

Assessment of Lactational Performance in Dairy Cows Receiving a Rumen Protected B Vitamin Blend during Lactation: Part 1: A Meta-Analysis of 50 Studies

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Abstract

The objective of this research was to evaluate the impact of a dietary supplemental rumen protected B vitamin blend (RPBV: Folic acid, B12, pyridoxine, pantothenic acid, and biotin; Jefo, St. Hyacinthe, QC Canada) on milk, fat, and protein yield in cows past peak lactation. Results from 50 double reversal design trials (control-test-control) were evaluated using meta-analyses. All herds participated in monthly milk recording services, and all were Holstein herds. Within each of the 50 trials, cows included in the statistical analysis were over 45 days in milk (DIM) at the first milk test, averaged 159 ± 26 DIM on the first test date, and were available for all milk collection periods. A total of 6483 cows from 7 countries were represented in the 50 studies, with an average herd size of 129.7 ± 118.0 . Meta-analyses were conducted to determine effect size for milk, fat, and protein yield based on the treatment differences and standard errors (SE) from the individual trials. Results indicated that dairy cows responded positively to the inclusion of the rumen protected B vitamin blend. Forest plots revealed trial-to-trial variation and might be related to age, level of milk production, and stage of lactation of the cows.

Keywords

Meta-Analysis, Dairy Nutrition, Rumen Protection, B Vitamins

1. Introduction

The water soluble “B” vitamins are indispensable nutrients for all mammalian species. The functions of these nutrients are wide ranging and impact all aspects

of health and productivity. The primary source of B vitamins for lactating dairy cattle is from the microbial synthesis in the rumen [1] [2] as most of the B vitamins from feed ingredients are quickly destroyed upon exposure to rumen microbes [1] [3] [4]. Early research, conducted in an era when productivity per cow was lower than now, demonstrated that microbial synthesis of B vitamins was adequate to support requirements [5] [6] [7] [8]. The findings were largely unchallenged until the turn of the century. The contemporary dairy cow has the genetic potential to produce greater quantities of milk.

Thus almost 50 years later, exploratory research conducted by Girard *et al.* [9] demonstrated improvements in production with folic acid injections. Bonomi *et al.* [10], dosing cows with pyridoxine noted improvements in milk production and reproductive performance. Biotin supported hoof health and increased milk yield in high producing cows [11] while thiamin was likewise shown to influence milk yield [12]. Recent research has shown that the amounts of B vitamins synthesized are variable and are altered by feeding conditions such as forage to concentrate ratio [13] [14] [15], rumen nitrogen availability [16], and even the ingredients per-se [16] [17]. The fact that rumen microbes may not provide adequate amounts of B vitamins to allow optimum productivity is now being accepted as true [1].

Because of microbial destruction for many of the B vitamins, research to determine post-ruminal metabolic responses requires the feeding of very high dietary concentrations or frequent intramuscular injections of the vitamins, to permit small quantities to escape to the intestines. With neither being of practical purpose, research regarding the actual requirements of B vitamins for functional ruminants has lagged. This difficulty in providing B vitamins has in recent times been overcome with rumen protection technology. The technology allows affordable concentrations of vitamins to be supplied and evaluated.

Lactation VB is a rumen protected B vitamin (RPBV) blend developed by Jefe, (St. Hyacinthe, QC, Canada) and contains folic acid, B12, pyridoxine, pantothenic acid, and biotin. The product was formulated to provide the vitamins most likely to be deficient in lactating dairy cows for optimum performance [18] [19].

In a previous series published in this Journal [19] [20] individual cow results from double reversal trials were combined to assess the impact of the same RPBV blend using regression models. The results revealed greater milk, fat, and protein yield overall. All studies were conducted in Canada and the United States of America. Since that time, additional trials have been conducted over a broader geographic range allowing more insight into the potential for supplementation of B vitamins in a rumen protected form. The current evaluation is based on a meta-analysis of 50 feeding experiments conducted in 7 countries.

2. Materials and Methods

2.1. Background and Description of Individual Trials

Data from past on-farm trials in which the RPBV blend (Jefe, St. Hyacinthe, QC

Canada) was compared to a control diet containing no added B vitamins in any format were obtained. Fifty, three-period double reversal feeding studies conducted between 2005 and 2015 were included in these analyses. Treatments were allocated in the sequence control-test-control. The two control periods were compared to the test period, as described by Sanders and Gaynor [21]. The treatment consisted of adding the test product to an existing diet. The test product, which provided rumen protected folic acid, B12, pyridoxine, pantothenic acid, and biotin imbedded in a lipid matrix was added during the test period at the feeding rate of 3 g/cow/day.

