Livestock Science 167 (2014) 186–194

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Milk production and composition, and body measurements of dairy cows receiving intramuscular injections of folic acid and vitamin B-12 in commercial dairy herds

M. Duplessis [a,b], C.L. Girard [b,*], D.E. Santschi [c], D.M. Lefebvre [c], D. Pellerin [a]

[a] Département des Sciences Animales, Université Laval, QC, Canada G1V 0A6
[b] Agriculture et Agroalimentaire Canada, Centre de Recherche et Développement sur le Bovin Laitier et le Porc, Sherbrooke, QC, Canada J1M 0C8
[c] Valacta, Ste-Anne-de-Bellevue, QC, Canada H9X3R4

ARTICLE INFO

Article history:
Received 3 March 2014
Received in revised form
20 May 2014
Accepted 27 June 2014

Keywords:
Dairy cow
Commercial herd
Folic acid
Vitamin B-12
Energy partitioning

ABSTRACT

The purpose of this study was to measure the effects of a supplementation in folic acid and vitamin B-12 given before calving and in early lactation on milk production and components within the first 60 days in milk (DIM) as well as the 305-d yield, and on indicators of energy balance for dairy cows in commercial dairy herds. A total of 805 dairy cows (271 primiparous and 534 multiparous) in 15 commercial dairy herds were involved. From February to December 2010, every 2 mo and within each herd, cows were assigned, according to parity, predicted 305-d milk production, and calving interval to receive weekly intramuscular injections (5 mL) of either (1) saline 0.9% NaCl (Control) or (2) 320 mg of folic acid + 10 mg of vitamin B-12 (Vitamins). Treatments began 21 d (SD 8) before the expected calving date and lasted until 60 (SD 4) DIM. For the first 60 DIM, average milk yield was 35.0 kg/d and was not affected by treatment. On average, milk fat concentration was decreased in early lactation for cows in the vitamin group as compared with control, from 42.1 to 40.3 g/kg whereas milk protein concentration was increased by the supplement, from 30.9 to 31.5 g/kg. Milk lactose and milk urea nitrogen concentrations were unaffected by treatment. No treatment effect was found on 305-d milk and protein yields. The vitamin supplement reduced 305-d milk fat yield in primiparous cows as compared with controls whereas no treatment effect was observed for multiparous cows. As indicators of energy balance, the fat:protein ratio was decreased by 0.06 and body condition score losses after calving tended to be smaller for cows in the vitamin group as compared with control. The decrease of the fat:protein ratio by the vitamin supplement was greater in primiparous cows than in multiparous cows. Cows receiving the vitamin supplement lost less body weight (estimated by heart girth circumference) during the first 60 DIM than control cows. Estimated body weight losses of 22.8 and 30.3 kg were recorded for vitamin and control cows, respectively. The observed reduction in estimated body weight loss coupled with a reduction of the fat:protein ratio without effect on milk yield suggest that supplementary folic acid and vitamin B-12 could have an effect on energy partitioning in early lactation.

* Correspondence to: Dairy and Swine Research and Development Centre, Agriculture and Agri-Food Canada, 2000 College Street, Sherbrooke, QC, Canada J1M 0C8. Tel.: +1 819 780 7233; fax: +1 819 564 5507.
E-mail address: Christiane.Girard@agr.gc.ca (C.L. Girard).

http://dx.doi.org/10.1016/j.livsci.2014.06.022
1871-1413/

1. Introduction

It is well known that rumen microorganisms can synthetize B vitamins including folic acid and vitamin B-12 (Bechdel et al., 1928; National Research Council, 2001). Previous studies showed that ruminal bacteria from healthy ruminants produce B vitamins in sufficient amounts to avoid the displaying signs of clinical deficiency and concluded that those animals do not need an exogenous supply of these vitamins (Eckles and Williams, 1925; Kon and Porter, 1954; National Research Council, 2001). Parturition and the onset of lactation are considered as the most demanding time for dairy cows. This period is characterized by a depression of the immune system combined with endocrine and physiological changes and a reduction of dry matter (DM) intake (Goff and Horst, 1997). Nadir of serum folate concentration in dairy cows is observed at calving (Girard et al., 1989) and at 8 wk after parturition regarding serum vitamin B-12 concentration (Girard and Matte, 1999). Those results demonstrated that supply is not sufficient to overcome fluctuations of serum folates and vitamin B-12 concentrations in early lactation. Moreover, milk and milk component yields per cow increased by 33% whereas DM consumption increased by only 15% (Weiss and Ferreira, 2006). Under these conditions, it is possible that synthesis of folates and vitamin B-12 by ruminal bacteria might not be sufficient to meet the cow needs and to optimize milk yield and components (Girard and Matte, 2005b).

In a previous study, a dietary supplement of folic acid, given alone or combined with a vitamin B-12 supplement, increased milk yield by 3.4 kg/d in multiparous dairy cows during the first 8 wk of lactation (Graulet et al., 2007). However, these authors concluded that metabolic efficiency of dairy cows was improved when they received the combined supplement of folic acid and vitamin B-12 as plasma glucose increased and hepatic concentrations of lipids decreased even if lactational performance and DM intake were similar to cows fed supplementary folic acid alone. Furthermore, milk production was increased by 12% in early lactation for multiparous cows receiving a parenteral supplement of both folic acid and vitamin B-12 as compared with control (Preynat et al., 2009a). Milk fat and protein yields were increased by the combined supplement of folic acid and vitamin B-12 without effect on DM intake (Preynat et al., 2009a). Previous studies concluded that a combined supplement of folic acid and vitamin B-12 improved efficiency of energy metabolism in early lactating dairy cows (Girard and Matte, 2005a; Graulet et al., 2007; Preynat et al., 2009a, 2010). However, these studies did not look at the effects of the combined vitamin supplement given during the periparturient period and early lactation further in the lactation, even after the end of the supplementation. An excessive negative energy balance (EB) in early lactation had negative effect on mature-equivalent 305-d milk yield at the herd level (Ospina et al., 2010). Therefore, it was hypothesized that a folic acid and vitamin B-12 supplement would enhance production performance during the first 60 days in milk (DIM) and on 305-d yield and would have a positive impact on indicators of EB of primiparous and multiparous cows in commercial dairy herds.

The objective of this study was to evaluate, in commercial dairy herds, the effect of a folic acid and vitamin B-12 supplementation given from 21 d before the expected calving date until 60 DIM to primiparous and multiparous cows on milk and component yields within the first 60 DIM and during the 305-d lactation, and on indicators of EB (such as body condition score (BCS) and the fat: protein ratio). The purpose to collect data even if the supplementation was finished was to study possible effects further in the lactation. Moreover, the large number of dairy herds involved in this study allowed comparison of treatment responses among herds.

