# Update on B Vitamins for Lactating Dairy Cows

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## Introduction

For B vitamins as for all other nutrients, the ideal situation is when the supply is equal to the needs. On the one hand, there is deficiency as soon as the supply is lower than the needs; even sub-clinical deficiency has a metabolic cost because to survive, the cells have to use alternate, less efficient metabolic pathways. On the other hand, if the supply is greater than the needs, then you have losses in feces and urine and there is also a metabolic cost to dispose of surpluses. Dairy nutritionists balance rations in order to meet this ideal situation for major nutrients but B vitamins are seldom taken into account. But what do we know about B-vitamin needs and supply in dairy cows?

## Estimating the Needs or the Requirements?

In humans, B-vitamin requirements are defined as the amount needed to sustain good health. In a high producing dairy cow, two supplementary components have to be taken into account: the objective to maximize metabolic efficiency and, for some B vitamins, the heavy drain imposed by their secretion in colostrum and milk. For example, concentrations of folates and vitamin $B_{12}$ were 6 and 9 times greater in colostrum than in milk 39 days after calving, respectively (Duplessis et al., 2016). In humans and non-ruminants, estimation of the minimum requirement, i.e. the lowest intake to support normal function, is essential to define a dietary recommendation for a specific nutrient. The first step to quantify the minimum requirement is to identify a marker, often the activity of an enzyme or the vitamin concentration in a specific tissue, which will respond early to a lack of the studied vitamin. The second step is to feed a basal diet deficient only in this vitamin and supplemented with increasing doses of this nutrient in order to obtain a dose-response curve for the chosen marker (Combs, 2012). Obviously this approach is not working in ruminants because even when feeding a diet deficient in B vitamins, an unknown but not negligible amount of B vitamins synthesized in the rumen are available for the cow (Bechdel et al., 1928).

Consequently, in dairy cows as opposed to non-ruminants, B-vitamin needs and requirements are not the same. The need is the amount of vitamin requested by the tissues to maintain an optimal metabolic activity whereas the requirement is the amount to include in the diet to reach this objective. This difference between need and requirement is the amount of B vitamins synthesized by the ruminal microflora (Bechdel et al., 1928), generally in amounts sufficient to avoid apparition of deficiency symptoms. This situation probably explains why there were so few attempts to define dairy cow

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requirements. Nevertheless, there is evidence in the literature that dairy cows could benefit from B-vitamin supplements. Although these results do not allow one to quantify a requirement, they indicate that the supply does not always equate the needs. There is no or only very limited information on the effects of thiamin, riboflavin, pantothenic acid, and vitamin $B_6$ supplements on production and metabolic activity of dairy cows. Consequently, only results from experiments using niacin, biotin, folic acid, and vitamin $B_{12}$ supplements are briefly described in this section.

*Niacin.* The name "niacin" is used for two active molecules, nicotinic acid and nicotinamide. Niacin is the essential component of nicotinamide adenine dinucleotide (**NAD**) and nicotinamide adenine dinucleotide phosphate (**NADP**) involved with more than 100 enzymes in all oxidation-reduction reactions. Niacin does not completely fit the definition of a vitamin because the molecule is synthesized from the amino acid tryptophan, although the importance of endogenous synthesis of niacin differs greatly among species (Combs, 2012). In preruminant calves, endogenous synthesis of niacin is sufficient to avoid apparition of deficiency symptoms if the diet provides a sufficient amount of tryptophan (Hoppner and Johnson, 1955). Interestingly, endogenous synthesis of niacin from tryptophan is suppressed in rats by ketone bodies (Shastri et al., 1968) and fatty liver (Fukuwatari and Shibata, 2013). Nevertheless, the importance of the tryptophan-niacin pathway for dairy cows across the gestation-lactation cycle is still unknown.

