

EVALUATION OF ENERGY STATUS OF DAIRY COWS USING MILK FAT, PROTEIN AND UREA CONCENTRATIONS

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ABSTRACT

Energy status of dairy cows may be estimated using results for concentrations of fat, protein and urea (MUN) in milk samples obtained from bulk tank or individual cows. Using individual cow milk samples is recommended on dairy farms in our geographical region due to the unhomogeneity of cows in the herds in respect to their genetic potential for milk production. Depression of milk fat occurs as a consequence of heat stress, underfeeding of periparturient cows, overfeeding concentrate with reduced ration fiber levels or overfeeding with dietary fat. High milk fat content is usually combined with severe negative energy balance. Nutrition and feeding practices have great impact on milk protein level. A deficiency of crude protein in the ration may depress protein in milk. Feeding excessive dietary protein does not significantly increase milk protein. MUN analyses point out potential problems in feeding program on dairy farm. High MUN values may reflect excessive dietary crude protein and/or low rumen degradable nonfiber carbohydrates intake. Also, MUN levels is impacted by heat stress since its value is increased during the summer season. Low MUNs indicate a possible dietary protein deficiency. Additionally, low MUNs concentration may indicate excess in dietary nonstructural carbohydrates. On the basis of the interrelationships between protein and urea concentrations, as well as protein and fat concentrations in individual milk sample, estimation of energy balance of dairy cows may be done more accurately.

Key words: *milk organic components, energy status, dairy cows*

INTRODUCTION

High yielding dairy cows are at an increased risk for many metabolic diseases, especially during early lactation when milk production increases, but with a lagging of feed intake. Namely, this combination leads to a negative energy balance (NEB). The animal attempts to supply the energy needs for milk production by increased lipolysis of adipose tissue. Nonesterified fatty acids (NEFA) that are released from the stored triacylglycerol (TAG) in the adipose tissue are readily taken up by the liver where they provide energy for liver function. If more NEFA arrive at the liver, than needed for energy purposes, the excess may be oxidized incompletely and gener-

ate ketone bodies as well as be converted to TAG for deposition. Under normal conditions, TAG is secreted from the liver as very-low-density lipoproteins (VLDL). Intensive lipolysis that occurs in the state of severe negative energy balance of early lactation dairy cows may lead to inadequate secretion of TAG through VLDL, its accumulation in the liver and development of fatty liver (1). It is established that fatty liver is one of the major health problems in high yielding dairy cows in early lactation that is followed by reduced milk production, other metabolic disorders, reproductive disturbances and infertile diseases. Cows that are overconditioned at calving are most likely to develop severe postparturient NEB combined with fatty liver, due to decreased

appetite around calving. However, obesity in dry cows is not the only etiological factor that causes fatty liver, indicating that there are risk factors other than obesity that predispose the cow to develop fatty liver (2).

Anyway, increased frequency of fatty liver and other metabolic diseases related to energy disturbances in high yielding dairy cows open a question of profitability of dairy farms, due to increased costs related to medication of diseases and reduced income because of decreased milk production. To improve profitability of dairy farms it was necessary to implement reliable, as well as low cost methods, for the evaluation of energy status and early diagnosis of metabolic disorders related to energy disturbances in early lactation dairy cows. Implementations of such methods should reduce metabolic disorders in early lactation cows and improve profitability of dairy farms.

Methods that are widely used for the evaluation of energy status of dairy cows are body condition scoring (BCS) of the cows (3), metabolic profile testing (4), determination of blood hormones concentrations and determination of milk organic components concentrations. The last one is strongly recommended for application due to its simplicity and reliability. The aim of this review is to explain how to estimate energy status of lactating dairy cows using results for concentrations of fat, protein and urea in milk samples.

MATERIALS

Biological bases for using of milk components (fat, protein and urea) for estimation of energy balance of dairy cows

