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Original article

Utilization of milk urea concentration as a tool to evaluate dairy herd management

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Abstract — The milk urea (MU) content variation was monitored in fourteen herds over a year to verify the reliability of this parameter as an indicator of the nutritional protein status of dairy cows. The relationships between MU concentrations and the nutritional value of the feed, pasture characteristics and lactation parameters were also investigated. For the single regressions, the average MU concentration had a positive relationship ($R^2 = 0.42$) with the difference between degradable proteins and fermentable energy (OEB) in the rumen, while the combination of crude protein (CP), net energy (VEM) and week of lactation (WL) more accurately explains the MU variation. The regression of MU prediction obtained with the data file of 14 farms was as follows: MU ($mg \cdot dL^{-1}$) = 2.56 + 0.130 CP ($g \cdot d^{-1}$) – 0.00154 VEM ($g \cdot d^{-1}$) + 0.407 WL (weeks) ($R^2 = 0.52$; s = 6.933; n = 250). This model was significant ($P < 0.05$) for eleven herds out of fourteen. The results of this study suggest that MU content depends essentially on the protein/energy balance of the diet but that many other factors can influence ureogenesis.

urea / milk / dairy cows

Résumé — Utilisation de la teneur en urée du lait comme indicateur de la conduite du troupeau laitier. Au cours d'une année, la variation de la teneur en urée du lait (UL) a été étudiée dans quatorze troupeaux pour vérifier la fiabilité de ce paramètre comme indicateur de l'alimentation protéique des vaches laitières. Le taux d'UL a été mis en relation avec la valeur alimentaire des rations, les caractéristiques du pâturage et les paramètres de lactation. Pour les régressions simples, c'est l'OEB (différence entre l'azote dégradable et l'énergie fermentescible dans le rumen) de la ration qui est le paramètre le mieux corrélé à la teneur en UL ($R^2 = 0{,}42$), alors que pour les régressions multiples, c'est la combinaison de l'ingestion de matières azotées totales (MAT), d'énergie nette (VEM) et du stade de lactation (SL) qui explique le plus précisément la variabilité de la teneur en UL. L'équation générale obtenue à partir de la base de données des 14 exploitations est la suivante : UL ($mg \cdot dL^{-1}$) = 2,56 + 0,0130 MAT ($g \cdot j^{-1}$) – 0,00154 VEM ($g \cdot j^{-1}$) + 0,407 SL (sem) ($R^2 = 0{,}52$; s = 6,933 ; n = 250).

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Ce modèle est significatif ($P < 0{,}05$) pour 11 des 14 fermes étudiées. Les résultats de cette étude suggèrent que la teneur en UL dépend principalement de l'équilibre azote/énergie de la ration, mais que de multiples autres facteurs sont susceptibles d'influencer l'uréogenèse.

urée / lait / vaches laitières

1. INTRODUCTION

Due to the small area available for forage growing in Belgium, milk production has had to be intensified in recent decades in order to maintain farm profitability. Intensification has led to a higher proportion of concentrate in the diet fed to dairy cows and reduced protein efficiency. In order to ensure a profitability and reduce nitrogen discharges, dairy farmers will in the future need to manage the effective use of nitrogen input on the farm. Several studies have shown that milk urea (MU) concentration could indicate a protein feed imbalance [11, 13, 14]. Urea, which is synthesized in the liver, comes from surplus ammonia released from the fermentation of the nitrogen fraction in the rumen and excess digestible protein in the small intestine. Following synthesis, urea quickly spreads through all the aqueous phases of the body. The MU concentration, which correlates closely with the plasma urea level [3, 9, 20], thus reflects the efficiency of use of degradable proteins by the micro-organisms present in the rumen and the proteins digestible by the animal. Besides the diet balance, the MU level could also indicate impaired fertility due to excess dietary protein [4, 12, 14, 17] or excessive nitrogen losses to the environment [7, 11, 13]. However, most of the studies concerned with MU have been carried out at research stations. To enable urea concentration to be a relevant parameter for dairy farmers, we propose to evaluate this indicator in practical conditions, which obviously vary much more due to different feeding conditions and the effects of geographical area, time of year and farming practices. The study was spread over a full year, with cattle both at pasture and in stalls, in conditions specific to the Walloon Region. The farms concerned are scattered through Condroz, where the soil is fertile and suited to large-scale arable farming, and the Ardennes, a more wooded area where most of the farmland is grassland. In both of these areas, stock farming is mainly cattle rearing. The first objective was to determine the main factors affecting the MU concentration and the second was to check how reliable the infrared method, which is widely used in Belgium, is for predicting urea concentrations.

