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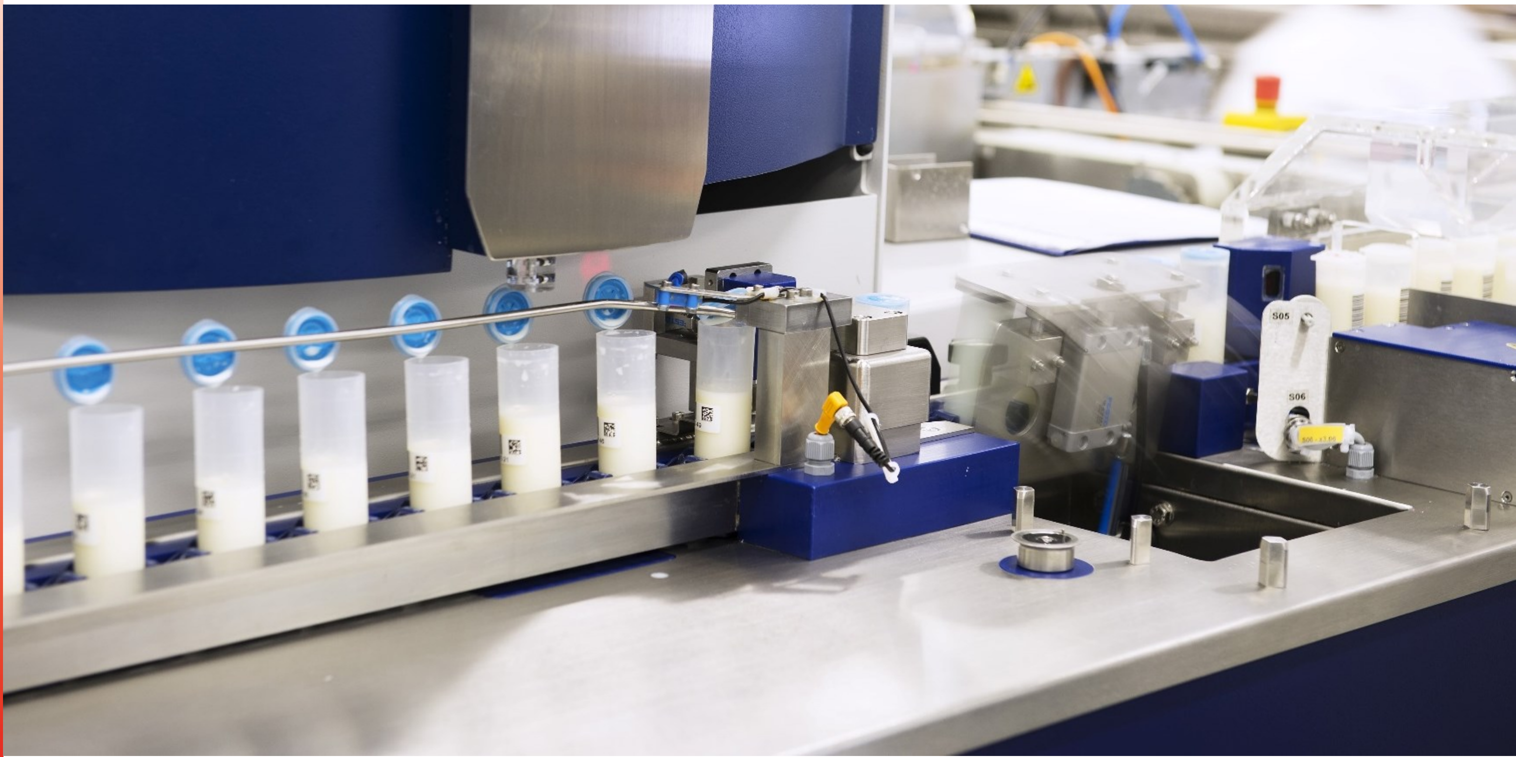

Application of a unified European conversion factor for the transformation of Bactoscan FC values

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Content

1.1 Introduction

The determination of total bacterial counts (TBC) is one of the standard measures to check the milk quality and hygiene. In Section IX of Regulation (EC) N°853/2004 [1], criteria have been fixed for raw milk (Chapters I, III) and for dairy products (Chapters II, III-criteria for the use of raw cow's milk for further processing). They include criteria on plate count at 30°C for raw cow's milk and for raw milk from other species.

