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Monitoring Feeding Adequacy in Dairy Cows Using Milk Urea and Milk Protein Contents under Farm Condition

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ABSTRACT : The experiment was conducted on 264 crossbred Karan-Fries (Holstein Friesian×Tharparkar) cows, over one year to explore the possibility of using milk urea (MU) concentration and milk protein content to monitor feeding adequacy under farm condition and to investigate the effects of different animal factors and season on MU concentration. Individual noon (1200 to 1300 h) milk samples were collected once in every month and analysed for urea and protein contents. Representative feed samples were also collected on the same day of milk collection and were analysed for CP content. A significant positive association ($p<0.01$) between MU concentration and milk yield was observed. MU concentrations (mg/dl) were found to be significantly ($p<0.01$) higher and lower in first lactation (44.8±0.7) and in early lactation stage (40.7±0.5), respectively. Average MU values were found to be significantly ($p<0.01$) higher in winter (50.7±0.3) and lower in summer (32.9±0.6). During the investigation, of the total MU observations, 50.3% were within the range of 30 to 50 mg/dl, 21.4% were <30 mg/dl and only 7.5% were >60 mg/dl. MU concentration was found to be associated significantly ($p<0.05$) with CP content of forages rather than concentrate. A close positive association ($p<0.01$) between MU level and daily milk protein (DMP) yield was observed during the investigation. The regression equation, DMP yield (g) = -24.6+33.5 daily milk yield (kg) +0.9 MU (mg/dl) was developed to establish the reference level of DMP yield. The result indicates that the effect of parity and stage of lactation may be ignored while interpreting MU values. However, reference MU values may be standardised separately for high milk yielders as level of milk yield contributes significantly to the variation of MU. The study revealed that the MU values together with DMP yield and milk protein content could be used as a potential non-invasive pointer to monitor feeding adequacy in dairy cows under farm conditions. (**Key Words :** Milk Urea, Milk Protein, Milk Yield, Parity, Stage of Lactation, Season)

INTRODUCTION

The urea concentration in milk and blood has been realised as a valuable farm managemental tool to monitor the protein and energy feeding efficiency in dairy cows (Baker et al., 1995; Jonker et al., 2002). Many developed countries have tried to recognise the reference values of urea in blood and milk. The concentration of urea in milk is more practical to use as a pointer. Because, milk is not only easy to collect, it also avoids the stress of blood collection. The urea concentrations in milk and blood are readily affected by protein intake, type of protein and protein-energy balance in diet (Oltner and Wiktorsson, 1983; Roseler et al., 1993; Gouda and Lindberg, 1994; Baker et al., 1995; Whitaker et al., 1995). It is reported that the high urea concentration depresses dairy cow's reproductive efficiency (Ferguson et al., 1993; Butler et al., 1996). A particular level of milk urea (MU) could be due to excess rumen degradable protein (RDP) intake with adequate fermentable metabolisable energy (FME) or intake of required RDP with inadequate FME (Whitaker et al., 1995). This indicates more than one possible explanation for a particular MU level. Therefore, with out a method of assessing energy intake or balance at the same time, MU may not act as an efficient indicator. It has been found that milk protein content reflects energy intake in lactating dairy cows (Coulon and Remond, 1991; Sato, 1998). Previous reports indicate the possibility of using milk protein content and MU concentration in either blood or milk to monitor dietary energy and protein intake in dairy cows (Paulicks, 1992; Hwang et al., 2000). However, the concept has not been

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validated under farm condition so far.

The season (Carlsson et al., 1995; Hwang et al., 2000) and few animal factors like parity (Oltner et al., 1985), stage of lactation (Broderick and Clayton, 1997) and milk yield (Oltner et al., 1985; Carlsson et al., 1995) also have been found to influence MU concentration. Hence, to utilise MU concentration as an efficient farm managemental pointer and to avoid misinterpretation, the effect of these factors are needed to be evaluated under farm condition.

The objectives of the current study were to explore the possibility of using MU concentration and milk protein content to monitor the protein and energy feeding adequacy in dairy cows and to investigate the influences of different animal factors and season on MU concentration under farm condition.

