

Effects of a rumen protected B vitamin blend upon milk production and component yield in lactating dairy cows

Essi Evans^{1*}, David T. Mair²

¹Essi Evans Technical Advisory Services Inc., Bowmanville, ON, Canada;

*Corresponding Author: essievans@sympatico.ca

²Jefo Nutrition Inc., Saint-Hyacinthe, QC, Canada

Received 4 November 2012; revised 8 December 2012; accepted 18 December 2012

ABSTRACT

Results from 12 switchback field trials involving 1216 cows were combined to assess the effects of a protected B vitamin blend (BVB) upon milk yield (kg), fat percentage (%), protein %, fat yield (kg) and protein yield (kg) in primiparous and multiparous cows. Trials consisted of 3 test periods executed in the order control-test-control. No diet changes other than the inclusion of 3 grams/cow/day of the BVB during the test period occurred. Means from the two control periods were compared to results obtained during the test period using a paired T test. Cows include in the analysis were between 45 and 300 days in milk (DIM) at the start of the experiment and were continuously available for all periods. The provision of the BVB resulted in increased ($P < 0.05$) milk, fat %, protein %, fat yield and protein yield. Regression models showed that the amount of milk produced had no effect upon the magnitude of the increase in milk components. The increase in milk was greatest in early lactation and declined with DIM. Protein and fat % increased with DIM in mature cows, but not in first lactation cows. Differences in fat yields between test and control feeding periods did not change with DIM, but the improvement in protein yield in mature cows declined with DIM. These results indicate that the BVB provided economically important advantages throughout lactation, but expected results would vary with cow age and stage of lactation.

Keywords: B Vitamin Blend; Biotin; Folic Acid; Pyridoxine; Pantothenic Acid

1. INTRODUCTION

Older studies clearly showed that mature ruminant ani-

mals did not require B vitamin supplementation as these vitamins are synthesized in ample quantities by rumen microbes [1-6]. This research was so complete and so elegantly executed that B vitamin needs remained relatively unexplored until the last decade or so.

Research conducted in recent times suggests that levels of water-soluble B vitamins may be insufficient to meet the needs of dairy cows during lactation. Bonomi *et al.* [7] determined that protect pyridoxine at 40 mg or unprotected pyridoxine at 100 mg/day improved milk yield and milk component yield in cows during the first 6 months of lactation. Girard and Matte [8] noted that supplementation with folic acid had the ability to increase milk and milk protein yield, and that the response was tied to B12. Biotin, being investigated as a tool to improve hoof health, was shown to improve milk production as well [9].

Much of the B vitamin content of feed ingredients is degraded in the rumen, with duodenal appearance of the vitamins principally from rumen microbes [10,11]. Recent studies have therefore involved feeding overwhelmingly high levels of the vitamin or vitamins being tested [12], injections of B vitamins [13] or rumen-protected B vitamins [7]. Even still results have not been consistent.

Sacadura *et al.* [14] tested a B vitamin blend (BVB) consisting of biotin, folic acid, pyridoxine and pantothenic acid in two feeding trials. In the first trial involving mature cows in early lactation, milk and component yields increased. In the second study involving mid lactation primiparous and multiparous cows, only milk protein increased significantly although there was a tendency for milk yield to increase. The differences in response might have been associated with stage of lactation or age of the cows. Results from the individual feeding trials used in the current compilation revealed that responses to the same BVB varied and might depend upon differences due to production level, stage of lactation and cow ma-

turity. The purpose of this study is to evaluate the body of information available and to learn when responses are anticipated and potentially from there to evaluate mechanisms by which vitamins support lactation performance.

2. MATERIALS AND METHODS

Twenty-three feeding experiments were conducted in Canada and The United States of America. Trials were conducted at existing, operating, privately-owned dairy farms, and not research facilities. Consulting nutritionists and veterinarians monitoring the trials were instructed to verify that the herds were provided with a consistent (ingredient and nutrient) diet for 3 consecutive feeding periods. Feeding periods were defined as the length of time between Dairy Herd Improvement Association (DHIA) milk tests from herds on a monthly, twice-daily testing program. The only feed change that was permitted was the addition of 3 grams of a protected BVB (Vicomb P+[®], Jefo Nutrition Inc., St. Hyacinthe, QC) previously shown to escape rumen degradation and support milk production [14]. The BVB was mixed into the concentrate portion of the total mixed ration and the test article was added based upon the average daily dry matter intake of the cows on each individual farm. The BVB was added no later than first 3 days after the first period DHIA measurements were taken, and was removed within 3 days after the second DHIA test.