Today, in most cases fluorescence flow cytometric methods are used to enumerate the total microbiota in milk in European countries and worldwide. Because of the legal context and the commonly use, the term "total flora" instead of total microbiota is used in this work. In Switzerland, milk samples are currently analyzed with Bactoscan FC instruments (FOSS Instruments, Foss Allé 1, 3400 Hillerød, Denmark). The electronic impulses (IBC) obtained with the Bactoscan FC are then converted into colony forming units in order to check compliance with legal limits of EC Regulation 853/2004, expressed in the unit of the reference method e.g. colony forming units (cfu) determined by the agar plate method.

There is a statistically close relationship between Bactoscan values (IBC/mL) and the values for colony forming units per milliliter (cfu/mL) obtained with the classic enumeration on agar plates. But for individual cases, values for the same milk sample can be very different. This depends, inter alia, on the fact that the two methods do not measure the same parameter. The targets of the fluorescence optical method are, in principle, single germs, including dead or not cultivable microorganisms, whereas with the agar plate method only microorganisms which are able to grow and form colonies in or on a solid medium after an incubation at defined conditions are detected. Depending on the type of microorganism, one to more than 10 individual nuclei may be equivalent to one colony forming unit. Hence, IBC values are always higher than cfu values for the same milk sample [2]. TBC expressed in colony forming units are calculated from IBC values by the linear regression equation $\log(cfu_{cal}) = \alpha * \log(IBC) + \beta$. Numerical values for α and β have to be established with analyses in parallel of a sufficient number of samples with the reference and the alternative method (ISO 21187 | IDF 196, 2004) [3]. Because TBC values converted from IBC to cfus are not really equivalent to colony forming units determined by plate count methods, the unit calculated colony forming units (cfu_{cal}) is used for these values in this report.

Commission Regulation (EC) 2074/2005 [4] modified by Commission Regulation (EC) N° 1664/2006 [5] includes in Annex VIa the description of testing methods for raw milk and heat-treated milk, including the reference method for total flora at 30°C, Standard EN ISO 4833-1:2013 [6] as well as conditions for the use of alternative methods.

The use of alternative methods for the determination of total flora in raw milk is allowed in Regulation (EC) 2074/2005 modified by Regulation (EC) N° 1664/2006 under the following conditions:

- if the alternative methods are validated against the reference method, in accordance with the protocol set out in Standard EN ISO 16140 [7; 8] or other similar internationally accepted protocols;
- in particular if the conversion relationship between an alternative method and the reference method is established according to ISO Standard EN ISO 21187:2004 [3] and ISO 16140-2:2016 [8].

The conversion equation for the analysis of cow's milk samples in Switzerland with Bactoscan FC instruments was evaluated and validated in 2006 [9]. This validation study resulted in the Swiss conversion factor: $\log(cfu_{cal}/mL) = \log(IBC/mL) * 0.95343 – 0.32695$. Since 1 March 2008 [10; 11], this conversion factor is used in Switzerland for bacterial cell counting within the official milk quality control scheme. In other countries, different conversion factors were established [12].

At the 2006 workshop of members of the European Union Reference Laboratory for Milk and Milk Products and National Reference Laboratories (EURL/NRLs MMP), it has been agreed, that one conversion factor per country should be established per apparatus and per animal species, according to ISO 21187:2004, under the responsibility of each NRL and with EURL coordination [12].