2. MATERIALS AND METHODS

2.1. Experimental conditions

The changing MU concentration was studied on fourteen participating farms in different parts of the Walloon Region, in Condroz and the Ardennes. Farm visits were made monthly during the winter period and twice a month during the grazing season, in order to take into account the considerable variations in grass quality. A total of 266 visits were made, that is 19 per farm, during which all the feed making up the diet was sampled and analyzed, as was a sample of milk (100 mL) from the tank. The nutritional value of the diet was calculated with respect to each farm visit. Monthly milk production, stage of lactation and average herd age were obtained from dairy inspectorate data sheets.

2.2. Laboratory analyses

Part of the milk sample was frozen for urea determination by differential pH-metry

(EFA-Hamilton, Bonaduz, Switzerland) after thawing, while part was stored at 4 °C with the aid of potassium dichromate ($K_2Cr_2O_7$), in order to predict the urea concentration, fat and protein content by the infrared method (Milkoscan 6000, Foss). pH-metry is an accurate enzymatic method based on measuring the pH variation occurring when urea breaks down into NH_3 and CO_2. The principle of the infrared method depends on the capacity of organic components such as fat, protein and urea to absorb light in the middle infrared range (wavelength 2 500 nm to 10 000 nm). Infrared MU determinations are predicted by means of a multilinear regression, using several wavelengths, which is calibrated by comparison to results available in a database, determined by pH-metry.

The dry matter at 60 °C (DM) in the feed was determined after drying the samples for 48 hours [1]. After comminution through a 1 mm screen, the CP content (N × 6.25) of the forage and concentrate was determined by the Kjeldahl method [1]. The nutritional value of the forage (VEM: net energy ($g \cdot d^{-1}$), OEB: difference between degradable protein and rumen fermentable energy ($g \cdot d^{-1}$), DVE: intestine digestible protein ($g \cdot d^{-1}$), FOM: fermentable organic matter ($g \cdot d^{-1}$), DOM: digestible organic matter ($g \cdot d^{-1}$)) was predicted by near infrared spectrometry (NIRS, NIR System 5000). This is an indirect method for estimating the feed composition. Using this technique requires calibration for each type of feed analyzed and depends on a large number of reference analyses performed by conventional chemical methods. Concentrates were also analyzed by NIRS, but since these are composed of different raw materials, this technique did not supply some values, such as VEM, OEB and DVE, which were therefore obtained from the feed manufacturers. The pasture composition (% leaves, % stems, % clover) was also estimated by means of the NIRS technique, whereas average grass height was estimated by making regular measurements (60 measurements per ha) throughout the plot, using a platform herbometer.

2.3. Calculation methods

The total quantity of dry matter ingested (TDMI) by the dairy cows was calculated by taking the mean results of the Faverdin [10] (1) and Caird and Holmes [5] (2) equations:

$$\text{TDMI} = \frac{-1.63 + 0.129\ \text{LW}^{0.75} + 0.22\ \text{MP} - 0.49\ \text{A}}{\text{TFOM}} \times 100 \qquad (1)$$

$$\text{TDMI} = 3.476 + 0.404\ \text{C} + 0.013\ \text{LW} - 0.129\ \text{WL} + 4.120\ \log\text{WL} + 0.140\ \text{MP} \qquad (2)$$

where A = age in years; C = quantity of concentrate consumed in kg $DM \cdot day^{-1}$; MP = production of 4% fat milk in $kg \cdot day^{-1}$; LW = live weight in kg; WL = number of weeks of lactation; TFOM = forage organic matter content in% of DM; TDMI = total DMI (dry matter ingested) in $kg \cdot day^{-1}$.

Grass ingestion was calculated as the difference between TDMI and the quantities of dry matter provided by the other components of the diet, calculated from information supplied by the farmer.