2. Materials and methods

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

2.1. Herds and cows

Herds and cows were previously described by Duplessis et al. (2014). Briefly, 15 commercial dairy herds located around Québec City, Canada were involved in this study. A total of 805 dairy cows were involved in this experiment; 271 primiparous and 534 multiparous cows; 780 Holstein and 25 Jersey cows. The number of cows per herd ranged between 25 and 120. All cows were housed in tie-stall barns and milked twice daily. Diet management differed among herds; 7 herds used a total mixed ration and 8 herds had an individual concentrate feeding system. Rations among herds were mainly based on legume-grass silage, corn silage, and concentrate as ground corn. Culling rate, disease, and reproduction data were presented elsewhere (Duplessis et al., 2014).

To join the study, herds were required to record milk production on a monthly basis through Valacta (Dairy Production Center of Expertise, Québec and Atlantic Provinces, Ste-Anne-de-Bellevue, QC, Canada). Moreover, herds had to be visited at least once a month by a local veterinarian and timed artificial insemination must not be used routinely. Veterinarians working close to Québec city, Canada were asked to contact dairy producers that met the above conditions and participation was on a voluntary basis.

In 2010, average dairy herds in Québec, Canada had 57 cows which produced in average 8800 kg of milk for a 305-d lactation (Valacta, 2011). Size of herds involved in this study corresponded to average dairy herds in Québec, but milk production was higher. For the lactation preceding the study, the average 305-d milk, fat, and protein yields were 9662 ± 114 kg, 423 ± 6 kg, and 345 ± 5 kg, respectively, and did not differ between treatment groups ($P > 0.64$). The calving interval preceding the experiment was 393 ± 3 d for both treatment groups ($P = 0.40$).

2.2. Treatments

The study lasted 14 mo and herds were visited from February 2010 to April 2011. During this period, each herd was visited by the same individual every other week on

the same schedule. Every 2 mo from February to December 2010 and within each herd, cows were assigned, based on parity (primiparous vs. multiparous), predicted 305-d milk yield, and calving interval to weekly intramuscular injections of either 5 mL of (1) saline 0.9% NaCl (Control group) or (2) 320 mg of folic acid + 10 mg of vitamin B-12 (Vitamin group; pteroylmonoglutamic acid, MP Biomedicals, Solon, OH, USA and cyanocobalamin, 5000 μg/mL, Vetoquinol, Lavaltrie, QC, Canada). The injected amount of vitamins was chosen according to the experiment of Girard et al. (1989) and Preynat et al. (2009a). The treatments began 21 ± 8 d before the expected calving date and lasted until 60 ± 4 DIM. Producers were asked to inject the cows during the weeks between visits. Disposable syringes containing the studied solutions were prepared every 2 wk and were kept refrigerated in a box to protect them from light.

2.3. Data collection

Individual milk yield and composition determined by mid infrared reflectance spectrometry (fat, protein, lactose, and urea) were recorded monthly in each herd, and monthly test-day, lactation length, and 305-d milk, fat, and protein yield data were provided by Valacta, the Dairy Herd Improvement agency. Even if the supplement was given until the first 60 DIM, monthly test-day data were collected for the entire lactation.

Body condition score was evaluated every other week by the same individual within herds and throughout the study according to a 1 (very thin) to 5 (very fat) scale with quarter points (Ferguson et al., 1994; Wildman et al., 1982) starting 21 ± 8 d before the expected calving date and lasted until 93 ± 6 DIM. Body condition score was measured even after the end of the supplementation period to evaluate possible long-term effect of the supplement. Body weight (BW) of cows was estimated by the same individual using calibrated weight tapes measuring heart girth circumference at 21 ± 8 d before calving, at the first visit after calving (7 ± 4 DIM) and at 55 ± 13 DIM. Because weigh scales were not available on farms, heart girth circumference was chosen to estimate BW as this body size measurement is highly correlated with BW of dairy cows (Yan et al., 2009). Even though BW measurements using a calibrated tape undoubtedly introduced some imprecision into the weight recording, it was assumed that the loss of precision was similar between treatments, especially as this measurement was done by the same individual.

For each herd, feed samples were taken when forages fed to dairy cows changed (for example switched over first to second cut), immediately put on ice for transportation, and sent to Valacta for analysis. Ration and nutrient compositions based on these feed sample analyses were obtained from herd nutritionists throughout the study. Average nutrient composition of diets fed before calving (from 21 d before parturition to calving) and during early lactation (from calving to 60–100 DIM) are described in Table 1. All dairy herds used a precalving and an early-lactation diets. However, data regarding precalving diet from one herd were lacking and were not included (Table 1). Among herds, rations were computed to meet or exceed Co requirements (0.11 mg/kg of DM) according to the National Research Council (2001).

Table 1
Average composition (range) of pre-calving and early lactation diets in the studied herds.[a]

Composition	Precalving diet	Early-lactation diet
Herds, *n*	14	15
CP[b], % DM	14.5 (12.8–16.2)	17.2 (16.3–18.5)
RDP[b,c], % DM	9.1 (7.5–11.1)	10.7 (9.5–11.4)
RUP[b,c], % CP	30.1 (26.3–34.0)	37.5 (32.0–42.6)
NE_L[b,c], Mcal/kg	1.49 (1.37–1.61)	1.63 (1.58–1.69)
Fat, % DM	2.8 (2.0–3.6)	4.0 (3.3–4.5)
ADF[b], % DM	26.9 (21.3–32.1)	20.4 (17.1–23.3)
NDF[b], % DM	44.9 (35.8–53.8)	33.7 (25.6–37.6)
Roughage NDF, % DM	40.9 (31.5–51.9)	27.3 (23.6–30.4)
NFC[b,c], % DM	32.5 (27.4–39.1)	38.6 (32.1–42.4)
Ca, % DM	0.86 (0.52–1.34)	0.91 (0.80–1.00)
P, % DM	0.39 (0.27–0.73)	0.43 (0.39–0.51)
Mg, % DM	0.39 (0.22–0.55)	0.32 (0.27–0.35)
K, % DM	1.53 (1.24–1.75)	1.50 (1.24–1.70)
Co, mg/kg DM	0.55 (0.11–1.21)	0.56 (0.26–0.94)
DCAD[d], mEq/kg	53.0 (−56.3–264.2)	253.0 (160.5–372.0)
Concentrate, % DM	28.8 (19.7–41.5)	41.8 (36.1–50.7)

[a] Diet compositions were provided by each herd nutritionist, based on individual forage analyses done in the Valacta laboratory. Data from one herd regarding precalving diet were lacking.

[b] CP = crude protein; DM = dry matter; RDP = rumen-degradable protein; RUP = rumen-undegradable protein; NE_L = net energy for lactation; ADF = acid detergent fiber; NDF = neutral detergent fiber; NFC = nonfiber carbohydrates.

