ASSESSMENT OF NUTRITIONAL ADEQUACY IN DAIRY COWS THROUGH DIET CHARACTERISTICS AND ANIMAL RESPONSES

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1. INTRODUCTION

Many productive or health disorders in dairy production can be due or favored by diet inadequacy, so that the control of the ability of diet to sustain normal physiological functions is often necessary. Diet inadequacy can relate to different steps: diet calculation, diet preparation, diet consumption, or digestion and metabolic efficiency of nutrients. The evaluation of the first steps can be direct, and the first part of this paper will discuss the limits of diet control. These limits explain the interest of animal criteria, which are also useful for the evaluation of digestive and metabolic processes, and can aid to reveal discrepancies between physiological requirements of animals and feeding management. Because nutritional imbalances are important risk factors for digestive and metabolic diseases, these diseases can be considered as animal responses to diet abnormalities, so that some of the criteria presented in this paper can also be used for detection of health problems in herds.

2. CONDITIONS, BENEFITS AND LIMITATIONS OF DIET EVALUATION

2.1 Steps of evaluation

2.1.1 Calculated diet

Diet calculation is based on information relative to feed composition and nutrient value, and animal requirements. Evaluation of the adequacy of these basic data is necessary.

The first step is to control the nutritional value of feedstuffs. Data can come from feed tables. This can be correct for feedstuffs that represent only a little part of the diet, or whose composition is rather constant. On the contrary, use of table values can be a major source of error for forages which represent a large part of the diet. For example, in French tables of feeds composition, corn silage contains, per kg of dry matter (DM), 82 to 87 g of crude protein (CP), 3.5 g of Ca and 2.5 g of P (INRA, 1988). Current values from corn silage laboratory analysis are 60 to 80 g of CP, 1.9 g of Ca and 1.8 g of P (Corneloup, 2001; Beguin, 2003), which can result in severe nutrient deficiencies when the diet is calculated with table values.

A precise evaluation of the calculated diet requires an analysis of forages, but this does not provide an absolute guarantee of accuracy because of possible errors due to sampling or determination of feeding value. Average relative variations between sampling areas of a corn silo are over 11% for DM and CP, and over 8% for fibre, and maximal relative deviations can reach 55% for DM, making it better to test DM on a weekly basis and chemical composition on a monthly basis (Stone, 2004b). Laboratory analysis provides values for crude constituents of feedstuffs. The determination of the energy or protein value for animals requires estimation of digestive and metabolic efficiency of feeds, which is calculated with regression equations which cannot fit with all situations. For example, results from equations used in France for prediction of energy value of corn silage can differ by 5% from values calculated from production responses of growing cattle (Brunschwig *et al.* 1996). Moreover, current analytical procedures do not determine the concentrations of trace minerals or vitamins.

Chemical analysis can be associated with a visual evaluation of feedstuffs or an analysis of conservation quality, which can reveal storage alterations, or problems in fibre particle size or corn kernel processing in silages.

Evaluation of the calculated diet also needs to retrospectively evaluate the management of diet changes, due to forage changes or transitioning from dry to lactating period.

The control of the calculated diet also involves checking the determination of animals requirements. Some variation factors are sometimes forgotten in calculations, for example variations of energetic requirements due to movements, ambient temperature, or milk composition. Moreover, when the concentrates are distributed on an individual basis, the true milk yield of each cow is to be precisely estimated; however, day-to-day variation of individual milk yield is on average 6-8%, which means that a cow producing 25 kg milk can deviate ± 4 kg (95% confidence interval; Svennertsen-Sjaunja *et al.* 1997).

