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Comparison of urea content in milk, measured in different laboratories

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ABSTRACT

Urea content was determined by the analysis of ten milk samples with 2 to 3 successions in each of the 6 laboratories (3 in Slovenia, 3 in Czech Republic). Determination method was different, but the principle of urea determination in these laboratories was based on enzymatic and/or photometric method. The average urea content varied from 2.38 mmol/l in the laboratory E to 3.43 mmol in the laboratory D. The highest standard deviation was found in the laboratory A, and the lowest in the laboratory F. The highest VC percent was noticed in the laboratory E (51.0%) and laboratory A (44.7%). The analysis results for the difference in urea content in milk measured in different laboratories indicated the lowest difference between the laboratories A and C, and the highest between the laboratories D and E. Standard deviation of differences ranged from 0.12 and 0.46. With t-test we examined the differences between laboratories, which were in almost all instances highly statistically significant. We also calculated the correlation coefficients among laboratories, ranging from 0.96 to 0.99, and were highly statistically significant. We determined regression coefficients among these laboratories and with linear regression equation predicted values for each laboratory if a certain trait was measured in any of the other laboratories.
(Keywords: cows, milk, urea content, determination methods)

ZUSAMMENFASSUNG

Vergleichende Untersuchung des Harnstoffgehaltes der Milch in unterschiedlichen Labors

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In sechs Labors (drei in Slowenien und drei in Tschechien) wurde der Harnstoffgehalt von zehn Milchproben mit zwei bis drei Wiederholungen bestimmt. Es wurden unterschiedliche Methoden benutzt, alle basierten auf enzymatischer oder photometrischer Basis. Der durchschnittliche Harnstoffgehalt variierte von 2,38 mmol/l im Labor E bis 3,43 mmol/l im Labor D. Die höchste Standardabweichung wurde im Labor A, die niedrigste im Labor F festgestellt. Der höchste Variationskoeffizient wurde in Labor E (51%) und Labor A (44,7%) festgestellt. Die höchste Differenz zwischen zwei Labors bestand zwischen Labors D und E, die niedrigste zwischen A und C. Die Standardabweichungen der Differenzen zwischen den Labors variierte von 0,12 bis

0,46. Die t-Teste zeigten hoch signifikante Unterschiede zwischen den meisten Labors. Die berechneten Korrelationskoeffizienten in den Labors variierten von 0,96 bis 0,99 und waren statistisch hoch signifikant. Es wurden auch lineare Regressionskoeffizienten berechnet, um den Harnstoffgehalt der Milch aus den Daten in einem Labor auch in anderen Labors schätzen zu können.
(Schlüsselwörter: Kuh, Milch, Harnstoffgehalt, Methode)

INTRODUCTION

Urea content in milk and serum of cows is very closely related. Both contents change when daily ratio of cows is changed. They are either increased or decreased in the same amount. Urea content in milk and in serum is dependent on protein supply, but where energy supply plays its major part. If energy supply is sufficient, the ammonia in the rumen changes to microbe proteins, which are for the animals, of very high quality. Surplus of ammonia is in the form of urea through liver secreted to blood stream and from here to milk. If the energy and protein supply is sufficient, urea content varies from 15 and 25 mg/dl (*Paulicks*, 1992). If there is a protein surplus and /or lack of energy in the ration, urea content in milk increases, reaching more than 25 mg/dl. And when there is a lack of proteins in the ration of dairy cows, and/or protein surplus, then urea content drops below 15 mg/dl.

Undesired consequences for the animals occur in the case of surplus of proteins in daily ration, and the increased urea content can cause (*Nage*l, 1994):

- Liver damages and health problems
- Productive and reproductive disorders
- Increased somatic cell count
- Increased veterinary aid expenses
- Increased nitrogen release and thus air pollution
- Increased feed costs due to irrational protein intake
- Increased energy need of the ration
- Increased losses in milk production process