MATERIALS AND METHODS

Animals and management

The study was conducted on crossbred Karan-Fries (Holstein Friesian×Tharparkar) cows maintained at the Institute dairy farm, National Dairy Research Institute, Karnal, India. Only healthy animals with out mastitis problem were selected for the study. The experimental animals were maintained under loose house system in small groups of 40 to 50 animals. Farm grown mixed green fodders and silage were offered *adlibitum* to all the experimental animals twice daily (0900 to 0930 h and 1500 to 1530 h). The animals were provided a let down ration of 0.5 kg concentrate and an additional amount of 1.0 kg concentrate was fed for every 2.5 kg milk produced above 5.0 kg daily yield. The daily concentrate requirement was divided into three equal parts and fed during each milking in morning (0500 to 0600 h), noon (1200 to 1300 h) and evening (1800 to 1900 h). Animals had free access to water throughout the day.

Collection of data and samples and analysis

Individual noon (1200 to 1300 h) milk samples were collected monthly from all the experimental animals over one year. The animals were machine milked during the experimental period. During the study, a total of 1,864 milk samples were collected from 264 cows. Samples were collected from the milk weighing bucket after complete milking and thorough mixing and were kept at 4°C until analysed for urea and protein contents on the same day. Representative samples of green fodders, silage and concentrate mixture that fed to the animals were collected on the same day of milk sampling. Feed samples were subjected to dry matter (DM) and crude protein (CP) analysis as per the procedure of AOAC (1984). Milk samples were analysed for urea content using a colorimetric p-dimethylaminobenzaldehyde (DMAB) procedure (Dhali et al., 2005a). Milk protein N in the form total protein was calculated as the difference between Kjeldahl total N and Kjeldahl NPN (Barbano et al., 1991). For individual animal the information on test day milk (TDM) yield, test day noon milk (TDNM) yield, milk fat content, parity and stage of lactation were collected from the farm record.

Statistical analysis

For analysis TDNM and TDM yields were categorised into 4 and 5 groups respectively, according to mean and SD. The group interval was 1SD. Four different lactation stages were defined for analysis. These were 1 to 3 months, 4 to 6 months, 7 to 9 months and 10 months and above. The whole study period was divided into four different seasons according to the variability of meteorological components. These were winter (Dec. to Mar.), summer (Apr. to Jun.), rainy (Jul. to Sep.) and autumn (Oct. to Nov). To determine whether effects were significant in explaining variations in MU concentration, milk protein content and daily milk protein (DMP) yield the data were analysed using GLM procedure (SPSS, 1999). The model included season, TDNM yield, TDM yield, parity and stage of lactation as sources of variations. To study the seasonal variation in CP content of forages and concentrate the data were analysed

Table 1. Effect of milk yield on milk urea concentration, milk protein content and daily milk protein yield

Particulars	Milk urea (mg/dl ±SE)	Milk protein content (%±SE)	Daily milk protein yield (g±SE)
Test day noon milk yield			
≤1.6 kg/d	37.7±0.8 [a]	3.0±0.04	151.8±4.2[a]
1.7-3.6 kg/d	42.0±0.3 [b]	3.0±0.01	297.4±2.8[b]
3.7-5.5 kg/d	44.7±0.5 [c]	3.0±0.02	462.3±5.0[c]
≥5.6 kg/d	44.1±0.9 [c]	2.9±0.03	657.5±11.3[d]
Test day milk yield			
≤7.0 kg/d	40.3±0.7 [a]	3.0±0.03	161.2±3.9[a]
7.1-12.2 kg/d	38.6±0.4 [b]	3.0±0.01	292.9±2.4[b]
12.3-17.5 kg/d	41.6±0.6 [a]	3.0±0.02	449.8±4.1[c]
17.6-22.8 kg/d	42.4±0.9 [a]	3.0±0.03	593.9±7.8[d]
≥22.9 kg/d	47.6±1.3 [c]	2.9±0.04	773.7±17.6[e]

[a, b, c] Means values with different superscripts within column differ significantly ($p<0.01$).

