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REGULAR ARTICLES

Association of bulk tank milk urea nitrogen concentration with elevated individual cow values and investigation of sampling frequency for accurate assessment

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Abstract
Individual milk urea nitrogen (MUN) levels ≥ 19.63 mg/dL have been recently reported to significantly affect fertility. The objectives of the present study were to (a) predict the percentage of cows with elevated MUN within a herd using bulk tank (BTMUN) levels, in the absence of individual MUN records, and (b) establish a sampling frequency protocol for the assessment of actual BTMUN levels. A database of 17,687 monthly individual MUN and concurrent 229 monthly BTMUN records from 24 dairy herds was used. A ROC analysis was performed to determine the BTMUN threshold over which cows in the herd have elevated MUN concentrations that, based on literature, affect fertility. Moreover, a regression was run to predict the percentage of cows with elevated MUN within a herd from BTMUN values. A second database of 10,687 daily BTMUN records from 29 herds was used to identify an appropriate sampling frequency to assess the actual BTMUN levels. Eleven different sampling frequencies ranging from once to 8 times per month were assessed. A BTMUN value of 15.76 mg/dL was the optimum threshold over which cows with elevated MUN concentrations are included in a herd. The percentage of cows with elevated MUN values can be accurately predicted using BTMUN values ($R^2 = 0.872$; $P < 0.001$). A bulk tank sampling frequency of once per week seems appropriate for most herds in order to assess the actual BTMUN levels, in case daily BTMUN values are not available from milk processors.

Keywords Bulk tank · Cattle (dairy) · Milk urea nitrogen · Sampling frequency

Introduction

In lactating dairy cows, milk urea nitrogen (MUN) concentration is considered a valuable tool in evaluating nutritional imbalances and predicting urinary nitrogen excretion to the environment (Jonker et al. 1998). It is affected by breed and parity of the cow, milk production and components' content, stage of lactation, and individual cow variation; however, MUN concentration is mainly affected by nutritional factors such as dietary crude protein (CP) content, ratio of rumen degradable protein (RDP) to rumen undegradable protein (RUP) fractions, ruminally available energy to protein ratio, and total amino acids reaching the small intestine (Patton et al. 2014). A herd-level MUN concentration target of 8–12 mg/dL, resulting from individual cow values, was proposed by Kohn et al. (2002) for intensive dairy farms.

The negative effect of excess dietary nitrogen on cow fertility and the underlying mechanisms have been thoroughly described (Rhoads et al. 2008). In a recent meta-analysis of published studies investigating the effect of elevated MUN on fertility of dairy cows, Raboisson et al. (2017) proposed an individual milk urea threshold of 420 mg/L (which corresponds to 19.63 mg/dL MUN), above which cows have a 43% reduction in conception risk (odds ratio = 0.57; 95% CI 0.45–0.73) compared to cows with lower urea levels, without excluding significant negative effects with lower cutoffs. Obviously, when a large percentage of cows have MUN values above the aforementioned threshold a herd-level impairment of reproduction performance may result.

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Individual cow sampling is expensive and labor intensive to be implemented on a daily basis. In several countries, individual cow MUN values are monthly recorded in dairies enrolled in national dairy herd improvement programs and the percentage of elevated MUN values can be calculated; however, whether this monthly testing accurately represents the true risk is unknown.

Bulk tank milk sampling appears a reliable, cost-effective alternative, and daily bulk tank MUN (BTMUN) values are usually available from milk processors. Determining the association between BTMUN and the percentage of elevated individual cow values would enable the identification of herds in risk of impaired reproduction. If BTMUN values are not available from milk processors on a daily basis, a standardized BTMUN sampling frequency must be established in order to accurately estimate the risk.

Therefore, the objective of this study were to (a) test the hypothesis that the percentage of cows with elevated MUN within a herd can be predicted by examining BTMUN levels, in the absence of individual MUN records, and (b) establish a sampling frequency protocol for the assessment of actual BTMUN levels.