2.1.2 Distributed diet

Two steps are important for controlling the distributed diet. The first one is the conformity between calculated and prepared diet: precision, accuracy and method of utilisation of weighing and mixing materials are important in this scope. The second step is the control of the particle distribution of the diet, which is useful for determining the ability of the diet to stimulate mastication. Among tools that are available for this determination, the PennState Forage Particle Separator has 2 sieves with 1.92 and 0.8 cm pore sizes. The most recent model has an additional 0.13 cm sieve (Heinrichs & Kononoff, 2004). For total mixed rations, the recommendation is between 30 and 50% of the diet retained on the middle sieve and between 30 and 50% retained on the lower sieve.

2.1.3 Consumed diet

Both quantitative and qualitative aspects need evaluation. On a quantitative point of view, most diet formulation systems assume and *ad libitum* feed consumption, so that it is necessary to evaluate the real availability of the diet. Heinrichs (2004) proposed a method based on feed bunk scoring one hour before the next feeding, whatever the number of daily meals, and considered that less than 5% of the amount fed in the bunk is indicative of underfeeding.

If diet is not *ad libitum* available, an evaluation of the amount distributed to animals will be necessary. Limitation of diet availability usually results in competition between animals, with poor feed intake by dominated cows. This increases heterogeneity among cows, which is always observed, even with an *ad libitum* diet, and particularly in early lactation. Finally, when diet

availability is restricted, cows can eat very rapidly after feeding, which results in a risk of lower post-feeding ruminal pH.

Qualitatively, the cows are able to sort feedstuffs in their diets, specifically particles over 5 cm, and usually the really eaten diet contains more concentrate and fine particle forages than the distributed diets. The difference between bunk contents before and after distribution can reveal a lack of physical structure for the animals, at least during the first meal after distribution, when cows first eat fine particles. Moreover, sorting behaviour can largely vary among cows, and sorting cows can move to minimally sorted ration in order to consume all day long fine particles (Leonardi & Armentano, 2003). Adding long particle hay to the diet can be inefficient for chewing stimulation on these cows (Armentano & Leonardi, 2003). Cows with induced acidosis and given free choice alfalfa hay or pellets preferentially eat hay (Keunen *et al.* 2002), which was interpreted by the authors as an attempt to attenuate acidosis. However, this safe behaviour has not been studied when the cause of ruminal acidosis is an individual preference for short particles.

2.2 What problems can and cannot be revealed by evaluation of diet?

Evaluation of diet only gives information on the possible gaps between nutritional recommendations and the available diet, bad transitions between diets, or a bad choice of feedstuffs, particularly when an important part of the diet contains high amounts of rapidly fermentable carbohydrates. It also can demonstrate discrepancies between what is supposed to be fed to cows and what is really eaten.

It can partly give suspicions on the ability of the diet to be efficiently and safely digested in the rumen, but the response of the animal can largely complete this first information.

3. COMPLEMENTARY BENEFITS CARRIED BY ANIMAL RESPONSES FOR DIET EVALUATION

3.1 Digestive efficiency

The main point in evaluation of low digestive efficiency is the evaluation of ruminal digestion, the main problem in dairy cows being subacute ruminal acidosis (SARA). Some indications on the risk of SARA can be obtained from carbohydrate composition of the diet: a diet with more than 35% Neutral Detergent Fibre (NDF) or 27% of forage NDF, or less than 30% of Non Fiber Carbohydrates (NFC% = 100 - NDF - CP - Fat - Ash), or less than 25% of sugar + starch will result in a low risk of SARA; on the contrary, less than 25% NDF, 16% of forage NDF, or more than 45% of NFC or 35% of starch + sugar will result in a high risk (Stones, 2004a). The cut-off point can be lowered when forage have long particles, when concentrates are mixed with forages or distributed frequently, or when starch fermentability is low (Allen, 1996).

