnost specifických kalibrací podle druhu mléka pro věrohodné výsledky složení mléka pomocí infračervené (IR) analýzy. Výsledky druhově irelevantních kalibrací poskytovaly většinou nevěrohodné výsledky pro F, P i L. Například CW kalibrace podhodnocovala P u mléka GT a SP (o 0,30 a 0,26 %). V případě potřeby by WN mléko mohlo být změřeno na F a P prostřednictvím GT a CW kalibrace s poměrně věrohodným výsledkem. Jsou nezbytné specifické kalibrace IR metody (F, P a L) ve smyslu biologického druhu mléka pro zajištění věrohodných výsledků. Pro SCC byly zjištěny korelace od 0,997 (CW) do 1,0 (GT) mezi referenčními a instrumentálními výsledky při jednom kalibračním nastavení na mléko CW. Je možné měřit použité biologické druhy mléka na SCC s jedním přístrojovým nastavením s věrohodnými výsledky.

kráva; koza; ovce; lidské mléko; analytická metoda; reference; kalibrace; složky; počet somatických buněk

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