In a second step, since the 2011 workshop, EURL MMP, in collaboration with volunteering NRLs, gathered in a working group, has conducted a study on harmonization at European level of conversion equations between instrumental and reference method for the quantification of total flora in raw milk.
Based on all data gathered, the working group of the EURL and volunteering NRLs MMP agreed at their meeting before the 2015 EURL/NRLs MMP Workshop (7 October 2015), that a unified conversion equation could be derived at European level for cow's milk, irrespective of type of flow cytometer. To reach this goal, the next intermediate step is to envisage the practical feasibility of this change. NRLs agreed to investigate the impact of implementation of EU equation at national level, based on the report of this study and the outcome of this enquiry should be discussed at the 2016 workshop.

1.2 Comparison of total bacterial cell count results using the Swiss conversion factor or the proposed unified European conversion factor for the data of 2015

The bacterial cell count results for the year 2015 were downloaded from the TSM database (TSM Treuhand GmbH, Weststrasse 10, 3000 Bern) [06/02/2016]. Of a total of 488'816 records 1'571 records were eliminated (values missing or values ≤ 0). Finally, 487'245 individual records could be included in the evaluation. In a first step, the bacterial counts (cfu/mL) were transformed into IBC/mL according to the equation
log(IBC/mL) = (log(cfu/mL) + 0.32695) / 0.95343.
These values then were re-converted again into colony forming units per ml (cfu_{cal}/ml), using the proposed unified conversion equation (ucf):
log(cfu_{cal}ucf/mL) = log(IBC/mL) * 0.850 + 0.185 [12].
Figure 1 shows the comparison of bacterial counts (cfu_{cal}/mL) calculated using the Swiss conversion factor (Scf) (log(cfu_{cal}Scf/mL) = log(IBC/mL) * 0.95343 – 0.32695) vs bacterial counts per milliliter calculated using the unified conversion factor. The regression line for the Swiss conversion equation is slightly steeper with a lower intercept. The intersection point is at about 89'000 IBC/mL (table 1).

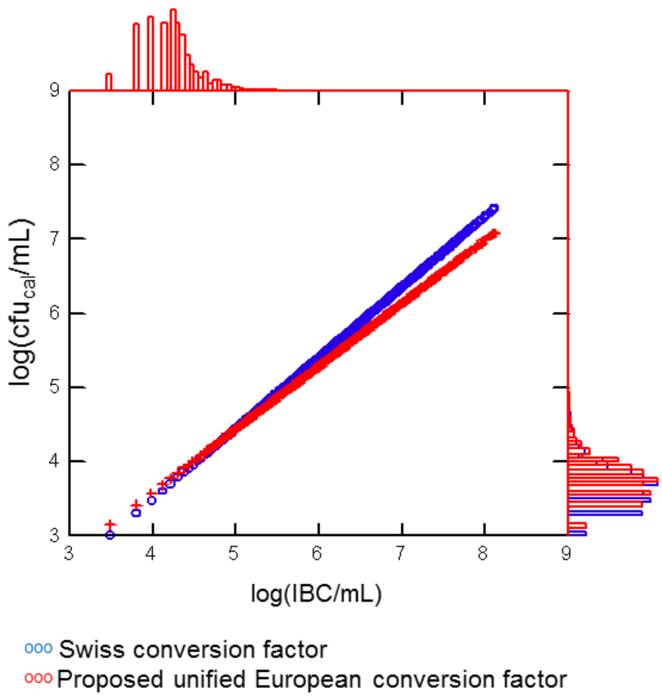

Figure 1: Regression lines of total bacterial counts (log(cfu_{cal})/mL) converted using the Swiss conversion factor (blue) versus total bacterial counts converted using the proposed unified conversion factor (red). Histograms show the distribution of the respective values.