[c] Calculated according to National Research Council (2001).

[d] Based on the equation: dietary cation-anion difference (DCAD; mEq/kg) = [(%Na × 435) + (%K × 256)] − [(% Cl × 282) + (%S × 624)] (Ender et al., 1971).

2.4. Calculations

Calculation of energy-corrected milk (ECM) was made as follows: ECM (kg/d) = 12.55 × fat (kg/d) + 7.39 × protein (kg/d) + 5.34 × lactose yield (kg/d), based on National Research Council (2001) and energy value of milk of 0.74 Mcal/kg (Tyrrell and Reid, 1965). For a given monthly test day, the percentages of milk fat and milk protein of each cow were used for the calculation of the fat:protein ratio (Buttchereit et al., 2010; Duffield et al., 1997).

2.5. Energy balance and fat mobilization indicators

Energy balance was not computed in the present experiment because it was not possible to measure accurate DM intake as it is not a common practice in commercial dairy herds. Nevertheless, some indicators correlated with fat mobilization as BCS and the fat:protein ratio could provide a good estimation of EB of dairy cows in early lactation (Buttchereit et al., 2010; de Vries and Veerkamp, 2000; Grieve et al., 1986) and they were considered as indicators of EB in the present experiment. Body weight changes after parturition could be considered as an indicator of fat mobilization (Tamminga et al., 1997; Weber et al., 2013).

Table 2
Milk production and components according to parity and treatments for the first 60 DIM (adjusted mean ± SE).

Item	Treatment[a] (Trt)				*P*-value		
	Primiparous		Multiparous				
	Control	Vitamins	Control	Vitamins	Trt	Parity	Trt × Parity
Number of cows	136	135	263	271			
Milk yield, kg/d	29.9 ± 0.5	29.6 ± 0.5	40.3 ± 0.3	40.3 ± 0.3	0.68	< 0.0001	0.68
ECM[b], kg/d	30.0 ± 0.5	28.7 ± 0.5	39.7 ± 0.4	39.3 ± 0.4	0.06	< 0.0001	0.21
Fat							
Concentration, g/kg	42.5 ± 0.6	40.1 ± 0.7	41.6 ± 0.5	40.4 ± 0.5	0.004	0.59	0.12
Yield[c], kg/d	1.26 ± 0.02	1.18 ± 0.03	1.66 ± 0.02	1.63 ± 0.02	0.42	< 0.0001	0.08
Protein							
Concentration, g/kg	30.6 ± 0.3	31.1 ± 0.3	31.1 ± 0.3	31.7 ± 0.2	0.04	0.05	0.83
Yield, kg/d	0.91 ± 0.02	0.90 ± 0.02	1.24 ± 0.01	1.25 ± 0.01	0.88	< 0.0001	0.43
Lactose							
Concentration, g/kg	46.6 ± 0.3	46.4 ± 0.3	45.6 ± 0.2	45.3 ± 0.2	0.22	< 0.0001	0.62
Yield, kg/d	1.40 ± 0.02	1.38 ± 0.03	1.84 ± 0.02	1.84 ± 0.02	0.77	< 0.0001	0.72
Fat:protein ratio[d]	1.41 ± 0.02	1.34 ± 0.02	1.36 ± 0.01	1.32 ± 0.01	0.001	0.18	0.07
MUN[e], mg/dL	8.49 ± 0.25	8.63 ± 0.26	8.39 ± 0.19	8.11 ± 0.19	0.79	0.20	0.15

[a] 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 B-12.
[b] Energy-corrected milk (ECM; kg/d) = 12.55 × fat (kg/d) + 7.39 × protein (kg/d) + 5.34 × lactose yield (kg/d). Adapted from National Research Council (2001) and Tyrrell and Reid (1965).
[c] No treatment effect for multiparous cows ($P = 0.23$) but the vitamin supplement reduced milk fat yield in primiparous cows as compared with control ($P = 0.007$).
[d] Vitamin supplement decreased the fat:protein ratio at a higher extent in primiparous cows ($P = 0.0005$) than in multiparous cows ($P = 0.02$).
[e] MUN = milk urea nitrogen.

2.6. Statistical analysis

Lactation curves for the whole lactation (0–400 DIM) were estimated from individual test-day records using a model as per Wilmink (1987) and Santschi et al. (2011), i.e. regression on DIM, and a regression on the exponential coefficient expDIM (calculated as $e^{-0.05\mathrm{DIM}}$) and the MIXED procedure of SAS (version 9.2, SAS Institute, 2008). Fixed effects of the model were treatment, parity, DIM, expDIM, block, and herd as well as the following interactions: treatment × parity, treatment × DIM, treatment × expDIM, parity × DIM, and parity × expDIM. Random regression effects per cow were intercept, DIM, and expDIM. Blocks meant 2-mo assignation intervals and parity referred to primiparous and multiparous cows after calving. ESTIMATE statements were created for each DIM for prediction of lactation curves. Cows with two or less individual test-day records were excluded for calculation of whole lactation curves. This random regression analysis allowed estimating lactation curves according to each DIM although individual test-day records were not taken at the same DIM within animals and herds.

Average milk production, and milk components i.e. fat, protein, and lactose yields and concentrations as well as the fat:protein ratio and milk nitrogen urea for the first 60 DIM were evaluated with the MIXED procedure of SAS using same fixed and random regression effects as for lactation curves. Appropriate ESTIMATE statements (summed and averaged over the first 60 DIM) were used in order to calculate effects of treatment for the first 60 DIM using the whole random regression lactation curve approach of Wilmink (1987) as described above. This analysis allows estimating production within the first 60 DIM using whole lactation individual test-day records.

Statistical analysis for the current 305-d milk, fat, and protein yields and lactation length were performed with the MIXED procedure of SAS including treatment, parity, block, herd, and treatment × parity interaction as fixed effects. Data on cows having a lactation length less than 250 d were not included in the analysis.

Data on BCS were analyzed with the MIXED procedure of SAS using repeated measures with treatment, parity, block, herd, time as well as treatment × parity, treatment × time, parity × time, and treatment × parity × time interactions as fixed effects. Time periods were defined as follows: (1) 27–14 d before calving; (2) 13–0 d before calving; (3) 1–14 DIM; (4) 15–28 DIM; (5) 29–42 DIM; (6) 43–56 DIM; (7) 57–70 DIM; (8) 71–84 DIM; and (9) 85–98 DIM. Seven covariance structures were tried out (CS, CSH, AR(1), ARH(1), TOEP, TOEPH, and UN). Unstructured covariance structure was chosen because fit statistics were the smallest.