Table 2. Relationship among milk yield, milk urea concentration, milk protein content and daily milk protein yield

Particulars	Correlation coefficient (r)
Milk urea (mg/dl)	
Test day milk yield (kg)	0.27**
Test day noon milk yield (kg)	0.23**
Milk protein content (%)	0.03
Daily milk protein yield (g)	0.33**
Milk protein content (%)	
Test day milk yield (kg)	-0.03
Test day noon milk yield (kg)	-0.02

** Indicates the r value is significant at $p<0.01$.

using GLM procedure (SPSS, 1999). The model included season as source of variation. When effects were found significant, Duncan multiple range test was used to test the differences among means (SPSS, 1999). To investigate the association between different traits the Pearson correlation analysis was performed (SPSS, 1999). To study the association of DMP yield with TDM yield and MU concentration the regression analysis was performed (SPSS, 1999). Only those TDM yield and MU values were considered for regression analysis, when MU values were found between 24.4 to 37.7 mg/dl with 3% or more milk protein content.

RESULTS

Effects of milk yield, parity and stage of lactation on MU concentration

The MU concentration, milk protein content and DMP yield in different milk yield groups are in Table 1. MU concentration was found to be significantly ($p<0.01$) associated with TDNM and TDM yields. MU values were increased with increasing milk production. Though milk protein content did not vary significantly among the groups but DMP yield was increased significantly ($p<0.01$) with increasing milk yield (Table 1). The correlation coefficients among milk yield, MU concentration, milk protein content and DMP yield are presented in Table 2. A close association ($p<0.01$) between MU concentration and DMP yield was observed. The effects of parity and lactation stage on MU concentration were significant ($p<0.01$). The concentration was found highest in first lactation and it was increased in advanced lactation stage (Table 3). The effects of parity and stage of lactation were significant ($p<0.01$) on DMP yield. It was lowest during first parity and was highest during early stage of lactation (Table 3). However, the effect of parity was not significant on milk protein content, but the effect of lactation stage on milk protein content was found significant ($p<0.01$). During experimental period the average milk fat content of experimental animals was 3.6±0.1%.

Table 3. Effect of parity and stage of lactation on milk urea concentration, milk protein content and daily milk protein yield

	Milk urea (mg/dl±SE)	Milk protein content (%±SE)	Daily milk protein yield (g±SE)
Parity			
1	44.8±0.7 ^{a}	2.9±0.02	165.9±11.7^{a}
2	43.5±0.6 ^{c}	3.0±0.02	217.7±10.1^{b}
3	42.4±0.7 ^{bc}	3.1±0.02	274.3±11.3^{b}
4	42.5±0.7 ^{bc}	3.0±0.02	262.5±12.6^{b}
5	41.7±0.7 ^{dbc}	3.0±0.03	276.2±13.7^{b}
6	39.7±1.1 ^{d}	3.0±0.04	309.4±17.5^{b}
7 and above	40.4±1.0 ^{db}	3.0±0.04	283.6±19.5^{b}
Stage of lactation (month)			
1-3	40.7±0.5 ^{a}	2.9±0.01^{a}	313.4±10.2^{a}
4-6	41.6±0.5 ^{a}	3.0±0.02^{b}	286.0±10.1^{a}
7-9	43.5±0.5 ^{b}	3.0±0.02^{b}	214.6±10.4^{ab}
10 and above	42.7±0.6 ^{b}	3.0±0.02^{b}	208.7±12.0^{b}

$^{a, b, c, d}$ Means values with different superscripts within column differ significantly ($p<0.01$).

Seasonal variation in MU concentration, milk protein content and DMP yield

Different feeds offered to the experimental animals in different months and their CP contents are presented in Table 4. The effect of season was significant ($p<0.01$) on MU concentration (Table 5). MU concentration was highest in winter and lowest in summer. The DMP yield was significantly ($p<0.01$) higher in winter and lower in summer (Table 5). To study the seasonal distribution, the MU concentrations were categorised into 5 different levels according mean and SD. The group interval was 1 SD. These levels were, L1 (<30 mg/dl), L2 (30-40 mg/dl), L3 (40.1-50 mg/dl), L4 (50.1-60 mg/ dl) and L5 (>60 m/dl). Seasonal distribution of different MU levels is shown in

Table 4. CP content (% DM) of different green forages, silages and concentrate mixture fed during experimental period

