Effects of folic acid and vitamin B₁₂ supplementation on culling rate, diseases, and reproduction in commercial dairy herds

M. Duplessis,*† C. L. Girard,†¹ D. E. Santschi,‡ J.-P. Laforest,* J. Durocher,‡ and D. Pellerin*

*Département des Sciences Animales, Université Laval, Québec, G1V 0A6, Canada

†Agriculture et Agroalimentaire Canada, Centre de recherche et développement sur le bovin laitier et le porc, Sherbrooke, Québec, J1M 0C8, Canada

‡Valacta, Ste-Anne-de-Bellevue, Québec, H9X 3R4, Canada

ABSTRACT

This study was undertaken to determine the effect of a combined folic acid and vitamin B₁₂ supplement given in early lactation on culling rate, metabolic disorders and other diseases, and reproduction in commercial dairy herds. A total of 805 cows (271 primiparous and 534 multiparous cows) in 15 commercial dairy herds were involved. Every 2 mo from February to December 2010 and within each herd, cows were assigned according to parity, previous 305-d milk production, and calving interval to 5 mL of either (1) saline 0.9% NaCl (control group) or (2) 320 mg of folic acid + 10 mg of vitamin B₁₂ (vitamin group). Treatments were administered weekly by intramuscular injections starting 3 wk before the expected calving date until 8 wk after parturition. A total of 221 cows were culled before the next dry period. Culling rate was not affected by treatment and was 27.5%; culling rate was greater for multiparous (32.2%) than for primiparous cows (18.8%). Within the first 60 d in milk (DIM), 47 cows were culled, representing 21.3% of total culling, and no treatment effect was noted. Ketosis incidence based on a threshold ≥ 100 $\mu\text{mol/L}$ of β -hydroxybutyrate in milk was $38.3 \pm 2.9\%$ for the vitamin group and $41.8 \pm 3.0\%$ for the control group and was not affected by treatment. The combined supplement of folic acid and vitamin B₁₂ did not decrease incidence of retained placenta, displaced abomasum, milk fever, metritis, or mastitis. However, the incidence of dystocia decreased by 50% in multiparous cows receiving the vitamin supplement, although no effect was observed in primiparous cows. The first breeding postpartum for multiparous cows occurred 3.8 d earlier with the vitamin supplement compared with controls, whereas no treatment effect was seen for primiparous cows. Days open, first- and second-breeding conception rates, number of breedings per conception, and percentage of cows pregnant at 150 DIM were not

affected by treatment. The reduced percentage of dystocia combined with the earlier DIM at first breeding for multiparous cows receiving the combined supplementation in folic acid and vitamin B₁₂ indicates that the vitamin supplement had a positive effect in older cows.

Key words: dairy cow, folic acid, vitamin B₁₂, commercial herds

INTRODUCTION

It is well known that ruminal bacteria can synthesize B vitamins, including folic acid and vitamin B₁₂ (Bechdel et al., 1928; Lardinois et al., 1944; NRC, 2001). Santschi et al. (2005) and Schwab et al. (2006) estimated apparent ruminal synthesis of folic acid and vitamin B₁₂ to be between 16.5 and 21.0 mg/d, and 73.0 and 79.8 mg/d, respectively. According to NRC (2001), synthesis of B vitamins in the rumen is sufficient to meet requirements of dairy cows. Nevertheless, ruminal synthesis of folic acid and vitamin B₁₂ was not sufficient to avoid fluctuations of serum concentrations of these vitamins around parturition in dairy cows (Girard et al., 1989; Girard and Matte, 1999). Moreover, a supplement of folic acid and vitamin B₁₂ increased milk production by approximately 12% when administered to multiparous cows 3 wk before the expected calving date until 16 wk of lactation (Preynat et al., 2009a).