Theoretical maintenance, yield and growth requirements were calculated in accordance with Dutch standards for dairy cows [22, 23]. To estimate maintenance requirements, the assumed live weight was 550, 600 and 650 kg respectively for cows in their first, second and third or later lactation. Milk production, meanwhile, was corrected for standard 4% fat milk production with the aid of the following formula [18]:

$$\text{MP}_{std} = [(0.337 + 0.116\ \text{BC}) + 0.06\ \text{PC}]\ \text{MP}$$

where MP_{std} = standard 4% fat milk production in kg; MP = milk production before adjustment in kg; BC = butterfat content in %; PC = protein content in %.

The difference between the nutrition supplied by the diet and theoretical requirements provides an estimate of the excess nutrient balance, if the value is positive, or deficiency, if it is negative (VEM balance and DVE balance).

2.4. Statistical analysis

For each farm visit (n = 14 farms × 19 visits = 266 observations), the MU concentrations determined by differential pH-metry were correlated with the following parameters: ingestion of CP, DOM, FOM, VEM, DVE, OEB, milk production (MP), WL, milk protein content (PC) and buttermilk content (BC), parity, number of days elapsed since 1st June (d > 01/06), percentage of clover (% clover) and leaves (% leaves) in the field grazed. Grass parameters were only correlated with respect to the grazing period.

Furthermore, farm-level results (19 milk and feed samples taken from each farm) and overall results (n = 266) were processed by multiple regressions using the "Best Subset Regression" procedure of MINITAB version 13 [19]. This model selects the combination of parameters with the most significant influence on MU content, eliminating non-significant parameters from the regression equation. The software initially calculates the two most precise regressions, choosing one of the proposed parameters, then two parameters, then three and so on until all the proposed parameters are used in the equation. Each of the regressions is then tested using the "Regression" procedure in the same software. The equation selected is the one where R^2 is the highest and the parameters are significant ($P < 0.05$) within the regression. The respective importance (I) of each variable in the regression equations was calculated as follows, using a ratio of deviation sums of squares (DSS):

$$I = \frac{DSS_{parameter}}{DSS_{residual}} \times 100.$$

3. RESULTS AND DISCUSSION

3.1. Methods for determining MU content

Predictions of milk urea concentrations by the infrared method (IR) are highly correlated with the values measured by pH-metry ($R^2 = 0.813$, n = 180). However, the infrared method appears to be reliable in the optimum concentration range (17.5 and 30.0 mg·dL^{-1}) [8], but less accurate above 30 mg·dL^{-1}. This may be attributed to a smaller reference database for higher MU concentrations. Also, some of the predictions are decidedly overestimated in relation to the concentrations measured by pH-metry. These values have been omitted on the grounds that they are the result of incorrect sample packaging, due to exposure to excessive temperatures on hot summer days.

3.2. Milk urea content trend

Table I shows the characteristics of the fourteen farms in the study and mean MU concentrations in the winter season and the grazing season. The data reveal the diverse nature of the farms concerned and the variability of the MU content. On average, MU concentrations are significantly higher in the grazing season (28 mg·dL^{-1} as opposed to 21 mg·dL^{-1}). However, the eight farmers who provided supplementary maize silage as an energy food throughout the grazing months thus maintained their herd's mean MU content below 26 mg·dL^{-1}. High milk urea concentrations, however, corresponded to grass-based feeding supplemented by prewilted forage or without supplementary forage. This feeding system in fact produces mean MU concentrations over 29 mg·dL^{-1} and even, in an extreme case, as high as 37 mg·dL^{-1}. These levels are too high for mean values, since they lie at the limit of or beyond the normal range of variation (17.5 and 30.0 mg·dL^{-1}) proposed by De

Table I. Characteristics of the 14 participant farms, means and standard deviations for milk urea (MU) concentration ($mg \cdot dL^{-1}$) determined by differential pH-metry.

