

Review / Derleme

# The role of micronutrients in high-yielding dairy ruminants: Choline and vitamin E

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**Abstract:** This review addresses the potential role of antioxidants and methyl-group sources in optimising the metabolic health of dairy ruminants. The productivity of high-yielding dairy cows has increased over the past 40 years and the milk yield has doubled. Such increases in milk production have been observed not only in dairy cows but also to some extent in other dairy ruminants such as ewes, goats and buffaloes (*Bubalus bubalis*). As a consequence, in all specialized dairy ruminants it is essential to optimize the macro and micro-nutrient supply, especially during the most critical period in the animals' production cycle i.e. from parturition until the peak of lactation. In this critical phase, an array of factors can enhance the balance between the intake and demand for nutrients, although the availability and supply of the selected micronutrients is also important. The supplementation of dietary antioxidants or boosting the endogenous methyl group status, via vitamin E, selenium and choline are proposed as possible strategies in maintaining stable metabolic health and optimising milk production.

**Keywords:** Choline, dairy cows, health, milk production, vitamin E.

## Introduction

In high-yielding dairy ruminants the antioxidant and methyl group status are important in defining the metabolic health of the animals. However, the supplementation of both methyl group sources and antioxidants are usually addressed separately. This review highlights the key knowledge on vitamin E and choline supplementation in dairy ruminants and their role in optimizing milk production and metabolic health.

## Vitamin E

Historically, deficiencies in vitamin E or selenium have been associated with high somatic cell counts (SCCs) in bulk and individual cow milk, and also with increased incidence and severity of intramammary infection (IMI) and mastitis. The positive effect of vitamin E on the SCC depends on adequate dietary Se levels, as reported since the 1980s. When dietary selenium was adequate, vitamin E supplementation significantly reduced the incidence of IMI and clinical mastitis (38). The supplemental vitamin E also improves the killing ability of neutrophils and enhances macrophage function in cows (6, 8, 16, 17, 33).

The current requirement for supplemental vitamin E is approximately 500 and 1000 IU/day for lactating and dry cows, respectively. Although NRC requirement published in the 2001 was substantially higher than that of the 1989, other data suggest that higher supplementation rates may be warranted in some situations. Cows supplemented with 4000 IU of vitamin E per day (in combination with 0.1 ppm of supplemental selenium) during the last two weeks before calving and 2000 IU/day during the first week of lactation were found to have significantly fewer mammary gland infections and clinical mastitis compared with 1000 and 500 IU/day during the dry and early lactation periods (42). Subsequent studies (3, 7, 23) confirmed these findings.

A meta-analysis (23) encompassing around 34 published papers between 1984 and 2003 on the relationship between vitamin E and mammary gland health, established that different supplementation levels, up to a maximum of 4000 IU during the dry and early lactation periods, were associated with lower somatic cell counts (SCCs), IMI, and clinical mastitis. Overall, vitamin E supplementation reduced milk SCC by a factor of 0.70,