Materials and methods

A database consisting of 17,687 monthly individual MUN and concurrent 229 monthly BTMUN records from 24 dairy herds was used to determine the association of bulk tank with elevated individual MUN values within a herd. Samples were collected by technicians of Thessaly's regional council of the Holstein Association of Greece and processed by Fourier transform infrared technology using MilkoScan FT6000 (Foss Electric, Hillerød, Denmark). In all herds, cows were housed indoors in free stalls. No grouping strategy was applied for lactating cows in these herds and only cows contributing to bulk tank were individually sampled. Mean (± SD) number of lactating cows was 78.8 (± 51.3) per herd and mean (± SD) daily milk production was 25.8 (± 4.1) kg per cow. To validate our database, monthly BTMUN records were paired with weighted-by-milk-production mean herd MUN (WHMUN) values and agreement between the two was assessed using the Bland–Altman graphical procedure and Lin's concordance correlation coefficient (CCC).

A receiver operating characteristic (ROC) analysis was performed to determine the optimum BTMUN threshold (OT), according to Youden index, above which individual cows in the herd have elevated MUN concentrations [≥ 19.63 mg/dL; as proposed by Raboisson et al. 2017] affecting their reproductive performance. Moreover, a univariate regression model analysis was run to predict the percentage of cows with elevated MUN (≥ 19.63 mg/dL) from the BTMUN values.

A second database consisting of 10,687 daily BTMUN records from 29 different dairy herds was used to identify an appropriate sampling frequency for representative assessment of the actual BTMUN levels. Mean (± SD) number of lactating cows was 73.5 (± 39.4) per herd and mean (± SD) daily milk production was 23.0 (± 4.9) kg per cow. Milk samples from all herds were collected by a technician from the milk processing company (Kri Kri S.A., Serres, Greece) and analyzed with Fourier transform infrared technology using MilkoScan FT6000 (Foss Electric, Hillerød, Denmark).

For each herd, monthly mean BTMUN values were calculated (total of 377 records) and classified in four clusters: low, moderate, high, and very high (< 8.00 mg/dL, 8.00–12.00 mg/dL, 12.01 mg/dL–OT, and > OT, respectively). A series of monthly sampling frequencies were assessed: once, twice (2 consecutive days; once per week for 2 consecutive weeks; once every 2 weeks), three times (3 consecutive days; once per week for 3 consecutive weeks), four times (2 consecutive days for 2 consecutive weeks; once per week), six times (2 consecutive days for 3 weeks), and eight times (2 consecutive days every week; twice per week with 3 days apart). Mean BTMUN values were calculated for multiple monthly samplings.

Overall, a total of 68,434 possible combinations were tested considering the following criteria: (a) the percentage of mean BTMUN values of each sampling frequency protocol classifying a herd in the same cluster as the monthly mean BTMUN, and (b) the accuracy of each sampling frequency protocol in identifying herds with very high monthly BTMUN (> OT). The chi-square (χ^2) test of independence was used to compare percentages for criterion A, among the studied sampling frequencies. Bonferroni's correction for multiple comparisons was used. For criterion B, sensitivity (Se), specificity (Sp), positive and negative predictive value (PPV and NPV, respectively), and Cohen's kappa coefficients were calculated. Data were analyzed using MedCalc v.17.8.6 (MedCalc Software bvba, Ostend, Belgium) and IBM SPSS v.22 (Armonk, NY: IBM Corp.).

Results

Mean (± SD) BTMUN and WHMUN levels in the first database were 15.72 (± 2.5) mg/dL and 15.77 (± 2.4) mg/dL, respectively. Bland–Altman plot of BTMUN against WHMUN showed a satisfactory agreement with a minor systematic bias (defined as the mean difference between tested methods) of − 0.1 mg/dL (Fig. 1). Ninety-four percent of paired differences were within the 95% limits of agreement (− 1.5–1.4 mg/dL). Regression of paired differences on average values of both methods was nonsignificant, revealing no proportional bias. The CCC was 0.96 (95% CI 0.94–0.97), representing substantial agreement between the two levels (Fig. 2).