The measurement of ruminal pH is the key indicator of SARA, and experimentally, SARA is often assessed through the duration of time at which rumen pH is under 6.0. Ruminal contents can be sampled via ruminocentesis, or via oral sampling. Ruminocentesis is well tolerated by animals (Enemark *et al.* 2004), and sampling with a stomach tube results in higher pH values than ruminocentesis: the difference varies among studies from 0.28 (Garrett *et al.* 1999) to 0.76 (Enemark *et al.* 2004) or 1.1 (Nordlund *et al.* 1995), but relationship between these two values is very low ($r^2 = 0.11$) (Enemark *et al.* 2004). Differences between sampling techniques and among studies are due to the contamination of ruminal samples by saliva, or the variations of the sampling site with stomach tube, so that sensitivity of oro-ruminal sampling for detection of low rumen pH is much lower than that of ruminocentesis (Duffield *et al.* 2004). Detection of SARA can be made by ruminal pH measurement in samples obtained via ruminocentesis in 12 cows in a herd; if at least 3

cows are below 5.5, SARA can be declared (Garret *et al.* 1999). The best time for sampling is 3 to 4 hours after the main meal when concentrate and forage are separated, and 5 to 8 hours after distribution of a total mixed ration.

Ruminal sampling also allows determination of volatile fatty acids, and SARA is associated with a high propionate/acetate ratio. However, this method presents the same sampling drawback as pH measurement and, to our knowledge, no cut-off point has been tested for sensitivity and specificity.

Empirical criteria based on observation of cows have been proposed for evaluation of ruminating activity, based on at least 50% of cows chewing among cows lying down comfortably in stalls (Nelson, 1996a), or 40% of cows chewing among cows lying or standing undisturbed (Eastridge, 2000).

Fecal pH is not related to ruminal pH (Enemark *et al.* 2004). High starch diets both result in low rumen and fecal pH, but replacing rapidly fermented starch by slowly fermented starch, i.e. wheat by corn, can be expected to increase ruminal pH and lower hindgut pH, due to hindgut fermentation of more starch (Eastridge, 2000).

Fecal observation is another possible way to evaluate ruminal digestion, because any ruminal disturbance will result in more nutrient in the lower digestive tract, and more hindgut fermentation. During SARA feces are bright, yellowish, with a sweet-sour smell (Kleen *et al.* 2003), foamy with gas bubbles, and contain more undigested fibre or grain (Hall, 2002). Moreover, because of insufficient fibre ruminal mat, fibre is not effectively retained in the rumen so that feces contain fibre particle with a 1-2 cm size instead of 0.5 cm (Hall, 2002). This can be seen after washing on a screen with 1.5-2 mm openings (Stone, 2004b), but is not specific of SARA because long particle can also be due to a lack of ruminally degradable nitrogen (Vagneur, 2003). Finally, feces from cows with SARA are usually soft, but the correlation coefficient between dietary fibre and fecal consistency score is low (Ireland-Perry & Stallings, 1993).

Lowered milk fat content is frequently used in farms as an indicator of SARA, and is described as “low milk-fat syndrome” or “milk fat depression”, so that milk fat depression has been used as a basis for systems predicting effectiveness of diet structure for chewing (Mertens, 1997; DeBrabander *et al.* 1999). The correlation coefficient between ruminal pH and milk fat content is only 0.305 (Enemark *et al.* 2004). Similarly, across 90 treatments in 23 experiments, Allen (1997) reported that milk fat percentage explains 39% of the variations of ruminal pH. Although the relationship can be expected to be closer within a herd, this low spectificity is due to other factors than rumen pH that can affect milk fat content: milk fat can decrease without rumen pH change with changes in starch fermentability; increasing starch fermentability in a diet with 30% starch results in a 15% decrease of milk fat without altering ruminal pH (Oba & Allen, 2003), dietary fat addition, particularly with unsaturated fatty acids (Doreau & Chilliard, 1992).

Moreover, the effect of a low ruminal pH on milk fat content is enhanced when the diet contains added unsaturated fat, because a low pH results in the production of *trans*Δ10 intermediates of ruminal biohydrogenation, which are associated with low milk fat (Griinari *et al.* 1998; Piperova *et al.* 2000). The effect of these biohydrogenation intermediates is higher in low producing than in high producing cows (Bradford & Allen, 2004). Consequently, the interpretation of a low milk fat content has to take into account the use of dietary lipids, and their level of unsaturation. Interpretation of milk fat in early lactation dairy cows is not possible because of body fat mobilization.