Biological bases for using milk fat, protein and urea concentrations for the estimation of energy status of dairy cows lies in the fact that their concentrations in milk depends on the interrelationship between protein and energy metabolism in dairy cows. Namely, crude proteins (CP) in bovine nutrition mainly consists of rumen degradable proteins (RDP). Minor part of CP in bovine nutrition are rumen undegradable proteins (RUP) that escape ruminal degradation. RDP are broken down, through enzymatic action, by microbes into ammonia, aminoacids and peptides that are used by other rumen bacteria for protein synthesis. Unused ruminal am-

monia is absorbed across the rumen wall into the portal circulation and detoxified by liver into urea (5) that immediately enters the blood. Since urea is a freely diffusible molecule it readily diffuses from the blood in to milk. Therefore, blood urea nitrogen is highly correlated to MUN. When there is an excess of nitrogen relative to energy in the rumen, ruminal ammonia and consequently BUN and MUN increase. Namely, decreased dietary energy intake inhibits rumen microbial activity and bacterial protein synthesis leading to absorption of unused ammonia. Simultaneously, due to decreased microbial protein synthesis there is a lack of aminoacids and proteins in the blood. Decrease of their blood concentrations leads to decreased milk protein synthesis. It should be emphasized that energy released from glucose is an important contributor to milk protein synthesis (6).

Milk fat is synthesized from the fatty acids (FA) that originate from blood lipids and *de novo* synthesis within the mammary epithelial cells. Blood lipids are primarily derived from very low density lipoproteins (VLDL) which are mainly composed of triglycerides and synthesized in the intestine or liver. The FAs contained in VLDL are dependent upon dietary lipids and mobilized fat from adipose tissue. The carbon sources used for *de novo* FA synthesis in the mammary gland are acetate (the most important one) and beta-hydroxybutyrate (BHBA). Acetate and butyrate (which is converted to BHBA in rumen wall tissue) are mainly produced in the rumen by digestion of fiber.

Milk sampling

Energy status of dairy cows may be estimated using results for concentrations of organic components in milk samples obtained from bulk tank or individual cows. It is widely accepted that milk samples from morning milking should be used for this purpose, but our results showed that both morning and evening milk samples should be analyzed (7).

Concentrations of milk organic components in bulk tank milk samples may be a good indicator of metabolic status of lactating dairy cows on a dairy farm (8). Five to sixty milliliters of milk should be collected from bulk tank after mixing. Milk samples should not be taken from the superficial milk layer, since separated milk fat in the top layer may interfere with the result. The main advantage of using bulk tank sample for estimation of energy status of dairy

cows is that it is a simple low cost method, since only one sample is used. But, the main disadvantage is that the obtained result from bulk tank sample can not show the difference in energy status of cows that belongs to different lactation groups (early, middle and late lactation group), as well as the difference in energy status of cows that are in the same lactation group. Due to the fact that the main characteristic of newly formed dairy farms in our geographical region is unhomogeneity of cows in the herd in respect to their genetic potential for milk production, using of bulk tank milk sample for estimation of energy status of dairy cows is not recommended in our region. Additionally, technological improprieties on dairy farms in our region, mainly the impossibility of precise feed intake measurement within one lactation group, make estimation of energy status of dairy cows from bulk tank samples more difficult.

Individual cow milk samples should be taken from all or some cows from each lactation group during milking, using devices that provide sampling a representative milk sample (9). It is very important to get representative milk samples from cows since chemical composition of milk is changed during the milking process. It refers mainly on milk fat concentration since it is relatively low at the beginning and relatively high at the end of milking. Devices attached to milking machine usually separate 25 mL per 1L of milked milk. On mini dairy farms it is necessary to analyze milk samples from each cow, while on large dairy farms it should be analyzed at least 10 % of cows from each lactation group. Due to unhomogeneity of dairy herds in our geographical region, using individual cow milk samples for the estimation of energy status is strongly recommended. Namely, this method provides observation of each milked cow and establishing not only its energy status, but the rate of unhomogeneity of energy status of cows within same group (that are usually on the same feeding regimen). The main disadvantage of this method is higher cost of analyses due to the higher number of milk samples that should be analyzed. But, this conclusion should be taken with caution because if changes in feeding that could be made on the basis of obtained results decrease the incidence of metabolic diseases on farm, then more money will be saved than is the price of milk analyses. Based on our experience, estimation of energy status of dairy cows using individual cow milk samples is recommended on newly established dairy farms, as well as on farms with unhomogeneous lactation groups.

RESULTS AND DISCUSSION

Interpretation of the results

Milk fat

Milk fat percent ranges from 3.2 to 3.6 % in Holstein and 3.6 to 4.0 % in Simmental breed. Factors that can affect milk fat are nutrition and feeding practices, age, stage of lactation, season and others. There is milk fat depression during hot, humid months in the summer season. As the animal becomes older, milk fat declines. Just after freshening, in colostrums, the highest amount of fat is found in milk. The level drops to its lowest point between 25 to 50 days after calving and peaks at 250 days as milk production begins to decrease (10).