Methylmalonyl-CoA mutase is a vitamin B₁₂-dependent enzyme that transforms methylmalonyl-CoA into succinyl-CoA; this step is required for the entry of propionate into the Krebs cycle (Scott, 1999). In ruminants, propionate is the major substrate for gluconeogenesis (Danfær et al., 1995). A lack of vitamin B₁₂ impedes this reaction and methylmalonyl-CoA is instead transformed into methylmalonic acid (Scott, 1999). Girard and Matte (2005) reported a lower concentration of methylmalonic acid in serum of cows supplemented with folic acid and vitamin B₁₂ compared with folic acid alone. Furthermore, a combined supplement of folic acid and vitamin B₁₂ given to dairy cows during the transition period increased the mRNA abundance of methylmalonyl-CoA mutase in liver (Preynat et al.,

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¹Corresponding author: Christiane.Girard@agr.gc.ca

2010). These results indicate that a combined supplement of folic acid and vitamin B₁₂ improved the entry of propionate into the Krebs cycle to provide energy. This is supported by Graulet et al. (2007) and Preynat et al. (2009a), who observed an increase in plasma concentration of glucose and whole-body irreversible loss rate of glucose, respectively, in response to the supplement of folic acid and vitamin B₁₂ to dairy cows. An increased blood concentration of glucose led to a reduced blood BHBA concentration and reduced the risk of ketosis in dairy cows (Nielsen and Ingvarsen, 2004). Ketotic cows had lower blood glucose concentration than nonketotic cows (Tehrani-Sharif et al., 2012). Furthermore, cows with subclinical ketosis are subsequently more likely to develop metritis, clinical ketosis, and displaced abomasum (Suthar et al., 2013) and are at greater risk of being culled (Roberts et al., 2012). Previous findings have shown that, when given together, these vitamins improved energy balance in postpartum dairy cows (Girard and Matte, 2005; Graulet et al., 2007; Preynat et al., 2009a). Negative energy balance (NEB) in early lactation increases the risk of disorders such as ketosis, displaced abomasum, and retained placenta (LeBlanc, 2010b) and decreases ovarian activity in dairy cows (Butler, 2003; Walsh et al., 2007).

To our knowledge, no research has been done to measure the effect of supplementation of folic acid and vitamin B₁₂ on reproduction in commercial dairy herds. However, Juchem et al. (2012) reported a significant improvement of conception rate 42 d after first breeding with a dietary supplement of B vitamins protected from ruminal degradation in dairy cows. Furthermore, in litter-bearing species, it has been reported that folic acid supplements increase litter size by improving embryo survival (Potier de Courcy and Terroine, 1979; Habibzadeh et al., 1986; Tremblay et al., 1989).

Given the effects of folic acid and vitamin B₁₂ on energy balance and reproduction in the studies described above, we hypothesized that a combined folic acid and vitamin B₁₂ supplement would reduce culling rate and incidence of metabolic disorders and other diseases and, hence, result in better reproductive performance. The purpose of this study was therefore to determine the effects of a supplement of folic acid and vitamin B₁₂ given from 3 wk prepartum until 8 wk of lactation on culling rate, incidence of metabolic disorders and diseases, and reproduction performance of cows in 15 commercial dairy herds.

MATERIALS AND METHODS

All procedures of this experiment were approved by the Animal Care Committee from Université Laval, Quebec, Canada following the guidelines of the Canadian Council on Animal Care (2009).

Experimental Procedures

A total of 805 dairy cows (271 primiparous and 534 multiparous cows; 780 Holstein and 25 Jersey cows) located in 15 commercial dairy herds around Quebec City, Canada, were enrolled. Herd size ranged from 25 to 120 cows. All lactating cows were kept in tiestall barns and milked twice daily. Average 305-d milk yield for the lactation preceding the study for multiparous cows was $9,662 \pm 114$ kg, and average calving interval was 393 ± 3 d, with no difference between treatments ($P = 0.97$ and 0.40 , respectively). In 2010, on average, a dairy herd in Quebec had 57 cows producing 8,800 kg of milk during a 305-d lactation (Valacta, 2011). Therefore, dairy herds participating in this experiment corresponded to average dairy herds in Quebec, except that cows in the study produced slightly more milk. All cows from each herd were included in the project except those with an estimated calving interval greater than 500 d. To join the study, herds were required to be visited at least once per month by a local veterinarian to increase accuracy of animal health and reproduction records. Veterinarian visits consisted of pregnancy checks using transrectal palpation or ultrasonography and evaluation and treatment of any sick cows. Timed AI was not used routinely.