Within each trial, treatment comparisons consisted of adding the test product to an existing diet. All diets were assumed to be nutritionally adequate, but no instructions were issued to alter the diets or usual feeding practices for the benefit of the trials. The test product, which provided rumen protected folic acid, B12, pyridoxine, pantothenic acid, and biotin was added to the current diet during the test period at the feeding rate of 3 g/cow/day.

Trials were invigilated by consulting nutritionists and veterinarians and those included in the analyses were determined to meet the following criteria: All trials supplied data by the cow for cow name or number, lactation number, days in milk at the first milk collection period, milk production, milk fat yield and milk protein yield for three consecutive periods. Periods were defined as the length of time between individual cow monthly monitoring, typically 28 - 33 days in length. Cows included in each analysis were present and tested in all three periods and were greater than 45 DIM at the first milk test date.

All herds participated in registered monthly recording services, and all were Holstein herds. No instructions were issued to alter the diets or the normal farm feeding routine to benefit the trial outcome. Trials were included in the analysis if confirmed by both the farm staff and the formulating nutritionist that no dietary changes to rations had been made over the course of the experiment, and there were no known management issues that would impact the diet, feeding, or health of the cows

2.2. Meta-Analysis of the 50 Available Trials

Trial result data were analyzed using Meta-Essentials Software (Creative Commons, Mountain View, CA, USA) as outlined by Suurmond *et al.* [22] for a random-effects model. Effect size was calculated for milk, fat and protein yield based on the treatment differences and standard errors (SE) from the individual trials. Forest plots were developed from the results and provide information on the effect size by trial and the 95% confidence interval (mean \pm two standard deviations). Results were declared significant if the P value was less than 0.05 (*i.e.*, the probability that the differences occurred by chance is under 5%).

3. Results and Discussion

Trials were conducted globally (Australia-6, Brazil-6, Canada-28, Chile-3, Co-

lombia-1, Mexico-4, Spain-1 and USA 16 trials). A total of 6483 cows were represented with an average of 129.7 ± 118.0 participants/study and an average parity of 2.2. Cows were largely mid to late lactation, with DIM averaging 159 ± 26 at the first test date.

Table 1 provides the results for effect size, confidence intervals, prediction intervals and statistical significance (P values). Milk production was increased by 0.88 kg/cow/day with the inclusion of the RPBV ($P < 0.05$). Yields of fat and protein were likewise increased when the diets were supplemented with the RPBV. The findings from these analyses are consistent with results determined from a limited number of trials conducted in North America [19] and support the fact that lactating dairy cows respond positively to the inclusion of RPBV.

Figures 1-3 provide results graphically in forest plot format. The results indicate that, while largely positive, there is considerable between trial variations in herd responses to RPBV blend. Frequency distributions for milk yield, fat yield and protein yield are provided in histogram format in **Figures 4-6** respectively. These data show that while variable, results were largely positive when vitamin supplementation is provided over the wide range in conditions.

The changes are modest, but suggest that, though no visible signs, cows are experiencing deficiencies in B vitamins. Girard and Graulet [1] remarked that a deficiency occurs any time a supply is lower than a need. A deficiency may not result in clinical signs of poor health or performance, but rather some metabolic distress that can resolve itself as lower growth or milk yield production, lower response to stress or reduced reproductive capabilities.

In some studies, individually administered vitamins have been demonstrated to improve milk production parameters. Chen *et al.* [23] noted that biotin was not completely degraded in the rumen, and identified 11 studies in which supplemental biotin was given to lactating dairy cows. The researchers found supplementation of diets with biotin significantly improved milk yield in 6 of the 11 experiments. Girard and Matte [24] found that intramuscular injections of folic acid increased milk yield in multiparous, but not primiparous cows.

Other biological uses of vitamins may supersede their use for milk production and further contribution to variability in results. Morrison *et al.* [25] determined that RPBV reduced anovulation in dairy cattle from 23% to 11% by 8 weeks post

Table 1. Results of meta-analyses for milk, fat and protein yield from 65 trials evaluating the inclusion of rumen protected B vitamins (RPBV) in diets for cows in mid-lactation.