For these reasons, although milk fat depression and SARA can arise in similar situations, milk fat depression cannot be simply considered as a sequel of SARA (Kleen *et al.* 2003).

3.2 Energy balance

Diet evaluation can provide some information about energy imbalance, specially when strong discrepancies exist between the calculated and the consumed diets. Negative energy balance (NEB) in early lactation is a risk factor for metabolic diseases, particularly ketosis, and for poor reproductive performance.

Body weight of animals cannot be used to evaluate energy balance in early lactation cows, mainly because they loose tissue while simultaneously increasing feed intake, which would result in underestimated body weight loss. On the contrary, scoring body condition is accurate for monitoring energy balance. According to scoring methods, body condition score ranges from 0 to 5 (Bazin, 1984) or 1 to 5 (Edmonson, 1989), the latter 5 points scale being the most widely used in the literature. The usual targets are around 3.5 at calving (Waltner *et al.* 1993), and condition loss in early lactation must not exceed 1 point for optimal reproductive performance (Lopez-Gatius *et al.* 2003). A 3-3.5 body condition score is the goal at dry-off. Under- or over-conditioning resulting from under- or overfeeding, and herd heterogeneity, can reveal problematic control of diet distribution, problematic access to feeding or competition between animals.

In the beginning of lactation, NEB and resulting subclinical ketosis suppress peak milk or result in an reversed peak milk (Gutafsson *et al.* 1993), but this criterion is not specific, because a low peak milk can also be due to protein deficiency. Moreover, lactation curves drawn on a monthly measurement basis do not allow an accurate interpretation.

NEB in early lactation also can be revealed by high milk fat percentage. Because the first test day can vary from 7 to 35 days in milk, and because days in milk are a strong variation factor for milk fat, individual interpretation of this parameter is difficult, but it can be used at a herd level. Holstein herds with more than 10% of cows under 60 days in milk having a milk fat percentage over 5.5% are suggestive of ketosis or fatty liver (Nordlund & Cook, 2004), consecutive to NEB. Because NEB simultaneously decreases milk protein content, a low protein content or a high fat/protein ratio also are indicators of NEB: however, protein/fat ratio under 0.75 as a test to identify subclinically ketotic cows as a limited interest because sensitivity is only 58% and specificity 69% (Duffield *et al.* 1997). Moreover, the highest prevalence of subclincal ketosis is during the two weeks after calving, when most cows have still not been controlled for milk composition. At a herd level, the test considering subclinical ketosis when more than 40% of cows at first control have a protein/fat ratio under 0.75 has been reported to have a sensitivity of 69% and a specificity of 83% (Duffield, 2003).

The fat/lactose ratio has also proven to be of value for detection of NEB (Reist *et al.* 2002). However, detection of early lactation NEB at herd-level requires large herd sizes, over 100 to 400 cows according to the length of calving season (Reist, 2002).

In mid and late lactation, several models for the relationship between NEB and milk production have been proposed. For example, a 3MCal NEB on a cow producing 30 kg milk would result in a production decreased by 1.4 o 3.2 kg of milk (INRA, 1988; Kirkland *et al.* 2001). This effect can be more important on cows with insufficient body reserves. NEB in mid-lactation also reduces milk protein percentage, but other factors, for example amino-acids insufficiency, can result in low milk protein.