If milk fat percent is out of the "normal range", it may indicate to metabolic disturbances in dairy cows.

Thus, depression of milk fat may occur as a consequence of underfeeding of periparturient cows, meaning during the period close to calving. On the other hand, milk fat content may be reduced as a consequence of overfeeding concentrate with reduced ration fiber levels. This usually leads to lower acetate production in the rumen and the state of ruminal acidosis that is usually combined with milk fat depression. Reduced milk fat content during heat stress is probably a consequence of decreased feed intake during high environmental temperatures and/or higher incidence of ruminal acidosis during the summer season (11). Additionally, milk fat may be reduced at high levels of dietary fat, especially with polyunsaturated oils. There are two reasons for that. First, linoleic acid in excess may inhibit the mammary synthesis of the milk fat (10) thus potentially accounting for the depressed milk fat percent. Second, adding fat to the ration in excess may reduce fiber digestibility (12).

High milk fat content is usually combined with state of severe negative energy balance that provoke lipomobilisation. Mobilised FAs are then used by the mammary gland for milk fat synthesis (13). Therefore, cows that may have ketosis problems usually have a transient increase in milk fat.

Milk protein

Different breeds of cattle vary in average milk protein levels. Thus, average milk protein content in

Holsteins is 3.06 %, while in Simmentals is 3.4 %. Factors affecting milk protein content in cows are almost the same as those that affect milk fat content. Thus, nutrition and feeding practices have great impact on milk protein level. A deficiency of crude protein in the ration may depress protein in milk. However, feeding excessive dietary protein does not significantly increase milk protein. Stage of lactation, age, and season affect milk protein content in almost the same manner as milk fat content. Additionally, it has to be mentioned that udder infections may increase milk protein percent (14).

Milk urea nitrogen (MUN)

MUN normal values range from 2 to 6 mmol/L. MUN and blood urea concentrations (BUN) are highly correlated (15), but according to Marenjak et al (2004) (16) it is much better to determine MUN than BUN for the estimation of metabolic status of cows. Namely, collecting milk samples is much more convenient than drawing a blood sample.

The concentration of urea in milk is influenced by nutrition, stage of lactation, body weight, age and season. The most important nutritional factor that affects MUN concentrations is the concentration of nutrients provided in a balanced diet and the manner in which these nutrients are presented to the rumen microorganisms. For the optimum utilization of ammonia by the rumen microorganisms for microbial protein synthesis, the correct proportions of degradable intake protein and nonstructural carbohydrates must be presented at the correct time (17). Diets which are high in degradable protein and do not contain adequate amounts of nonstructural carbohydrates will result in higher concentrations of MUN. Carbohydrates must be available for bacteria at the correct time for optimum utilization of ammonia. Thus, MUN concentration increases even in the case of optimal dietary protein intake if diet is poor in energy. MUN concentration tends to be higher in the summer months due to increased involvement of green conveyor, that is rich in RDP and poor in energy. There is no agreement in literature about the influence of age on MUN. Some authors indicate that multiparous cows have lower MUN. Nevertheless, Johnson and Young (2003) (18) indicate that MUN concentration is highest in primiparous cows. The stage of lactation influences the level of MUN. A low level of MUN was noticed at the start of lactation, probably due to decreased appetite at

that period. MUN concentration reaches its highest level at the peak of lactation and then drops at the end of lactation (18). But when cow's ration at the end of lactation is changed in a way that concentrate intake is decreased (due to preparation of cows for dry period), MUN concentration may maintain high until the end of lactation. In that case, due to increased consumption of forage, besides increased MUN concentrations, milk fat concentration increases, too.

Due to the fact that many different factors may affect MUN concentration, there is some doubt in the interpretation of results and some unacceptability in the incorporation of MUN testing on dairy farms. In order to avoid all suspicions related to interpretation of results it is necessary to establish the baseline MUN for a dairy farm. Namely, normal range for MUN concentrations in a dairy farm can be established after 6 months of once a monthly measuring the morning MUN. Thereafter, analysis of milk for MUN should be done once at three month intervals (16; 8). According to our results (7), MUN should be tested both in morning and evening milking since some additional conclusions related to energy status of cows may be brought out. The same recommendation refers to milk protein and fat determination.