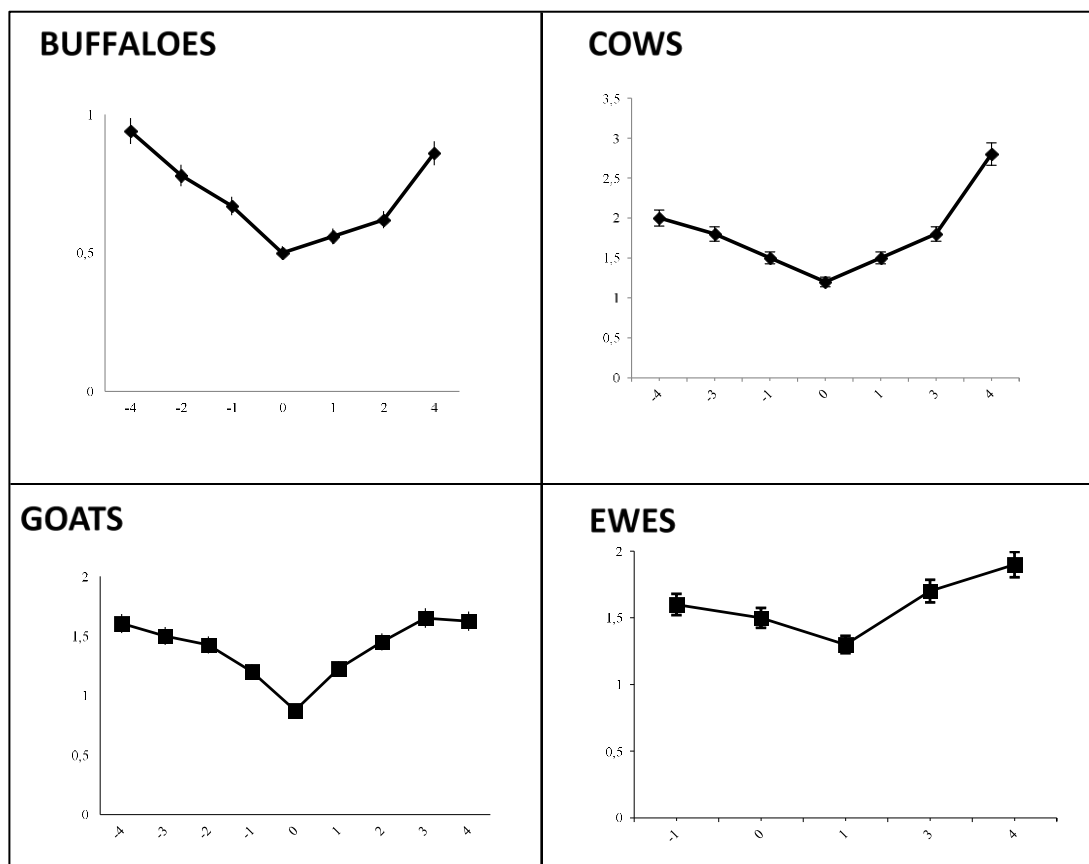
the risk of IMI by 14%, and the occurrence of clinical mastitis by 30%.

A further disorder in which antioxidant status plays an important role is the retention of fetal membranes (RFM). RFM was one of the first conditions to be attributed to depleted antioxidant defenses and may also be related to impaired neutrophil function. RFM in dairy cows is a cause of endometritis, subsequent ovarian cycle delay and hence delayed pregnancy, resulting in serious economic losses (19). In fact, there is considerable evidence that oxidative stress is enhanced in RFM compared to animals with non-retained placenta. Thus, cows with low fast-acting antioxidants (including alpha-tocopherol) in plasma, and low glutathione peroxidase in red blood cells have been shown to have a higher incidence of retained placenta than cows with higher antioxidant levels (10, 11). LeBlanc et al. (21) showed that vitamin E (in association with selenium) was involved in RFM, but also highlighted that there are other important contributors, one of which may be the energy supply for immune function.

Supplementing dairy cattle with adequate amounts of vitamin E and Se is thus now a widely-accepted practice. However, adequate levels of these micronutrients do not completely control oxidative stress around calving (3, 6, 27). Numerous studies in dairy cows (34) have shown that

the plasma vitamin E concentration decreases gradually around parturition, reaching the lowest values around calving and then increasing gradually afterwards. The inadequacy of micronutrient supplementation in this situation is probably due to increased milk yields, and a greatly reduced feed intake, resulting in a substantial increase in the recommended intake levels for this animal (24, 43).