Data on estimated BW were analyzed with the MIXED procedure of SAS using repeated measures with unequal time intervals. Fixed effects were as described previously for BCS analysis. Eight different covariance structures were compared (SP(POW), SP(GAU), SP(EXP), SP(LIN), SP(LINL), SP(SPH), ANTE(1), and UN); ANTE(1) was chosen because fit statistics were the smallest. Estimated BW changes between 21 d before calving until 7 d after calving and 7 d after calving until 55 DIM were also analyzed separately with the MIXED procedure of SAS with treatment, parity, block, herd, and treatment × parity interaction as fixed effects.

When the interaction treatment × parity was significant or a tendency, the SLICE option in the LSMEANS

statement of SAS was used to help interpretation. Results were considered significant when $P \leq 0.05$ and as a tendency at $0.05 < P \leq 0.10$.

3. Results

3.1. Milk yield and components

Average milk yield during the first 60 DIM was 35.0 ± 0.3 kg/d and was unaffected by treatment ($P=0.68$). As expected, multiparous cows had a greater milk production than primiparous cows ($P < 0.0001$; Table 2); 29.7 ± 0.5 and 40.3 ± 0.4 kg/d for primiparous and multiparous cows, respectively. Energy-corrected milk tended to be lower in cows receiving the vitamin supplement than in control cows ($P=0.06$). Indeed, for the first 60 DIM, ECM was 34.0 ± 0.3 and 34.9 ± 0.3 kg/d for the vitamin group and control, respectively. The vitamin supplement decreased milk fat concentration from 42.1 to 40.3 ± 0.4 g/kg ($P=0.004$; Fig. 1a) but increased milk protein concentration from 30.9 to 31.5 ± 0.2 g/kg ($P=0.04$; Fig. 1b). No treatment effect on milk lactose concentration was observed ($P=0.22$; Table 2). Daily yields of protein and lactose secreted in milk did not differ between treatments ($P > 0.42$; Table 2) and were greater for multiparous cows than primiparous cows ($P < 0.0001$). A tendency for a lower daily milk yield of fat for primiparous cows that received the vitamin supplement was observed as compared with controls but no effect was observed in multiparous cows (treatment × parity interaction; $P=0.08$).

The average fat:protein ratio was decreased by 0.06 by the folic acid and vitamin B-12 supplement ($P=0.001$; Table 2). However, the fat:protein ratio decrease in response to the vitamin supplementation tended to be greater for primiparous than for multiparous cows (treatment × parity interaction; $P=0.07$). No effects of treatment, parity as well as treatment × parity interaction on milk urea nitrogen were observed ($P > 0.15$; Table 2).

Lactation length was not different between treatments and parity (treatment × parity interaction, $P=0.33$). The current 305-d milk yield was not affected by treatment nor was 305-d protein yield ($P \geq 0.31$; Table 3). Pertaining to 305-d milk fat yield, a tendency treatment × parity interaction was observed ($P=0.08$; Table 3). The 305-d milk fat yield for primiparous cows was decreased by 16 kg by the vitamin supplement ($P=0.03$) but no effect was noted for multiparous cows ($P=0.94$). Curves of ECM for the 305-d yield for primiparous and multiparous cows are shown in Fig. 2a,b (treatment × parity interaction, $P=0.20$). Primiparous cows had a lower ECM peak but a better persistency than multiparous cows.

3.2. Body condition score and estimated body weight

At 21 ± 8 d before calving, average BCS were 3.76 and 3.23 ± 0.03 for primiparous and multiparous cows, respectively, and were not different between treatment groups ($P=0.69$). The BCS at 7 ± 4 DIM were not affected by treatment ($P=0.12$; Table 4). The vitamin supplement tended to increase BCS over time (treatment × time interaction, $P=0.07$). There was no treatment effect on BCS before calving ($P \geq 0.45$) but BCS of cows receiving the vitamin supplement tended to be higher by 0.04 unit after calving ($P \leq 0.08$) as compared with control cows. Despite BCS being higher for primiparous cows throughout the studied period, they lost more BCS after calving than did multiparous cows (0.92 ± 0.03 vs. 0.72 ± 0.02 for primiparous and multiparous cows, respectively; parity × time interaction, $P < 0.0001$).

As expected, at 21 ± 8 d before calving, primiparous cows were lighter ($P < 0.0001$); 644.2 ± 4.9 and 696.5 ± 3.5 kg of estimated BW for primiparous and multiparous cows, respectively. Treatment × time interaction on estimated BW was significant ($P=0.01$). Estimated BW losses among time periods were subsequently calculated to understand this interaction. Estimated BW losses 21 d before calving to 7 d postpartum did not differ between treatments ($P=0.29$;

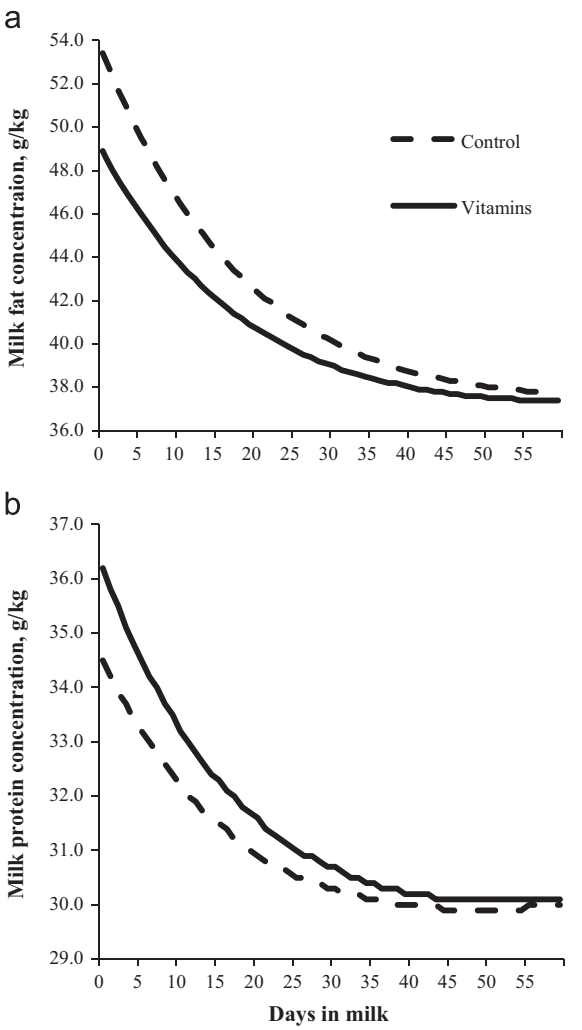

Fig. 1. Effects of a supplementation in folic acid and vitamin B-12 during the first 60 days in milk on milk fat (a; $P=0.004$) and milk protein (b; $P=0.04$) concentrations. Control = 5 mL of saline 0.9% NaCl; Vitamins = 3 mL of 320 mg of folic acid and 2 mL of 10 mg of vitamin B-12. The average standard errors were 0.4 and 0.2 g/kg for milk fat concentration and milk protein concentration curves, respectively.