Farm	Agricultural land (ha)	Dairy herd (n)	Milk production ($kg \cdot d^{-1}$)	Forage components of diet (+ grass)	MU concentration ($mg \cdot dL^{-1}$)		
					Stalls	Grazing	Year
1	104	27	17.9	maize*, gs	16 ± 6	25 ± 7	21 ± 8
2	66	70	25.0	maize, gs*	22 ± 10	29 ± 5	26 ± 8
3	88	65	20.1	maize*, gs	16 ± 4	24 ± 6	21 ± 7
4	116	55	22.7	maize*, gs, pulp	26 ± 5	24 ± 8	25 ± 7
5	62	35	17.1	maize, gs, pulp*	17 ± 5	32 ± 15	26 ± 14
6	92	12	20.8	maize, gs*, pulp	14 ± 5	30 ± 10	25 ± 12
7	110	42	21.2	maize*, gs, pulp*	21 ± 6	19 ± 10	20 ± 8
8	72	40	22.0	maize*, gs, pulp	24 ± 5	24 ± 6	24 ± 5
9	46	53	17.2	maize*, gs	22 ± 6	24 ± 8	24 ± 8
10	72	49	17.5	maize*, gs	24 ± 6	25 ± 9	25 ± 10
11	140	61	16.0	maize, gs*	17 ± 6	32 ± 9	28 ± 10
12	89	88	23.4	maize*, gs*	23 ± 3	22 ± 10	23 ± 8
13	85	44	20.8	pulp, gs	21 ± 2	37 ± 10	32 ± 11
14	42	38	20.0	gs	32 ± 5	34 ± 12	34 ± 12
Means	85	48	20		21 ± 8	28 ± 10	25 ± 10

*: given all year round; gs = grass silage (± 50% DM); pulp = beet pulp; maize = maize silage.

Brabander et al. [8]. Given the variation of the milk urea concentration during grazing, the values recorded for these farms are frequently over 40 or even 50 $mg \cdot dL^{-1}$. Milk urea concentrations at this level should be avoided, since they are detrimental to herd fertility [4, 12, 14, 17] and to the environment, due to higher nitrogen discharges [7, 11, 13]. As mentioned by De Brabander et al. [7] and Holden et al. [15], supplementary maize silage feeding in the summer is therefore advisable.

3.3. Determination of factors affecting the MU content

3.3.1. Parameters correlated with MU content

Table II shows the parameters studied and their correlation with the MU concentration. Among dietary factors, the OEB diet has a predominant influence on the MU concentration measured by pH-metry ($R^2 = 0.42$). This confirms the findings of De Brabander et al. [6] and Hof et al. [14]. The correlation between OEB and MU content is significant for 11 out of the 14 farms. As in the literature, [3, 9, 13], CP also has a high correlation coefficient with the MU content, as does ingested DVE. The effect of the latter factor is more pronounced, however, taking into consideration the effect of excess DVE to the animals' requirements, or the DVE/VEM ratio. These results suggest that protein feed has a strong influence on MU content irrespective of the form of nitrogen supplied, whether as rumen degradable protein or small intestine degradable protein [2, 16]. As far as energy parameters are concerned, the FOM available to the rumen micro-organisms and VEM available to the cow do not correlate significantly with the milk urea concentration. The influence of energy input on MU content has nevertheless been revealed by some tests [3, 6, 21].

Although much less closely correlated with MU concentration than feed factors, some lactation parameters, specifically the

Table II. The means and variability of parameters studied, correlations with MU concentration (n = 266) and number of farms where the parameter has a significant influence ($P < 0.05$).

	Mean value of parameter	r	Probability	Significant farms
Feed parameters:				
TDMI (kg DM·d^{-1})	17.9 ± 0.13	+ 0.06	NS	0 / 14
DOM (g·d^{-1})	12558 ± 1523	+ 0.13	*	5 / 14
FOM (g·d^{-1})	10268 ± 1484	+ 0.10	NS	4 / 14
VEM (g·d^{-1})	16577 ± 2124	+ 0.08	NS	3 / 14
CP (g·d^{-1})	2810 ± 60	+ 0.55	***	7 / 14
DVE (g·d^{-1})	1492 ± 280	+ 0.37	***	5 / 14
DVE balance (g·d^{-1})	200 ± 273	+ 0.47	***	5 / 14
DVE/VEM (-)	0.09 ± 0.01	+ 0.44	***	4 / 14
OEB (g·d^{-1})	223 ± 345	+ 0.65	***	11 / 14
Lactation parameters:				
Stage of lactation (weeks)	28.8 ± 6.0	+ 0.29	***	7 / 14
Protein content (%)	3.42 ± 0.26	+ 0.18	**	4 / 14
Somatic cells (10^3·mL^{-1})	242 ± 125	+ 0.18	**	3 / 14
Yield (kg)	22.3 ± 3.3	– 0.14	*	3 / 14
Grass parameters:				
Stems (% of biomass)	20.9 ± 8.0	– 0.31	***	4 / 14
Leaves (%)	55.7 ± 14.5	+ 0.25	**	4 / 14
Clover (%)	6.0 ± 7.8	+ 0.07	NS	0 / 14
Field days (d)	6.2 ± 6.7	– 0.05	NS	1 / 14
Height (cm)	7.5 ± 2.4	– 0.01	NS	4 / 14