Herds were visited from February 2010 to April 2011 every other week on the same schedule. Every 2 mo and within each herd, cows were randomly assigned to treatments, based on parity (primiparous vs. multiparous), predicted 305-d milk yield, and calving interval. Treatments consisted of weekly intramuscular injections of 5 mL of either (1) saline 0.9% NaCl (control group) or (2) 320 mg of folic acid + 10 mg of vitamin B₁₂ (vitamin group; pteroylmonoglutamic acid, MP Biomedicals, Solon, OH; cyanocobalamin, 5,000 µg/mL, Vetoquinol, Lavaltrie, QC, Canada) administered from 3 wk before expected calving date until 8 wk after parturition. In the weeks between visits, dairy producers were asked to inject treatments. Disposable syringes containing the studied solutions were prepared every 2 wk and kept refrigerated in a box to protect them from light until use.

About 3 wk before calving, BW was 667.0 and 673.7 ± 4.3 kg, and BCS was 3.45 and 3.47 ± 0.04 for dairy cows receiving the vitamin supplement and the control cows, respectively ($P > 0.27$). During the first 60 DIM, milk yield averaged 35.0 ± 0.3 kg/d and no effect of treatment was observed ($P = 0.68$).

Data Collection

Reasons for culling and DIM when cows left the herd were obtained from producers and Valacta (Dairy

Production Center of Expertise, Quebec and Atlantic Provinces, Ste-Anne-de-Bellevue, Quebec, Canada). Only culling data from the studied lactation were kept for analysis; cows being culled after the studied lactation were not included. Culling reasons were considered as intentional or unintentional. Intentional culling reasons included cows being removed from the herd because they no longer met dairy producer standards, mainly for milk production and conformation, whereas unintentional culling reasons included cows leaving the herd due to illness, reproduction problems, or injury.

Within each herd, producers recorded calving difficulty (no assistance, light assistance, difficult calving, surgery, or nonfavorable calf presentation), number of calves born, and calf size (small, medium, or large) for each calving according to the classification defined by Jamrozik et al. (2005). Dystocia was defined as calving requiring human intervention and included difficult calving, surgery, and nonfavorable calf presentation (Mee, 2008). In addition, producers had to record any health problems, treatments, or abnormal events. Metabolic disorder and other disease data were obtained from producer and veterinary records.

Ketosis was assessed from milk using Keto-Test strips (Elanco Animal Health, Guelph, ON, Canada) for each cow between 3 and 21 DIM on the day of the visit. As described by the manufacturer, 1 drop of milk from 1 quarter was placed on the strip for 3 s and reading was done after a waiting period of 60 s. If BHBA concentration was $<100 \mu\text{mol/L}$, cows were declared nonketotic; if BHBA concentration was ≥ 100 and $<200 \mu\text{mol/L}$, cows were considered mildly ketotic; and BHBA concentration $\geq 200 \mu\text{mol/L}$ indicated severe ketosis. If a cow was tested on 2 consecutive visits, the highest result was kept for analysis.

Definitions of metabolic disorders and other diseases were standardized within herds as previously described by Santschi et al. (2011). Briefly, occurrences of retained placenta, metritis and endometritis, mastitis, milk fever, and displaced abomasum were recorded during the visit following each calving. A retained placenta was described as a failure to expel fetal membranes partly or completely within 24 h after calving. Metritis and endometritis were defined by abnormal and suppurating vaginal discharges within the first 25 DIM. Mastitis was determined by producers as cows giving abnormal milk and requiring treatment, and was noted until 60 DIM for the current study. Milk fever was classified as none (cow without milk fever symptoms), mild (feeble cow whose symptoms were improved after calcium or other mineral doses), or severe (cow cannot get up). In the current paper, milk fever refers to mild and severe milk fevers. Displaced abomasum corresponded to veterinary diagnosis and surgery.