Beyond direct observation of animals or production results, biochemical parameters, are widely used as alarms for metabolic diseases, and also can be used for detection of NEB. The most common measurement is blood β-hydroxybutyrate: more than 10% of tested cows with over

1400 μmole of BHB/l of blood is indicative of subclinical ketosis (Oetzel, 2004) consecutive to an excessive NEB. Other circulating metabolites (non-esterified fatty acids, acetone) can be monitored, and milk acetone or milk β-hydroxybutyrate measured with cow-side tests, also can be useful to reveal and important NEB (Enjalbert *et al.* 2001; Geishauser *et al.* 2000).

A comparison of methods for estimating severe NEB in early lactation cows showed that, at a herd level, milk fat/protein over 1.5 had a better sensitivity than milk fat over 4.8%, whereas milk ketone over 100 μmol/l and blood β-hydroxybutyrate over 1.2 mmol/l had sensitivities under 30%. Specificities were over 85% for the four tests (Heuer *et al.* 2000). However the use of this model required herds with over 160 cows. Another study showed that a milk fat/protein ratio over 1.5 in early lactation is associated with a 1.8-fold higher risk of loosing more than 0.5 point of body condition score (Heuer *et al.* 1999).

3.3 Nitrogen imbalance

Lack of protein in early lactation results in low peak milk. However, a low peak milk is not specific of a low protein yield, and for example can relate to a high NEB.

Nitrogen imbalance always results in modifications of blood or milk urea. Urea is the final molecule for nitrogen excretion in mammals, and results from elimination of endogenous nitrogen, ammonia nitrogen absorbed from the rumen and originating in microbial degradation of dietary N, and from deamination of the part of absorbed amino acids that cannot be incorporated into proteins because of essential amino-acids imbalance, because of utilization of amino-acids for neoglucogenesis, or because absorption exceeds requirements. The main variation factors of urea hepatic synthesis are ruminally absorbed ammonia and excess absorption of amino-acids. Because urea easily diffuses across mammary tissue, blood urea nitrogen is strongly correlated with milk urea nitrogen, but values can be different: Broderick & Clayton (1997) determined, on a data set of 2231 observations in 35 trials and 106 diets, the following relationship:

$$\text{Milk Urea N (mg/l)} = 0.62\ \text{Blood urea N} + 47.5\ (r^2=0.842)$$

These authors also demonstrated that blood and plasma urea concentrations are equal.

Milk urea is commonly measured by dairy industries or dairy herd improvement in many countries, and near-infrared analysis provides suitable results (Godden *et al.* 2000), but which can depend on instruments (Kohn *et al.* 2004). Two modes of expression for urea are used in the literature or in field evaluations: total urea and urea nitrogen. The conversion is easy, urea containing 46.6%N. Evaluation of milk urea also can use cow-side tests. The most usual one has been shown to overestimate values, failing to detect some normal or low values, and classifying as high many samples with normal values (Godden *et al.* 2003).

Diurnal variations of blood urea are important, peak values being observed about 3 hours after meal (Gustafsson and Palmquist, 1993), but diurnal variations are much lower for milk urea (Rodriguez *et al.* 1997). Use of total mixed rations lowers diurnal variations (Geerts *et al.* 2004). Other non-nutritional variation factors like milk production, days in milk, milk composition, body weight or parity (Jonker *et al.* 1999) have to be considered when individual milk samples are interpreted.

The main variation factors for milk urea are dietary CP, either expressed on a DM or a net energy basis, and excess N intake. A 1% increase of concentration of dietary protein (DM basis) results in an 18 to 37 mg/l increase of milk urea (Broderick & Clayton, 1997; Nousianen *et al.* 2004). On the contrary, the correlation coefficient between ruminal ammonia and milk urea is somewhat lower, and experimentally, the intake of undegradable protein elevates plasma and milk urea to a similar

extent as intake of degradable protein (Roseler *et al.* 1993). However in field evaluation, high values for milk urea often are interpreted as resulting from an excess of degradable N, which can lead to inaccurate corrections of the diet.

Excess milk urea originating in excess ruminal ammonia not only can result from an excessive degradability of dietary protein, but also from a lack of fermentable energy: in this last case, due to energy restriction, milk protein content can be expected to be low (Nelson, 1996b).