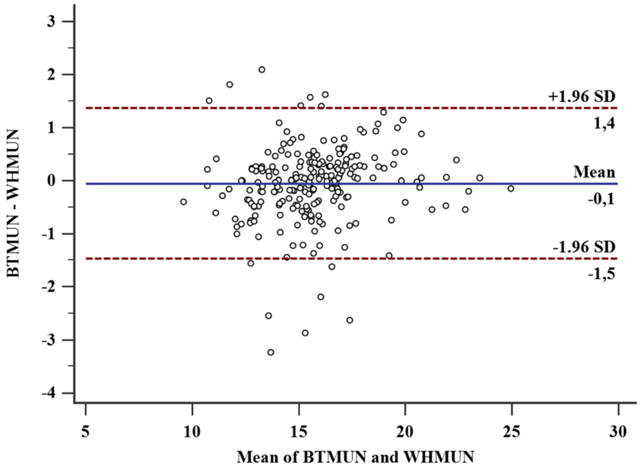

Fig. 1 Bland–Altman plot of the mean herd MUN levels measured as bulk tank MUN (BTMUN) and weighted-by-milk-production mean herd MUN (WHMUN) values on *x*-axis against paired differences between BTMUN and WHMUN on *y*-axis. Solid line indicates the systematic bias and dash lines the 95% limits of agreement

A BTMUN value of > 15.76 mg/dL was identified as the OT over which cows with individual MUN concentration ≥ 19.63 mg/dL are present in a herd (Fig. 3) Corresponding Se was 76.15%, Sp was 88.89%, and area under the ROC curve was 0.89 ($P < 0.001$). Regression analysis showed that the percentage of cows with MUN values ≥ 19.63 mg/dL within a herd can be accurately predicted ($R^2 = 0.872$; $P < 0.001$) by BTMUN values based on a quadratic model (Fig. 4). At BTMUN values of 17 mg/dL and 20 mg/dL, the percentage of cows with elevated individual values is predicted to be 11.3% and 43.9%, respectively.

Mean (± SD) BTMUN across herds was 12.85 (± 3.1) mg/dL in the second database, which was used to establish optimum sampling frequency. The year-round coefficient of variation for BTMUN values ranged from 7.2 to 34.4% for

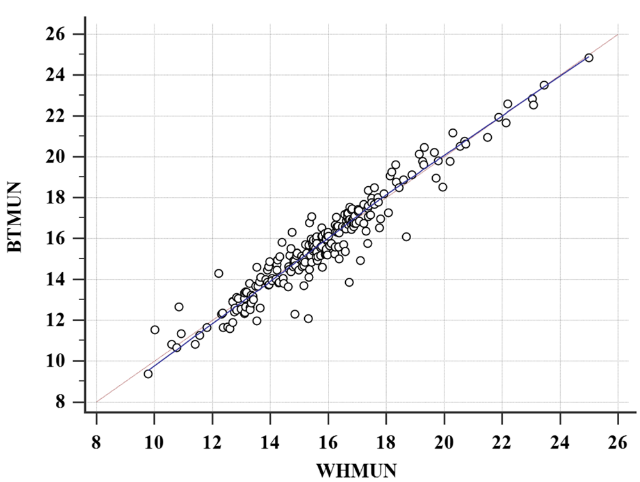

Fig. 2 Concordance correlation plot for 229 paired weighted-by-milk-production mean herd MUN (WHMUN) values (mg/dL) against bulk tank MUN (BTMUN) values (mg/dL) showing substantial agreement; concordance correlation coefficient = 0.96 (95% CI 0.94–0.97)