Information was recorded on milk yield (kg), milk fat percentage (%), milk protein %, milk fat yield (kg) and milk protein yield (kg) by cow. Data were obtained from DHIA as comma delineated ASCII files and were placed into a Microsoft Excel[®] spread sheet for sorting. Results from period 1 and period 3 were compared to period 2 for all variables using a paired, two sided T test.

Each farm study was analysed separately. Individual cow records were used in the analyses. All cows were assumed to be participants in the study if they had been on a consistent diet for at least 21 days before the first DHIA test, were at least 45 days and no more than 300 days in milk (DIM) at the first test date and were available for all three testing periods. Because the magnitude of response varied from trial to trial, data from valid studies were combined in order to produce regression models from the population of cows employed in valid experiments. Regression models were developed from the data set using Minitab 16 statistical software (Minitab Inc., State College, PA).

3. RESULTS AND DISCUSSION

The BVB evaluated in this experiment provided biotin, pantothenic acid, pyridoxine and folic acid, and was shown to provide over 90% escape after 24 hour incuba-

tion [14]. Sacadura *et al.* [14] calculated that the B vitamins likely to be deficient in lactating dairy cows would be pantothenic acid, pyridoxine, and folic acid. Biotin was not calculated to be limiting, but was included in the BVB based on recent research findings [15-17].

Of the 23 trials conducted, 12 were determined to be suitable for inclusion in the final analysis (Tables 1 and 2). Individual data were available for 1216 cows of which 467 were primiparous, and the remainders were from lactation 2 or greater.

Although results varied from the individual trials, the combined analysis showed that both primiparous and multiparous cows responded to the BVB (Table 3).

Table 1. Description of data used in the analyses.

Farm	Country	Cows Milking, N	Cows Used, N	Average Yield, kg
1	Canada	215	171	30.3
2	Canada	35	29	39.3
3	Canada	35	29	36.4
4	Canada	60	50	30.4
5	Canada	40	28	33.4
6	Canada	95	75	35.0
7	Canada	110	84	30.0
8	USA	425	317	37.0
9	USA	110	80	35.2
10	USA	200	145	38.5
11	USA	175	134	29.1
12	USA	95	74	35.8

Table 2. Reasons for excluding farms.

Farm	Reason for Exclusion
13	Vitamins added before milk pick up date
14	Diet was changed during the experiment
15	Diet was changed during the experiment
16	Milk was tested from one milking/day only
17	Farm remained on product after test period
18	Farm remained on product after test period
19	Unsure of start date
20	Diet was changed during the experiment
21	BST treatment imposed during test period
22	Diet contained biotin during the control period
23	Herd was tested every two months

Ferreira *et al.* [17] found that biotin improved milk production in high producing but not low producing cows. Similar results were expected with the BVB. The regression model predicting the effects of current milk yield on the change in milk yield obtained with the BVB (**Table 4**) revealed that cows in lactation 2 or higher responded regardless of production level, while first lactation cows were more likely to respond to the BVB if their level of production was low, quite the opposite of the findings of Ferreira *et al.* [17] with biotin alone.

The difference in milk yield response to the BVB and the control feeding programs was significantly reduced by DIM for mature cows (**Table 5**) indicating changes in milk yield are more likely to occur in early stages of

Table 3. Effects of B vitamin supplementation upon lactation performance.