MUN analyses point out potential problems in the feeding program on a dairy farm. In particular, high MUN values may reflect excessive dietary crude protein and/or low rumen degradable non-fiber carbohydrates intake. Also, MUN levels are impacted by heat stress since its value is increased during the summer season. Namely, heat stress causes increased insulin action, with the net effect of increased glucose uptake by systemic tissues. As a consequence, the heat stressed cow becomes increasingly dependent on glucose. Glucose is provided by gluconeogenesis that uses, among others precursors, amino acids (AA) as substrate. More AA is provided by increased proteolysis which is followed by increased BUN and MUN concentrations (10).

Low MUNs indicate a possible dietary protein deficiency, especially RDP. Additionally, low MUNs concentration may indicate an excess in dietary nonstructural carbohydrates. In that case, rumen pH decreases and ammonia molecules are converted into ammonia ions which are poorly absorbed through rumen wall. Therefore, liver urea synthesis from ammonia is decreased (17).

It is important to emphasize that increased MUN values are always combined with decreased profitability of dairy farms, since it indicates excessive dietary crude protein consumption (19). As known, CP is the most expensive component of the ration. Additionally, high MUN indicates increased energy cost associated with the conversion of excess ammonia to urea by the liver, and this is at the expense of energy use for other productive purposes. Increased MUN also leads to decreased glycemia since common substrate i. e. oxaloacetate is used for gluconeogenesis and synthesis of aspartate that is needed for urea synthesis. Some authors believe that increased urea concentration in the blood leads to lipolysis, even in the case when there are no reasons for that (20). High MUN values after calving are associated with an altered uterine environment and decreased fertility meaning reduced conception rate and decreased pregnancy rate (21).

Determination of fat to protein and protein to urea ratio in individual cows milk samples as a model for evaluation of energy status

On the basis of the interrelationships between protein and urea concentrations in each cow's milk sample, estimation of the degree of dietary and energy supply of dairy cows may be estimated (Figure 1). Namely, if MUN value in individual milk sample is lower than 4 mmol/L and milk protein (MP) concentration higher than 32 g/L, cow is adequately supplied with both dietary crude protein and energy. If MP is higher than 32 g/L, but MUN is over 4 mmol/L it indicates overfeeding of dietary protein. If MUN is higher than 4 mmol/L, but MP concentration lower than 32 g/L there is relative excess in dietary protein supply which means that supply with proteins is probably in accordance with cows' needs but there is not enough dietary energy to maintain ruminal bacterial activity for converting ammonia to microbial proteins. If MUN is lower than 4 mmol/L and MP lower than 32 g/L cow is probably insufficiently supplied with both dietary energy and protein.

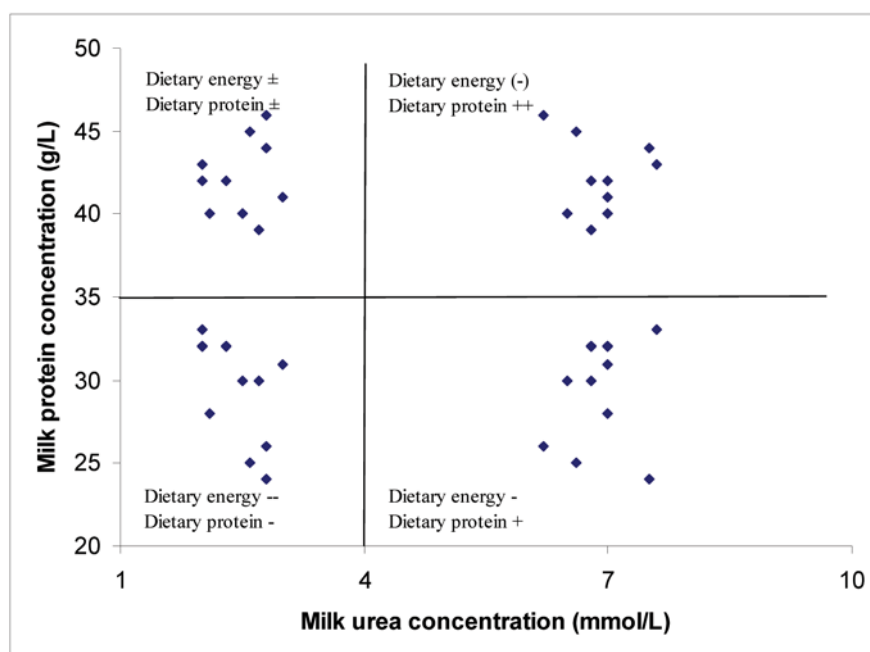


Figure 1. Estimation of dietary protein and energy intake based on interrelationship between milk protein and milk urea concentrations in individual milk samples