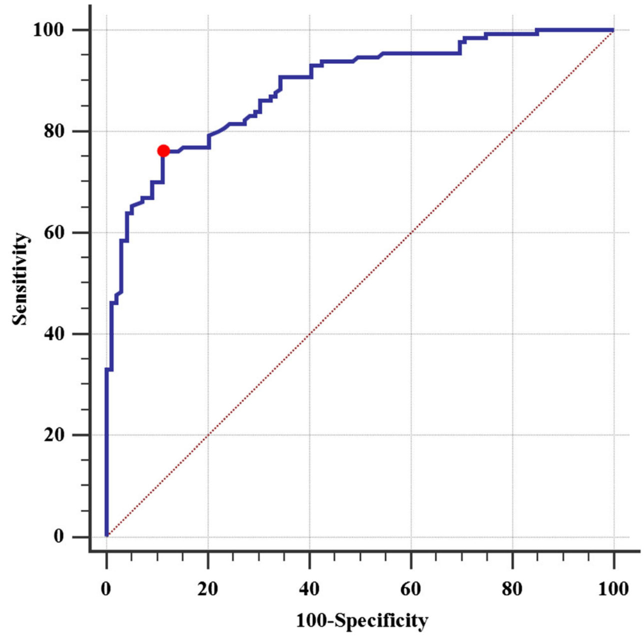

Fig. 3 Receiver operating characteristic (ROC) plot of BTMUN values for shows the optimum threshold over which cows with a MUN concentration ≥ 19.63 mg/dL are included in a herd. Area under the ROC curve 0.89 ($P < 0.001$); sensitivity 76.15% (95% CI 67.9–83.2); specificity 88.89% (95% CI 81.0–94.3); criterion (Youden index) > 15.76 mg/dL

different herds. The percentage of mean BTMUN values of each sampling frequency studied classified herds in the same cluster as the monthly mean BTMUN (criterion A) are presented in Table 1. Sampling once per month resulted in ca. one quarter of the values falsely classifying a herd into a different cluster than monthly mean BTMUN. Correct classification improved as sampling frequency increased. Sampling once per week resulted in ca. 90% correct classifications and did

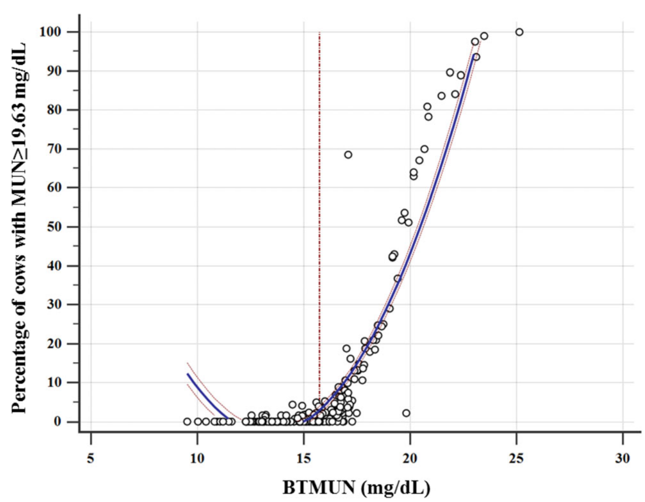

Fig. 4 Scatter diagram and regression line of the percentage of cows with milk urea nitrogen (MUN) ≥ 19.63 mg/dL in a herd (*y*-axis), against the bulk tank MUN (BTMUN) levels of the herd (*x*-axis). Regression equation: $y = 183.70 - 27.99x + 1.05 \times {}^{2}$ ($R^2 = 0.872$, $P < 0.001$). The vertical line refers to BTMUN value of 15.76 mg/dL

Table 1 Comparison of sampling frequencies for criteria A [the percentage of mean bulk tank milk urea nitrogen (BTMUN) values of each sampling frequency classifying a herd in the same as the monthly mean BTMUN cluster] and B (the accuracy of each sampling frequency in identifying herds with very high monthly BTMUN; cutoff derived from ROC curve > 15.76 mg/dL)

Sampling frequency	Criterion A (%)	Criterion B					
		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Kappa coefficient	
						Value	Interpretation
1×	75.82 a	80.94	93.02	57.14	97.70	0.625*	Substantial
2 × a	78.41 b	81.98	94.41	62.74	97.85	0.673*	Substantial
2 × b	82.96 c	85.85	96.28	72.43	98.36	0.759*	Substantial
2 × c	82.85 c	85.29	96.35	72.27	98.33	0.756*	Substantial
3 × a	80.66 d	84.09	95.31	67.30	98.12	0.715*	Substantial
3 × b	87.05 e	88.19	97.55	80.07	98.67	0.820*	Good
4 × a	85.03 f	86.87	96.88	76.01	98.48	0.788*	Substantial
4 × b	89.70 g	87.81	98.63	87.50	98.67	0.863*	Good
6×	88.80 g	88.05	98.29	85.16	98.67	0.851*	Good
8 × a	91.83 h	90.08	98.92	90.08	98.92	0.890*	Good
8 × b	92.92 h	96.97	99.45	94.12	99.72	0.951*	Good