	Parameter		
	Milk	Fat	Protein
Effect size, kg/cow/day	0.88	0.043	0.033
Standard error	0.14	0.01	0.01
Confidence interval (95%)	0.60 - 1.17	0.02 - 0.05	0.02 - 0.05
Prediction interval	-1.05 - 2.81	-0.04 - 0.11	-0.05 - 0.11
P value	<0.001	<0.001	<0.001

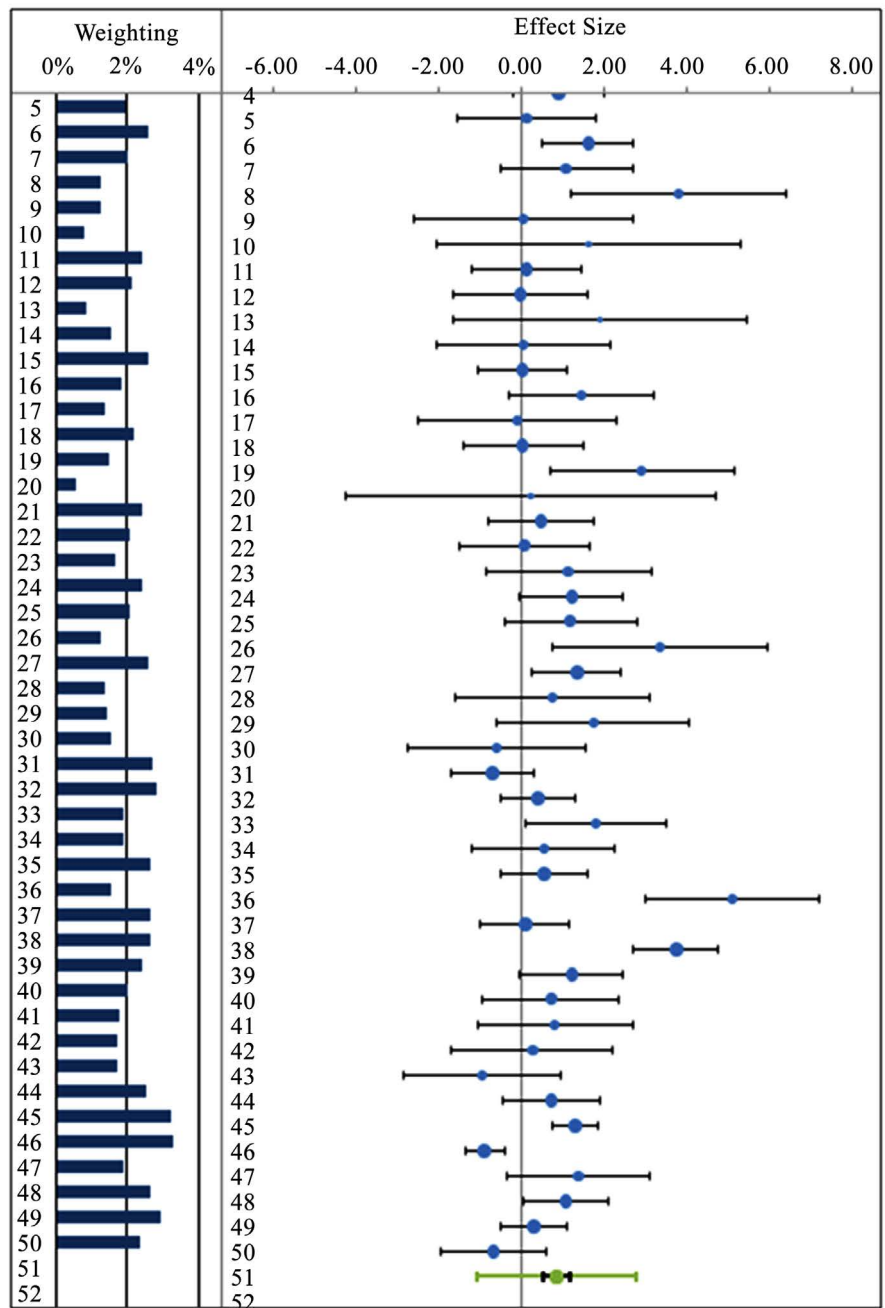


Figure 1. Forest plot for milk yield. Trial weighting is determined by the standard error. Plots provide individual trial effect size and confidence interval. The lowermost bar shows the overall effect size, confidence interval (inner bar) and prediction interval (outer bar).