Feed	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Concentrate mixture	23. 2	21.1	20.8	18.0	23.1	22.1	24.9	22.9	25.2	21.3	19.0	21.0
Berseem	-	-	-	-	18.5	16.9	17.9	16.7	22.7	16.3	-	-
Mustard	-	-	-	-	20.4	21.0	-	-	-	-	-	-
Maize	12.4	12.1	13.8	12.3	-	-	-	-	-	-	8.7	-
Sorghum	-	10.7	10.5	10.4	-	-	-	-	-	-	-	8.9
Oat	-	-	-	-	-	-	10.5	-	-	11.3	-	-
Maize silage	-	-	-	-	-	-	14.7	-	-	-	-	-
Oat silage	-	-	-	-	12.0	-	-	-	-	-	-	-

Table 5. Seasonal variation in milk urea concentration, milk protein content, daily milk protein yield, forage CP content and concentrate CP content

Season	Milk urea (mg/dl±SE)	Milk protein content (%±SE)	Daily milk protein yield (g±SE)	Concentrate CP content (%±SE)	Forage CP content (%±SE)
Winter (Dec.-Mar.)	50.7±0.3 [a]	3.2±0.01[a]	463.4±7.6[a]	23.7±0.9	17.2±1.3[A]
Summer (Apr.-Jun.)	32.9±0.6 [b]	2.7±0.01[b]	339.5±6.2[b]	20.4±1.0	11.3±1.7[B]
Rainy (Jul.-Sep.)	41.3±0.4 [c]	3.0±0.02[a]	308.9±6.2[b]	21.7±1.0	11.9±1.6[B]
Autumn (Oct.-Nov.)	43.5±0.5 [d]	3.0±0.02[a]	347.0±9.9[c]	20.5±1.2	14.7±1.6[AB]

[a, b, c, d] Means values with different superscripts within column differ significantly (p<0.01).
[A, B, C, D] Means values with different superscripts within column differ significantly (p<0.05).

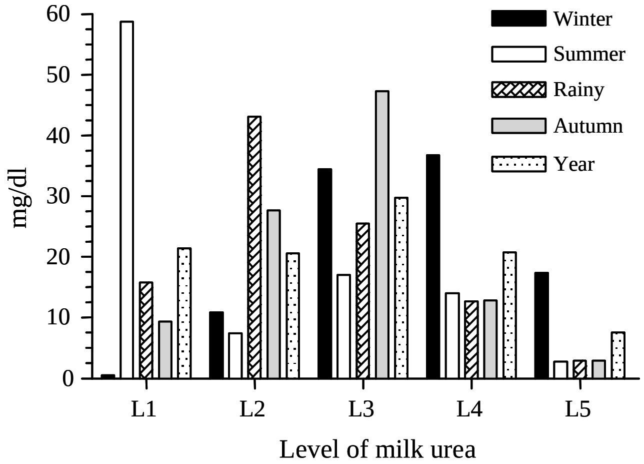

Figure 1. Distribution (% of total observation) of different milk urea levels L1 (<30 mg/dl), L2 (30-40 mg/dl), L3 (40.1-50 mg/dl), L4 (50.1-60 mg/dl) and L5 (>60 mg/dl) in different seasons (n = 1,864).