Although there is no clear consensus about normal values, milk urea concentrations between 240 and 330 mg/l of milk can be considered normal, according to milk production. High values are indicative of excess protein, but because of the potential of cows to adapt to high protein diets, this does not preclude that negative effects, particularly on reproduction, will be observed (Weston *et al.* 1998).

3.4 Mineral nutrition

Due to lack of determination of minerals in most regular feed analysis, and due to high variability of mineral contents in feedstuffs, the precise assessment of mineral nutrition through control of diet is difficult, so that biochemical parameters are useful for the evaluation of nutritional status.

Due to homeostatic regulation, plasma calcium is poorly dependent on dietary allowances. Plasma inorganic phosphorus is lowered when dietary phosphorus is insufficient (Underwood & Suttle, 2000), and borderline deficient phosphorus supply is associated with low urinary phosphorus concentration (Wu *et al.* 2001). Low urinary magnesium is associated with a low plasma concentration, due to a low supply or a poor absorption (Underwood & Suttle, 2000).

Animal response also can be useful for adjustment of specific mineral nutrition. In late gestation dairy cows, a low dietary cation-anion difference (DCAD) decreases the risk of milk fever in early lactation. Adjustment of DCAD by dietary calculation is difficult because Na, K, Cl and S contents of feedstuffs are usually unknown, but a urinary pH around 6.5 is indicative of effective anion addition (National Research Council, 2001).

With regard to trace-minerals, most imbalances are not sufficient to cause specific symptoms, but cause impairment of defences or production losses (Enjalbert *et al.* 2006). Digestive of metabolic efficiencies are highly susceptible to interaction with other dietary constituents, which can result in normal allowances but secondary deficiencies. Methods for assessment of trace mineral status via blood, urine, milk or tissue measures, and their limitations, have been reviewed (Kincaid, 1999; Herdt *et al.* 2000). According to the criteria, they can provide useful information on the short or long term status of animals, or information on interferences, for example high plasma Cu associated with low plasma ceruloplasmin is usually consecutive to a high Mo intake.

4. CONCLUSION

Diet evaluation is mainly limited by knowledge about nutritional value of feedstuffs and true feed consumption. Dietary inadequacy can result in various animal responses, which can be observed (body condition score, feces), measured (milk production) or analysed (milk, blood or urine constituents). However most of these parameters lack specificity, so that both diet evaluation and interpretation of animal responses will be necessary for evaluation of nutritional status.

5. SUMMARY

This paper presents methods for assessment of dietary adequacy in dairy herds. The evaluation of the diet needs to check basis and accuracy of the calculated diet, conformity of distributed diet, and consumption by animals. Complementary information is provided by observation of animals and their feces, critical analysis of performance and milk composition, of data relative to composition of ruminal contents, blood or urine. Because of lack of specificity for many criteria, dietary and animal parameters are both necessary for validation of a suspicion of dietary imbalance.

6. KEY WORDS

Diet, nutrient imbalance, dairy cow, animal response.

7. RESUME

Cet article présente les méthodes permettant d'évaluer l'adéquation de la ration aux besoins nutritionnels et physiologiques des vaches laitières. Le contrôle direct de la ration doit, au-delà de la vérification de la ration calculée et de ses bases, s'assurer que la ration distribuée est conforme, et que la ration est correctement consommée par les animaux. En complément, l'observation des animaux ou de leurs bouses, les performances zootechniques, la composition du lait et des paramètres de composition ruminale, sanguine ou urinaire peuvent mettre en évidence des déséquilibres énergétiques, azotés ou minéraux. Eu égard au manque de spécificité de nombreux paramètres, c'est la conjonction de présomptions qui permettra de valider une suspicion de déséquilibre.

8. MOTS CLES

Ration, déséquilibre alimentaire, vache laitière, réponse animale.

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