Table 3
Lactation length, 305-d milk and component yields according to parity and treatments (adjusted mean ± SE).

Item	Treatment[a] (Trt)				*P*-value		
	Primiparous		Multiparous				
	Control	Vitamins	Control	Vitamins	Trt	Parity	Trt × Parity
Number of cows[b]	118	113	202	218			
Lactation length[c], *d*	351 ± 6	356 ± 5	361 ± 5	355 ± 5	0.99	0.40	0.33
Milk yield (305 d)[c], kg	8782 ± 124	8655 ± 126	10,396 ± 94	10,306 ± 91	0.31	< 0.0001	0.86
Fat yield (305 d)[c,d], kg	357 ± 5	341 ± 5	405 ± 4	405 ± 4	0.07	< 0.0001	0.08
Protein yield (305 d)[c], kg	286 ± 4	281 ± 4	335 ± 3	333 ± 3	0.35	< 0.0001	0.61

[a] 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 B-12.
[b] Cows with lactation length less than 250 d were not included.
[c] Herd effect, $P < 0.0001$.
[d] The vitamin supplement reduced 305-d milk fat yield in primiparous cows as compared with control ($P = 0.03$) whereas no treatment effect was observed for multiparous cows ($P = 0.94$).

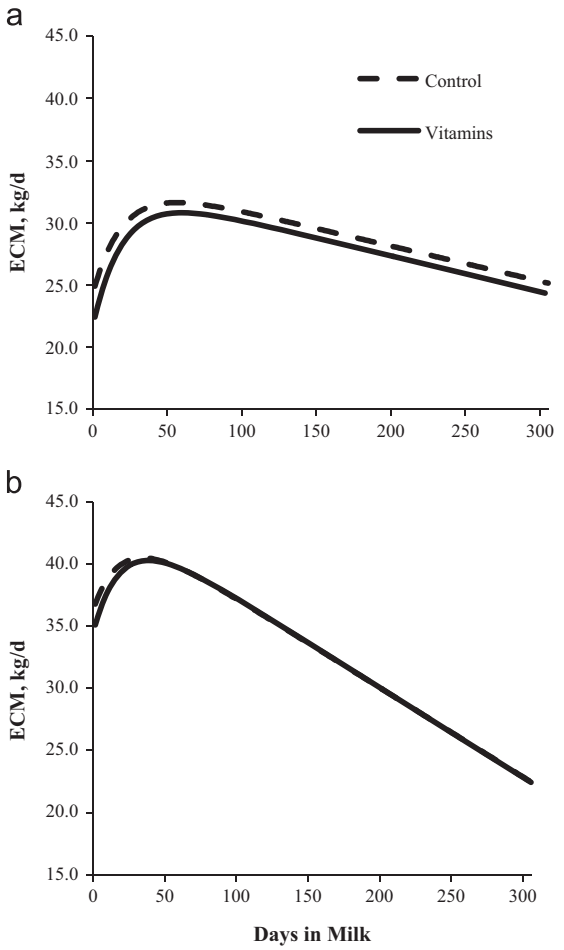

Fig. 2. Energy corrected milk (ECM) 305-d lactation curves for primiparous (a) and multiparous (b) cows according to treatments (treatment × parity interaction, $P = 0.20$). Control = 5 mL of saline 0.9% NaCl; Vitamins = 3 mL of 320 mg of folic acid and 2 mL of 10 mg of vitamin B-12. ECM (kg/d) = 12.55 × fat (kg/d) + 7.39 × protein (kg/d) + 5.34 × lactose yield (kg/d). The average standard errors were 0.5 and 0.3 kg/d for primiparous and multiparous curves, respectively.

Table 4) and averaged 26.0 and 28.7 ± 2.7 kg for vitamin and control groups, respectively. The vitamin supplement reduced estimated BW losses after calving, from 7 to 55 DIM, by 7.5 kg ($P = 0.007$) as compared with control cows. Estimated BW losses were 22.8 and 30.3 ± 2.9 kg for vitamin and control groups, respectively.

3.3. Variation among herds

Responses to treatments among the 15 herds involved in this experiment are illustrated in Figs. 3 and 4. Milk fat concentration and estimated BW loss decreased in 12 herds out of 15 and milk protein concentration increased in 9 herds out of 15 following the vitamin supplementation (Figs. 3a, b and 4). The variability among herd responses is huge, especially for milk fat concentration and estimated BW loss differences. Among herds in which the vitamin supplement decreased milk fat concentration and estimated BW loss, the diminution varied from −0.6 to −5.5 g/kg and from −1.4 to −21.0 kg, respectively. On the other hand, the vitamin supplement increased the protein concentration of milk from 0.3 to 2.9 g/kg in 9 herds.

4. Discussion

The folic acid and vitamin B-12 supplement administrated by intramuscular injections did not increase milk yield within the first 60 DIM and 305-d lactation period in commercial herds. Milk fat concentration was decreased during the first 60 DIM for both primiparous and multiparous cows receiving the vitamin supplement. In contrast, in a previous study, a significant increase of milk production of 3.4 kg/d during the first 8 wk of lactation was observed for multiparous cows fed dietary folic acid, alone or in combination with vitamin B-12 given around parturition and in early lactation (Graulet et al., 2007). Moreover, Preynat et al. (2009b) observed that a combined supplement of folic acid and vitamin B-12 injected intramuscularly every week tended to increase milk production during the first 16 wk of lactation in multiparous cows. In these two studies, the combined supplement of folic acid and vitamin B-12 had no effect on milk fat concentration (Graulet et al., 2007; Preynat et al., 2009b). The discrepancy among these trials is difficult to explain as, in the current study, no blood measurements were performed to analyze folic acid and vitamin B-12 status of cows. Responses among herds

Table 4
Body condition score (BCS) and estimated body weight (BW) after calving and estimated BW losses before and after calving (adjusted mean ± SE).

Item	Treatment[a] (Trt)				*P*-value		
	Primiparous		Multiparous				
	Control	Vitamins	Control	Vitamins	Trt	Parity	Trt × Parity
Number of cows	136	135	263	271			
BCS at 7 DIM[b,c,d]	3.41 ± 0.05	3.43 ± 0.05	2.97 ± 0.05	3.03 ± 0.05	0.12	< 0.0001	0.55
Estimated BW at 7 DIM[c,d,e], kg	599.7 ± 5.6	592.1 ± 5.6	664.7 ± 4.0	664.2 ± 4.0	0.56	< 0.0001	0.73
Estimated BW loss,[e] kg							
Before calving until 7 DIM[d,f]	33.9 ± 3.5	30.6 ± 3.6	23.4 ± 2.9	21.4 ± 2.9	0.29	0.0001	0.81
After calving[d,g]	32.5 ± 3.8	24.7 ± 3.8	28.1 ± 3.1	21.0 ± 3.1	0.007	0.14	0.89

[a] 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 B-12.
[b] According to a 1 (very thin) to 5 (very fat) scale with quarter points (Wildman et al., 1982; Ferguson et al., 1994).
[c] Measured at the first visit after calving on average at 7 ± 4 days in milk (DIM).
[d] Herd effect, $P \leq 0.002$.
[e] Estimated using calibrated tapes measuring heart girth circumference.
[f] Loss in estimated BW between 21 ± 8 d before calving until 7 ± 4 d after calving.
[g] Loss in estimated BW 7 ± 4 d after calving until 55 ± 13 DIM.