Table 1: Possible effects on bacterial cell count results depending on the used conversion factor

Bactoscan FC values (IBC/mL)	Total bacterial cell counts using the Swiss conversion factor ($cfu_{cal}Sfc$/mL)	Total bacterial cell counts using the unified conversion factor ($cfu_{cal}ucf$/mL)
10'000	3'067	3'846
30'778		10'000
34'538	10‘000	
50'000	14'229	15'105
80'000	22'274	22'523
89'000	24'657	24'659
100'001	27'555	27'227
200'000	53'359	49'077
300'000	78'542	69'271
305'844	80‘000	
355'381		80‘000
500'000	127'826	106'936
1'000'000	247'531	192'752
1'223'402	300'000	
1'682'771		300'000
1'500'000	364'352	272'068
2'000'000	479'337	347'437
3'000'000	705'556	490'403

$cfu_{cal}Scf$ = Total bacterial cell counts calculated with the Swiss conversion factor;
$cfu_{cal}ucf$ = Total bacterial cell counts calculated with the unified European conversion factor

The application of the unified conversion factor would have led to higher bacterial counts ($cfu_{cal}ucf$/mL) for 467'624 (95.97 %) samples and to lower bacterial counts for 19'621 (4.03 %) samples (table 2). But it is essential to note, that only Bactoscan values lower than 89'000 IBC/ml (about 25’000 cfu_{cal}/mL) would result in higher cfu_{cal} using the unified conversion factor. For Bactoscan values greater than 89'000 IBC/mL the converted values for cfu_{cal} per milliliter calculated with the proposed unified conversion factor would always be lower (table 1). A direct conversion from one value into the other can be done by using the equations:

$\log(cfu_{cal}Scf) = ((\log(cfu_{cal}ucf) – 0.185) / 0.850 * 0.95343) – 0.32695$ and
$\log(cfu_{cal}ucf) = ((\log(cfu_{cal}Scf) + 0.32695) / 0.95343 * 0.850) + 0.185$

Using the Swiss conversion factor, 4’382 (0.90 %) samples exceeded the lower noncompliance threshold of 80’000 cfu_{cal}/mL [10] and 1’284 (0.26 %) samples the higher noncompliance threshold of 300’000 cfu_{cal}/mL [11; 13], whereas only 3‘793 (0.78 %) samples and 1’012 (0.21 %) samples respectively would have exceeded these thresholds using the unified conversion factor (table 2).
Again using the Swiss conversion factor, 412’719 (84.72 %) samples met the criteria for premium milk quality ≤ 10‘000 cfu_{cal}/mL [13], whereas only 376’814 (77.34 %) samples would have fulfilled the criteria of this category using the unified conversion factor.

Table 2: Changes in proportions of samples related to relevant bacterial counts determined by Bactoscan FC and converted with the Swiss conversion factor or a unified European conversion factor			
Criteria	**Factor used**	**Number of samples [-]**	**Percentage of samples [%]**
All samples			
cfu_{cal}**Scf** > cfu_{cal}**ucf**		19'621	*4.03*
cfu_{cal}**Scf** < cfu_{cal}**ucf**		467'624	*95.97*
Total		487'245	*100.00*
Noncompliance			
Lower threshold ≥ 80'000	cfu_{cal}Scf/mL	4'382	*0.90*
	cfu_{cal}ucf/mL	3'793	*0.78*
Upper threshold ≥ 300'000	cfu_{cal}Scf/mL	1'284	*0.26*
	cfu_{cal}ucf/mL	1'012	*0.21*
Bonus			
Threshold ≤ 10'000	cfu_{cal}Scf/mL	412'731	*84.71*
	cfu_{cal}ucf/mL	376'826	*77.34*

cfu_{cal}Scf = Total bacterial cell counts calculated with the Swiss conversion factor;
cfu_{cal}ucf = Total bacterial cell counts calculated with the unified European conversion factor