partum in dairy herds is considered to be optimally managed. Similarly, Duplessis *et al.* [26] found that intramuscular injections of vitamin B12 and folic acid in periparturient cows resulted in lower dystocia in multiparous cows (incidence rates of 10.8% vs. 5.3% for control as compared to vitamin injected) and reduced body weight loss in adequately managed Quebec dairy herds. Reduced involuntary culling [27] was witnessed in a California dairy when rumen protected B vitamins were included in the diet in early lactation. Additionally, vitamins may be

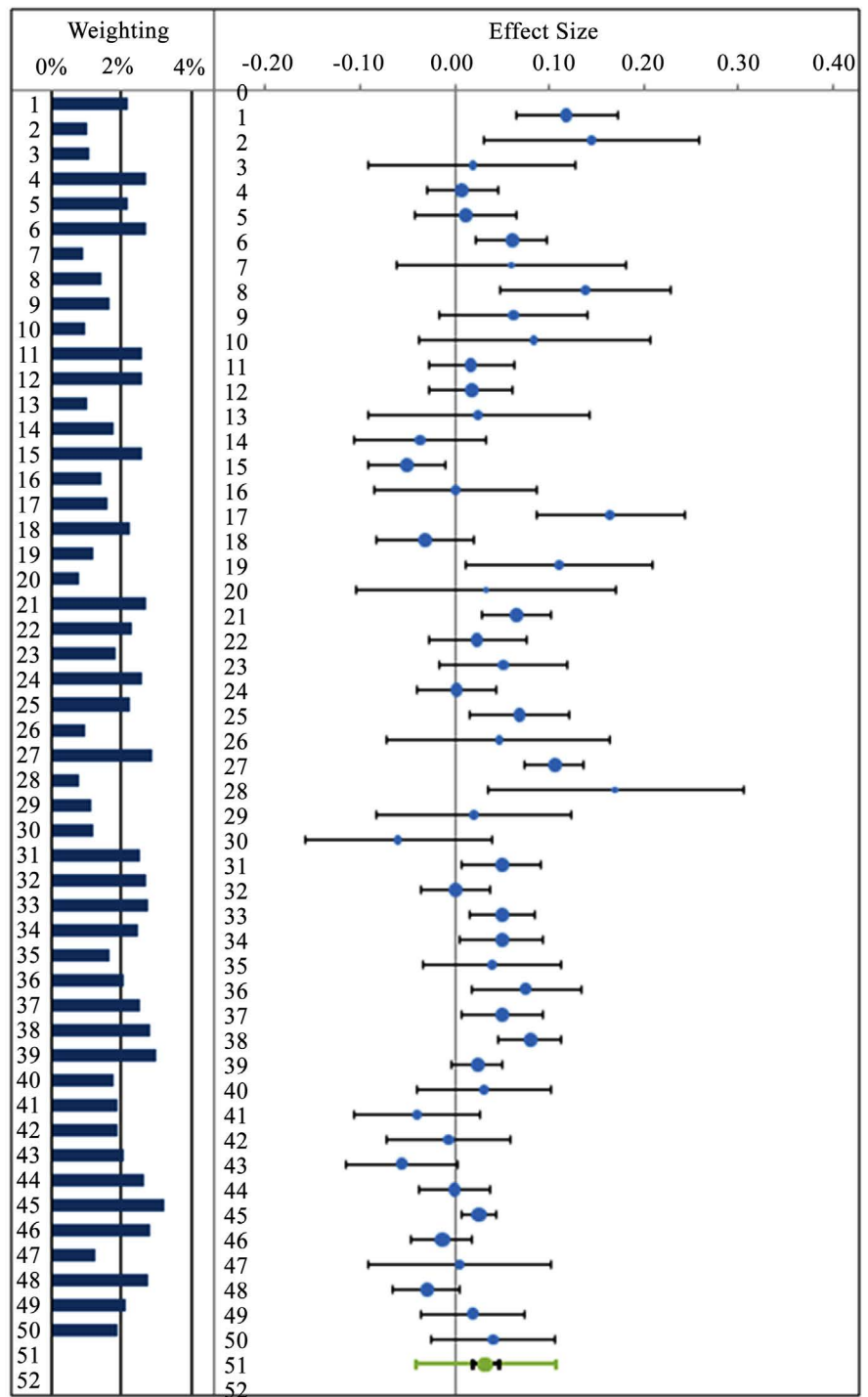


Figure 2. Forest plot for fat yield. Trial weighting is determined by the standard error. Plots provide individual trial effect size and confidence interval. The lowermost bar shows the overall effect size, confidence interval (inner bar) and prediction interval (outer bar).

needed to prevent reduce stresses support other biological functions. These results demonstrate that over a wide geographic area, with many types of feed ingredients provided, well maintained dairy cows respond to the inclusion of rumen protected B vitamins in their diets with improved milk production.