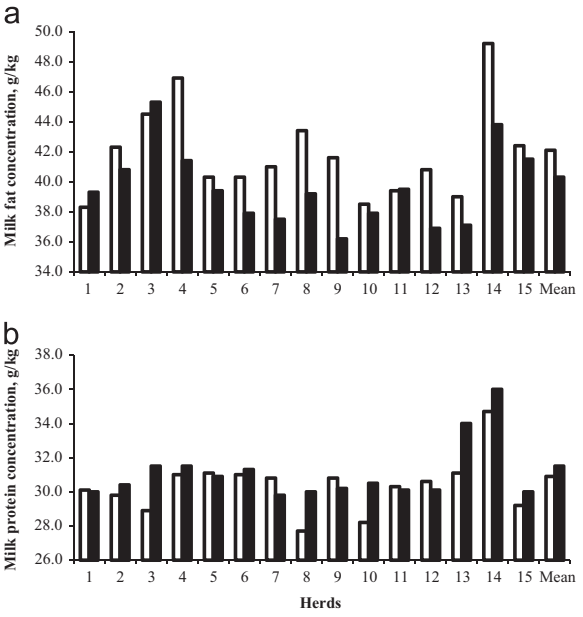

Fig. 3. Milk fat (a) and milk protein (b) concentrations during the first 60 days in milk according to treatments (open bars = control (5 mL of saline 0.9% NaCl) and closed bars = vitamins (3 mL of 320 mg of folic acid and 2 mL of 10 mg of vitamin B-12)) among the 15 herds involved and the mean (herd effect; $P < 0.0001$). Number of cows per herd involved in the experiment was: 1 = 30; 2 = 50; 3 = 81; 4 = 47; 5 = 129; 6 = 47; 7 = 48; 8 = 45; 9 = 44; 10 = 54; 11 = 27; 12 = 31; 13 = 75; 14 = 14; and 15 = 83.

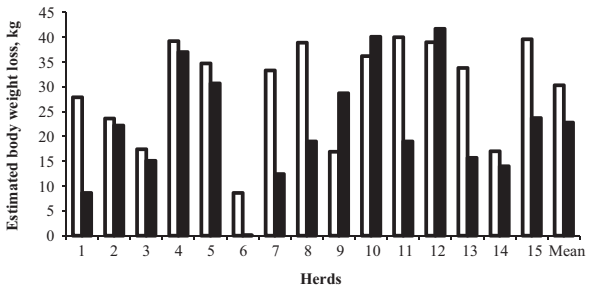

Fig. 4. Estimated body weight loss from 7 to 55 days in milk according to treatments (open bars = control (5 mL of saline 0.9% NaCl) and closed bars = vitamins (3 mL of 320 mg of folic acid and 2 mL of 10 mg of vitamin B-12)) among the 15 herds involved and the mean (herd effect; $P = 0.002$). Number of cows per herd involved in the experiment was: 1 = 30; 2 = 50; 3 = 81; 4 = 47; 5 = 129; 6 = 47; 7 = 48; 8 = 45; 9 = 44; 10 = 54; 11 = 27; 12 = 31; 13 = 75; 14 = 14; and 14 = 83.

were consistent regarding milk fat concentration within the first 60 DIM but the extent of the response was variable among herds. This variability among herds highlights the fact that the actual state of knowledge does not allow predicting ruminal synthesis and supply of folic acid and vitamin B-12 based on diet characteristics.

Milk protein concentration increased within the first 60 DIM for cows receiving the vitamin supplement as compared with control ones for the majority of herds involved in this experiment. Similarly, several studies reported that a supplement of folic acid in combination with vitamin B-12 increases milk protein concentration, yield, or both (Graulet et al., 2007; Preynat et al., 2009b).

Girard and Matte (2005a), Graulet et al. (2007) and Preynat et al. (2010; 2009a) concluded that a combined supplement of folic acid and vitamin B-12 given during the transition period and in early lactation improved metabolic efficiency of dairy cows. An explanation could be that vitamin B-12 is involved as a coenzyme for the entry of propionate into the Krebs cycle for providing energy (Scott, 1999), and subsequently being used for gluconeogenesis. Propionate is the major glucogenic precursor (Reynolds, 2006) and accounts for 50–60% of glucose flow in ruminants (Danfær et al., 1995).

In the literature, it is reported that cows with an excessive negative EB in early lactation generally produce a higher milk fat concentration and lower milk protein concentration (de Vries and Veerkamp, 2000; Grieve et al., 1986; Gross et al., 2011). In early lactation, milk production requires more energy than what can be provided by DM intake. This results in a negative EB leading to a mobilization of body fat reserves

to meet requirements for milk production and maintenance (Butler and Smith, 1989). Fat from body reserves can be taken up by the mammary gland and secreted in milk (Bauman and Griinari, 2003; Remppis et al., 2011) and can increase thereafter milk fat concentration. On the other hand, protein synthesis in the mammary gland requires ATP (Lemosquet et al., 2010). Coulon and Rémond (1991) reported that, in early lactation, milk protein concentration increases linearly with energy supply. By decreasing milk fat concentration and increasing milk protein concentration as compared with control cows, it could be hypothesized that the vitamin supplement changed energy partitioning in early lactation.

The fat:protein ratio is a good indicator of the energy status of dairy cows in early lactation (Buttchereit et al., 2010; Heuer et al., 2000). Therefore, in the present study, the lower fat:protein ratio among cows receiving the vitamin supplement could indicate a better energy status as compared with control cows. The reason why the decrease of the fat:protein ratio over the first 60 DIM in response to the vitamin supplement was greater for primiparous cows as compared to multiparous cows remains unclear.

As indicators of body fat mobilization and EB, the reduced losses of estimated BW and the higher BCS of cows receiving the vitamin supplement observed in early lactation are in accordance with the lower milk fat concentration as compared with control cows. These results suggest that there was possibly less mobilization of body fat reserves for cows in the vitamin group and the response was consistent among herds. However, average differences of BCS and estimated BW changes between treatments are low, especially for BCS. As weigh scales were not available on farms, BW was estimated by heart girth circumference measurements. Yan et al. (2009) concluded that, in lactating dairy cows from different parities and stages of lactation, heart girth circumference had a strong relationship with BW as the correlation coefficient between these two variables was 0.88.