Percentages with different letters within the same column differ significantly ($P < 0.05$)

*$P < 0.001$

PPV positive predicted value, *NPV* negative predicted value, *1×* once a month, *2 × a* 2 consecutive days, *2 × b* once a week for 2 consecutive weeks, *2 × c* once every 2 weeks, *3 × a* 3 consecutive days, *3 × b* once a week for 3 consecutive weeks, *4 × a* 2 consecutive days for 2 consecutive weeks, *4 × b* once every week, *6×* 2 consecutive days for 3 weeks, *8 × a* 2 consecutive days every week, *8 × b* twice a week with 3 days apart

not differ from sampling six times per month. Sampling twice a week (2 consecutive days every week and twice per week with 3 days apart) differed significantly from sampling once per week and six times per month and gave the best results: ca. 92% and 93% correct classifications, respectively. Calculations of Se, Sp, PPV, NPV, and kappa statistics for each sampling frequency with regard to identification of herds with monthly mean BTMUN > 15.76 mg/dL (threshold derived from ROC curve) are shown in Table 1. Measures of Sp were constantly > 90%. Measures of Se improved as sampling frequency increased, exceeding 90% for sampling 8 times per month. Several sampling frequencies had a good categorized agreement (kappa coefficient > 0.80) with monthly mean BTMUN values (Table 1). However, agreement was best for the twice per week sampling frequencies (kappa coefficient of 0.89 and 0.95, respectively).

Discussion

Increasing ration CP content is a commonly implemented practice to increase milk production in cattle, both in temperate (Godden et al. 2001; Colmenero and Broderick 2006) and tropical (Wanapat et al. 2017; Naveed-ul-Haque et al. 2018) dairy farming systems. The dietary CP content is positively correlated with MUN concentration (Broderick and Clayton 1997), while over-feeding protein decreases the already low nitrogen efficiency in ruminants (Huhtanen and Hristov 2009), thereby compromising farm profitability.

High ammonia and urea concentrations in blood and milk have been associated with impaired reproductive performance (Carroll et al. 1988; Butler 2000). Several relevant mechanisms have been described including (1) the energetic cost for ammonia transformation to urea and its excretion, which aggravates the negative energy balance of the cow in early lactation (Staples et al. 1993; Butler 1998; Leroy et al. 2008); (2) the direct toxic effect of ammonia and urea on gametes, follicles, and oviducts (Visek 1984; Ferguson et al. 1988; Staples et al. 1993); (3) the increased urea concentration in uterine secretions (Jordan et al. 1983); (4) the reduction of uterine lumen pH (Elrod and Butler 1993); and (5) the reduction in plasma progesterone levels (Jordan and Swanson 1979; Sonderman and Larson 1989).

Generally, diets resulting in mean MUN concentration of < 8 mg/dL are considered deficient in protein supply, while MUN > 12 mg/dL represents excess nitrogen over milk production requirements and/or concurrent energy deficiency (Jonker and Kohn 2001). Notable negative effects on cow fertility are likely to occur at individual MUN values higher than the herd-level target range proposed to satisfy both production and nitrogen efficiency utilization. In the present study, we used the individual cow threshold previously proposed by Raboisson et al. (2017).

In many countries, the only way to monitor the MUN status of a herd is by sampling the bulk tank milk. Therefore, the development of a function that reliably connects the herd's BTMUN value with the percentage of cows at risk of impaired fertility due to high MUN concentrations was essential. To the best of our knowledge, a similar retrospective approach has only been applied for predicting the percentage of cows with high somatic cell counts from bulk tank milk (Lievaart et al. 2009).