Parameter	Test	Control	P Level
All cows (N = 1216)			
Milk, l	35.3	34.2	<0.05
Fat, %	3.65	3.56	<0.05
Protein, %	3.21	3.15	<0.05
Fat yield, kg	1.27	1.19	<0.05
Protein yield, kg	1.13	1.07	<0.05
First lactation cows (N = 467)			
Milk, l	32.4	31.6	<0.05
Fat, %	3.63	3.57	<0.05
Protein, %	3.21	3.14	<0.05
Fat yield, kg	1.14	1.09	<0.05
Protein yield, kg	1.02	0.97	<0.05
Cows lactation ≥ 2 (N = 749)			
Milk, l	37.1	35.9	<0.05
Fat, %	3.67	3.55	<0.05
Protein, %	3.21	3.16	<0.05
Fat yield, kg	1.33	1.24	<0.05
Protein yield, kg	1.17	1.11	<0.05

lactation. However, the model indicates that primiparous cows sustain a response to the BVB throughout their lactation.

Table 6 provides predictions of response when both yield and DIM are included in the regression model. When the effects of DIM were taken into account, the increase in yield with the BVB diminishes with higher levels of production all cows. It would appear that the natural reduction in yield with increased lactation is of greater importance in higher lactation cows than in first lactation cows.

Girard *et al.* [13] reported an increase in milk production in late lactation cows when folic acid was administered by weekly injection, and later Girard and Matte [8] confirmed this response in cows given very high daily doses of unprotected folic acid. This indicates that this vitamin is of greater importance in late lactation pregnant cows. In the latter study [8], milk from multiparous, but not primiparous cows increased with folic acid. This may partially explain the lack of change in milk yield with level of production in primiparous cows. Graulet *et al.* [18] found higher milk yield in early lactation with high levels of dietary folic acid, and speculated that the folic acid supplementation improved synthesis of purine and pyrimidine compounds in the mammary gland.

Table 4. Effects of current milk yield on change in milk yield when a B vitamin blend was provided to lactating cows.

Group	Intercept	Slope	R ²	P Level
All Cows	1.46	-0.0119	0.1	=0.40
First Lactation	2.79	-0.0626	3.4	<0.05
Lactation ≥ 2	1.39	-0.0051	0.0	=0.78

Table 5. Effect of days in milk on change in milk yield when a B vitamin blend was provided to lactating dairy cows.

Group	Intercept	Slope	R ²	P Level
All Cows	1.77	-0.00475	1.4	<0.05
First Lactation	1.11	-0.00192	0.2	=0.18
Lactation ≥ 2	2.24	-0.00689	2.2	<0.05

Table 6. Effects of current milk yield and days in milk on change in milk yield when a B vitamin blend was provided to dairy cows.

Group	Intercept	Slope Yield	P Level	Slope DIM	P Level	R ²
All Cows	3.50	-0.0441	<0.05	-0.00625	<0.05	2.1
First Lactation	3.75	-0.0777	<0.05	-0.00316	=0.09	0.2
Lactation ≥ 2	4.83	-0.0607	<0.05	-0.00963	<0.05	3.5

Milk fat % on average increased when cows were given the BVB (Table 3), but did not increase in all of the individual trials. Regression curves in Table 7 predicting the magnitude of the increase in fat % show that a greater response would be expected when current fat % is low. For example, the regression model would predict no response if current milk fat was 3.9%, but a response of 0.11% if current fat was 3.5%. These changes were not influenced by level of milk production (Table 8), but were impacted by DIM (Table 9) in multiparous cows. Therefore the increase in fat % would be expected in later lactation in these cows. The equations in Table 10 again support the fact that, the improvement in milk fat % when the BVB was used in the ration would be expected to be greater in cows with a low milk fat test, and would increase as lactation progressed. Improvements in milk fat have not been reported with folic acid [12,13, 18,19] or with biotin [15-17]. Bonomi *et al.* [7] found higher milk fat % when rumen protected pyridoxine was added to the ration, and it is suggested that that vitamin might be responsible for the improvement in fat percentage.

In similar fashion, the equations giving the change in milk protein % relative to current milk protein show that responses would be less likely to occur in cows giving milk that was currently high protein % (Table 11). The model revealed that when milk protein was less than 3.64% in first lactation cows, and less than 3.70% in later lactation cows, a response could be expected. This parameter appeared to be unrelated to milk yield (equations not shown, $P = 0.48$ for all cows). Considering the relationship between the alteration in protein % and DIM revealed that % was more likely to increase as lactation advanced in multiparous cows (Table 12). The change in

milk protein % with the BVB was confirmed to be greater in cows with lower protein %, and the responses were determined to be greater in latter stages of lactation (Table 13). Although milk protein declines with advancing lactation, the equations provided in Table 13 clearly show that they are independent responses, and are not confounded.