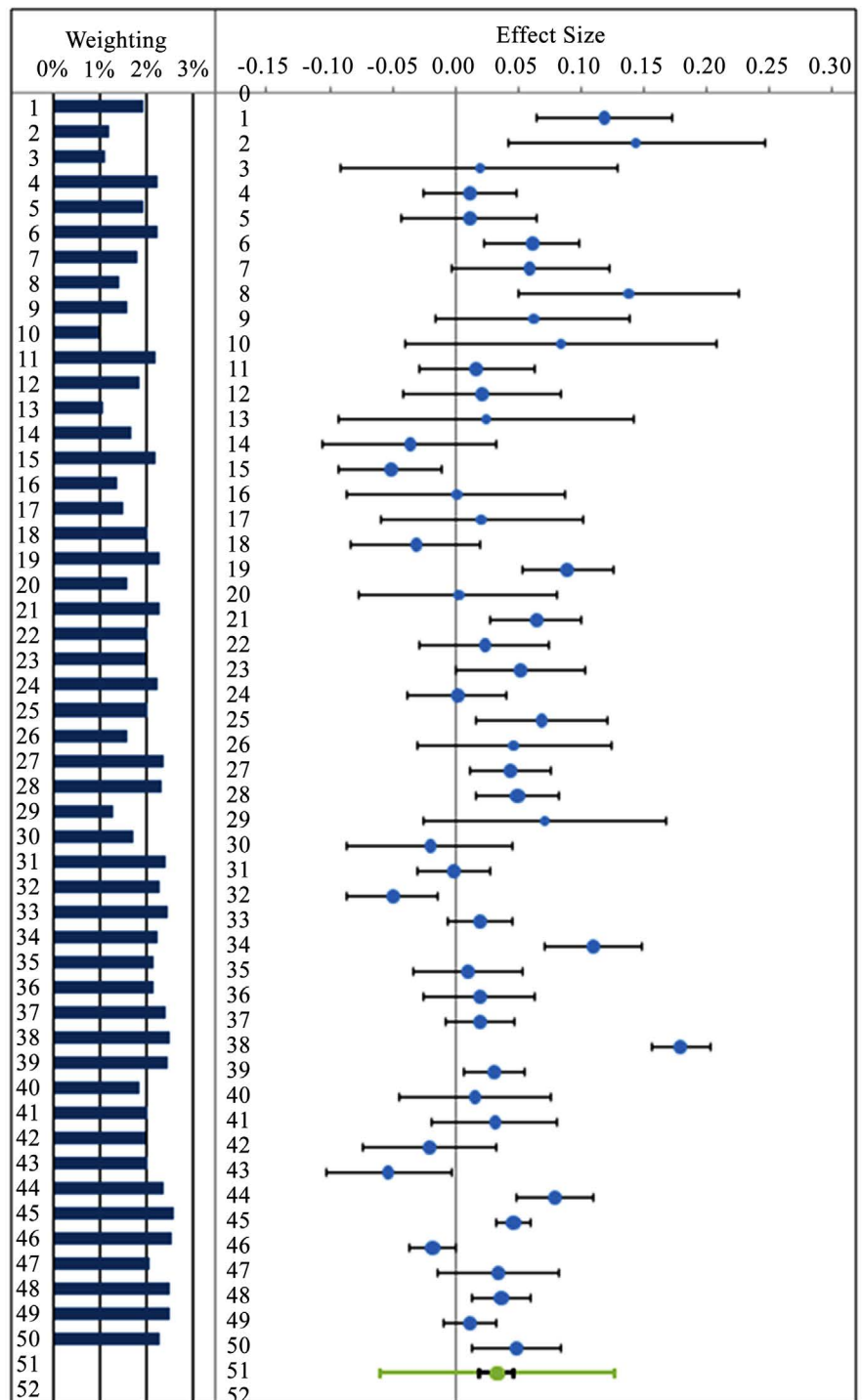


Figure 3. Forest Plot for protein yield. Trial weighting is determined by the standard error. Plots provide individual trial effect size and confidence interval. The lowermost bar shows the overall effect size, confidence interval (inner bar) and prediction interval (outer bar).