Data for DIM at first and last breedings (the latter being the number of days open) and number of breedings per conception were obtained from Valacta (Ste-Anne-de-Bellevue, Québec, Canada). Days in milk at last breeding were used for computation of days open only for cows confirmed pregnant by the veterinarian before being culled and for nonculled cows. In some herds, to increase chance of pregnancy per estrus, cows were bred 2 times within 3 d. In these cases, the second insemination was not taken into account to standardize data among herds. These breedings were considered to be unrelated to treatments. First-breeding conception rate (**CR**) was defined as the percentage of cows confirmed pregnant after the first service, whereas second-breeding CR represented the percentage of cows confirmed pregnant after the second service, excluding cows confirmed pregnant after the first service.

Statistical Analysis

Culling rate was analyzed with the GLIMMIX procedure of SAS (version 9.2; SAS Institute, 2008) using treatment, parity, block, herd, and treatment $\times$ parity interaction as fixed effects. Parity refers to primiparous and multiparous cows after calving, and block refers to 2-mo assignments. Logit-transformation after conversion to binomial distribution was performed on these data. Proc FREQ (SAS Institute, 2008) was used to compute proportions of cows among culling reasons. A chi-squared test was performed for detecting any difference between treatments.

Incidence of metabolic disorders and other diseases, dystocia, and twin birth and calf size at birth were analyzed with the GLIMMIX procedure of SAS (SAS Institute, 2008) using the same model as described above. Incidence of ketosis was transformed into a binomial distribution to facilitate analysis. In a first analysis, cows were considered nonketotic when BHBA level was $<100 \mu\text{mol/L}$ and ketotic when BHBA concentration was $\geq 100 \mu\text{mol/L}$. In a second analysis, severely ketotic cows had a BHBA level $\geq 200 \mu\text{mol/L}$ compared with all others with a BHBA level $<200 \mu\text{mol/L}$. Proc FREQ was used to calculate proportions of calf size among cows suffering from dystocia and those that did not.

Days in milk at first breeding, days open, and breedings per conception were compared with the MIXED procedure of SAS (SAS Institute, 2008). Treatment, parity, block, herd, and the interaction treatment $\times$ parity were fixed effects of the model. Conception rates at first and second breedings and percentage of cows pregnant at 150 DIM were computed with the GLIMMIX procedure of SAS using the same fixed effects described above. Survival curves for days to pregnancy

Table 1. Proportion and number of culled cows during the entire studied lactation and within the first 60 d in milk

Item	Proportion, % (no.)	
	Entire lactation ¹	First 60 DIM ²
Intentional culling		
Poor milk production	7.7 (17)	12.8 (6)
Poor conformation	5.4 (12)	2.1 (1)
Sold to another producer	0.9 (2)	0.0 (0)
Unintentional culling		
Reproduction	17.2 (38)	0.0 (0)
Mastitis or high SCC	16.7 (37)	10.6 (5)
Feet and leg problems	14.0 (31)	12.8 (6)
Other diseases	10.4 (23)	27.7 (13)
Injury	9.0 (20)	17.0 (8)
Milking problems	2.7 (6)	0.0 (0)
Age	1.8 (4)	2.1 (1)
Dystocia	0.5 (1)	2.1 (1)
Other or unknown	13.6 (30)	12.8 (6)

¹Proportion based on 221 culled cows; culling data are from calving to the beginning of the next dry period.²Proportion based on 47 culled cows.

were computed using the LIFETEST procedure of SAS (SAS Institute, 2008).

When the interaction treatment $\times$ parity was significant or a tendency, the SLICE option in the LSMEANS statement of SAS (SAS Institute, 2008) was used to help interpretation. Results were considered significant when $P \leq 0.05$ and as a tendency at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Culling Rate

Culling rate averaged 27.5% and was not affected by treatment ($P = 0.58$). Among the 805 dairy cows involved in this study, 221 cows were culled or sold before the next dry period. Reasons for culling are presented in Table 1, and no treatment effect was observed for proportion of cows among culling reasons ($P = 0.48$). The primary culling reason was reproduction (17.0%), followed by mastitis or high SCC (16.6%). As expected, significantly more multiparous than primiparous cows were culled ($P < 0.0001$). Culling rates were 18.8 and 32.2% for primiparous and multiparous cows, respectively. During the first 60 DIM, 21.3% of total cullings occurred (47 culled cows) and was not affected by treatment ($P = 0.55$). Primary culling reasons during this period were diseases such as displaced abomasum (27.7%), injury (17.0%), feet and leg problems (12.8%), and poor milk production (12.8%; Table 1).