Urea content in milk is an important parameter that indicates correctly balanced protein and energy diet of dairy cows. To use the results of urea content in milk for the estimation of correct energy and protein supply in the ration, these results have to be accurate. Several authors report that urea content indicates, in many instances, the correct protein and energy supply in the ration of dairy cows (*Herzog*, 1994 ; *Herre*, 1998). The analysis result of urea content in a sample of milk from the pool provides the estimation of herd ration regarding the relation between proteins and energy supply. Prediction promptness varies from 60% when milk from the pool is analysed (showing the herd ration situation), and up to 80% when the results are obtained for separate dairy cows (*Hanuš*, 1995). Usually milk analysis for urea content are performed by different methods and procedures, on various machines, often adjusted for the determination of urea content. Punctuality and correctness of urea content determination procedures are often estimated by succession. For the usage of the obtained results of urea content in milk, the most important are the punctuality and correctness of the results, analyses estimation and the efficiency of machines used for the determination of urea content in milk (number of samples – analysis per hour). These three criteria were deciding factors for the development of new methods of routine determination

of urea content in milk, the so called UREAKVANT, which was developed in the Czech Republic (*Hanuš*, 1998).

In Germany, after the acceptance of urea content determination in milk on Milkoscan using infrared procedure, the accuracy of the results were compared to the analysis performed on Autoanalyzer – previously used for the determination of urea content in milk. The comparison of Autoanalyzer analysis with reference method was very good. The average deviation was less than 2 mg urea per 100 ml of milk. 93% of samples with protein surplus, and 74% samples with a lack of proteins in the ration were measured correctly (*Herre*, 1998). For the majority of animals the Autoanalyzer protein supply was correct. Yet the results of Milkoscan method compared to the reference method, the deviation was much higher or it varied. Especially in the case of protein surplus in the ration, where the average urea content was 11.5 mg/100 ml lower than the actual value. In the group of samples with protein surplus, where urea value exceeded 30 mg/100 ml, only 43% of samples were correctly measured. In the other 57% milk samples urea content was incorrect. In the case of lack of protein supply, where urea content was lower than 15 mg/100 ml, only 64% samples were measured correctly. Due to the fact that the results of urea content were correctly measured by milkoscan only for the good half (54%) of samples, this method and procedure is at the moment not suitable for the urea content determination in milk. For the interpretation and exploitation of the analysis results, better and more expensive analysis should be used as they will be more accurate than the cheaper analysis giving incorrect results (*Herre,* 1998).

In 1998 *Hanuš* compared four different procedures performed in three laboratories. Correlation coefficients among these four methods ranged between 0.76 (the comparison of enzymatic method on Ureakvant and infrared method on FOSS 4000) and 1.00 (the comparison of enzymatic method with NADH and enzymatic UV method COBAS MIRA).

MATERIALS AND METHODS

In the reference laboratory of the Biotechnical Faculty , Department of Animal Science, 10 different samples of milk were prepared in 6 successions and sent to 6 different laboratories with the purpose to determine urea content in milk. Milk samples were preserved by bronopol. The analysis for urea content were performed in the mentioned 6 laboratories, where different methods for the urea content determination were used. The following methods for the urea content determination in milk were used:

A: *In the laboratory A* milk analysis for urea content with 3 parallel measurements were performed. The analysis were carried out on biochemical analyzer COBAS MIRA and by enzymatic UV test (ureaza method/GLDH).

B: *In the laboratory B* milk analysis were performed in 2 successions on UREAKVANT 2 (SD<1.5%; w=±3mg) – enzymatic method and conductibility measurement.

C: *In the laboratory C* milk analysis were carried out in 2 successions using UREAKVANT 1 (SD<1.5%; w=±3mg) by enzymatic method and conductibility measurement.

D: *In the laboratory D* milk analysis were carried out by using photometric method on milkoscan 133 B.

E: *In the laboratory E* the analysis were performed with 3 successions using enzymatic UV method.

F: *In the laboratory F* the analysis were carried out in two successions and enzymatic method on CL-10 (Eurochem). (SD=1.7% ; w=4.2%).