Energy status on dairy cows may be easily estimated on the bases of interrelationship between MP and milk fat (MF) concentrations (Figure 2). If MP is higher than 32 g/L and MF between 35 and 45 g/L, energy status of cows is optimal and in accordance with its milk production. However, if MF is higher than 45 g/L, but MP is lower than 32 g/L, that

means that cow suffers from severe negative energy balance. Namely, severe NEB is associated with uncontrolled lipomobilisation and increased blood NEFA that are used for milk fat synthesis. If both MF and MP are lower than 35 and 32 g/L, respectively, the cow is underfed.

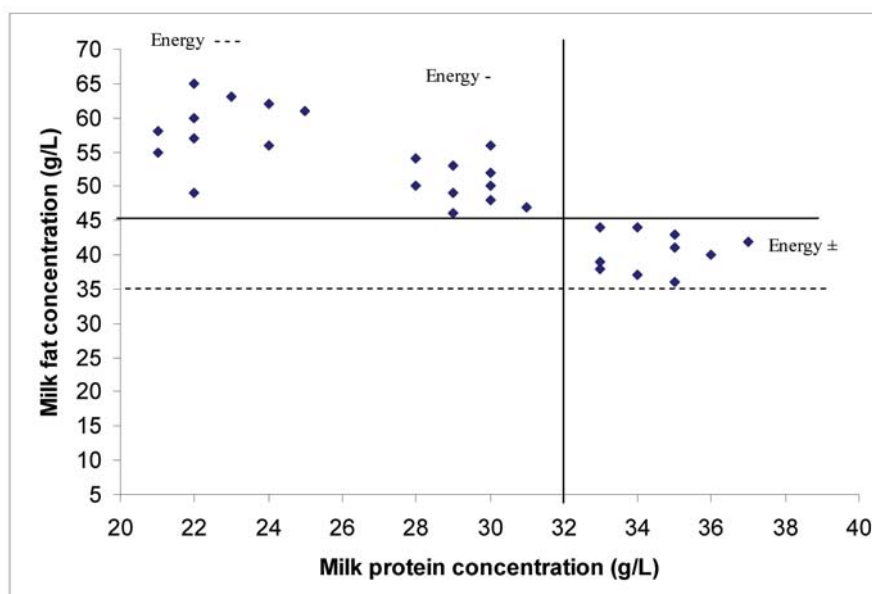


Figure 2. Estimation of energy balance of dairy cows based on interrelationship between milk protein and milk fat concentrations in individual milk samples

Although this model is highly applicative for early lactation cows, it may be used for estimation of energy status of middle and late lactation cows, too. Due to feeding regiment, MP is usually higher in late than early lactation cows. If late lactation cows have increased MF, too, it may indicate on excessive forages intake with lack of concentrate. This situation is usually combined with higher MUN in those samples. But, if increased MP in late lactation cows is combined with lower MF it may indicate on overfeeding with concentrate meaning that those cows have tendency to be obese at dry period with a risk of development of fat cow syndrome.

In conclusion, the average value for MUN, MP and MF, as well as their interrelationships in individual cows milk samples, can be used effectively to detect when major inadequacies in protein and energy nutrition are occurring. When evaluating the feeding program, the protein fractions and amount

of nonstructural carbohydrates supplied in the balanced ration first should be reevaluated.

REFERENCES

1. Bobe, G., J.W. Young, D.C. Beitz. (2004). Invited review: pathology, etiology, prevention, and treatment of fatty liver in dairy cows. *Journal of Dairy Science* 87: 3105-3124.
2. Šamanc, H., V. Stojić, D. Kirovski, M. Jovanović, H. Cernescu, I. Vujanac. (2010). Thyroid hormones concentrations during the mid-dry period: an early indicator of fatty liver in Holstein – Friesian dairy cows. *Journal of Thyroid Research* 2010 : ID 897602
3. Šamanc, H., D. Kirovski, M. Jovanović, I. Vujanac, S. Bojković-Kovačević, D. Jakić-Dimić,