In 2010, the average culling rate from dairy herds subscribing to DHI in Quebec was 36.1% (10th percentile = 50.8% and 90th percentile = 22.5%) according to Valacta (2011). The lower culling rate obtained in this trial could be explained by cows with previous calving intervals >500 d not being enrolled. It could be hypoth-

esized that those cows had previous reproduction issues combined with extended days open and a longer dry period. Extended days open and a longer dry period in the previous lactation have been reported to increase death and culling risk in the next lactation (Pinedo and De Vries, 2010). Moreover, dairy herds involved in the present study had good management practices and were visited at least once a month by a veterinarian. In spite of the difference in culling rate, the pattern of culling reasons was similar between this study and Valacta data for 2010 (Valacta, 2011).

Metabolic Disorders and Other Diseases

Based on the threshold of ≥ 100 $\mu\text{mol/L}$ BHBA from Keto-Test results, ketosis incidence was $38.3 \pm 2.9\%$ and $41.8 \pm 3.0\%$ for vitamin and control cows, respectively ($P = 0.37$; Table 2). Incidence of severe ketosis defined by Keto-Test results ≥ 200 $\mu\text{mol/L}$ BHBA was about $12.8 \pm 1.9\%$ and also did not differ between treatments ($P = 0.91$). Ketosis incidences in the current study were slightly higher than previously reported in commercial dairy herds in Quebec using the same method and the same cut-off points (from 16.4 to 35.5% for the lowest and highest treatment means, respectively; Santschi et al., 2011). That could be explained by the poor quality forage harvested during summer 2009 because of inadequate weather conditions (Valacta, 2010). In previous studies, no treatment effect was found on plasma BHBA concentrations for dairy cows receiving weekly folic acid plus vitamin B₁₂ supplement around parturition compared with control cows (Preynat et al., 2009a,b). Furthermore, plasma BHBA concentrations of dairy cows receiving weekly injections of vitamin B₁₂ alone did not differ from those of control cows (Akins

et al., 2013). However, a vitamin B₁₂ supplement increased plasma glucose concentration of dairy cows fed folic acid during the transition period but had no effect in cows not fed a folic acid supplement (Graulet et al., 2007). Moreover, a combined supplement of folic acid and vitamin B₁₂ increased glucose irreversible loss rate by 160 g/d, suggesting enhancement of gluconeogenesis in dairy cows receiving the vitamin supplement (Preynat et al., 2009a). Based on these previous results, it could be expected that a combined supplement of folic acid and vitamin B₁₂ would reduce ketosis. However, results from the current study do not support this hypothesis.

Surprisingly, primiparous cows had a higher incidence of severe ketosis than multiparous cows: $17.4 \pm 3.0\%$ versus $10.3 \pm 1.8\%$, respectively ($P = 0.005$; Table 2). This finding is not in accordance with van der Drift et al. (2012) and McArt et al. (2013), who reported that cows in first and second lactations had a lower prevalence of hyperketonemia determined by a plasma BHBA concentration $\geq 1,200$ $\mu\text{mol/L}$.

We observed no effect of treatment ($P \geq 0.53$) or parity ($P \geq 0.16$) on retained placenta, displaced abomasum, milk fever, metritis, or mastitis during the first 60 DIM (Table 2). Experiments studying the effects of a supplement of folic acid and vitamin B₁₂ on metabolic and other diseases are lacking. However, a study in which vitamin B₁₂ injections were given weekly to dairy cows reported that metabolic and uterine disorders recorded were unlikely to be related to treatment (Akins et al., 2013). Previous studies concluded that a supplement of folic acid and vitamin B₁₂ given to dairy cows around parturition could enhance energy balance (Girard and

Matte, 2005; Graulet et al., 2007; Preynat et al., 2009a). Negative energy balance in the periparturient period increases the risk of developing metabolic disorders in early lactating cows (LeBlanc, 2010b; Ingvarsen and Moyes, 2013). Even though results previously reported (Girard and Matte, 2005; Graulet et al., 2007; Preynat et al., 2009a) suggested that the vitamin supplement enhanced energy metabolism efficiency in early lactation, no significant decreases of metabolic disorders and other diseases were observed in the current study.