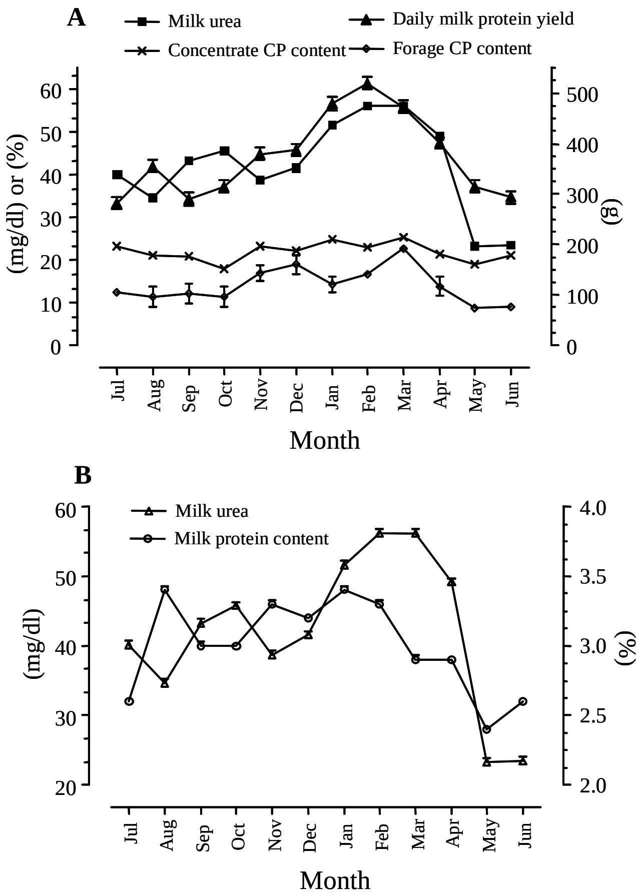

Figure 2. Variation in milk urea concentration (mg/dl±SE), daily milk protein yield (g/day±SE), milk protein content (%±E), forage CP content (%) and concentrate CP content (%) during experimental period; Panel A: Month wise variation in milk urea concentration, daily milk protein yield, forage CP content and concentrate CP content; Panel B: Month wise variation in milk urea concentration and milk protein content.

Figure 1. During investigation, of the total observed MU concentrations, 50.3% were between 30 to 50 mg/dl, 21.4% were <30 mg/dl and only 7.5% were >60 mg/dl (Figure 1). The month wise distribution of MU concentration, milk protein content, DMP yield, forage CP content and concentrate CP content are depicted in Figure 2. The association of MU concentration, milk protein content and DMP yield with CP contents of concentrate and forages are in Table 6. MU level was found to be significantly (p<0.05) associated with CP content of forages than concentrate. In contrast, DMP yield was found to be significantly (p<0.05) associated with CP content of forages and concentrate. A similar trend was also observed for milk protein content but was not significant. The association of DMP yield (g) with daily milk yield (DMY, kg) and MU concentration (mg/dl) was described by the regression equation, DMP yield = -24.6 (9.8)+33.5 (0.6) DMY+0.9 (0.6) MU, where values in parenthesis are SE and R^2 = 0.93.

DISCUSSION

The study was conducted to explore the possibility of using MU concentration and milk protein content to monitor the energy and protein feeding adequacy in dairy cows and to explore the possible influences of different animal factors and season on MU concentration under farm condition. It is reported that urea concentration in plasma and milk can be used as an indicator of nitrogen utilisation and feeding adequacy in dairy cows (Baker et al., 1995; Jonker et al., 2002). The main factor influencing these concentrations is the ratio of protein and fermentable energy in diet (Oltner and Wiktorsson, 1983). However, previous reports also indicate the possible effects of different animal

Table 6. Relationship of milk urea concentration, milk protein content and daily milk protein yield with CP content of forages and concentrate

Particulars	Correlation coefficient (r)
Milk urea (mg/dl)	
Forage CP content (%)	0.70*
Concentrate CP content (%)	0.54
Milk protein content (%)	
Forage CP content (%)	0. 44
Concentrate CP content (%)	0. 36
Daily milk protein yield (g)	
Forage CP content (%)	0.70*
Concentrate CP content (%)	0.64*

* Indicates the r value is significant at p<0.05.

factors on MU concentration. These are milk yield (Oltner et al., 1985), stage of lactation (Carlsson et al., 1995; Whitaker et al., 1995; Broderick and Clayton, 1997) and parity (Oltner et al., 1985; Broderick and Clayton, 1997).

During investigation a significant positive association between MU concentration and milk yield was observed. A similar trend also has been indicated previously (Oltner et al., 1985; Carlsson et al., 1995; Yoon et al., 2004). The current investigation revealed that in cows, which yielded 22.9 kg or more milk daily, MU concentration was increased by 12.2 to 23.3% than the cows with comparatively lower daily milk yield. The result indicates that for high yielding cows, the reference MU values may be standardised separately. MU concentration was found to be significantly higher in first calvers than multiparous cows. A similar finding has been reported previously by Broderick and Clayton (1997). However, Oltner et al. (1985) has found lower MU nitrogen level (0.76 M mol/lit) in first calvers than multiparous cows. In present study, the higher MU concentration in first calvers might be due to the fact that the cows were still in growing phase with comparatively higher nutritional requirement and were probably under energy deficient condition at first calving. A trend for high MU concentration in early stages of lactation was observed. A similar trend also has been reported previously (Carlsson et al., 1995; Whitaker et al., 1995; Broderick and Clayton, 1997; Yoon et al., 2004). The low MU values in early stages of lactation were probably due to the fact that animals were on energy deficient condition during this period. In dairy cows it has been observed that animals are prone to energy deficiency in first 100 days of lactation and have energy over supply in the last third of lactation (Kalchreuter, 1990). However, in current investigation the variation in MU concentration among different parities and stages of lactation were found small and within the range of 0.2 to 10.8% and 2.1 to 6.4%, respectively. The result indicates that the effect of parity and stage of lactation may not be considered while interpreting MU values. A significant variation in MU concentration was observed in different seasons. The proportion of MU values, which were above 40 mg/dl was found highest in winter and lowest in summer. The effect of season on MU concentration was probably due to the changes in feeding (Carlsson et al., 1995) as the average CP contents of concentrate and forages were found highest in winter and lowest in summer.