Table 7. Effects of current milk fat % on change in milk fat % when a B vitamin blend was provided to dairy cows.

Group	Intercept	Slope	R ²	P Level
All Cows	1.02	-0.260	8.3	<0.05
First Lactation	1.19	-0.318	13.5	<0.05
Lactation ≥ 2	0.935	-0.230	6.3	<0.05

Table 8. Effects of current milk yield on change in milk fat % when a B vitamin blend was provided to dairy cows.

Group	Intercept	Slope	R ²	P Level
All Cows	0.125	-0.00084	0.1	=0.66
First Lactation	-0.134	0.00609	0.6	=0.09
Lactation ≥ 2	0.279	-0.00447	0.5	=0.06

Table 9. Effects of days in milk on change in milk fat % when a B vitamin blend was provided to dairy cows.

Group	Intercept	Slope	R ²	P Level
All Cows	0.0461	0.000330	0.4	<0.05
First Lactation	0.0001	0.000207	0.1	0.498
Lactation ≥ 2	0.0471	0.000478	0.7	<0.05

Table 10. Effects of current fat % and days in milk on change in milk fat % when a B vitamin blend was provided to dairy cows.

Group	Intercept	Slope Fat %	P Level	Slope DIM	P Level	R ²
All Cows	0.986	-0.274	<0.001	0.000571	<0.05	9.5
First Lactation	1.170	-0.329	<0.001	0.000393	<0.05	14.2
Lactation ≥ 2	0.889	-0.247	<0.001	0.000208	<0.05	7.8

Table 11. Effects of current milk protein % on change in milk protein % when a B vitamin blend was provided to dairy cows.

Group	Intercept	Slope	R ²	P Level
All Cows	0.408	-0.111	2.9	<0.05
First Lactation	0.503	-0.138	3.2	<0.05
Lactation ≥ 2	0.349	-0.0943	2.8	<0.05

Table 12. Effects of days in milk on change in milk protein % when a B vitamin blend was provided to dairy cows.

Group	Intercept	Slope	R ²	P Level
All Cows	0.0270	0.000200	1.1	<0.05
First Lactation	0.0466	0.000138	0.4	0.17
Lactation ≥ 2	0.130	0.000248	2.8	<0.05

Changes in component yield can be more revealing of the influence of a nutritional modification than percentage, and are generally of greater economic importance. **Table 3** clearly shows that milk fat yield increased overall with the feeding of the BVB to dairy cows. However, based on additional analyses, the increase in yield was found to occur at a diminishing rate, with responses witnessed in both mature cows and first lactation cows producing levels of fat below 1.66 and 1.39 kg, respectively (**Table 14**). Alterations in milk fat yield were not related to milk yield (equations not shown, $P = 0.98$ for all cows in the study). Likewise, stage of lactation was found to not be an important modeling factor relating the alteration in fat with the inclusion of the BVB in the diet ($P = 0.42$ for all cows in the study, equations not shown). However, DIM could be used to predict response when considered in conjunction with milk fat yield (**Table 15**).

Existing milk protein yield did not influence the extent of change in milk protein in mature cows, but did in

cows in their first lactation (**Table 16**). This might be related to growth changes in the younger cows. Whether or not the BVB influenced the deposition of protein in first lactation cows could not be discerned from this study. Similar to the alterations in milk protein %, a relationship between milk yield and the alteration in yield that occurred when the BVB was supplied could not be established ($P = 0.79$ for all cows), suggesting that a response can be anticipated at all levels of milk production. As with milk protein %, the increase in milk protein yield was not influenced by milk yield. DIM was not a factor that could be related to the increase in milk protein yield obtained in this study with first lactation cows, but the difference between control and test declined as lactation progressed in mature cows either as a single independent variable (**Table 17**), or when included with protein yield (**Table 18**). Modeling protein yield response to the added BVB need to consider lactation number, based on these analyses.

Table 13. Effects of current protein % and days in milk on change in milk protein % when a B vitamin blend was provided to dairy cows.