In dairy cows the intake of vitamin E is generally considered adequate when alpha-tocopherol plasma levels are over 3-3.5 mg/ml or above 2 mg/mg relative to plasma cholesterol. Beyond these levels, no further benefits are observed (3). Reaching an adequate vitamin E status however, is difficult in dairy cows. Plasma vitamin E levels, expressed as alpha-tocopherol, fall significantly around parturition in all dairy ruminants (cows, ewes, goats, buffaloes - Figure 1), and it is difficult to maintain levels that are thought to be adequate for health. It has been suggested that this is one of the reasons for the decline in peripartum innate and acquired immune defense in dairy ruminants. Supplementation levels should therefore be re-considered, choosing high bioavailability sources. In this respect, the bio-potency and bioactivity of naturally occurring RRR- $\alpha$ -tocopherol are known to be higher than its synthetic all-rac counterpart (34, 41).



**Figure 1.** Changes in plasma alpha-tocopherol around parturition (day 0 in x axis) in various dairy ruminant species. Data from Hogan et al. (17), Panda et al. (25), Pinotti et al. (32), Toker (40). x-axes = days relative to parturition; y- axes = alpha-tocopherol, mg/l.

Advances in feed manufacturing technology (including microencapsulation, and polymer coat protection) now make it possible to design products that deliver specific nutrients to the absorption sites of the small intestine. It is also clear that the formulation (natural vs. synthetic; inorganic vs. organic form; protected vs. unprotected) may not only influence intestinal bioavailability, but may also affect the uptake from target organs, tissue utilization, transfer to the new-born animals (1), and food quality (e.g. milk).

### Choline

Choline supplementation increases productivity as well as metabolic health in dairy ruminants (2, 29, 32). Choline is often considered as a vitamin however, unlike classical vitamins, its endogenous synthesis is possible, and a choline deficiency syndrome usually goes undetected in healthy mammals. It has therefore been suggested that choline may be an essential nutrient for mammals, irrespectively of whether or not it is classified as a vitamin-like compound. Choline has an important role in energy and protein metabolism especially for those pathways that take place in the liver. It is involved in lipid metabolism, and more precisely in lipid transport as a lipotropic agent.

Choline, an important labile methyl group donor, takes part in the biosynthesis of other methylated compounds in the body. This latter function is "code shared" with methionine, thus choline and methionine are interchangeable being the two primary methyl donors in animal metabolism.

However, the metabolic pathways of choline and methyl groups are different in ruminants. In adult ruminants, choline is extensively degraded in the rumen; and as a consequence, unable to contribute significantly to the choline body pool. The methyl group metabolism is generally conservative with a relatively low rate of methyl catabolism, and a high rate of *de novo* synthesis of methyl groups via the tetrahydrofolate (THF) system.

In ruminants there is a conservative methyl group metabolism, which works perfectly in positive energy balance animals (e.g. beef cattle). However, this can become exacerbated in lactating dairy ruminants, for which the availability of dietary choline is still limited, while the output of methylated compounds in milk is high, and precursors from the tetrahydrofolate pathway (such as glucose and other gluconeogenetic precursors) are limiting, especially at the beginning of lactation.