In the present study, BTMUN values had a substantial agreement with WHMUN (CCC = 0.96), better than previously reported (CCC = 0.91; Arunvipas et al. 2004). McBride (2005) proposed the following interpretation of CCCs: poor (CCC < 0.90), moderate (0.90 ≤ CCC ≤ 0.95), substantial (0.95 < CCC ≤ 0.99), and almost perfect agreement (CCC > 0.99). In our study, the systematic error derived from Bland–Altman plot is slightly higher (− 0.1 mg/dL vs. + 0.05 mg/dL), but the 95% limits of agreement are narrower (− 1.5 to + 1.4 mg/dL vs. − 2.46 to + 2.56 mg/dL) than those of Arunvipas et al. (2004), respectively. The latter also reported that the strong agreement observed between the two methods extends to both pasture and nonpasture seasons, various herd sizes, and sampling regimen applied.

The BTMUN value of 15.76 mg/dL that was identified as the threshold, above which cows within a herd may have impaired fertility according to the study by Raboisson et al. (2017), is within the range commonly observed. This result practically coincides with the mean BTMUN and WHMUN values of the first database in the present study. Moreover, similar or even higher mean herd values have been previously reported (15.5 mg/dL, Johnson and Young 2003; 16.0 mg/dL, Fatehi et al. 2012; 17.9 mg/dL, Aguilar et al. 2012; 15.54 mg/dL, Siachos et al. 2017). The aforementioned threshold can be considered as an alert starting-point. However, the scatter plot of the percentage of cows with high MUN against the herd BTMUN values indicates that considerable concerns regarding herd-level reproduction efficiency should arise at a BTMUN level of > 17 mg/dL where the percentage of cows at risk of impaired fertility is clearly over 10% and rises abruptly thereafter. This percentage can be quite safely predicted by evaluating BTMUN records, following the quadratic function developed.

Our analysis was based on the assumption that cows with elevated MUN values are equally distributed across lactation; however, grouping strategies influence the range and distribution of MUN levels across lactation in a herd (Patton et al. 2014). Early lactation cows are normally expected to have higher MUN values than cows at mid or late lactation, as an effect of higher CP diets and lower nitrogen utilization efficiency and/or periparturient muscle protein catabolism (Jonker et al. 1998; Patton et al. 2014). Milk-line sampling collectors are commercially available and have been evaluated for obtaining representative pooled samples of different groups of cows within a herd (Godden et al. 2002). They can be applied in large dairies for monitoring MUN status in high-risk groups or pens of cows. Patton et al. (2014) have proposed specific recommendations on MUN levels for different lactation groups to evaluate dietary protein efficiency.

At herd level, BTMUN values exhibit significant variation due to season, month, and even test-day. This variation results mainly from yearly calving distribution, variability in forage quality, periods of transition from nongrazing to grazing and vice-versa, periods of transition to different forage feeds, and an overall inconsistency in diet formulation and distribution (Arunvipas et al. 2004; Wattiaux et al. 2005). Additionally, alterations in the temperature-humidity index account for variations in blood and milk urea nitrogen levels (Kekana et al. 2018).

In less-developed countries, dairy farmers usually do not have access to herd improvement programs. When access is feasible, milk testing is conducted only in monthly intervals. Although essential, an optimal sampling frequency to credibly monitor herd MUN status from BTMUN values had not been reported before this study. Eleven different sampling frequency protocols were evaluated in the present study. With once per month sampling, the number of falsely low BTMUN values (false negatives) obtained would be low, as Sp was constantly high. On the other hand, sampling once per month would result to 25% false positives, which would incorrectly lead farmers, nutritionists, and veterinarians to the conclusion that diet formulation accounts for reproductive inefficiency. A sampling frequency of once per week seems the most suitable frequency, approaching 90% of correct classifications. Of course, twice per week sampling gave the best results; practical differences with sampling once per week were small, though. Nevertheless, in herds with very high BTMUN variation, twice per week sampling would probably be prudent; in this case, sampling twice per week with 3 days apart seems to be preferable.

Conclusions and recommendations

The percentage of cows at risk of impaired reproduction efficiency due to elevated MUN concentrations within a herd can be accurately predicted by BTMUN values in farms not participating in herd milk testing schemes and therefore, lacking individual cow MUN values. Sampling once per week from the bulk tank is recommended for most herds, for an actual assessment of the BTMUN status, considering the variation across day-to-day measurements, in the absence of daily measurements reported by milk processors.

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Compliance with ethical standards

Statement of animal rights The manuscript does not contain clinical studies or patient data.