Urea concentration in milk and blood is readily affected by protein intake, type of protein and protein-energy balance in diet (Oltner and Wiktorsson, 1983; Roseler et al., 1993; Gouda and Lindberg, 1994; Whitaker et al., 1995). But a particular level of MU could be due to excess rumen degradable protein (RDP) intake with adequate fermentable metabolisable energy (FME) or intake of required RDP with inadequate FME (Baker et al., 1995). This indicates more than one possible explanation for any particular MU level. Hence, to use MU as an effective indicator to assess nutritional adequacy, a simultaneous measure of energy balance is also required at the same time. Milk protein content is of practical importance at this juncture. A positive relationship has been found between the amount and concentration of ME and, milk protein content and milk protein yield (Depeters and Cant, 1992). Paulicks (1992) reported that the milk protein content is a useful supplementary indicator while interpreting MU for dietary protein-energy balance. It is because that milk protein responds almost exclusively to decreased-energy supply independent of protein intake. Through an extensive literature survey Hwang et al. (2000) has established the reference level of MU nitrogen 11 to 17 mg/dl (equivalent to 24.4 to 37.7 mg/dl MU) and 3% milk protein content for monitoring the nutritional adequacy of dairy cows in Taiwan. Milk protein content below 3% indicates the inadequate dietary energy and MU value below or above the reference range indicates the deficiency or surplus of dietary protein, respectively. The basis of using 3% milk protein content as reference is that milk fat is set at 3.5% in Taiwan and the percentage ratio of protein to fat in milk is 0.85 to 0.88 under normal nutrition status for lactating cows (Mahanna, 1995). In Karan-Fries cows during experimental period the average milk fat recorded was 3.6±0.1%. The morning, noon and evening MU values have been found within the range of 24.2 to 35.4 mg/dl in Karan-Fries cows when fed berseem and green maize based diet according to NRC (1989) standard (Dhali et al., 2005b). At this context the reference values for MU and milk protein content as proposed by Hwang et al. (2000) was found suitable to interpret nutritional adequacy in Karan-Fries cows.

During investigation MU values were found more closely associated with forage CP content. In summer milk protein content was found less than 3%, which indicated the low energy intake in experimental animals. However, the average MU value was found within the reference range that indicated the cows were on adequate protein diet. It was observed that the low MU values during summer and in May and June were coincided with low forage CP content in comparison to other seasons and months. But in the other three seasons, milk protein content above 3% and MU values above the reference range indicated that cows were fed on adequate energy and surplus protein. The highest MU values were observed during winter and in March, when forage CP content was highest. During investigation, less than 3% milk protein content was found in first three months of lactation, in highest yielding cows and in first calvers, which indicated an energy deficient condition. In all these cases MU values above reference range indicated that animals were on surplus protein diet. A significant association of MU with DMP yield than milk protein content was found in current study. This indicated that the DMP yield could be used more precisely to monitor feeding adequacy in dairy cows. Hence, the regression equation was developed to establish the reference level of DMP yield on the basis of daily milk yield and MU. For developing the equation only that milk yield and MU values were considered which satisfied the reference levels of MU and milk protein content as established by Hwang et al. (2000) to exclude the possible values due to faulty dietary condition.

The study revealed that the MU values together with DMP yield and milk protein content could be used as a potential non-invasive pointer to monitor feeding adequacy in dairy cows under farm condition, when individual animals are not being monitored for nutrient intake. The parity and stage of lactation may be ignored while interpreting MU values. Because the contribution of these two factors is less in variation of MU. However, reference MU values may be standardised separately for high milk yielders as the level of milk yield contributes a higher variation in MU concentration.

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