In addition, the variation in response to the B vitamins witnessed in this analysis might also be related to the age, level of milk production and stage of lactation of the cows, and further analyses are required to address these items.

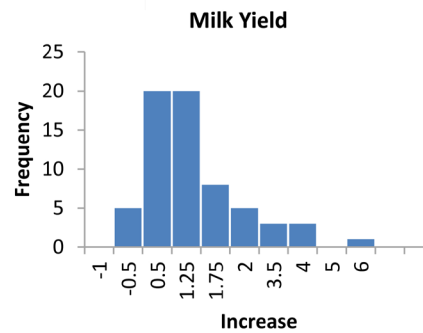


Figure 4. Histogram of change in milk yield with the inclusion of rumen protected B vitamins (RPBV) in diets for cows in mid-lactation.

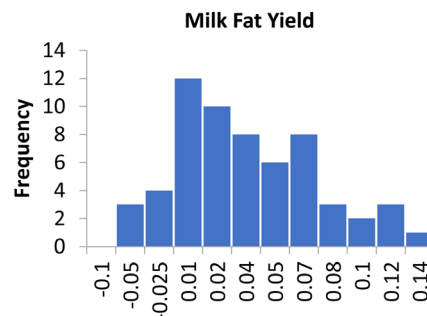


Figure 5. Histogram of change in milk fat yield with the inclusion of rumen protected B vitamins (RPBV) in diets for cows in mid-lactation.

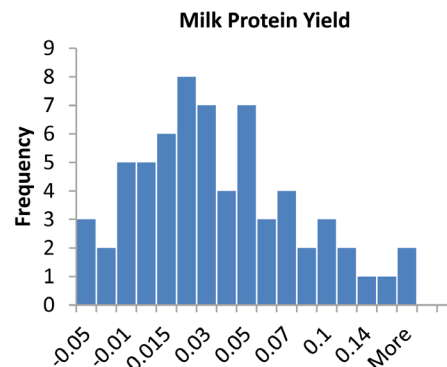


Figure 6. Histogram of change in milk yield with the inclusion of rumen protected B vitamins (RPBV) in diets for cows in mid-lactation.

4. Conclusion

This meta-analysis revealed that the inclusion of RPVB in diets for dairy cows maintained in many parts of the world resulted in increased milk yield, fat yield and protein yield, and that the expectation of using RPBV would be positive. Effective rumen protection of these vitamins offers significant advantages relative to intramuscular injections or feeding high doses of unprotected vitamins by being more cost-effective and labor-effective. The results of this meta-analysis do not adequately reveal the causes for the trial-to-trial variation, and further analyses are needed. Such results would permit more judicious use of such products.

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Conflicts of Interest

There are no conflicts of interest with respect to this study.