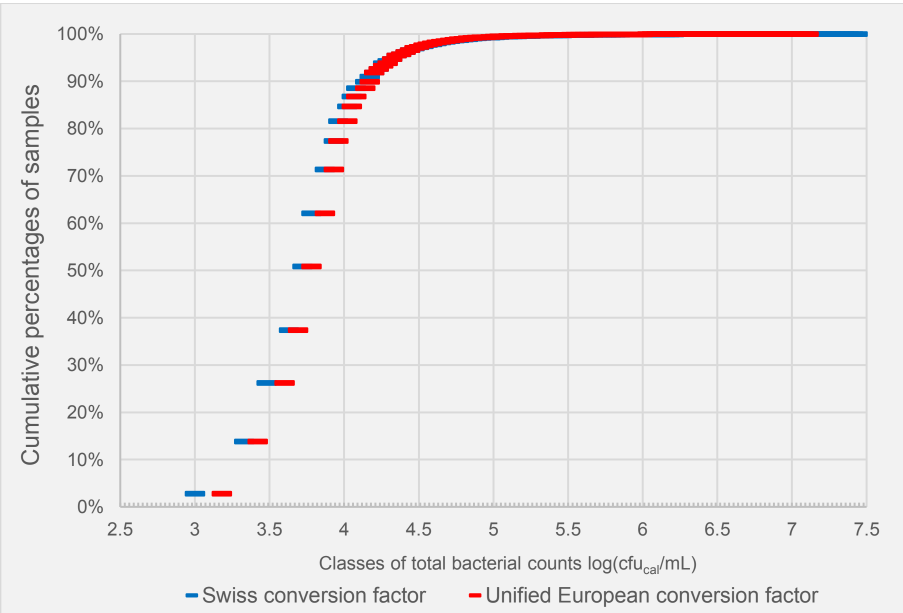

Figure 2: Cumulative histogram of total bacterial cell counts converted from Bactoscan FC values using the Swiss conversion factor and the proposed European conversion factor

Figure 3 and figure 4 show the percentages of bulk milk samples with TBC < 80'000 cfu_{cal}/mL and TBC < 10'000 cfu/mL for each month of the year 2015. There are no obvious variations in the monthly proportions visible.

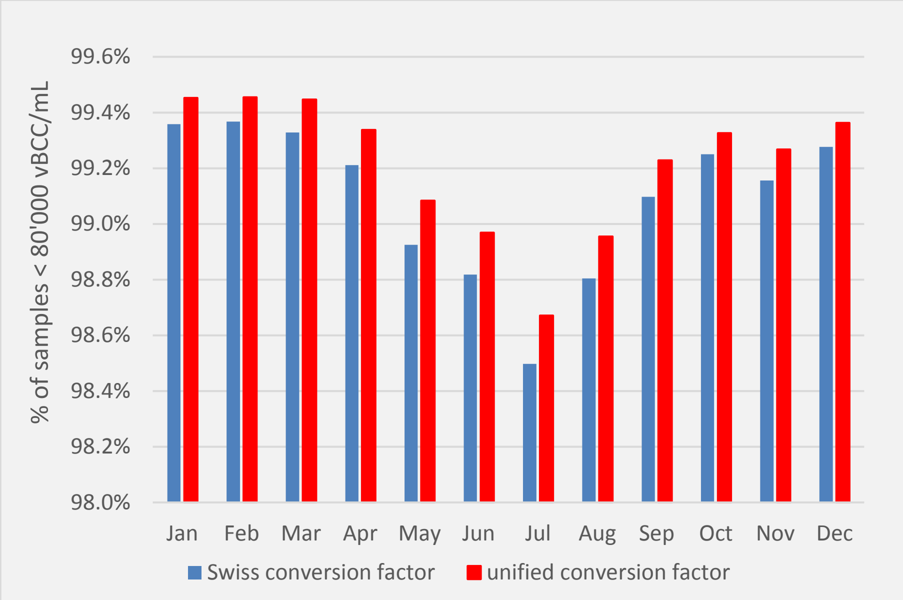

Figure 3: Percentages of bulk milk samples with total bacterial counts below the threshold of 80'000 cfu_{cal}/mL throughout the year 2015