Nicotinic acid supplements reduce lipolysis in normal and ketotic cows (Waterman and Schultz, 1972; Waterman et al., 1972). Decreased plasma concentrations of non-esterified fatty acids and β-hydroxybutyrate and increased plasma glucose are the most frequently reported responses during use of nicotinic acid supplements, although the response is highly variable among experiments (Schwab et al., 2005; Niehoff et al., 2009; Yuan et al., 2012; Pires et al., 2016). Moreover, in *in vitro* and *in vivo* experiments, niacin supplements are frequently reported to increase the number of protozoa as well as microbial protein synthesis (Schussler et al., 1978; Riddell et al., 1980; Riddell et al., 1981; Dennis et al., 1982; Shields et al., 1983; Brent and Bartley, 1984; Horner et al., 1988a and b; Erickson et al., 1990; Ottou and Doreau, 1996; Aschemann et al., 2012; Niehoff et al., 2013). These effects on ruminal fermentation and lipolysis led to numerous experiments on the effects of niacin supplements on cow metabolism and production performance. According to a meta-analysis (Schwab et al., 2005) using data from 27 studies published between 1980 and 1998, a dietary supplement of 6 g of nicotinic acid per day had no effect on lactation performance of dairy cows. However, at a dose of 12 g/d, supplementary nicotinic acid increased fat yield and tended to increase 3.5% fat-corrected milk and protein yield. As there was no effect of the vitamin supplement on dry matter intake, feed efficiency tended also to be increased. Use of supplementary nicotinic acid has been also studied for its pharmacological effects on vasodilation to alleviate the consequences of heat-stress on lactating dairy cows. For production and metabolic responses, the responses differs among experiments (Di Costanzo et al., 1997; Wrinkle et al., 2012; Zimbelman et al., 2010 and 2013; Lohölter et al., 2013; Rungruang et al., 2014; Pineda et al., 2016).

*Biotin.* Biotin is likely to be of great importance in ruminants because it is a coenzyme for two essential enzymes for gluconeogenesis and it is involved in regulation of gene expression of many enzymes critical for glucose metabolism. In addition, biotin plays key roles in lipid and amino acid metabolism. Two meta-analyses on the effects of dietary supplements of biotin on milk production and composition of Holstein dairy cows were published in 2011 (Chen et al., 2011; Lean and Rabiee, 2011). Each of them used data from 11 comparisons with only 6 comparisons shared by both studies; the biotin supplement dose was generally 20 mg/d and exclusion and inclusion criteria differed between these studies. In spite of these differences, the conclusions were similar (**Table 1**). Such results illustrate that for dairy cows, biotin supply is frequently lower than the need although they partially hide the variability among experiments. Ferreira and collaborators (2007) stressed that supplementary biotin was more likely to increase milk and milk component yields in high-producing cows than in low-producing ones because the metabolic demand was greater in the former. However, in some experiments even high-producing cows did not respond to biotin supplementation (Rosendo et al., 2004). Biotin supplements at doses varying from 10 to 20 mg/d frequently improved hoof health.

*Folic acid.* The term “folic acid” is used either as the generic name of the vitamin or specifically, for the synthetic form of the vitamin. The term “folates” applies to the numerous biologically active forms. Folates have the single biochemical function of accepting and releasing one-carbon units for DNA synthesis and replication and thus, cell division. Folate coenzymes also provide one-carbon units for *de novo* formation of methyl groups essential to, for example, DNA methylation (which controls gene transcription and genetic stability) and synthesis of phosphatidylcholine, choline, creatine, and many neurotransmitters. Folic acid supplements, given orally or by intramuscular injections, increased milk production and milk protein yield during the first part of the lactation in multiparous cows (Girard and Matte, 1998; Graulet et al., 2007; Girard et al., 2009, Li et al., 2016). Except for one (Li et al., 2016), none of these experiments observed an increase in dry matter intake suggesting that supplementary folic acid increased efficiency of protein metabolism. Moreover, folate metabolism in mammary epithelial cells seems to be a critical regulatory point for synthesis of milk protein in many species, including dairy cows (Menzies et al., 2009). The absence of effects of the folic acid supplements on lactation performance observed in some experiments could be due to a low vitamin $B_{12}$ supply (Girard et al., 2005; Preynat et al., 2009a).

*Vitamin $B_{12}$.* Vitamin $B_{12}$ acts as a coenzyme in only two metabolic reactions. The vitamin is a coenzyme for methionine synthase; this interface between folic acid and vitamin $B_{12}$ metabolism is so critical that a lack of vitamin $B_{12}$ causes a secondary folate deficiency, even in presence of a sufficient folic acid supply (Scott, 1999). Besides this role, the other vitamin $B_{12}$-dependent enzyme, methylmalonyl-coenzyme A mutase, plays a major role in ruminants for the entry of propionate in the Krebs cycle and gluconeogenesis (McDowell, 2000).