Principle of the enzymatic method

$$\text{Urea} + H_2O \longrightarrow 2\ NH_3 + CO_2$$

$$\text{alfa-Ketoglutarat} + \text{NADH} + NH_4^+ \xrightarrow{\text{GLDH}} \text{L-Glutamat} + NAD^+ + H_2O$$

Principle of measurement with enzymatic method on CL-10

$$\text{Urea} + H_2O \longrightarrow 2\ NH_3 + CO_2$$

$$2\ NH_3 + 2H_2O \longrightarrow 2\ NH_4^+ + 2\ OH^-$$

$$CO_2 + H_2O \longrightarrow H_2CO_3 \Longleftrightarrow HCO_3^- + H^+$$

Milk analysis results for urea content were processed using SAS programme. Simple statistical parameters were estimated (x, SD, CV). Differences in mean values among the laboratories were compared by using the method of difference. We calculated the correlation and determination coefficient, as well as the regression coefficient.

RESULTS AND DISCUSSION

Table 1 presents statistical parameters for urea content, measured in different laboratories. The results indicate that the average urea content is the lowest in the laboratory E (2.38 mmol/l), with the highest variability coefficient (51.0%):The highest urea content was measured in the laboratory D (3.43 mmol/l), where urea content was determined photometrically on milkoscan. Variability coefficient was the lowest in this laboratory (33.5%). Standard deviation ranged from 0.98 in the laboratory F, where urea content was measured by enzymatic method on CL – 10, and 1.43 in the laboratory A, where urea content was determined by using enzymatic method on biochemical analyzer COBAS MIRA.

Table 1

Statistical parameters for urea content (mmol/l), measured in different laboratories

Laboratory (1)	Number of samples (2)	$\bar{x}$	SD	CV%	Min	Max
A	10	3,19	1,43	44,7	1,35	5,09
B	10	3,29	1,19	36,3	1,70	4,90
C	10	3,21	1,14	35,4	1,70	4,85
D	10	3,43	1,15	33,5	1,78	4,66
E	10	2,38	1,21	51,0	0,91	4,41
F	10	2,90	0,98	33,7	1,59	4,26

1. Tabelle: Statistische Parameter des Harnstoffgehaltes (mmol/l), gemessen in verschiedenen Labors

Labor(1), Anzahl der Proben(2)

Table 2 presents the difference in results for urea content in milk in different laboratories. The average difference ranges from 0.015 between the laboratories A and C, and 1.052 between the laboratories D and E. Standard deviation for differences (SD4) shows the random error of differences among laboratories, expressed absolutely. Standard deviation for differences ranges from 0.12 (B – C) and 0.46 (A - F). The variability coefficient for differences (VC_d) is also the indicator of differences among the laboratories and is expressed relatively. Variability coefficient for differences in four comparisons among the laboratories exceeds the value of 10**%** and in seven comparisons this coefficient is lower than 7**%**. The successions of results is specially expressed in the examples where VC_d is lower than 7**%**. Differences among the laboratories were tested by t-test. In almost all instances of the tested differences, these differences are statistically highly significant. Statistically insignificant are the differences among the laboratories A and B, A and C, A and D, and B and C.

Table 2

The analysis of different results for urea content in milk, measured in different laboratories (n=10)

Difference	$\bar{d}$	SD_d	VC_d	t_{exp}	t_{tab}
A – B	- 0,095	0,24	7,52	1,22898	n.s.
A – C	- 0,015	0,33	10,34	0,1437	n.s.
A – D	- 0,237	0,33	10,34	2,2411	n.s.
A – E	0,815	0,31	9,72	2,6316	*
A – F	0,290	0,46	14,42	8,3217	***
B – C	0,080	0,12	3,65	1,9979	n.s.
B – D	- 0,142	0,18	5,47	2,4411	*
B – E	0,910	0,182	5,53	15,8261	***
B – F	0,385	0,22	6,69	5,4241	***
C – D	- 0,222	0,21	6,54	3,375399	**
C – E	0,830	0,17	5,30	15,44186	***
C – F	0,305	0,20	6,23	4,8328	***
D – E	1,052	0,33	9,62	10,096	***
D – F	0,527	0,25	7,29	6,7391	***
E – F	- 0,525	0,279	11,72	5,9436	***

2. Tabelle: Analyse der unterschiedlichen Meßergebnisse zum Harngehalt der Milch in verschiedenen Labors (n=10)

Table 3 presents correlation coefficient, determination and regression coefficients for the differences among different laboratories. The lowest correlation coefficient is established for the difference between the laboratory D and E (r=0.96) and the highest for the difference between the laboratory A and B. All the correlation are statistically highly significant. The determination coefficients are showing similar picture, where the determination coefficients R vary between 0.926 (D – E) and 0.997 (A – B). Table 3 also presents the partial regression coefficients; a 1 and b 1 are partial regression coefficients when the laboratory X value is an independent variable, and laboratory Y

value dependant variable. Regression coefficients a 2 and b 2 are partial regression coefficients, when the values of laboratory Y are independent variables, and the values of laboratory X dependant variable.