Graulet et al. (2007) and Preynat et al. (2009b) observed no treatment effect on pre- and post-calving BW and BCS for cows receiving a combination of folic acid and vitamin B-12 supplement. However, in the present study, the folic acid and vitamin B-12 supplement significantly decreased BW losses from 7 until 55 DIM and tended to diminish BCS losses. These differences among experiments could be partially explained by the number of animals involved in each study. The BCS at calving for multiparous cows was similar to figures reported by Santschi et al. (2011). Řehák et al. (2012) observed similar BW changes from 1 to 8 wk after parturition. As in the current study, Adrien et al. (2012) and Janovick and Drackley (2010) reported that before calving, multiparous cows were heavier and had a lower BCS than primiparous cows. The reason why primiparous cows lost more BCS than multiparous cows in the present experiment remains difficult to explain as results from VandeHaar et al. (1999) showed opposite results. However, it could be explained by primiparous cows having a higher BCS at calving than multiparous cows. A review made by Broster and Broster (1998) revealed that the larger is the BCS at calving, the greater the loss of BCS would be over the first 60–70 DIM.

A limitation of this study is that DM intake could not be recorded. However, several studies showed that folic acid and vitamin B-12 supplementation did not affect DM intake in early lactation (Girard and Matte, 2005a; Graulet et al., 2007; Preynat et al., 2009a, 2009b).

5. Conclusion

Supplementation of folic acid and vitamin B-12 given 21 d before the expected calving date until 60 DIM did not increase milk yield of dairy cows in early lactation and during the 305-d lactation period in commercial dairy herds. However, the decrease of milk fat concentration and the increase of milk protein concentration leading to a lower fat:protein ratio in cows receiving the vitamin supplement suggest that the supplement changed energy partitioning in early lactation. The reduced loss of estimated BW after calving in dairy cows receiving folic acid and vitamin B-12 as compared with control cows is in agreement with that statement. However, as the experimental design did not allow measuring DM intake, it cannot be ruled out that the change in energy partitioning was due to an increased DM intake in cows receiving the folic acid and vitamin B-12 supplement.

Conflict of interest statement

None.

Acknowledgments

The authors acknowledge the invaluable participation of dairy producers, veterinarians and nutritionists in this project. We are also grateful to Professor Roger I. Cue (Department of Animal Science, McGill University, Ste-Anne-de-Bellevue, QC, Canada) for statistical advices. We thank Véronique Roy (Agriculture et Agroalimentaire Canada, Sherbrooke, QC, Canada) for preparing folic acid solutions, and Vétoquinol (Lavaltrie, QC, Canada) for generously providing vitamin B-12. This project has been made possible through the support from Conseil pour le développement de l'agriculture du Québec (CDAQ). Funding was provided through Agriculture and Agri-Food Canada's Advancing Canadian Agriculture and Agri-Food (ACAAF) program.

References

Adrien, M.L., Mattiauda, D.A., Artegoitia, V., Carriquiry, M., Motta, G., Bentancur, O., Meikle, A., 2012. Nutritional regulation of body condition score at the initiation of the transition period in primiparous and multiparous dairy cows under grazing conditions: milk production, resumption of post-partum ovarian cyclicity and metabolic parameters. Animal 6, 292–299.

Bauman, D.E., Griinari, J.M., 2003. Nutritional regulation of milk fat synthesis. Annu. Rev. Nutr. 23, 203–227.

Bechdel, S.I., Honeywell, H.E., Dutcher, R.A., Knutsen, M.H., 1928. Synthesis of vitamin B in the rumen of the cow. J. Biol. Chem. 80, 231–238.

Broster, W.H., Broster, V.J., 1998. Body score of dairy cows. J. Dairy Res. 65, 155–173.

Butler, W.R., Smith, R.D., 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. J. Dairy Sci. 72, 767–783.

Buttchereit, N., Stamer, E., Junge, W., Thaller, G., 2010. Evaluation of five lactation curve models fitted for fat:protein ratio of milk and daily energy balance. J. Dairy Sci. 93, 1702–1712.

Canadian Council on Animal Care, 2009. In: Rolfert, E. D., Cross, B. M., McWilliam A. A. (Ed.), Guide to the care and use of experimental animals, vol. 1. 2nd ed. Canadian Council on Animal Careal, Ottawa, Ontario, Canada.

Coulon, J.B., Rémond, B., 1991. Variations in milk output and milk protein content in response to the level of energy supply to the dairy cow: a review. Livest. Prod. Sci. 29, 31–47.

Danfær, A., Tetens, V., Agergaard, N., 1995. Review and an experimental study on the physiological and quantitative aspects of gluconeogenesis in lactating ruminants. Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 111, 201–210.

de Vries, M.J., Veerkamp, R.F., 2000. Energy balance of dairy cattle in relation to milk production variables and fertility. J. Dairy Sci. 83, 62–69.

Duffield, T.F., Kelton, D.F., Leslie, K.E., Lissemore, K.D., Lumsden, J.H., 1997. Use of test day milk fat and milk protein to detect subclinical ketosis in dairy cattle in Ontario. Can. Vet. J. 38, 713–718.

Duplessis, M., Girard, C.L., Santschi, D.E., Laforest, J.-P., Durocher, J., Pellerin, D., 2014. Effects of folic acid and vitamin B_{12} supplementation on culling rate, diseases, and reproduction in commercial dairy herds. J. Dairy Sci. 97, 2346–2354.

Eckles, C.H., Williams, V.M., 1925. Yeast as a supplementary feed for lactating cows. J. Dairy Sci. 8, 89–93.

Ender, F., Dishington, I.W., Helgebostad, A., 1971. Calcium balance studies in dairy cows under experimental induction and prevention of hypocalcaemic paresis puerperalis. The solution of the aetiology and the prevention of milk fever by dietary means. Z. Tierphysiol. Tierernahr. Futterm. 28, 233–256.

Ferguson, J.D., Galligan, D.T., Thomsen, N., 1994. Principal descriptors of body condition score in Holstein cows. J. Dairy Sci. 77, 2695–2703.

Girard, C.L., Matte, J.J., 1999. Changes in serum concentrations of folates, pyridoxal, pyridoxal-5-phosphate and vitamin B_{12} during lactation of dairy cows fed dietary supplements of folic acid. Can. J. Anim. Sci. 79, 107–113.

Girard, C.L., Matte, J.J., 2005a. Effects of intramuscular injections of vitamin B_{12} on lactation performance of dairy cows fed dietary supplements of folic acid and rumen-protected methionine. J. Dairy Sci. 88, 671–676.