Vitamin $B_{12}$ is synthesized by ruminal bacteria if the cobalt supply is sufficient (Martens et al., 2002). Incidentally, it has been observed that, in spite of a sufficient dietary cobalt supply in dairy cows, the lowest plasma concentrations of vitamin $B_{12}$ are observed during the first weeks of lactation (Elliot et al., 1965; Mykkänen and Korpela, 1981; Girard and Matte, 1999; Girard et al., 2005; Kincaid and Socha, 2007). Nevertheless, oral or parenteral supplements of vitamin $B_{12}$ generally fail to affect milk and milk component yields in cows (Frobish and Davis, 1977; Croom et al., 1981; Graulet et al., 2007; Akins et al., 2013). However, as compared to a supplement of folic acid alone, a combined supplement of vitamin $B_{12}$ and folic acid given to primiparous cows during the first weeks of lactation increased energy-corrected milk, packed cell volume and blood hemoglobin and decreased serum methylmalonic acid concentrations (Girard and Matte, 2005). The effect on blood hemoglobin and packed cell volume suggests that a low vitamin $B_{12}$ supply interferes with folate metabolism decreasing DNA synthesis and blood red cell formation (Bills et al., 1992). Accumulation of methylmalonic acid in serum indicates that a low vitamin $B_{12}$ supply also affects the other vitamin $B_{12}$-dependent enzyme, essential to propionate utilization. These observations support the hypothesis that a suboptimal vitamin $B_{12}$ supply, especially during early lactation may limit the effects of folic acid supplements. Indeed, a combined supplement of folic acid and vitamin $B_{12}$ has been reported to improve metabolic efficiency, especially energy metabolism (Graulet et al., 2007; Preynat et al., 2009b; Gagnon et al., 2015; Duplessis et al., 2014a). Moreover, possibly through an improvement of the energy balance in early lactation, the combined supplement of vitamins changes the expression of genes involved in differentiation of ovarian follicles (Gagnon et al., 2015), increases the number of large follicles and the size of the dominant follicle (Ghaemialehashemi, 2013) and decreases the interval between calving and the first insemination (Duplessis et al., 2014b). Nevertheless, production and metabolic responses to a combined supplement of folic acid and vitamin $B_{12}$ are variable as illustrated in **Table 2**.

In the 5 experiments described in Table 2, multiparous dairy cows received by intramuscular injections a combined supplement of folic acid and vitamin $B_{12}$ during the 3 to 4 weeks before the expected calving date and in early lactation. Dry matter intake and milk production of control cows were similar among these experiments; nevertheless, milk production responses to the supplement varied from a decrease of 1.7 kg/d to an increase of 3.6 kg/d (Table 2). Looking at the plasma concentrations of both vitamins as indicators of the vitamin status of the animals, it appears that the largest response was observed in experiment 3 where plasma concentrations of both vitamins were the lowest whereas the negative response was observed in experiment 5 where both concentrations were the highest. These observations suggest that at least part of the variability among experiments studying production and metabolic responses to B-vitamin supplements could be due to the vitamin status of the cow which reflects vitamin supply. Indeed, when the vitamin supply is adequate, a supplementation is likely to be useless.

## The Challenge: Estimating B-vitamin Supply

Table 3 illustrates the great variability of intake, duodenal flow and apparent synthesis of B vitamins in the rumen of dairy cows. Negative values for apparent ruminal synthesis indicate that the amount of vitamin destroyed in the rumen is greater than the amount of vitamin ingested. As B-vitamin absorption takes place mostly in the small intestine, the duodenal flow of B vitamins represents the amount of vitamins available for absorption by the cow.

In non-ruminants, B-vitamin supply can be calculated by multiplying B-vitamin concentrations in the diet by the intake. In ruminants, B-vitamin supply is the sum of the vitamins ingested and not destroyed by the ruminal microbial population and those synthesized in the rumen.

In the experiments reported in Figure 1, 6 diets based on alfalfa silage (range of 42 to 60% on a DM basis), dry corn (range of 34 to 39% on a DM basis) and soybean meal and/or SoyPlus (range of 4 to 13% on a DM basis) were fed to lactating dairy cows (Castagnino et al., 2016a, b, c). Folate intake was similar for diets C and D, 11 mg/d, but the amount of folates recovered at the duodenum was 75% with diet C compared with the amount recovered with diet D (**Figure 1a**). Moreover, as shown in Figure 1, all B vitamins did not respond alike to dietary changes. Among the studied diets, niacin intake was nearly ten times greater for diet C than A but the amount of niacin reaching the small intestine was similar, 1197 vs. 1268 mg/d for diets A and C, respectively (**Figure 1b**). In the present example, apparent ruminal synthesis of niacin seems to be inversely proportional to the amount ingested.