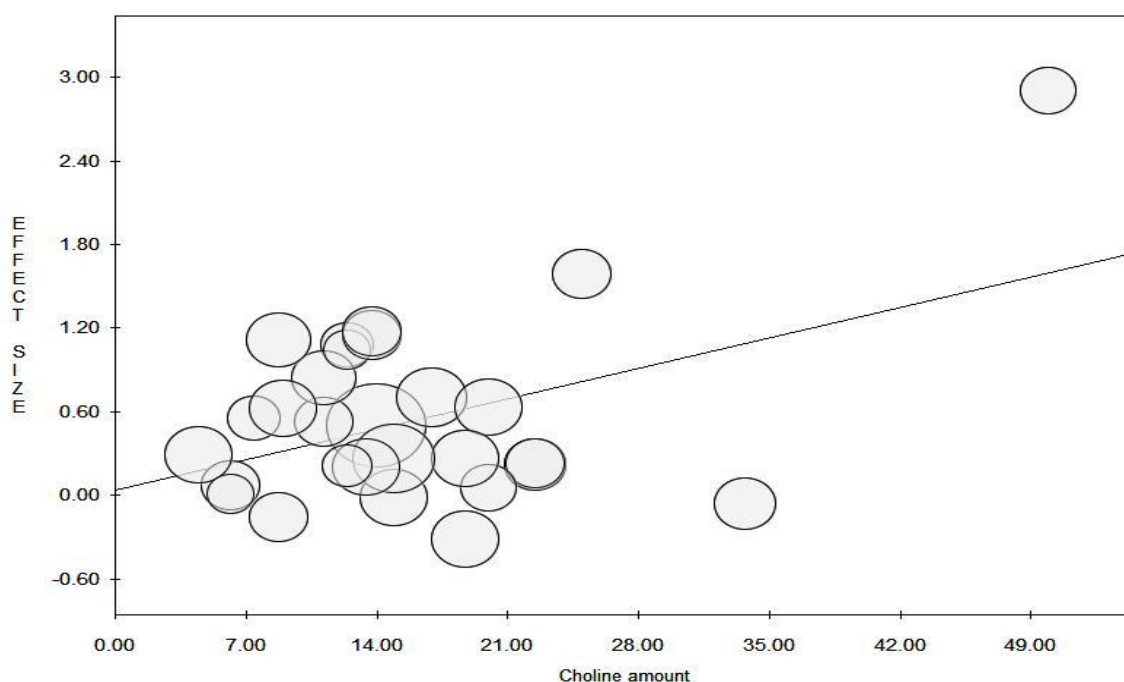
On the basis of these assumptions, the hypothesis that choline may be a limiting nutrient for milk production has been proposed (29, 32). A great number of studies conducted to assess the possible effects of choline in milk production, established that from a technical/feeding point of view, choline must be rumen-protected in order to be effective in ruminant nutrition.

Knowledge about transition and early lactation periods of dairy cows proposes that greater choline availability improves not only milk production (5, 9), but also lipid (12, 13, 26, 30, 32) and methyl group metabolism (5). These results have also been investigated in a few meta-analyses. In 2010, Sales et al. (37) quantified the dietary effects of RPC (rumen-protected choline) on the production traits of dairy cows. In Sales' review, by increasing RPC supplementation from 6 to 50 g/d, which can also be effective in increasing milk yield, no dose response was observed. Milk yield in fact, decreased from 131.5 to 0.037 g of milk for each g of dietary RPC supplemented in the diets from 6 up to 50 g/d. In the same supplementation range, milk fat content decreased linearly at a rate of 0.00339% for a 1g/d increase in dietary RPC, confirming that choline has no effect on the milk fat content. Although the reasons for this are not clear, an interaction between choline and methionine has been proposed (37).

More recently two other meta-analyses on choline in dairy cow nutrition have been published. In 2019 using data from 27 studies, Humer et al. (18) reported that choline supplementation increased postpartum dry matter intake by 4% and milk yield by 3.4% in dairy cows. A higher milk yield also induced a higher milk fat yield and milk protein yield, without affecting their concentration. However also in this meta-analysis, no dose/response effects were observed. Providing RPC had no effect not only on the main plasma metabolites (such as non-esterified fatty acids, beta-hydroxybutyrate, glucose, and cholesterol) but nor on the incidence of ketosis, and mastitis across all studies in this meta-analysis.

These results seem to be in contrast with several studies in which choline was shown to improve the metabolic health and liver function (5, 12, 13, 26, 30). In fact, Zenobi et al. (44) stated that feeding pregnant, non-lactating Holstein cows with increasing amounts of RPC (from 0 to 25.8 g/d) decreased the concentration of hepatic triglycerides in a linear manner. Dietary choline thus seems to improve fat metabolism in the liver and increase the apparent absorption/traffic of triglycerides in the bloodstream, further confirming the beneficial effects of supplemental RPC for adult ruminants.