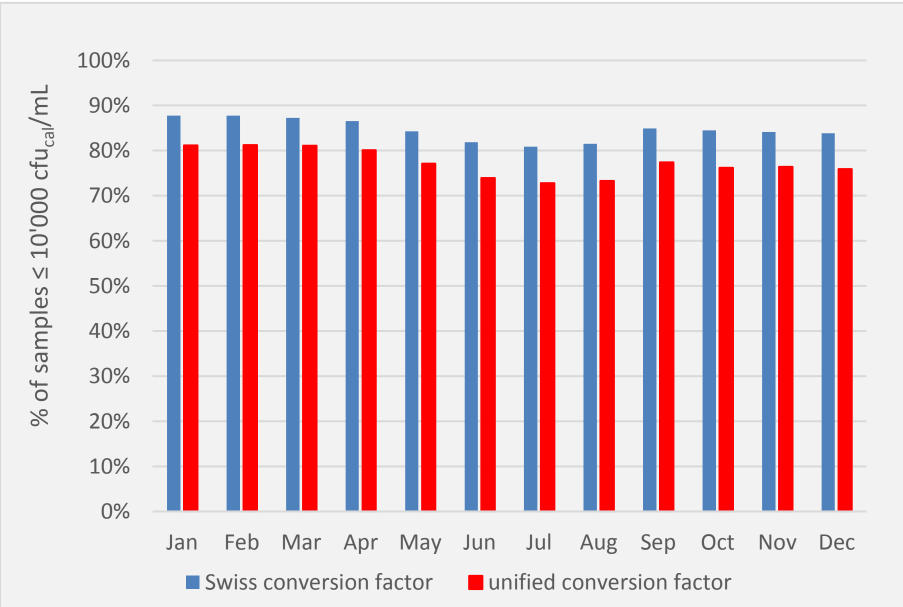

Figure 4: Percentages of bulk milk samples with total bacterial counts ≤ 10'000 cfu_{cal}/mL throughout the year 2015

1.3 Conclusions

The application of the proposed unified conversion factor would result in higher bacterial counts in the range below about 25’000 cfu_{cal}/mL or 89’000 IBC/mL, and in lower bacterial counts for samples in the in the range above 89’000 IBC/mL.
The percentages of milk samples violating the noncompliance limits (80’000 and 300’000 cfu_{cal}/mL) would have been lower (0.78 % vs. 0.90 % and 0.21 % vs. 0.26 %, respectively) in the year 2015 when applying the unified conversion factor. Thus, the application of the unified conversion factor would lead to results slightly in favour of milk producers delivering milk of poor microbial quality. But nonetheless, considering the fact, that in most cases bacterial counts above 80’000 cfu_{cal}/mL are only ‘accidental’ events, the impact on milk quality is assumed to be negligible.
The percentage of samples fulfilling the criteria to get a bonus according to the private milk quality payment scheme (≤ 10’000 cfu_{cal}/mL) would have been lower applying the unified conversion factor (77.34 % vs. 84.72 %). The use of the unified conversion factor would be slightly less favourable for milk producers considering the possibility to get a bonus for extra milk quality.
Over all, the impact on milk quality would be only marginal by replacing the Swiss conversion factor with the proposed European unified conversion factor.
Correct interpretations of the results (focusing e.g. on history and trend of results, comparison with other farms, elimination of possible sources for contaminations) and the milk quality payment scheme in force have a bigger effect on the quality of the delivered milk than the slight change in transforming IBC values into converted colony forming units using the proposed unified conversion factor.