References

- [1] Girard, C.L. and Graulet B. (2021) Methods and Approaches to Estimate B Vitamin Status in Dairy Cow: Knowledge Gaps and Advances. *Methods*, **186**, 52-58. <https://doi.org/10.1016/j.ymeth.2020.05.021>
- [2] National Research Council (NRC) (2001) Nutrient Requirements of Dairy Cattle. Seventh Revised Edition, National Academy Press, Washington DC.
- [3] Santschi, D.E., Berthiaume, R., Matte, J.J., Mustafa, A.F. and Girard, C.L. (2005) Fate of Supplementary B-Vitamins in the Gastrointestinal Tract of Dairy Cows. *Journal of Dairy Science*, **88**, 2043-2054. [https://doi.org/10.3168/jds.S0022-0302\(05\)72881-2](https://doi.org/10.3168/jds.S0022-0302(05)72881-2)
- [4] Ragaller, V., Lebzien, P., Bigalke, W., Südekum, K.H., Hüther, L. and Flachowsky, G. (2011) Effects of a Pantothenic Acid Supplementation to Different Rations on Ruminal Fermentation, Nutrient Flow at the Duodenum, and on Blood and Milk Variables of Dairy Cows. *Journal of Animal Physiology and Animal Nutrition*, **95**, 730-743. <https://doi.org/10.1111/j.1439-0396.2010.01103.x>
- [5] McElroy, L.W. and Goss, H. (1940) A Quantitative Study of Vitamins in the Rumen Contents of Sheep and Cows Fed Vitamin Low Diets: I. Riboflavin and Vitamin K, Four Figures. *Journal of Nutrition*, **20**, 527-540.
- [6] Mitchell, H.H., Hamilton, T.S. and Hainess, W.T. (1941) The Utilization by Calves of the Energy Contained in Balanced Rations Composed of Combinations of Different Feeds. *Journal of Nutrition*, **22**, 541-552. <https://doi.org/10.1093/jn/22.6.541>
- [7] Lardinois, C.C., Mills, R.C., Elveijem C.A. and Hart E.B. (1944) Rumen Biosynthesis of Vitamin B Complex as Influenced by Ration Composition. *Journal of Dairy Science*, **27**, 579-588. [https://doi.org/10.3168/jds.S0022-0302\(44\)92635-4](https://doi.org/10.3168/jds.S0022-0302(44)92635-4)
- [8] Agrawala, L.P., Huffman, C.F., Luecke R.W. and Duncan C.W. (1953) A Quantitative Study of Rumen Synthesis in the Bovine on Natural and Purified Rations: 3. Riboflavin, Pantothenic Acid and Niacin. *Journal of Nutrition*, **49**, 631. <https://doi.org/10.1093/jn/49.4.631>
- [9] Girard, C.L., Matte, J.J. and Tremblay, G.F. (1995) Gestation and Lactation of Dairy Cows: A Role for Folic Acid? *Journal of Dairy Science*, **78**, 404-411. [https://doi.org/10.3168/jds.S0022-0302\(95\)76649-8](https://doi.org/10.3168/jds.S0022-0302(95)76649-8)
- [10] Bonomi, A., Bonomi, B.M., Quarantelli, A., Sabbioni, A. and Superchi, P. (1998) Dairy Cattle Ration Integration with Rumen-Protected Pyridoxine. Effects on Milk Production and Reproductive Efficiency. *Rivista di Scienza dell'Alimentazione*, **27**, 201-211.
- [11] Zimmerly, C.A. and Weiss, W.P. (2001) Effects of Supplemental Biotin on Performance of Holstein Cows during Early Lactation. *Journal of Dairy Science*, **84**, 498-506. [https://doi.org/10.3168/jds.S0022-0302\(01\)74500-6](https://doi.org/10.3168/jds.S0022-0302(01)74500-6)
- [12] Shaver, R.D. and Bal, M.A. (2000) Effect of Dietary Thiamin Supplementation on Milk Production by Dairy Cows. *Journal of Dairy Science*, **83**, 2335-2340.