- R. Prodanović, S. Stajković. (2010). New insights into body condition score and its association with fatty liver in Holstein dairy cows, *Acta Veterinaria Beograd* 60 : 525-540.
4. Šamanc, H., D. Kirovski, V. Stojić, I. Vujanac, R. Prodanović, S. Bojković-Kovačević S. (2011). Application of the metabolic profile test in the prediction and diagnosis of fatty liver in Holstein cows. *Acta veterinaria Beograd*. 61: 543-553.
 5. Broderick, G.A., M.K. Clayton. (1997). A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. *Journal of Dairy Science*. 80 : 2964-2971.
 6. Rius, A.G., J.A. Appuhamy, J. Cyriac, D. Kirovski, O. Becvar, J. Escobar, M.L. McGilliard, B.J. Bequette, R.M. Akers, M. Hanigan. (2010). Regulation of protein synthesis in mammary glands of lactating dairy cows by starch and aminoacids. *Journal of Dairy Science*. 93 : 3114-3127.
 7. Šamanc, H., D. Kirovski, B. Dimitrijević, I. Vujanac, Z. Damjanović, M. Polovina. (2006). Energy status of dairy cows determined by biochemical analysis of organic components of milk. *Veterinarski glasnik*. 60 : 283-297.
 8. Zadnik, T, M. Klinkon, M. Nemec, M. Mesarić. (2000) Diagnosis of some bovine diseases by analysis of bulk milk samples. *Praxis veterinaria*. 48 : 55-63.
 9. Fouz, R., E. Yus, M.L. Sanjuán, F.J. Diéguez. (2009). Effect of the sampling device on fat and protein variation in cow milk samples obtained for official milk recording. *Journal of Dairy Science*. 92 : 4914-4918.
 10. Bauman, D.E., J.M. Griinari. (2003). Nutritional regulation of milk fat synthesis, *Anu Rev Nutr*. 23 : 203-227.
 11. Kadyere, C.T., M.R. Murphy, N. Silanikove, E. Maltz. (2002). Heat stress in lactating dairy cows: a review. *Livestock Production Science*. 77 : 59-91.
 12. Perfield, J.W. 2nd, A.L. Lock, J.M. Griinari, A. Saebo, P. Delmonte, D.A. Dwyer, D.E. Bauman. (2007). Trans-9, cis-11 conjugated linoleic acid reduces milk fat synthesis in lactating dairy cows. *Journal of Dairy Science*. 90 : 2211-2218.
 13. van Knegsel, A.T., H. van den Brand, J. Dijkstra, B. Kemp. (2007). Effects of dietary energy source on energy balance, metabolites and reproduction variables in dairy cows in early lactation. *Theriogenology*. 68 : S 274-280.
 14. Hortet, P., H. Seegers. (1998). Loss in milk yield and related composition changes resulting from clinical mastitis in dairy cows. *Prev Vet Med*. 37 : 1-20.
 15. Kampl, B., N. Pljičak-Milas, Đ. Francetić, E. Srebočan. (1993). Determination of the urea content in the deproteinized cows skim milk by the urease/glutamate dehydrogenase method. *Vet arhiv*. 63 : 5-60.
 16. Marenjak, T.S., N. Pljičak-Milas, Z. Stojić. (2004). The aim of urea determination in cow's milk. *Praxis veterinaria*. 52 : 233-241.
 17. Westwood, C.T., I.J. Lean, R.C. Kellaway (1998). Indications and implications for testing of milk urea in dairy cattle: A Quantitative review. Part 1. Dietary protein sources and metabolism. *New Zealand Veterinary Journal*. 46 : 87-96.
 18. Johnson, R.G., A. J. Young. (2003). The association between milk urea nitrogen and DHI production variables in commercial dairy herds. *Journal of Dairy Science*. 86 : 3008-3015.
 19. Van Horn, H.H., G.L. Newton, W.E. Kunkle (1996). Ruminant nutrition from an environmental perspective: factors affecting whole-farm nutrient balance. *Journal of Animal Science*. 74 : 3082-3102.
 20. Oetzel, G.R. (2004). Monitoring and testing dairy herds for metabolic diseases. *Vet Clin North Am Food Anim Pract*. 20 : 651-74.
 21. Tamminga, S. (2006). The effect of the supply of rumen degradable protein and metabolisable protein on negative energy balance and fertility in dairy cows, *Anim Reprod Sci*. 96 : 227-39.
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