Calf Size and Incidence of Dystocia

No treatment effect was found for calf sizes ($P \geq 0.18$). The proportion of small calves was greater ($P < 0.0001$) for primiparous than for multiparous cows, at 20.3 and 9.4%, respectively. The proportion of large calves followed the opposite trend: 23.6 and 32.4% for primiparous and multiparous cows, respectively ($P = 0.01$). The proportion of medium calves was 57.5% and no parity effect was observed ($P = 0.56$). These proportions are similar to those reported in the study of Santschi et al. (2011). The proportion of twins did not differ within treatments and parity ($P > 0.93$) and was 3.1%.

Incidence of dystocia was $10.3 \pm 1.8\%$ and $11.8 \pm 1.9\%$ for vitamin and control groups, respectively, and was not affected by treatment ($P = 0.53$). However, a significant treatment $\times$ parity interaction was observed ($P = 0.008$; Table 2). For multiparous cows, supplementation with folic acid and vitamin B₁₂ decreased the incidence of dystocia by 50% ($P = 0.02$), from $10.8 \pm 2.0\%$ to $5.3 \pm 1.4\%$, but it had no effect on primiparous

Table 2. Incidences of diseases and dystocia according to treatments and parity (adjusted means $\pm$ SE)

Item	Treatment ¹ (Trt)				<i>P</i> -value		
	Primiparous		Multiparous				
	Control	Vitamins	Control	Vitamins	Trt	Parity	Trt × parity
No. of cows	136	135	263	271			
Incidence of diseases (%)							
Ketosis ²	44.3 ± 4.8	41.8 ± 4.7	39.4 ± 3.4	35.0 ± 3.2	0.37	0.14	0.78
Ketosis, severe ³	17.3 ± 3.5	16.0 ± 3.4	9.5 ± 1.9	9.9 ± 1.9	0.91	0.005	0.77
Retained placenta	11.0 ± 2.7	10.3 ± 2.7	9.0 ± 1.8	10.5 ± 1.9	0.83	0.70	0.63
Displaced abomasum	7.7 ± 2.3	4.0 ± 1.7	3.6 ± 1.1	4.6 ± 1.3	0.56	0.32	0.17
Milk fever ⁴	—	—	3.5 ± 1.4	4.9 ± 1.9	1.00	0.92	1.00
Metritis	13.2 ± 2.9	11.6 ± 2.7	13.6 ± 2.1	19.1 ± 2.4	0.56	0.16	0.21
Mastitis	11.5 ± 2.8	12.3 ± 2.9	9.3 ± 1.8	10.6 ± 1.9	0.61	0.37	0.88
Incidence of dystocia ⁵ (%)	12.8 ± 3.0	19.1 ± 3.7	10.8 ± 2.0	5.3 ± 1.4	0.53	0.0007	0.008

¹Control = 5 mL of saline 0.9% NaCl; Vitamins = 3 mL of 320 mg of folic acid and 2 mL of 10 mg of vitamin B₁₂.

²Nonketotic cow if BHBA concentration <100 $\mu\text{mol/L}$ and mild or severe ketotic cow if BHBA >100 $\mu\text{mol/L}$.

³Severe ketosis if BHBA concentration >200 $\mu\text{mol/L}$.

⁴Incidence of milk fever for primiparous was $<0.01\%$ (data not shown) and was not affected by treatment ($P = 1.0$).

⁵No treatment effect for primiparous cows ($P = 0.16$) but the vitamin supplement reduced dystocia in multiparous cows compared with controls ($P = 0.02$).

cows ($P = 0.16$). The lower incidence of dystocia in multiparous cows in the vitamin group remains difficult to explain. It is unlikely to be related to genetic or non-genetic (such as nutrition or sex of calf) factors known to have an effect on dystocia (Meijering, 1984) because cows were equally distributed within treatments and herds. Moreover, no significant difference was observed for calf size according to calving difficulty ($P = 0.95$). Among multiparous cows, 31.9% of cows that suffered from dystocia had a large calf compared with 32.4% for cows that had an easy calving. The proportions of medium and small calves, respectively, were 57.5 and 10.6% for multiparous cows that suffered from dystocia and 58.2 and 9.2% for those that did not.