Group	Intercept	Slope Protein %	P Level	Slope DIM	P Level	R ²
All Cows	0.529	-0.170	<0.05	0.000421	<0.05	6.5
First Lactation	0.624	-0.195	<0.05	0.000370	<0.05	5.5
Lactation ≥ 2	0.471	-0.155	<0.05	0.000463	<0.05	7.9

Table 14. Effects of current milk fat yield on change in milk fat yield when a B vitamin blend was provided to dairy cows.

Group	Intercept	Slope	R ²	P Level
All Cows	0.276	-0.168	4.0	<0.05
First Lactation	0.255	-0.184	5.2	<0.05
Lactation ≥ 2	0.289	-0.161	3.8	<0.05

Table 15. Effects of current milk fat yield and days in milk on change in milk fat yield when a B vitamin blend was provided to dairy cows.

Group	Intercept	Slope Fat Yield	P Level	Slope DIM	P Level	R ²
All Cows	0.343	-0.197	<0.05	-0.000224	<0.05	4.7
First Lactation	0.272	-0.190	<0.05	-0.000073	<0.35	5.0
Lactation ≥ 2	0.394	-0.206	<0.05	-0.000334	<0.05	5.3

Table 16. Effects of current milk protein yield on change in milk protein yield when a B vitamin blend was provided to dairy cows.

Group	Intercept	Slope	R ²	P Level
All Cows	0.115	-0.0524	0.6	<0.05
First Lactation	0.130	-0.0820	1.8	<0.05
Lactation ≥ 2	0.104	-0.0371	0.3	0.120

Table 17. Effects of days in milk on change in milk protein yield when a B vitamin blend was provided to dairy cows.

Group	Intercept	Slope	R ²	P Level
All Cows	0.0686	-0.0000663	0.2	0.09
First Lactation	0.0489	0.000012	0.0	0.81
Lactation ≥ 2	0.0814	-0.000123	0.5	<0.05

Table 18. Effects of current milk yield and days in milk on change in milk protein % when a B vitamin blend was provided to dairy cows.

Group	Intercept	Slope Protein Yield	P Level	Slope DIM	P Level	R ²
All Cows	0.128	-0.452	<0.05	-0.000101	<0.05	0.9
First Lactation	0.130	-0.0819	<0.05	0.000002	0.973	1.8
Lactation ≥ 2	0.166	-0.674	<0.05	-0.000186	<0.05	1.5

4. IMPLICATIONS

These results indicate that dairy cows may require supplemental B vitamins. Cows respond to a BVB with increased milk production and components. These changes seem to be independent of level of production. Increases in components can be expected when the components are low, and there appears to be less opportunity for improvement when fat or proteins are currently high. Fat and protein appears to be more responsive to change on a % basis as lactation progresses with multiparous cows, with less effect of DIM in primiparous cows. The equations generated can be used to predict the likely outcome from the use of the BVB and can be used in the development of improved nutritional requirement models for lactating dairy cows.