Table 3

Correlation, determination and regression coefficients for differences in urea content among laboratories

Difference X - Y	r	a_1 (X – Y)	a_2 (Y – X)	b_1 (X – Y)	b_2 (Y – X)	R
A – B	0,99873***	0,6248	-0,7392	0,8339	1,1961	0,99746
A – C	0,99262***	0,6853	- 0,8079	0,7899	1,2474	0,98530
A – D	0,98976***	0,8886	- 1,0289	0,7958	1,2311	0,97964
A – E	0,98588***	- 0,2917	0,4286	0,8359	1,1627	0,97196
A – F	0,99694***	0,7215	- 1,0305	0,6829	1,4553	0,99389
B – C	0,99578***	0,0875	- 0,0638	0,9490	1,0449	0,99158
B – D	0,98835***	0,3008	- 0,2327	0,9516	1,0265	0,97683
B – E	0,98864***	- 0,9230	0,9728	1,0039	0,9736	0,97741
B – F	0,99804***	0,2103	- 0,2429	0,8188	1,2165	0,99609
C – D	0,98346***	0,2425	- 0,1309	0,9936	0,9734	0,96719
C – E	0,99151***	- 1,0111	0,9950	1,0565	0,9305	0,98309
C – F	0,99332***	0,1594	- 0,1413	0,8551	1,1539	0,98668
D – E	0,96233***	- 1,1031	1,2599	1,0149	0,9125	0,92608
D – F	0,98560***	0,0221	0,0724	0,8398	1,1568	0,97140
E – F	0,98988***	1,0001	- 1,1783	0,7997	1,2253	0,97985

Regression equation *(Regressionsgleichung)*: $\mathbf{y=a+bx_i}$
$\mathbf{x_i}$=Individual measurement in the laboratory. *(Individuelle Messung im Labor.)*
a, b=Regression coefficient *(Regressionskoeffizient)*
y=Estimated value of measurement i in the laboratory prediction, if the factors a and b are known. *(Schätzwerte der Messung i in der Laborschätzung, wenn die Faktoren a und b bekannt sind.)*

3. Tabelle: Korrelations-, Determinations- und Regressionskoeffizienten des Harngehaltes in der Milch zwischen den einzelnen Labors

CONCLUSIONS

- Systematic difference between laboratories is shown in the difference in mean values. As they are statistically significant it means that the laboratories are not adjusted or due to different methods or other reasons, give different results.
- Systematic environmental effects can be excluded:
  - by the adjustment of laboratories, based on their joint reference laboratory,
  - systematic error can be excluded with the regression coefficient estimation, factors a and b,
  - with the estimated straight line and consideration of estimated values, we can get the values that are comparable among the laboratories.

- Differences are higher if the methods are different (enzymatic, photometric), e.g. D – E and C – E.
- Standard deviation of differences (SD_d) indicate random error for the difference between the laboratories, expressed absolutely. This fact is supported by variability coefficient (VC_d), showing the relative difference. In absolute and relative difference we can notice that the successions of the results is the highest where SD_d and VC_d are having low values. Thus we can see the highest successions between B and C, B and D, B and E, B and F, C and D, C and E and C and F, where VC_d has low values, below 7**%**. The successions is worse especially between the laboratories A and F (VC_d=14.42), E and F (VC_d=11.72), A and C, as well as A and D, where VC_d is higher than 10**%** in all instances.
- Correlation coefficients and determination coefficients are among the laboratories and within the same samples relatively high, all above 0.98, except between the laboratories D and E, where the correlation coefficient is 0.96. Correlation coefficients indicate the possible successions among laboratories as mentioned in VC_d estimation. The estimations of separate laboratories show relatively high values of correlation coefficients that are higher than 0.96, and determination coefficients, that are higher than 0.92.

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