As previously observed by Fiedlerova et al. (2008), dystocia incidence in the current experiment was 2-fold higher in primiparous cows than in multiparous cows ($P = 0.0007$), averaging $15.7 \pm 2.5\%$ and $7.6 \pm 1.3\%$, respectively. Dystocia incidence for multiparous cows was higher than previously reported by Santschi et al. (2011) even though the same definition of dystocia was used.

Reproduction

Days in milk at first breeding in response to treatments tended to differ according to parity (interaction treatment $\times$ parity, $P = 0.07$; Table 3). No treatment effect was observed on DIM at first breeding for primiparous cows ($P = 0.44$). However, the first service occurred at an earlier time postpartum, by 3.8 d, for multiparous cows receiving the folic acid and vitamin B₁₂ supplement ($P = 0.05$). Two hypotheses could be proposed based on this observation. First, in a study by Eaglen et al. (2011), first breeding was significantly delayed in primiparous cows that had a difficult calving

compared with primiparous cows that had an easy calving. According to those authors, a difficult calving may exacerbate NEB in early lactation, which may delay the surge of LH required for ovulation (Eaglen et al., 2011). In the current study, a higher proportion of easy calving and an earlier first breeding date were observed in multiparous cows receiving the vitamin supplement. However, no significant difference on DIM at first breeding according to treatment and calving difficulty was observed in multiparous cows (interaction treatment $\times$ calving difficulty, $P = 0.77$). Second, vitamin B₁₂ is involved as a co-enzyme for methylmalonyl-CoA mutase in the metabolic pathway, allowing the entry of propionate into the Krebs cycle to provide energy (Scott, 1999). As mentioned above, vitamin supplement seems to improve energy balance in early lactation (Girard and Matte, 2005; Graulet et al., 2007; Preynat et al., 2009a). In those studies, cows receiving the vitamin supplement had better lactational performance without increasing DMI, NEFA, or BHBA. Furthermore, cows receiving a combined folic acid and vitamin B₁₂ supplement had reduced BW and BCS losses after calving combined with lower milk fat and higher milk protein contents, suggesting improved energy status for the vitamin group (Duplessis et al., 2012). Negative energy balance impairs reproductive performance (Butler, 2003; Remppis et al., 2011), and a strong relationship between NEB and delayed first ovulation postpartum is frequently reported (Butler et al., 1981; Canfield and Butler, 1990; Staples et al., 1990). In the current study, the earlier first breeding date in multiparous cows receiving the vitamin supplement could be explained by the supplement lessening NEB in early lactation.

Despite the effects of a combined supplement of folic acid and vitamin B₁₂ on energy metabolism previously reported (Girard and Matte, 2005; Graulet et al., 2007;

Table 3. Reproductive parameters according to treatments and parity (adjusted means $\pm$ SE)

Item	Treatment ¹ (Trt)				<i>P</i> -value		
	Primiparous		Multiparous				
	Control	Vitamins	Control	Vitamins	Trt	Parity	Trt × parity
DIM at first breeding ²	79.9 ± 1.8	81.8 ± 1.8	80.4 ± 1.4	76.6 ± 1.3	0.54	0.13	0.07
Days open	127.9 ± 6.5	133.5 ± 6.7	136.9 ± 5.2	134.1 ± 5.0	0.80	0.41	0.46
First-breeding CR ³ (%)	46.1 ± 5.2	36.1 ± 5.0	39.2 ± 4.1	36.7 ± 3.8	0.15	0.49	0.40
Second-breeding CR ⁴ (%)	46.7 ± 7.2	58.5 ± 6.9	43.3 ± 5.7	43.8 ± 5.3	0.31	0.14	0.35
First + second breeding CR (%)	72.0 ± 4.7	74.5 ± 4.6	68.3 ± 4.0	66.9 ± 3.8	0.88	0.17	0.62
Breedings per conception	2.2 ± 0.2	2.4 ± 0.2	2.4 ± 0.1	2.4 ± 0.1	0.67	0.45	0.47
Pregnant at 150 DIM (%)	78.0 ± 4.3	75.8 ± 4.5	66.0 ± 4.0	69.3 ± 3.7	0.95	0.03	0.51

¹Control = 5 mL of saline 0.9% NaCl; Vitamins = 3 mL of 320 mg of folic acid and 2 mL of 10 mg of vitamin B₁₂.