r: correlation coefficient; MU: milk urea; TDMI: total quantity of dry matter ingested; DOM: digestible organic matter; FOM: fermentable organic matter; VEM: net energy; CP: crude protein; DVE: intestine digestible protein; OEB: difference between degradable proteins and rumen fermentable energy; Field days: number of days between cows entering field and grass sampling; ***: $P \leq 0.001$; **: $P \leq 0.01$; *: $P \leq 0.05$; NS: not significant ($P > 0.05$).

stage of lactation, do have a significant influence on MU concentration. This finding is in contradiction with the literature [11, 16]. In the case of grazing animals this may partly be accounted for by reduced protein efficiency of the diet for cows at the end of the lactation period, given that grass-based feeding is still protein-rich. The correlation coefficient assigned to milk yield is negative, suggesting a dilution effect also observed by Broderick and Clayton [3].

In the case of the parameters relating to grass quality, the number of days spent in a field by the cows (field days), the grass height and the percentage of clover do not correlate with MU content. This is due to the great diversity of grazing practices prevailing on the fourteen farms in the study. Conversely, the proportions of leaves and stems in the grass sample do affect the MU concentration. The correlation is positive for the percentage of leaves and negative for the percentage of stems. These results may be explained by the greater availability and solubility of nitrogen in the leaves compared to the stems.

In contrast to the correlations, the best set regression equations reveal the actual influence of each parameter on the milk urea concentration, avoiding any bias due to interaction between the parameters.

3.3.2. Farm-level regression equations

The equations for each farm are shown in Table III. With the exception of farm 8,

Table III. Regression equations calculated for the 14 farms (n = 19 per equation).

Farm	Regression equations	I (%)	R^2 (%)
1	MU = – 37.0 + 0.0156 CP – 0.00209 VEM + 17.7 PC	78–11–11	0.87
2	MU = – 23.3 + 0.00310 VEM	100	0.57
3	MU = – 2.86 + 0.0169 DVE + 0.0153 OEB	41–59	0.59
4	MU = – 17.2 + 0.0126 CP – 0.833 WL	89–11	0.77
5	MU = 47.8 + 0.0209 CP – 3.75 MP	84–16	0.65
6	MU = – 2.59 + 0.0107 CP – 0.00456 VEMexc	86–14	0.77
7	MU = 24.0 + 0.0250 OEB	100	0.37
8	No significant variables	–	–
9	MU = 51.2 – 1.38 MP	100	0.47
10	MU = 20.8 + 0.0230 OEB	100	0.32
11	MU = – 6.3 + 0.0203 OEB	100	0.41
12	MU = – 189 + 62.0 PC	100	0.53
13	MU = – 13 + 0.0153 CP	100	0.65
14	MU = 87.5 + 0.0143 CP – 0.00781 DOM	35–65	0.79

I: respective importance of each variable in the regression equations in %; MU: milk urea determined by pH-metry ($mg \cdot dL^{-1}$); CP: total ingested protein ($g \cdot d^{-1}$); VEM: net ingested energy ($g \cdot d^{-1}$); PC: protein content (%); DVE: intestine digestible proteins ($g \cdot d^{-1}$); OEB: difference between degradable proteins and rumen fermentable energy ($g \cdot d^{-1}$); WL: stage of lactation in weeks; MP: standard milk ($kg \cdot d^{-1}$); VEM balance: difference between net ingested energy and net energy requirements ($g \cdot d^{-1}$); DOM: quantity of DOM ingested ($g \cdot d^{-1}$); R^2: coefficient of determination.

where the MU concentration did not vary significantly due to strictly controlled feeding, analysis of the determination coefficients suggests that depending on the farm, 32 to 87% of the MU concentration variation can be explained by the parameters investigated. This variability is partly attributable to the vagueness of the data supplied by some farmers, in particular daily feed amount.