1.4 Conclusions

L’utilisation du facteur de conversion uniforme proposé engendrerait, pour des nombres de germes jusqu’à environ 25'000 cfu_{cal}/ml ou 89'000 IBC/ml des teneurs en germes plus élevées et, pour des teneurs dépassant 89'000 IBC/ml, des teneurs moins élevées.
Avec l’utilisation du facteur de conversion uniforme, les pourcentages pour les échantillons de lait qui n’auraient pas rempli les exigences (80'000 cfu_{cal}/ml et 300'000 cfu_{cal}/ml) seraient moins élevées pour l’année 2015 (0,78 % vs. 0,90 % et 0,21 % vs. 0,26 %). Ainsi, l’utilisation du facteur de conversion uniforme engendrerait des résultats favorisant légèrement les producteurs de lait qui livrent du lait avec une mauvaise qualité microbienne. Si l’on considère le fait que des teneurs en germes dépassant 80'000 cfu_{cal}/ml dans la plupart des cas constituent des «accidents», l’impact sur la qualité du lait peut être tout de même considéré comme négligeable.
La part d’échantillons qui auraient rempli les critères pour un bonus conformément à la réglementation de droit privé pour le paiement selon des critères de qualité (≤ 10'000 cfu_{cal}/ml), aurait été moins élevée lors de l’utilisation du facteur de conversion uniforme (77,34 % contre 84,72 %). En conséquence, son utilisation engendrerait qu’un nombre un peu moins élevé de producteurs de lait toucherait un bonus pour une qualité de lait particulièrement bonne.
En somme, l’impact sur la qualité du lait du remplacement du facteur de conversion actuel en Suisse par le facteur de conversion européen uniforme ne serait que marginal.
Une interprétation correcte des résultats (par focalisation sur la tendance du nombre de germes, comparaison directe des valeurs à celles d’autres exploitations, l’élimination de potentielles sources de contamination) et la réglementation relative au paiement du lait selon des critères de qualité ont une plus grande influence sur la qualité du lait livré que la modification mineure des valeurs numériques lors de la transformation des valeurs IBC en unités formant des colonies avec l’utilisation du facteur de conversion proposé.

1.5 Schlussfolgerungen

Die Anwendung des vorgeschlagenen einheitlichen europäischen Umrechnungsfaktors würde für Keimzahlen bis ungefähr 25'000 cfu_{cal}/mL bzw. 89'000 IBC/mL zu höheren und bei Werten über 89'000 IBC/mL zu tieferen Keimzahlwerten führen.

Die Prozentsätze von Milchproben, welche die Qualitätsanforderungen (80'000 cfu_{cal}/mL bzw. 300'000 cfu_{cal}/mL) nicht erfüllt hätten, wären bei der Anwendung des einheitlichen Umrechnungsfaktors für das Jahr 2015 niedriger gewesen (0.78 % vs. 0.90 % bzw. 0.21 % vs. 0.26 %). Somit würde die Anwendung des einheitlichen Umrechnungsfaktors zu Ergebnissen führen, die leicht zugunsten derjenigen Milcherzeuger ausfallen, die Milch von schlechter mikrobieller Qualität liefern. In Anbetracht der Tatsache, dass Keimzahlen über 80'000 cfu_{cal}/mL in den meisten Fällen «Unfälle» sind, können die Auswirkungen auf die Milchqualität trotzdem als vernachlässigbar beurteilt werden.

Der Anteil der Proben, die die Kriterien für einen Bonus gemäss der privatrechtlichen Regelung für die Bezahlung nach Qualitätsmerkmalen (≤ 10'000 cfu_{cal}/mL) erfüllt hätten, wäre bei Anwendung des einheitlichen Umrechnungsfaktors niedriger gewesen (77.34 % gegenüber 84.72 %). Die Verwendung des einheitlichen Umrechnungsfaktors würde demnach dazu führen, dass etwas weniger Milcherzeuger einen Bonus für besonders gute Milchqualität erhalten würden.

Alles in allem wären aber die Auswirkungen auf die Milchqualität bei einem Ersatz des bisherigen Umrechnungsfaktors in der Schweiz durch den vorgeschlagenen europäischen Umrechnungsfaktor nur marginal.

Eine korrekte Interpretation der Ergebnisse (Fokussierung z.B. auf die Tendenz der Keimzahlwerte, direkter Vergleich der Werte mit denjenigen anderer Betriebe, die Elimination von möglichen Quellen für Kontaminationen) und die Regelung zur Bezahlung der Milch nach Qualitätsmerkmalen haben einen grösseren Einfluss auf die Qualität der abgelieferten Milch als die geringfügigen Änderung der numerischen Werte bei der Transformation der IBC-Werte in berechnete koloniebildende Einheiten.

1.6 Literature

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