²No treatment effect for primiparous cows ($P = 0.44$) but the vitamin supplement decreased DIM at first breeding for multiparous cows compared with controls ($P = 0.05$).

³CR = conception rate.

⁴Percentage of cows confirmed pregnant after the second service excluding cows confirmed pregnant after the first service.

Figure 1. Survival curves for days to pregnancy for (a) primiparous cows ($P = 0.35$) and (b) multiparous cows ($P = 0.61$) according to treatments. Control = 5 mL of saline 0.9% NaCl; Vitamins = 3 mL of 320 mg of folic acid and 2 mL of 10 mg of vitamin B₁₂.

Preynat et al., 2009a), no treatment effect ($P \geq 0.15$) was observed on days open, first-breeding CR, second-breeding CR, first + second breeding CR, breedings per conception, or percentage of cows pregnant at 150 DIM (Table 3). A higher percentage of primiparous cows were pregnant at 150 DIM compared with multiparous cows ($P = 0.03$): $76.9 \pm 3.2\%$ of primiparous cows were pregnant at 150 DIM compared with $67.7 \pm 2.8\%$ for multiparous cows. Reproductive data for multiparous cows obtained in this study were similar to those reported by Santschi et al. (2011).

Effects of folic acid supplement on reproduction have been reported in other species. Folic acid deficiency in female mammals during the preconception and gestational periods can impair fertility, folliculogenesis, and early embryogenesis (Laanpere et al., 2010). In a study

in which dietary folic acid was given to ewes around mating, the ovulation rate increased during the estrous season for the prolific breed receiving the supplement compared with prolific control ewes and ewes of a non-prolific breed (Méthot et al., 2008). Supplementary folic acid administered to sows before and during gestation significantly increased the number of piglets born and the number of piglets born alive (Matte et al., 1984) by decreasing the number of dead fetuses at 30 d of gestation (Tremblay et al., 1989). Research pertaining to the effects of a folic acid and vitamin B₁₂ supplement on reproduction in dairy cows is scarce. Nevertheless, in a study in which multiparous dairy cows were fed a B-vitamin supplement including folic acid and vitamin B₁₂ and protected from ruminal degradation in early lactation, first-service CR at 42 d after AI and at 150 DIM were significantly higher for the vitamin group compared with the control group (Juchem et al., 2012). Results from a study in which a supplement of folic acid and vitamin B₁₂ was administered 3 wk before the expected calving date until 9 wk after parturition suggests that this combined supplement increases the expression of genes related to ovulation, allowing dairy cows to have faster follicular growth, which can lead to earlier ovulation (Gagnon, 2012). Combined with the earlier first breeding date in multiparous cows receiving the vitamin supplement in the current study, the results described above imply that a supplement of folic acid and vitamin B₁₂ could improve reproductive performance of dairy cows.

The percentage of pregnant cows according to parity on a given DIM was represented by survival curves (Figure 1). According to LeBlanc (2010a), this is the most accurate method to analyze pregnancy data because culled cows are also included. In the current study, we observed no treatment effect on the percentage of pregnant cows with regard to parity ($P > 0.35$).

CONCLUSIONS

A combined supplement of folic acid and vitamin B₁₂ did not decrease culling rate or incidence of disorders such as ketosis, displaced abomasum, and retained placenta in early lactation. However, multiparous cows receiving the vitamin supplement experienced less difficulty at calving than did multiparous control cows. Although no treatment effect was observed for the majority of reproductive parameters, such as first-breeding CR and breedings per conception, the first breeding postpartum for multiparous cows occurred 3.8 d earlier in cows of the vitamin group and was not related to difficulty at calving.

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