REFERENCES

- [1] 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. *Journal of Nutrition*, **20**, 527-540.
- [2] McElroy, L.W. and Goss, H. (1940) A quantitative study of vitamins in the rumen contents of sheep and cows fed vitamin low diets. II. Vitamin B6. *Journal of Nutrition*, **20**, 541-550.
- [3] Lardinois, C.C., Mils, R.C., Elveijem, C.A. and Hart, E.B. (1944) Rumen synthesis of vitamin B complex as influenced by ration composition. *Journal of Dairy Science*, **27**, 579-583. [doi:10.3168/jds.S0022-0302\(44\)92635-4](https://doi.org/10.3168/jds.S0022-0302(44)92635-4)
- [4] 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**, 631-638.
- [5] Pearson, P.B., Struglia, L. and Lindahl, I.L. (1953) The fecal and urinary excretion of certain B vitamins by sheep fed hay and semi-synthetic rations. *Journal of Animal Science*, **12**, 213-218.
- [6] Hunt, C.H., Bentley, O.G., Hershberger, T.V. and Cline, J.H. (1954) The effects of carbohydrate and sulfur on B-Vitamin synthesis, cellulose and urea utilization by rumen microorganisms *in vitro*. *Journal of Animal Science*, **13**, 570-580.
- [7] 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 (experimental report). L'integrazione delle razioni per le bovine da latte con piridossina in forma rumino-protetta. Effetti sull'efficienza produttiva e riproduttiva *La Rivista di scienza dell'alimentazione*, **27**, 201-211.
- [8] 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. [doi:10.3168/jds.S0022-0302\(98\)75705-4](https://doi.org/10.3168/jds.S0022-0302(98)75705-4)
- [9] Bergsten, C., Greenough, P.R., Gay, J.M., Seymour, W.H. and Gay, C.C. (2003) Effects of biotin supplementation on performance and claw lesions on a commercial dairy farm. *Journal of Dairy Science*, **86**, 3953-3962. [doi:10.3168/jds.S0022-0302\(03\)74005-3](https://doi.org/10.3168/jds.S0022-0302(03)74005-3)
- [10] 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 dairy cows. *Journal of Dairy Science*, **89**, 174-187. [doi:10.3168/jds.S0022-0302\(06\)72082-3](https://doi.org/10.3168/jds.S0022-0302(06)72082-3)
- [11] Santschi, D.E., Berthiaume, R., Matte, J.J., Mustafa, A.F. and Girard, C.L. (2005) Fate of supplemental B-Vitamins in the gastrointestinal tract of dairy cows. *Journal of Dairy Science*, **88**, 2043-2054. [doi:10.3168/jds.S0022-0302\(05\)72881-2](https://doi.org/10.3168/jds.S0022-0302(05)72881-2)
- [12] Girard, C.L., Lapierre, H., Matte, J.J. and Lobley, G.E. (2005) Effects of dietary supplements of folic acid and rumen protected methionine on lactational performance and folate metabolism of dairy cows. *Journal of Dairy*

- Science*, **88**, 660-670.
[doi:10.3168/jds.S0022-0302\(05\)72730-2](https://doi.org/10.3168/jds.S0022-0302(05)72730-2)
- [13] 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.
[doi:10.3168/jds.S0022-0302\(95\)76649-8](https://doi.org/10.3168/jds.S0022-0302(95)76649-8)
- [14] 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. [doi:10.1016/j.anifeedsci.2007.10.005](https://doi.org/10.1016/j.anifeedsci.2007.10.005)
- [15] Zimmerly, C.A. and Weiss, W.P. (2001) Effects of supplemental dietary biotin on performance of Holstein cows during early lactation. *Journal of Dairy Science*, **84**, 498-506. [doi:10.3168/jds.S0022-0302\(01\)74500-6](https://doi.org/10.3168/jds.S0022-0302(01)74500-6)
- [16] Majee, D.N., Schwab, E.C., Bertics, S.J., Seymour, W.M. and Shaver, R.D. (2003) Lactation performance by dairy cows fed supplemental biotin and a B-vitamin blend. *Journal of Dairy Science*, **86**, 2106-2112.
[doi:10.3168/jds.S0022-0302\(03\)73800-4](https://doi.org/10.3168/jds.S0022-0302(03)73800-4)
- [17] Ferreira, G., Weiss, W.P. and Willet, L.B. (2007) Changes in measures of biotin status do not reflect milk yield responses when dairy cows are fed supplemental biotin. *Journal of Dairy Science*, **90**, 1452-1459.
[doi:10.3168/jds.S0022-0302\(07\)71630-2](https://doi.org/10.3168/jds.S0022-0302(07)71630-2)
- [18] Graulet, B., Matte, J.J., Desrochers, A., Doepel, L., Palin, M. and Girard, C.L. (2007) Effects of dietary supplements of folic acid and vitamin B12 on metabolism of dairy cows in early lactation. *Journal of Dairy Science*, **90**, 3442-3455. [doi:10.3168/jds.2006-718](https://doi.org/10.3168/jds.2006-718)
- [19] Girard, C.L. and Matte, J.J. (2005) Folic acid and vitamin B12 requirements of dairy cows: A concept to be revised. *Livestock Production Science*, **98**, 123-133.
[doi:10.1016/j.livprodsci.2005.10.009](https://doi.org/10.1016/j.livprodsci.2005.10.009)