Figure 1 illustrates why, in dairy cows, the amount of B vitamins ingested is not a reliable indicator of the amount of vitamins reaching the sites of absorption and available for the animal. It also highlights the fact that effects on one vitamin cannot be extrapolated to another one. Knowledge on the factors controlling the amounts of B vitamins escaping the rumen is very limited. It is likely that ingredient and diet composition and their consequences on ruminal fermentation pattern control the fate of B vitamins in the rumen. Increasing knowledge on these effects possibly offers the best approach to predict B vitamin supply for the dairy cow.

## Conclusions

Research on B-vitamin requirements of dairy cows is still in its very early stage. The number of published experiments on production and metabolic responses of dairy cattle to B-vitamin supplements is still very small. As described above, there is scientific evidence that B-vitamin supply from the diet and synthesis in the rumen is not always sufficient to meet the needs because increasing supply in niacin, biotin, folic acid and vitamin $B_{12}$ improves metabolic efficiency of dairy cows, especially during the critical period around calving and in early lactation. One has to remember however, that these values are the doses most frequently used in the published experiments; they are not requirements, they cannot even be considered as recommended intakes because even for the most studied vitamins, very few dose-response experiments have been conducted. Moreover, although quantification of the metabolic demand for B vitamins is still far from precise, variability among experiments is likely frequently due to differences in the amounts of B vitamins available for the cow. Consequently, recommendations for B-vitamin adequate intakes is dependent of our ability to predict their total supply, i.e. the amounts of vitamins from dietary sources escaping degradation in the rumen plus the amounts synthesized in the rumen. If supply and requirement are equal, a positive effect of a B-vitamin supplement is unlikely whereas a positive response to supplementation can be expected if the supply is sub-optimal.

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**Table 1.** Effects of biotin supplements on lactation performance of dairy cows – summary of two meta-analyses.

| | Lean and Rabie (2011) | | Chen et al. (2011) | |
|---|---|---|---|---|
| | Difference | *P* value | Difference | *P* value |
| Dry matter intake, kg/d | +0.70 | 0.09 | +0.87 | 0.01 |
| Milk production, kg/d | +1.29 | 0.002 | +1.66 | 0.002 |
| Milk fat, % | +0.05 | 0.23 | +0.01 | 0.53 |
| Milk protein, % | -0.09 | 0.33 | +0.03 | 0.55 |
| Milk fat, kg/d | 0.07 | 0.08 | +0.05 | 0.04 |
| Milk protein, kg/d | 0.02 | 0.09 | +0.05 | 0.001 |

**Table 2**. Milk production responses to the administration of a combined supplement of folic acid and vitamin $B_{12}$ in early lactation in 5 experiments.

| | Trial 1 | Trial 2 | Trial 3 | Trial 4 | Trial 5 |
|---|---|---|---|---|---|
| Control | | | | | |
| Dry matter intake, kg/d | 21.3 | 21.8 | 21.1 | 22.5 | 21.0 |
| Milk production, kg/d | 39.5 | 37.7 | 40.1 | 39.5 | 41.5 |
| Effect of the combined supplement of vitamins on milk production, kg/d | | | | | |
| | +1.4 | +1.2 | +3.6 | +1.2 | -1.7 |
| Control | | | | | |
| Plasma folates, ng/mL | 15 | 16 | 11 | 11 | 16 |
| Plasma vitamin $B_{12}$, pg/mL | 172 | 181 | 131 | 223 | 216 |

**Table 3**. Intake, duodenal flow and apparent ruminal synthesis of B vitamins (mg/kg of dry matter intake) [1].

| | Intake | Apparent synthesis in the rumen | Duodenal flow |
|---|---|---|---|
| Thiamin | 1.3 to 3.8 | -1.5 to 4.2 | 0.8 to 7.8 |
| Riboflavin | 4 to 106 | -50 to 29 | 3 to 87 |
| Niacin | 22 to 170 | -123 to 120 | 47 to 146 |
| Pantothenic acid [2] | | | |
| Vitamin $B_6$ | 2.6 to 17.6 | -14.1 to 1.3 | 0.7 to 7.7 |
| Biotin | 0.2 to 7.0 | -0.9 to 0.2 | 0.2 to 6.6 |
| Folates | 0.2 to 1.1 | 0.5 to 3.3 | 0.9 to 2.4 |
| Vitamin $B_{12}$ [3] | -[4] | 0.1 to 4.8 | 0.1 to 4.8 |

[1] Breves et al., 1981; Steinberg and Kaufman, 1977; Santschi et al., 2005; Schwab et al., 2006; Lebzien et al., 2006; Niehoff et al., 2013; Beaudet et al., 2016; Castagnino et al., 2016a, b, c; Seck et al., 2016.