Girard, C.L., Matte, J.J., 2005b. Folic acid and vitamin B_{12} requirements of dairy cows: a concept to be revised. Livest. Prod. Sci. 98, 123–133.

Girard, C.L., Matte, J.J., Tremblay, G.F., 1989. Serum folates in gestating and lactating dairy cows. J. Dairy Sci. 72, 3240–3246.

Goff, J.P., Horst, R.L., 1997. Physiological changes at parturition and their relationship to metabolic disorders. J. Dairy Sci. 80, 1260–1268.

Graulet, B., Matte, J.J., Desrochers, A., Doepel, L., Palin, M.-F., Girard, C.L., 2007. Effects of dietary supplements of folic acid and vitamin B_{12} on metabolism of dairy cows in early lactation. J. Dairy Sci. 90, 3442–3455.

Grieve, D.G., Korver, S., Rijpkema, Y.S., Hof, G., 1986. Relationship between milk composition and some nutritional parameters in early lactation. Livest. Prod. Sci. 14, 239–254.

Gross, J., van Dorland, H.A., Bruckmaier, R.M., Schwarz, F.J., 2011. Milk fatty acid profile related to energy balance in dairy cows. J. Dairy Res. 78, 479–488.

Heuer, C., Van Straalen, W.M., Schukken, Y.H., Dirkzwager, A., Noordhuizen, J.P.T.M., 2000. Prediction of energy balance in a high yielding dairy herd in early lactation: model development and precision. Livest. Prod. Sci. 65, 91–105.

Janovick, N.A., Drackley, J.K., 2010. Prepartum dietary management of energy intake affects postpartum intake and lactation performance by primiparous and multiparous Holstein cows1. J. Dairy Sci. 93, 3086–3102.

Kon, S.K., Porter, J.W.G., 1954. The intestinal synthesis of vitamins in the ruminants. Vitam. Horm. 12, 53–67.

Lemosquet, S., Guinard-Flament, J., Raggio, G., Hurtaud, C., Van Milgen, J., Lapierre, H., 2010. How does increasing protein supply or glucogenic nutrients modify mammary metabolism in lactating dairy cows? In: Proceedings of the 3rd EAAP International Symposium on Energy and Protein Metabolism, Parma, Italy.

National Research Council, 2001. Nutrient Requirements of Dairy Cattle, 7th revised ed. National Academy Press, Washington, DC.

Ospina, P.A., Nydam, D.V., Stokol, T., Overton, T.R., 2010. Association between the proportion of sampled transition cows with increased nonesterified fatty acids and β-hydroxybutyrate and disease incidence, pregnancy rate, and milk production at the herd level. J. Dairy Sci. 93, 3595–3601.

Preynat, A., Lapierre, H., Thivierge, M.C., Palin, M.F., Cardinault, N., Matte, J.J., Desrochers, A., Girard, C.L., 2010. Effects of supplementary folic acid and vitamin B_{12} on hepatic metabolism of dairy cows according to methionine supply. J. Dairy Sci. 93, 2130–2142.

Preynat, A., Lapierre, H., Thivierge, M.C., Palin, M.F., Matte, J.J., Desrochers, A., Girard, C.L., 2009a. Effects of supplements of folic acid, vitamin B_{12}, and rumen-protected methionine on whole body metabolism of methionine and glucose in lactating dairy cows. J. Dairy Sci. 92, 677–689.

Preynat, A., Lapierre, H., Thivierge, M.C., Palin, M.F., Matte, J.J., Desrochers, A., Girard, C.L., 2009b. Influence of methionine supply on the response of lactational performance of dairy cows to supplementary folic acid and vitamin B_{12}. J. Dairy Sci. 92, 1685–1695.

Řehák, D., Volek, J., Bartoň, L., Vodková, Z., Kubešová, M., Rajmon, R., 2012. Relationships among milk yield, body weight, and reproduction in Holstein and Czech Fleckvieh cows. Czech J. Anim. Sci. 57, 274–282.

Remppis, S., Steingass, H., Gruber, L., Schenkel, H., 2011. Effects of energy intake on performance, mobilization and retention of body tissue, and metabolic parameters in dairy cows with special regard to effects of pre-partum nutrition on lactation – A review. Asian–Aust. J. Anim. Sci. 24, 540–572.

Reynolds, C.K., 2006. Production and metabolic effects of site of starch digestion in dairy cattle. Anim. Feed Sci. Technol. 130, 78–94.

Santschi, D.E., Lefebvre, D.M., Cue, R.I., Girard, C.L., Pellerin, D., 2011. Complete-lactation milk and component yields following a short (35-d) or a conventional (60-d) dry period management strategy in commercial Holstein herds. J. Dairy Sci. 94, 2302–2311.

SAS Institute, 2008. User's Guide: Statistics. SAS Institute, Cary, NC (Version9.2).

Scott, J.M., 1999. Folate and vitamin B_{12}. Proc. Nutr. Soc. 58, 441–448.

Tamminga, S., Luteijn, P.A., Meijer, R.G.M., 1997. Changes in composition and energy content of liveweight loss in dairy cows with time after parturition. Livest. Prod. Sci. 52, 31–38.

Tyrrell, H.F., Reid, J.T., 1965. Prediction of the energy value of cow's milk. J. Dairy Sci. 48, 1215–1223.

Valacta., 2011. L'effet de la synergie. L'évolution de la production laitière québécoise 2010. Le producteur de lait québécois.

VandeHaar, M.J., Yousif, G., Sharma, B.K., Herdt, T.H., Emery, R.S., Allen, M.S., Liesman, J.S., 1999. Effect of energy and protein density of prepartum diets on fat and protein metabolism of dairy cattle in the periparturient period. J. Dairy Sci. 82, 1282–1295.

Weber, C., Hametner, C., Tuchscherer, A., Losand, B., Kanitz, E., Otten, W., Singh, S.P., Bruckmaier, R.M., Becker, F., Kanitz, W., Hammon, H.M., 2013. Variation in fat mobilization during early lactation differently affects feed intake, body condition, and lipid and glucose metabolism in high-yielding dairy cows. J. Dairy Sci. 96, 165–180.

Weiss, W.P., Ferreira, G., 2006. Water soluble vitamins for dairy cattle. In: Proceedings Tristate Dairy Nutrition Conference, Ft Wayne, IN. pp. 51–63.

Wildman, E.E., Jones, G.M., Wagner, P.E., Boman, R.L., Troutt Jr., H.F., Lesch, T.N., 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65, 495–501.

Wilmink, J.B.M., 1987. Adjustment of test-day milk, fat and protein yield for age, season and stage of lactation. Livest. Prod. Sci. 16, 335–348.

Yan, T., Mayne, C.S., Patterson, D.C., Agnew, R.E., 2009. Prediction of body weight and empty body composition using body size measurements in lactating dairy cows. Livest. Sci. 124, 233–241.