Conflict of interest The authors declare that they have no conflict of interest.

References

Aguilar, M., Hanigan, M.D., Tucker, H.A., Jones, B.L., Garbade, S.K., McGilliard, M.L., Stallings, C.C., Knowlton, K.F., and James, R.E., 2012. Cow and herd variation in milk urea nitrogen concentrations in lactating dairy cattle. Journal of Dairy Science, 95, 7261–7268.

Arunvipas, P., VanLeeuwen, J.A., Dohoo, I.R., and Keefe, G.P., 2004. Bulk tank milk urea nitrogen: seasonal patterns and relationship to individual cow milk nitrogen values. Canadian Journal of Veterinary Research, 68, 169–174.

Broderick, G.A., and Clayton, M.K., 1997. A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. Journal of Dairy Science, 80, 2964–2971.

Butler, W.R., 1998. Effect of protein nutrition on ovarian and uterine physiology in dairy cattle. Journal of Dairy Science, 81, 2533–2539.

Butler, W.R., 2000. Nutritional interactions with reproductive performance in dairy cattle. Animal Reproduction Science, 60/61, 449–457.

Carroll, D.J., Barton, B.A., Anderson, G.W., and Smith, R.D, 1988. Influence of protein intake and feeding strategy on reproductive performance of dairy cows. Journal of Dairy Science, 71, 3470–3481.

Colmenero, J.O., and Broderick, G.A., 2006. Effect of dietary crude protein concentration on milk production and nitrogen utilization in lactating dairy cows. Journal of Dairy Science, 89, 1704–1712.

Elrod, C.C., and Butler, W.R., 1993. Reduction of fertility and alteration of uterine pH in heifers fed excess ruminally degradable protein. Journal of Animal Science, 71, 694.

Fatehi, F., Zali, A., Honarvar, M., Dehghan-banadaky, M., Young, A.J., Ghiasvand, M., and Eftekhari, M., 2012. Review of the relationship between milk urea nitrogen and days in milk, parity, and monthly temperature mean in Iranian Holstein cows. Journal of Dairy Science, 95, 5156–5163.

Ferguson, J.D., Blanchard, T., Galligan, D.T., Hoshall, D.C., and Chalupa, W., 1988. Infertility in dairy cattle fed a high percentage of protein degradable in the rumen. Journal of the American Veterinary Medical Association, 192, 659–661.

Godden, S.M., Lissemore, K.D., Kelton, D.F., Leslie, K.E., Walton, J.S., and Lumsden, J.H., 2001. Relationships between milk urea concentrations and nutritional management, production, and economic variables in Ontario dairy herds. Journal of Dairy Science, 84, 1128–1139.

Godden, S., Bey, R., Reneau, J., Farnsworth, R., and LaValle, M., 2002. Field validation of a milk-line sampling device for monitoring milk component data. Journal of Dairy Science, 85, 2192–2196.

Huhtanen, P., and Hristov, A.N., 2009. A meta-analysis of the effects of dietary protein concentration and degradability on milk protein yield and milk N efficiency in dairy cows. Journal of Dairy Science, 92, 3222–3232.

Johnson, R.G., and Young, A.J., 2003. The association between milk urea nitrogen and dhi production variables in western commercial dairy herds. Journal of Dairy Science, 86, 3008–3015.

Jonker, J.S., and Kohn, R.A., 2001. Using milk urea nitrogen to evaluate diet formulation and environmental impact on dairy farms. The Scientific World Journal, 1, 852–859.

Jonker, J.S., Kohn, R.A., and Erdman, R.A., 1998. Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows. Journal of Dairy Science, 81, 2681–2692.

Jordan, E.R., and Swanson, L.V., 1979. Serum progesterone and luteinizing hormone in dairy cattle fed varying levels of crude protein. Journal of Animal Science, 48, 1154.

Jordan, E.R., Chapman, T.E., Holtan, D.W., and Swanson, L.V., 1983. Relationship of dietary crude protein to composition of uterine secretions and blood in high-producing dairy cows. Journal of Dairy Science, 66, 1854.