[2] No data available.

[3] Total dietary concentrations of cobalt: 0.17 to 2.5 mg/kg DM.

[4] Under or close to the level of detection.

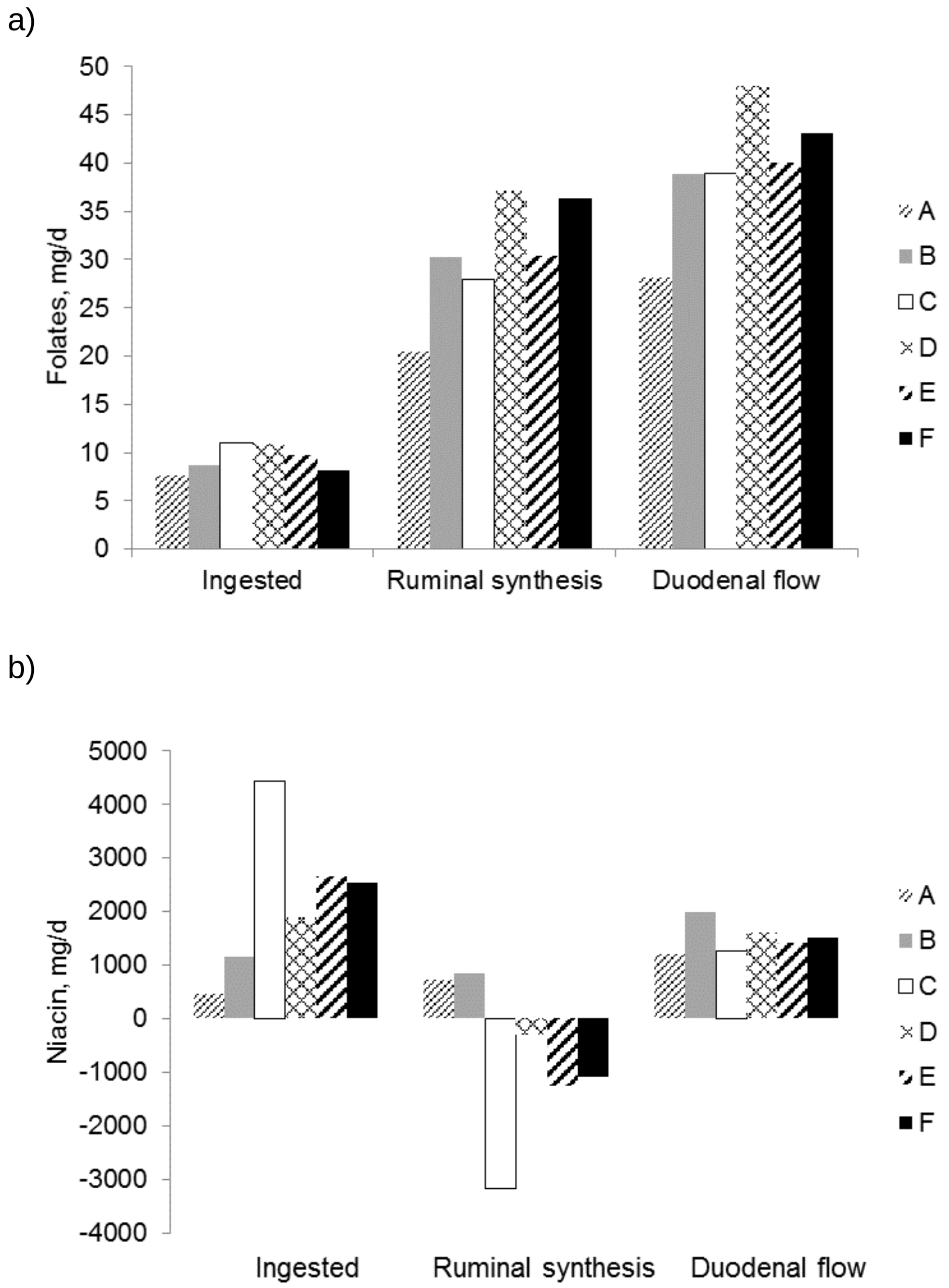

**Figure 1.** Daily intake, apparent ruminal synthesis and duodenal flow of a) folates and b) niacin in dairy cows fed 6 diets based on alfalfa silage.

# SESSION NOTES