An analysis of the equations for each farm shows that CP and OEB are the two most frequently occurring variables in the proposed models. The total or excess VEM ingested is mainly relevant to correcting CP. As observed with the correlations, the effect of MP was negative and the effects of PC and WL were positive.

3.3.3. General regression equation

The following general regression equation (1) was calculated from all the parameters for the whole study period:

$$MU = 2.56 + 0.0130\ CP - 0.00154\ VEM + 0.407\ WL \quad (1)$$

(R^2 = 0.52; s = 6.933; n = 256)

where MU = milk urea concentration determined by pH-metry ($mg \cdot dL^{-1}$); CP = total ingested nitrogen fraction ($g \cdot d^{-1}$); VEM = net ingested energy ($g \cdot d^{-1}$); WL = stage of lactation, in weeks.

In this equation, the three parameters selected have a very highly significant influence, accounting for 52% of the MU concentration variation. R^2 is not very high, due to a number of factors affecting the residue, chiefly probably the effect of the farm.

Based on the respective importance (I) of each variable in the regression equations, the variability of the MU concentration is in the main due to CP (71%) and to a lesser degree to VEM (18%) and WL (11%). OEB, which is highly correlated with CP ($R^2 = 0.79$; $P > 0.001$), is not included in the general regression equation, since it cannot significantly increase the precision of the model. Equation (1) shows the negative effect of the quantity of VEM ingested on the MU concentration. This was not thrown up by correlation analysis, due to the strong interaction between VEM and other parameters. While CP and WL are easy for farmers to estimate, the proportion of VEM in the different components of the diet is more difficult. In this respect, it would be useful to promote the development of the NIRS technique for analysis of the nutritional value of forage.

Equation (1) in this study suggests that the MU concentration reflects the protein/energy balance of the diet. However, the large number of factors that can influence MU content suggests that this is merely an indication of the nutritional status and can never take the place of dairy cattle forage analysis and diet calculation. Since the MU concentration is deemed satisfactory between 17.5 and 30 $mg \cdot dL^{-1}$ [8], it will thus be important to check and adjust the diet as soon as these limits are exceeded on a regular basis.

3.3.4. Usefulness and limits of farm-level observation

The study set out to look at the MU indicator in field conditions without in any way influencing farming practices, in contrast to station-based research where levels of nutritional factors are altered independently. The discriminatory capacity between parameters is thus less on the farm, since some variables sometimes correlate with one another.

To compare research station findings with measurements from the fourteen farms, the MU concentration was predicted by a regression equation (2) established by De Brabander et al. [7]:

$$MU = 19.6 - 0.0015\ VEM + 0.02\ DVE + 0.028\ OEB \quad (2)$$

R^2 for the De Brabander et al. data [7] = 0.94.

R^2 for the 14 farms throughout the one-year study = 0.45.

This equation (2), produced for grass and maize silage based diets, is logically less accurate than on the research station due to the diversity of situations encountered in the field, especially in the grazing season. A comparison between equation (1) and equation (2) is nevertheless interesting, since the coefficients of determination are fairly close ($R^2(1) = 0.52$ and $R^2(2) = 0.45$), which clearly shows the difficulty of accounting for over 50% of the MU concentration variation in practice. Moreover, the correlations between measured MU concentrations and those predicted by equation (2) are significant for ten of the fourteen farms. This result suggests that the equations derived from farms studied are verifiable in practice. Also, the equations based on field observations allow station results to be verified and supplemented.

4. CONCLUSION

The coefficient of determination of the farm-level regressions varies from 32 to 87%. Despite the diversity of the situations encountered in practice, the general equation accounts for over 50% of the MU concentration variation, taking into account protein and net energy ingested and the stage of lactation. The MU concentration is deemed satisfactory between 17.5 and 30 $mg \cdot dL^{-1}$ [8]. This measurement can serve to indicate a protein/energy imbalance in the feed and the diet should therefore be checked if these limits are regularly surpassed. Nevertheless, milk urea can never replace forage analysis and diet calculation for dairy cattle.

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