Kekana, T.W., Nherera-Chokuda, F.V., Muya, M.C., Manyama, K.M., and Lehloenya, K.C., 2018. Milk production and blood metabolites of dairy cattle as influenced by thermal-humidity index. Tropical animal health and production, 50, 921–924.

Kohn, R.A., Kalscheur, K.F., and Russek-Cohen, E., 2002. Evaluation of models to estimate urinary nitrogen and expected milk urea nitrogen. Journal of Dairy Science, 85, 227–233.

Leroy, J.L., Van Soom, A., Opsomer, G., Goovaerts, I.G., and Bols, P.E., 2008. Reduced fertility in high-yielding dairy cows: are the oocyte and embryo in danger? Part II. Mechanisms linking nutrition and reduced oocyte and embryo quality in high-yielding dairy cows. Reproduction in Domestic Animals, 43, 623–632.

Lievaart, J., Barkema, H.W., Hogeveen, H., and Kremer, W., 2009. Reliability of the bulk milk somatic cell count as an indication of average herd somatic cell count. Journal of Dairy Research, 76, 490–496.

McBride, G.B., 2005. A proposal for strength-of-agreement criteria for Lin's concordance correlation coefficient. NIWA Client Report HAM2005-062. http://www.medcalc.org/download/pdf/McBride2005.pdf. Accessed 20 January 2019.

Naveed-ul-Haque, M., Akhtar, M.U., Munnawar, R., Anwar, S., Khalique, A., Tipu, M.A., Ahmad, F., and Shahid, M. Q., 2018. Effects of increasing dietary protein supplies on milk yield, milk composition, and nitrogen use efficiency in lactating buffalo. Tropical Animal Health and Production, 50, 1125–1130.

Patton, R.A., Hristov, A.N., and Lapierre, H., 2014. Protein feeding and balancing for amino acids in lactating dairy cattle. Veterinary Clinics of North America: Food Animal Practice, 30, 599–621.

Raboisson, D., Albaaj, A., Nonne, G., and Foucras, G., 2017. High urea and pregnancy or conception in dairy cows: A meta-analysis to define the appropriate urea threshold. Journal of Dairy Science, 100, 7581–7587.

Rhoads, M.L., Bilby, T.R., Rhoads, R.P., Baumgard, L.H., 2008. Effects of nutrient metabolism and excess protein catabolism on dairy cow fertility. In: Proceedings of the 24th Annual Southwest Nutrition and Management Conference. Arizona: Department of Animal Sciences - The University of Arizona. http://www.dairyweb.ca/Resources/SWNMC2008/Rhoads.pdf. Accessed 5 November 2018.

Siachos, N., Panousis, N., Arsenos, G., and Valergakis, G.E., 2017. Investigation of milk urea nitrogen concentration and factors affecting its variation in Greek Holstein herds. Journal of the Hellenic Veterinary Medical Society, 68, 423–432.

Sonderman, J.P., and Larson, L.L., 1989. Effect of dietary protein and exogenous gonadotropin-releasing hormone on circulating progesterone concentrations and performance of Holstein cows. Journal of Dairy Science, 72, 2179.

Staples, C.R., Garcia-Bojali, C.M., Oldick, B.S., Thatcher, W.W., and Risco, C.A., 1993.Protein intake and reproductive performance of dairy cows: a review, a suggested mechanism, and blood and milk urea measurements. Pages 37-51 in Proceedings of 4th Annual Florida Ruminant Nutrition Symposium, Univ. Florida, Gainesville.

Visek, W.J., 1984. Ammonia: its effects on biological systems, metabolic hormones, and reproduction. Journal of Dairy Science, 67, 481–498.

Wanapat, M., Foiklang, S., Phesatcha, K., Paoinn, C., Ampapon, T., Norrapoke, T., and Kang, S., 2017. On-farm feeding interventions to increase milk production in lactating dairy cows. Tropical Animal Health and Production, 49, 829–833.

Wattiaux, M.A., Nordheim, E.V., and Crump, P., 2005. Statistical evaluation of factors and interactions affecting Dairy Herd Improvement milk urea nitrogen in commercial Midwest dairy herds. Journal of Dairy Science, 88, 3020–3035.