- [https://doi.org/10.3168/jds.S0022-0302\(00\)75121-6](https://doi.org/10.3168/jds.S0022-0302(00)75121-6)
- [13] Schwab, E.C., Schwab, C.G., Shaver, R.D., Girard, C.L., Putnam, D.E. and Whitehouse, N.L. (2006) Dietary Forage and Nonfiber Carbohydrate Contents Influence B-Vitamin Intake, Duodenal Flow, and Apparent Ruminal Synthesis in Lactating Dairy Cows. *Journal of Dairy Science*, **89**, 174-187. [https://doi.org/10.3168/jds.S0022-0302\(06\)72082-3](https://doi.org/10.3168/jds.S0022-0302(06)72082-3)
- [14] Santschi, D.E., Chiquette, J., Berthiaume, R., Martineau, R., Matte, J.J., Mustafa, A.F. and Girard, C.L., (2005) Effects of the Forage to Concentrate Ratio on B-Vitamin Concentrations in Different Ruminal Fractions of Dairy COWS. *Canadian Journal of Animal Science*, **85**, 389-399. <https://doi.org/10.4141/A05-012>
- [15] Seck, M., Linton, J.V., Allen, M.S., Castagnino, D.S., Chouinard, P.Y. and Girard, C.L. (2017) Apparent Ruminal Synthesis of B Vitamins in Lactating Dairy Cows Fed Diets with Different Forage-To-Concentrate Ratios. *Journal of Dairy Science*, **100**, 1914-1922. <https://doi.org/10.3168/jds.2016-12111>
- [16] Beaudet, V., Gervais, R., Chouinard, P.Y., Graulet, B., Martin, C., Doreau, M. and Girard, C.L. (2020) Effects of Increasing Amounts of Extruded Linseed in the Diet on Apparent Ruminal Synthesis of Some B Vitamins in Dairy Cows. *Animal*, **14**, 1885-1891. <https://doi.org/10.1017/S1751731120000671>
- [17] Castagnino, D.S., Seck, M., Beaudet, V., Kammes, K.L., Linton, J.V., Allen, M.S., Gervais, R., Chouinard, P.Y. and Girard, C.L. (2016) Effects of Forage Family on Apparent Ruminal Synthesis of B Vitamins in Lactating Dairy Cows. *Journal of Dairy Science*, **99**, 1884-1894. <https://doi.org/10.3168/jds.2015-10319>
- [18] Sacadura, F.C., Robinson, P.H., Evans, E. and Lordelo, M., (2008) Effects of a Ruminally Protected B-Vitamin Supplement on Milk Yield and Composition of Lactating Dairy Cows. *Animal Feed Science and Technology*, **144**, 111-124. <https://doi.org/10.1016/j.anifeedsci.2007.10.005>
- [19] Evans, E. and Mair, D.T. (2013) Effects of a Rumen Protected B Vitamin Blend upon Milk Production and Component Yield in Lactating Dairy Cows. *Open Journal of Animal Sciences*, **3**, 76-82. <https://doi.org/10.4236/ojas.2013.31011>
- [20] Evans, E. and Mair, D.T. (2013) Effects of a Rumen Protected B Vitamin Blend Substituted for Biotin upon Milk Production and Component Yield in Lactating Dairy Cows. *OPEN Journal of Animal Sciences*, **3**, 93-98. <https://doi.org/10.4236/ojas.2013.32014>
- [21] Sanders, W.L. and Gaynor, P.J. (1987) Analysis of Switchback Data Using Statistical Analysis System, Inc.® Software. *Journal of Dairy Science*, **70**, 2186-2191. [https://doi.org/10.3168/jds.S0022-0302\(87\)80273-4](https://doi.org/10.3168/jds.S0022-0302(87)80273-4)
- [22] Suurmond, R., van Rhee, H. and Hak, T. (2017) Introduction, Comparison, and Validation of Mets-Essentials: A Free and Simple Tool for Meta-Analysis. *Research Synthesis Methods*, **8**, 537-553. <https://doi.org/10.1002/jrsm.1260>
- [23] Chen, B., Wang, C., Wang, Y.M. and Liu, J.X. (2011) Effect of Biotin on Milk Performance of Dairy Cattle: A Meta-Analysis. *Journal of Dairy Science*, **94**, 3537-3546. <https://doi.org/10.3168/jds.2010-3764>
- [24] Girard, C.L. and Matte, J.J. (1998) Dietary Supplements of Folic Acid during Lactation: Effects on the Performance of Dairy Cows. *Journal of Dairy Science*, **81**, 1412-1419. [https://doi.org/10.3168/jds.S0022-0302\(98\)75705-4](https://doi.org/10.3168/jds.S0022-0302(98)75705-4)
- [25] Morrison, E.I., Reinhardt, H., Leclerc, H., DeVries, T.J. and Le Blanc, S.J. (2018) Effect of Rumen-Protected B Vitamins and Choline Supplementation on Health, Production, and Reproduction in Transition Dairy Cows. *Journal of Dairy Science*, **101**, 9016-9027. <https://doi.org/10.3168/jds.2018-14663>

- [26] Duplessis, M., Girard, C.L., Santschi, D.E., Lefebvre, D.M. and Pellerin, D. (2014) Milk Production and Composition, and Body Measurements of Dairy Cows Receiving Intramuscular Injections of Folic Acid and Vitamin B-12 in Commercial Dairy Herds. *Livestock Science*, **167**, 186-194.
<https://doi.org/10.1016/j.livsci.2014.06.022>
- [27] Juchem, S.O., Robinson, P.H. and Evans, E. (2012) A Fat Based Rumen Protection Technology Post-Ruminally Delivers a B Vitamin Complex to Impact Performance of Multiparous Holstein Cows. *Animal Feed Science and Technology*, **174**, 68-78.
<https://doi.org/10.1016/j.anifeedsci.2012.03.004>