The most recent meta-analysis conducted by Arshad et al. (2) was based on 21 experiments including a total of 1313 cows, with a range of prepartum choline supplementation from 5.0 to 25.0 g/d. The results confirm that milk yields and energy-corrected milk (ECM) in multiparous cows increase linearly with the amount of dietary choline supplemented during the transition period. In addition, across the studies included in this meta-analysis, feeding RPC tended to reduce retained placenta and mastitis, although the optimum dose of choline was not established. Arshad et al. (2) recommended more than



**Figure 2.** Meta-regression of choline amount (g/d of choline chloride) on effect size (MY) based on 17 studies.

13 g/d of choline, provided in a rumen-protected form. This quantity, however should be considered with caution since the dose–response relationship between dietary choline and milk yield needs to be addressed more thoroughly (Figure 2). Thus, several physiological and dietary factors are probably related to the obtained responses with dietary RPC supplementation necessitating further research to investigate the precise mechanism of choline action in lactating dairy cows.

In the case of other dairy ruminants, the effects of RPC have been less investigated. In dairy buffaloes 15 g/d of choline in a rumen-protected form were shown to increase milk yield by 15% (15). Using the same RPC dose, Kumar et al. (20) obtained a 20% increase in milk yield in choline-supplemented animals compared to the controls. The effect of RPC on milk yield recorded in these studies on dairy buffaloes are among the highest registered in dairy ruminants. Although the exact mechanism for such a large response is still unknown, the co-presence of supplemental fat in the diets cannot be ruled out. In small ruminants, Lobley et al. (22) studied the effects of choline infusion in sheep on transmethylation reactions, while Emmanuel and Kennelly (14) investigated methionine and choline incorporation into the plasma and milk of lactating goats. In both cases however, the main goal was to assess the fate and metabolism of methyl groups and the interchangeable nature between choline and methionine. Other researchers (4, 32) also assessed the efficacy of RPC supplementation in dairy goats. Pinotti et al. (32) supplemented periparturient dairy goats with 4 g/d of RPC

starting four weeks prior to expect kidding and continuing for five weeks after parturition. Supplemental choline dose was derived from the experiments with dairy cows (32) corrected for the metabolic body weight ( $BW^{0.75}$ ) of the goats at the beginning of the experiment. In RPC supplemented goats during early lactation, milk yield and fat-corrected milk (4% FCM) yield increased by 7 and 12%, respectively, compared to non-supplemented goats. RPC supplement also increased the milk fat concentration, and fat and protein yield while plasma metabolites did not differ between treatments. By contrast, in dairy ewes (39), RPC supplementation in combination with methionine and betaine had no effect on milk yield, although the dose of choline used was extremely low. It is worth noting that the number of studies on ovine species is still limited.

Overall these results suggest that greater choline availability is essential for optimizing milk production in dairy ruminants, although other factors such as dietary composition, dietary crude protein content, and post-ruminal methionine supply merit further investigation.

This review has highlighted that the dietary supply of antioxidants and methyl group sources may not always be sufficient to maximize milk production in dairy ruminants. Although the requirement of these nutrients may in theory be satisfied by specific supplementation (vitamin E and selenium) or by other nutrients (in the case of choline), it is unlikely that this happens in practice especially at the onset of lactation. The magnitude of the production response is likely to be affected by the basal diet composition, the dose of the nutrient, its form of

presentation/supply, and the stage of lactation (5, 24, 28, 41). However, it is also evident that our knowledge is incomplete regarding the potential role of antioxidants and methyl group sources in optimising metabolic health in dairy ruminants. For example, there are indications that both methyl group precursors (including choline) and other co-factors (folate and vitamin B12) are important for the optimal metabolic support of milk production, although methionine may not always be involved in this scenario (35). At the same time there are limited indications (*in vitro*, 36) that antioxidant and methyl sources interact positively in bovine mammary epithelial cells. In conclusion, a nutritional approach based on vitamin E and choline supplementation in dairy ruminants could be beneficial in terms of optimizing their milk production and metabolic health. However, the supply and use of individual nutrients should be reconsidered in order to meet the novel findings about the synergic effects of these compounds in ruminant diet.

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### Conflict of Interest

The authors declared that there is no conflict of interest.

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